الجمهورية الجزائرية الديمقراطية الشعبية

Ministry of Higher Education and Scientific Research

Ibn Khaldoun University–Tiaret-

Faculty of Nature and Life Sciences

Department of Biology


3rd Cycle Doctoral Thesis (L.M.D)

Option: Food Biotechnology

## Theme

# Study of combined effect of some medicinal plants and probiotics against *Helicobacter pylori* responsible for gastroduodenal diseases


Presented by:

**Mrs. BOUHENNI Hasna**


Date of defense : 15/03/2023

Approved by:

| | | | |
|---|---|---|---|
| President: | Mr. HAMMOUDI Abdelhamid | Professor | University of Tiaret |
| Thesis Director: | Mrs. DOUKANI Koula | Professor | University of Tiaret |
| Co-Director: | Mrs. TABAK Souhila | Associate Professor | University of Tiaret |
| Examinators: | Mr. BEKADA Ahmed Med Ali | Professor | University of Tissemsilt |
| | Mr. BAHRI Fouad | Professor | University of Mostaganem |
| | Mrs. ARABI Zohra | Associate Professor | University of Tiaret |

2022-2023

# Aknowledgements

First and foremost, praises and thanks to the God, the Almighty, "Allah" for blessings and help to complete the research successfully.

I would like to express my deep and sincere gratitude to my research supervisor, **Pr. DOUKANI Koula**, Professor at Ibn Khaldoun University, Tiaret, for the opportunity to do this work and providing valuable guidance. Which her dynamism, sincerity and motivation have deeply inspired me. He taught me the methodology to carry out the research and to present the research works as clearly as possible. I would also like to thank her for her friendship and empathy. I also extend my heartfelt thanks for her acceptance, comprehension, patience and unique support during all the process. It was a great privilege and honor to work and study under her guidance. I am extremely grateful for what she has offered to me. The time I had with her on research work and thesis preparation.

I wish to express my gratitude to **Pr. HAMMOUDI Abdelhamid, Pr. BEKADA Ahmed Mohamed Ali, Pr. BAHRI Fouad,** and **Dr. ARABI Zohra** for accepting judging this work.

I would like to thank **Dr. HEMIDA Houari** and **Dr. BOUMEZRAG Assia** for their help and constant encouragement.

Foremost, I would like to express my sincere gratitude to **Pr. ŞEKEROĞLU Nazım** and **Dr. GEZICI Sevgı** from the Advanced Technology Application and Research Center, Department of Food Engineering, Faculty of Engineering and Architecture, Kilis 7 Aralik University, Kilis, Turkey; and also **Pr. SPINU Marina** and **Dr. PALL Emoke** from the Department of Infectious Diseases and Clinical Sciences, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania for the continuous support to realize my PhD study and research.

I'd like to thank **Dr. DAHOU Abdelkader ElAmine**, **Dr. HELLAL Nouria** and **Dr. SOUDANI Leila** for their help to complete this project. Also I'd like to thank the technical and support staff in the Biology department, Faculty of Nature and Life Sciences, University of Ibn Khaldoun, Tiaret, especially **Mr. SAID Abdelkader** and **Mr. KHAN Mohamed** for all their assistance and continuous help.

I express my thanks to all my professors of Faculty of Nature and Life Sciences who gave me the necessary knowledge.

I am very much thankful to my family members for their love, understanding, prayers and continuing support to complete this research work.

Finally, my thanks go to all the people who have supported me to complete the research work directly or indirectly.

## Table of Contents

List of abbreviations — i

List of tables — iv

List of figures — vi

List of annexes — viii

Introduction

**Part I:**

**Literature Review**

**Chapter -I-**

**Generalities on *Helicobacter pylori***

I.1 History — - 4 -

I.2 Taxonomy and classification — - 5 -

I.3 Characteristics of *H. pylori* — - 6 -

I.3.1 Morphological and physiological — - 6 -

I.3.2 Cultural — - 8 -

I.3.3 Biochemical — - 10 -

I.3.4 Genetical — - 10 -

I.4 Habitat — - 12 -

I.5 Epidemiology of *H. pylori* infection — - 12 -

I.5.1 Prevalence — - 13 -

I.5.2 Transmission pathways — - 14 -

I.6 Pathogenesis — - 15 -

I.6.1 Adherence and colonisation — - 15 -

I.6.2 Virulence factors — - 16 -

I.6.2.1 Cytotoxin-associated gene pathogenicity island (cagPAI) — - 17 -

I.6.2.2 Vacuolating cytotoxin VacA — - 17 -

I.6.2.3 Outer membrane proteins — - 19 -

I.6.3 *Helicobacter*-induced gastric pathology in humans — - 20 -

I.6.3.1 Superficial gastritis — - 20 -

I.6.3.2 Peptic ulcer disease — - 21 -

I.6.3.3 Gastric adenocarcinoma and its precursor lesions — - 21 -

I.6.3.4 MALT lymphoma — - 23 -

I.7 Diagnosis of *H. pylori* infection — - 24 -

I.7.1 Invasive tests — - 24 -

I.7.1.1 Gastric biopsies — - 24 -

I.7.1.2 Molecular biology techniques — - 25 -

I.7.2 Non-Invasive tests — - 25 -

I.7.2.1 Urea breath tests (UBT) - 26 -
I.7.2.2 Serological tests - 26 -
I.7.2.3 Stool antigen test (HpSA) - 27 -
I.7.3 Other non-invasive tests - 28 -
I.8 Treatment - 28 -
I.8.1 First-line therapy - 28 -
I.8.2 Second-line treatment - 31 -
I.8.3 Alternative therapies - 32 -
I.8.3.1 Phytomedicines - 32 -
I.8.3.2 Probiotics - 35 -

**Chapter -II-**

**General facts on probiotics**

II.1 History - 37 -
II.2 Definition - 38 -
II.3 Taxonomy and classification - 39 -
II.3.1 Yeasts - 39 -
II.3.2 Lactic Acid Bacteria (LAB) - 39 -
II.3.2.1 Physiology and morphology - 42 -
II.3.2.2 Ecology and habitat - 43 -
II.3.2.3 Properties - 44 -
II.3.2.4 Different genus of LAB and their metabolic properties - 45 -
II.3.2.4.1 *Lactobacillus* *- 45 -*
II.3.2.4.2 *Bifidobacterium* *- 46 -*
II.3.2.4.3 *Leuconostoc* *- 48 -*
II.3.2.4.4 *Lactococcus* - 49 -
II.3.2.4.5 *Streptococcus* - 50 -
II.3.2.4.6 *Pediococcus* - 51 -
II.3.2.4.7 *Oenococcus* - 52 -
II.3.2.4.8 *Weissella* - 53 -
II.4 Beneficial effects - 53 -
II.4.1 Effects on gastrointestinal tract - 54 -
II.4.2 Effects on lactose intolerance and malabsorption - 54 -
II.4.3 Effects on diarrheal diseases - 55 -
II.4.4 Role of LAB in treating ulcer - 55 -
II.4.5 Effects of LAB on human immune system - 56 -
II.4.6 Antifungal activity - 57 -
II.4.7 Role of LAB in preventing colon cancer - 57 -

II.4.8 Inhibition of intestinal pathogens - 58 -
II.5 Mechanism action of LAB - 59 -

## Chapter -III-
## Medicinal Plants

III.1 *Allium sativum* .L (Garlic) - 60 -
III.1.1 History - 60 -
III.1.2 Origin and distribution - 60 -
III.1.3 Botanical description - 60 -
III.1.4 Taxonomy and classification - 61 -
III.1.5 Habitat and ecology - 62 -
III.1.6 Varieties - 63 -
III.1.7 Chemical composition - 63 -
III.1.7.1 Physicochemical - 63 -
III.1.7.2 Phytochemical - 65 -
III.1.8 Pharmacological properties - 67 -
III.1.8.1 Antioxidant - 69 -
III.1.8.2 Antibacterial - 70 -
III.2 *Allium cepa* L. (Onion) - 71 -
III.2.1 History - 71 -
III.2.2 Origin and distribution - 72 -
III.2.3 Botanical description - 72 -
III.2.4 Taxonomy and classification - 73 -
III.2.5 Habitat and ecology - 73 -
III.2.6 Varieties - 74 -
III.2.7 Chemical composition - 74 -
III.2.7.1 Physicochemical - 74 -
III.2.7.2 Phytochemical - 75 -
III.2.8 Pharmacological properties - 77 -
III.2.8.1 Antioxidant - 79 -
III.2.8.2 Antimicrobial - 80 -
III.3 *Trigonella Foenum-groecum* L. (Fenugreek) - 80 -
III.3.1 History - 80 -
III.3.2 Origin and distribution - 81 -
III.3.3 Botanical description - 82 -
III.3.4 Taxonomy and classification - 82 -
III.3.5 Habitat and ecology - 83 -
III.3.6 Varieties - 84 -

III.3.7 Chemical composition - 84 -
III.3.7.1 Physicochemical - 84 -
III.3.7.2 Phytochemical - 85 -
III.3.8 Pharmacological properties - 87 -
III.3.8.1 Antioxidant - 89 -
III.3.8.2 Antimicrobial - 89 -
III.4 *Cuminum cyminum* L. (Cumin) - 90 -
III.4.1 History - 90 -
III.4.2 Origin and distribution - 91 -
III.4.3 Botanical description - 91 -
III.4.4 Taxonomy and classification - 91 -
III.4.5 Habitat and ecology - 92 -
III.4.6 Varieties - 93 -
III.4.7 Chemical composition - 93 -
III.4.7.1 Physicochemical - 93 -
III.4.7.2 Phytochemical - 94 -
III.4.8 Pharmacological properties - 95 -
III.4.8.1 Antioxidant - 95 -
III.4.8.2 Antimicrobial - 97 -

**Part II:**

**Experimental Work**

**Chapter -I-**

**Material and methods**

I. Material and methods - 98 -
I.1 Objectives, site and duration of study - 98 -
I.1.1 Objectives of study - 98 -
I.1.2 Site and duration of study - 98 -
I.2 Material - 98 -
I.2.1 Plant material - 98 -
I.2.2 Probiotic strains - 100 -
I.2.3 *Helicobacter pylori* strains - 101 -
I.2.4 Animals - 102 -
I.3 Methods - 102 -
I.3.1 Experimental protocol - 102 -
I.3.2 Selection of varieties of fenugreek and cumin - 102 -
I.3.2.1 Weight of 1000 seeds - 104 -
I.3.2.2 Germination rate - 104 -

I.3.3 Sample preparation - 104 -
I.3.4 Physico-chemical analyses - 104 -
I.3.5 Phytochemical tests - 109 -
I.3.5.1 Determination of total phenolic content - 110 -
I.3.5.2 Determination of total flavonoids content - 110 -
I.3.5.3 Determination of condensed tannins content - 110 -
I.3.5.4 Determination of hydrolysable tannins content - 111 -
I.3.5.5 Phytochemical screening - 111 -
I.3.6 Determination of phenolic content by High Performance Liquid Chromatography (HPLC) analysis - 111 -
I.3.7 Antioxidant activity - 111 -
I.3.8 Antibacterial part - 113 -
I.3.8.1 Macroscopic examination - 113 -
I.3.8.2 Microscopic examination - 113 -
I.3.8.3 Biochemical identification - 113 -
I.3.8.3.1 Catalase - 113 -
I.3.8.3.2 Urease - 113 -
I.3.8.3.3 Oxidase - 113 -
I.3.8.3.4 API Campy - 114 -
I.3.8.4 Antibiogram - 115 -
I.3.8.5 Genetical identification using PCR - 116 -
I.3.8.5.1 Preparation of samples for PCR amplification - 116 -
I.3.8.5.2 Detection and analysis of amplified DNA products - 116 -
I.3.8.6 Evaluation of anti- *H. pylori* effect of plant extracts - 117 -
I.3.8.6.1 Determination of DZI of plant extracts using disc diffusion method - 117 -
I.3.8.6.2 Determination of MIC of plant extracts with agar dilution method - 117 -
I.3.8.6.3 Determination of MIC and MBC of plant extracts with broth dilution method - 117 -
I.3.8.6.4 Evaluation of growth kinetics of *H. pylori* in presence of plant extracts - 118 -
I.3.8.7 Evaluation of anti- *H. pylori* effect of probiotics (well diffusion assay) - 118 -
I.3.8.7.1 Preparation of cell-free supernatant (CFS) of probiotics - 118 -
I.3.8.7.2 Evaluation of growth kinetics of *H. pylori* in presence of probiotics - 118 -
I.3.8.8 Combined effect of medicinal plants with probiotics on *H. pylori* - 119 -
I.3.8.8.1 Determination of DZI of combined mixtures using disc diffusion method - 119 -
I.3.8.8.2 Evaluation of growth kinetics of *H. pylori* in presence of combined solutions - 119 -
I.3.9 *In vivo* study - 120 -

I.3.9.1 Protocol - 120 -
I.3.9.2 Histopathologic analysis of gastric tissue samples - 120 -
I.3.10 Statistical analysis - 122 -

**Chapter -II-**

**Results and discussion**

II Results and discussion - 98 -
II.1 Selection of varieties - 123 -
II.2 Physicochemical analysis - 123 -
II.3 Minerals analysis - 130 -
II.4 Phytochemical analysis - 132 -
II.5 Phytochemical screening - 138 -
II.6 Chromatographic analysis (HPLC) - 140 -
II.7 Antioxidant activity - 146 -
II.8 Results of isolation and identification of *H. pylori* - 149 -
II.8.1Results of macroscopic and microscopic observation, biochemical identification and antibiogram - 149 -
II.8.2 Results of identification of *H. pylori* by PCR - 151 -
II.8.3 Results of revivication and confirmation of probiotics - 152 -
II.8.4 Results of evaluation of anti- *H. pylori* effect of plant extracts - 156 -
II.8.4.1 Results of determination of DZI of plant extracts using disc diffusion method - 156 -
II.8.4.2 Determination of MIC and MBC of plant extracts - 157 -
II.8.4.3 Results of evaluation of growth kinetics of *H. pylori* in the presence of plant extracts - 157 -
II.8.5 Results of evaluation of anti- *H. pylori* effect of probiotics - 159 -
II.8.5.1 Determination of DZI of probiotics using well diffusion assay - 159 -
II.8.5.2 Results of evaluation of growth kinetics of *H. pylori* in the presence of probiotics - 160 -
II.8.6 Results of combined effect of medicinal plants with probiotics on *H. pylori* - 163 -
II.8.6.1 Determination of DZI of combined mixtures ( plant extracts with probiotics) using disc diffusion method - 163 -
II.8.6.2 Evaluation of growth kinetics of *H. pylori* in presence of combined mixtures (plant extracts with probiotics) - 164 -
II.9 Results of *invivo* study - 168 -
Conclusion - 172 -
References - 175 -
Annexes - 209 -

# List of abbreviations

| | |
|---|---|
| AASEA : | Algerian Association of Sciences in Animal Experimentation |
| ACSOs : | Salk(en)yl-L-cysteine sulfoxides |
| ADH : | Arginine dihydrolase |
| AGE : | Aqueous garlic extract |
| AGS cells : | Adenocarcinoma gastric cell line |
| ARE : | Antioxidant response element |
| *B.* : | *Bifidobacterium* |
| BabA : | Blood group antigen-binding adhesion |
| *C.* : | *Campylobacter* |
| Cag PAI : | Cytotoxin-associated gene pathogenicity island |
| CDX2 : | Caudal-type homeobox 2 |
| CE : | Catechin equivalent |
| CFS : | Cell-free supernatant |
| CLO : | *Campylobacter*-like organism |
| CM: | Cytoplasmic membrane of target cell |
| DCs: | Epithelial and dendritic cells |
| DDS : | Diallyl disulfide |
| DM : | Dry matter |
| DMS : | Diallyl monosulfide |
| DNA : | Deoxyribonucleic acid |
| DPPH : | Diphenyl-1-picrylhydrazyl |
| DTS : | Diallyl trisulfide |
| DTTS : | Diallyl tetrasulfide |
| DZI : | Diameter of zones of inhibition |
| EC : | Electerical conductivity |
| EHPSG : | European *Helicobacter pylori* Study Group |
| ELISA : | Enzyme linked immunosorbent assay |
| FDA : | Food and Drug Administration |
| FISH : | Fluorescence in situ hybridization |
| GAE : | Gallic acid equivalent |
| GCLM : | Glutamate-cysteine ligase modifier |

GP : Glycoprotein

GSH-Px : Decrease glutathione peroxidase

*H.* : *Helicobacter*

HDL : High-density lipoprotein

HE : Hematoxylin and eosin

*HP. :* *Helicobacter pylori*

HPLC : High-performance liquid chromatography

HpSA : Stool antigen test

IC : Inhibition concentration

IECs: Intestinal epithelial cells

IM : Inner bacterial membrane

LAB : Lactic acid bacteria

*Lb.*: *Lactobacillus*

*Lc.* : *Leuconostoc*

LDL: Low density lipoprotein-cholesterol

LPS : Lipopolysaccaride

MALT : Mucosa associated lymphoid tissue

MAPK : Mitogen-activated protein kinase

MBC : Minimum bactericidal concentration

MCSO : Allicin and S-methyl cysteine-sulfoxide

MIC : Minimum inhibitory concentration

NBT : Nitroblue tetrazolium

NC : Negative control

*O.* : *Oenococcus*

OBMT : Omeprazole, bismuth subcitrate, metronidazole, and tetracycline

OM : Outer bacterial membrane

OMPs : Outer membrane proteins

ORFs : Open reading frames

ORS: Oxygen-free radical species

*P.* : *Pediococcus*

PC : Positive control

PCR : Polymerase chain reaction.

PCSO : S-propyl-cysteine sulfoxide

PG : Peptidoglycan

PGE2 : Prostaglandin E2

PPI : Proton pump inhibitors

PPI-AC : Clarithromycin-based triple therapy

PPI-AL : Levofloxacin based triple therapy

PSCA : Prostate stem cell antigen

PUD: Peptic ulcer disease

QE : Quercitin equivalent

RANKL : Receptor activator of nuclear factor-kappa B ligand

REA : Rutin acid equivalent

RNA : Ribonucleic acid

Rt : Retention time

*S.:* *Saccharomyces*

SOD : Superoxide dismutase

T4SS: needle-like type 4 secretion system

TAE : Tanic acid equivalent

TBB : Treated with *B. breve*

TC : Total cholesterol

TCMs: Traditional chinese medicines

TFE1 : Treated with fenugreek extract 150 µg/kg

TFE2 : Treated with fenugreek extract 300 µg/kg

TG : Triglyceride

TSS : Total suspended solids

TV : Trichomoniasis

UBT: Urea breath tests

VacA : Vacuolating cytotoxin

VBNC : Viable but nonculturable

VVC: Vulvovaginal candidiasis

*W.* : *Weissella*

# List of tables

Table 1: History of the discovery of *H. pylori* ..................... - 5 -

Table 2: Classification of *H.pylori* ..................... - 6 -

Table 3: Biochemical and cultural characteristics of *H.pylori* ..................... - 10 -

Table 4: Anti-*H. pylori* activity of some medicinal plant extracts ..................... - 33 -

Table 5: Mechanisms of inhibition of *H. pylori* by probiotics *in vitro* ..................... - 36 -

Table 6: Descriptions of probiotics ..................... - 40 -

Table 7: Different ecological environments of LAB ..................... - 44 -

Table 8: World garlic production statistics in 2019 ..................... - 61 -

Table 9: Classification of *Allium sativum* ..................... - 62 -

Table 10: Varieties of garlic ..................... - 64 -

Table 11: Chemical composition of fresh garlic g/100g ..................... - 65 -

Table 12: Trace elements of fresh garlic ..................... - 65 -

Table 13: Structures of some sulfur-containing compounds isolated from garlic ..................... - 66 -

Table 14: Phenolic acid constituents in fresh garlic ..................... - 67 -

Table 15: Chemical structures of some flavonoid in garlic ..................... - 67 -

Table 16: Pharmacological activities of garlic ..................... - 68 -

Table 17: Classification of *Allium cepa* ..................... - 74 -

Table 18: Varieties of onion ..................... - 75 -

Table 19: Chemical composition of onion bulb ..................... - 76 -

Table 20: Phytochemical composition of various onion speices ..................... - 77 -

Table 21: Pharmacological activities of onion ..................... - 78 -

Table 22: Classification of *Trigonella Foenum-graecum* L. ..................... - 83 -

Table 23: Some ecological factors of fenugreek growth ..................... - 83 -

Table 24: Varieties of fenugreek ..................... - 84 -

Table 25: Nutrient composition of fenugreek seed ..................... - 86 -

Table 26: Major phytochemical constituents of fenugreek ..................... - 87 -

Table 27: Pharmacological activities of fenugreek ..................... - 88 -

Table 28: Classification of *Cuminum cyminum* ..................... - 92 -

Table 29: Varieties of cumin ..... - 93 -

Table 30: Nutritional factors cumin seeds ..... - 94 -

Table 31: Phytochemical composition of cumin seeds ..... - 95 -

Table 32: Pharmacological activities of cumin seeds ..... - 96 -

Table 33: Source and origin of probiotic strains used in the present study ..... - 100 -

Table 34: Phytochemical screening of garlic and red onion ..... - 112 -

Table 35: Weight of 1000 seeds and germination rate of cumin and fenugreek seeds ..... - 123 -

Table 36: Results of physicochemical analysis of plant samples ..... - 124 -

Table 37: Results of minerals analysis of plant samples ..... - 130 -

Table 38: Results of extraction yield, TPC, TFC, TCT and HTC of plant extracts ..... - 133 -

Table 39: Results of phytochemical screening of plant extracts ..... - 139 -

Table 40: Polyphenolic compounds of plant extracts analyzed by HPLC ..... - 143 -

Table 41: Results of evaluation of antioxidant activity of plant extracts ..... - 146 -

Table 42: Results of Antibiogram test against *H. pylori* ..... - 151 -

Table 43: Results of biochemical confirmation tests of probiotics ..... - 155 -

Table 44: Results of determination of DZI of plant extracts against *H. pylori* ..... - 156 -

Table 45: Results of determination of MIC and MBC of plant extracts against *H. pylori* - 157 -

Table 46: Results of determination of DZI of probiotics against *H. pylori* ..... - 160 -

Table 47: Results of determination of DZI of medicinal plants with probiotics against *H. pylori* ..... - 164 -

# List of figures

Figure 1: Phylogenetic tree representing the different taxons of the genus *Helicobacter* .... - 7 -

Figure 2: *Helicobacter pylori* under electron microscopy .................................................. - 8 -

Figure 3: Appearance of *H.pylori* colonies ....................................................................... - 9 -

Figure 4: Prevalence of *H. pylori* infection worldwide in 2017........................................ - 13 -

Figure 5: Assembly and interaction model of the cag type IV secretory apparatus........... - 18 -

Figure 6: Schematic representation of *vacA* pathogenesis ................................................. - 18 -

Figure 7*: H. pylori* infection and progression to gastric cancer ......................................... - 22 -

Figure 8: Micrographs of strains *Lactobacillus* by scanning electron microscope............ - 46 -

Figure 9: Micrographs of strains *Bifidobacterium* by scanning electron microscope......... - 47 -

Figure 10: Micrographs of strains *Leuconostoc* by scanning electron microscope ........... - 49 -

Figure 11: Micrographs of strains *Lactococcus* by scanning electron microscope............ - 50 -

Figure 12: Micrographs of strains *Streptococcus* by scanning electron microscope .......... - 51 -

Figure 13: Micrographs of strains *Pediococcus* by scanning electron microscope ........... - 52 -

Figure 14: Micrographs of strains *Oenococcus* by scanning electron microscope ............ - 53 -

Figure 15: Micrographs of strains *Weissella by* scanning electron microscope................. - 53 -

Figure 16: Mechanism action of LAB ................................................................................ - 59 -

Figure 17: *Allium sativum* L. plant ....................................................................................... - 61 -

Figure 18: *Allium cepa* L. plant ........................................................................................... - 73 -

Figure 19: *Trigonella Foenum-groecum* L. plant................................................................ - 82 -

Figure 20: *Cuminum cyminum* L. plant ................................................................................ - 92 -

Figure 21: Plant materials used in the present study .......................................................... - 99 -

Figure 22: Isolation steps of *H. pylori* strains .................................................................. - 101 -

Figure 23: Acclimatisation period of animals used in the present study.......................... - 102 -

Figure 24: Experimental protocol .................................................................................... - 103 -

Figure 25: Sample plants preparation............................................................................... - 105 -

Figure 26: Differents groups of *In vivo* experimentation ................................................ - 121 -

Figure 27: *In vivo* protocol steps ...................................................................................... - 122 -

Figure 28: HPLC chromatogram of onion dry extract .................................................... - 141 -

Figure 29: HPLC chromatogram of garlic dry extract .................................................... - 141 -

Figure 30: HPLC chromatogram of cumin dry extract ................................................... - 142 -

Figure 31: HPLC chromatogram of fenugreek dry extract ............................................. - 142 -

Figure 32: Results of isolation and identification of *H. pylori* ....................................... - 150 -

Figure 33: PCR-amplified products of the Ure C gene of *H.pylori* ................................ - 152 -

Figure 34: Results of macroscopic examination of probiotics ........................................ - 153 -

Figure 35: Results of microscopic observation (Gram straining) of probiotics ................ - 154 -

Figure 36: Results of evaluation of growth kinetics of *H. pylori* without plant extracts .. - 157 -

Figure 37: Results of evaluation of growth kinetics of *H. pylori* in the presence of extracts .....- 158 -

Figure 38: Results of evaluation of growth kinetics of *H. pylori* in the presence of supernatant of probiotics ........................................................................................................ - 160 -

Figure 39: Results of evaluation of growth kinetics of *H. pylori* in the presence of neutralized supernatant of probiotics ............................................................................................ - 161 -

Figure 40: Results of evaluation of growth kinetics of *H. pylori* in the presence of supernatant of probiotics + Catalase .......................................................................................... - 161 -

Figure 41: Results of evaluation of growth kinetics of *H. pylori* in the presence of probiotics supernatant + garlic extract ........................................................................................ - 165 -

Figure 42: Results of evaluation of growth kinetics of *H. pylori* in the presence of probiotics supernatant + onion extract ........................................................................................ - 165 -

Figure 43: Results of evaluation of growth kinetics of *H. pylori* in the presence of probiotics supernatant + cumin extract ....................................................................................... - 166 -

Figure 44: Results of evaluation of growth kinetics of *H. pylori* in the presence of probiotics supernatant + fenugreek extract ................................................................................. - 166 -

Figure 45: Histopathologic evaluation of antral gastric samples from *H. pylori* infected rat groups. ......................................................................................................................... - 170 -

# List of annexes

**Annexe 1:** Photos of plants used in the present study ........................................................ -210-

**Annexe 2:** Photos of commercialized used probiotics........................................................ -211-

**Annexe 3:** Steps of preperation of plants powder................................................................ -212-

**Annexe 4:** Reading table of API Campy ............................................................................. -215-

**Annexe 5:** Results of Phytochemical screening of plant extracts........................................ -216-

**Annexe 6:** Results of determination of DZI of plant extracts against *H. pylori*................. -217-

**Annexe 7:** Results of determination of MIC and MBC of plant extracts against *H. pylori* -218-

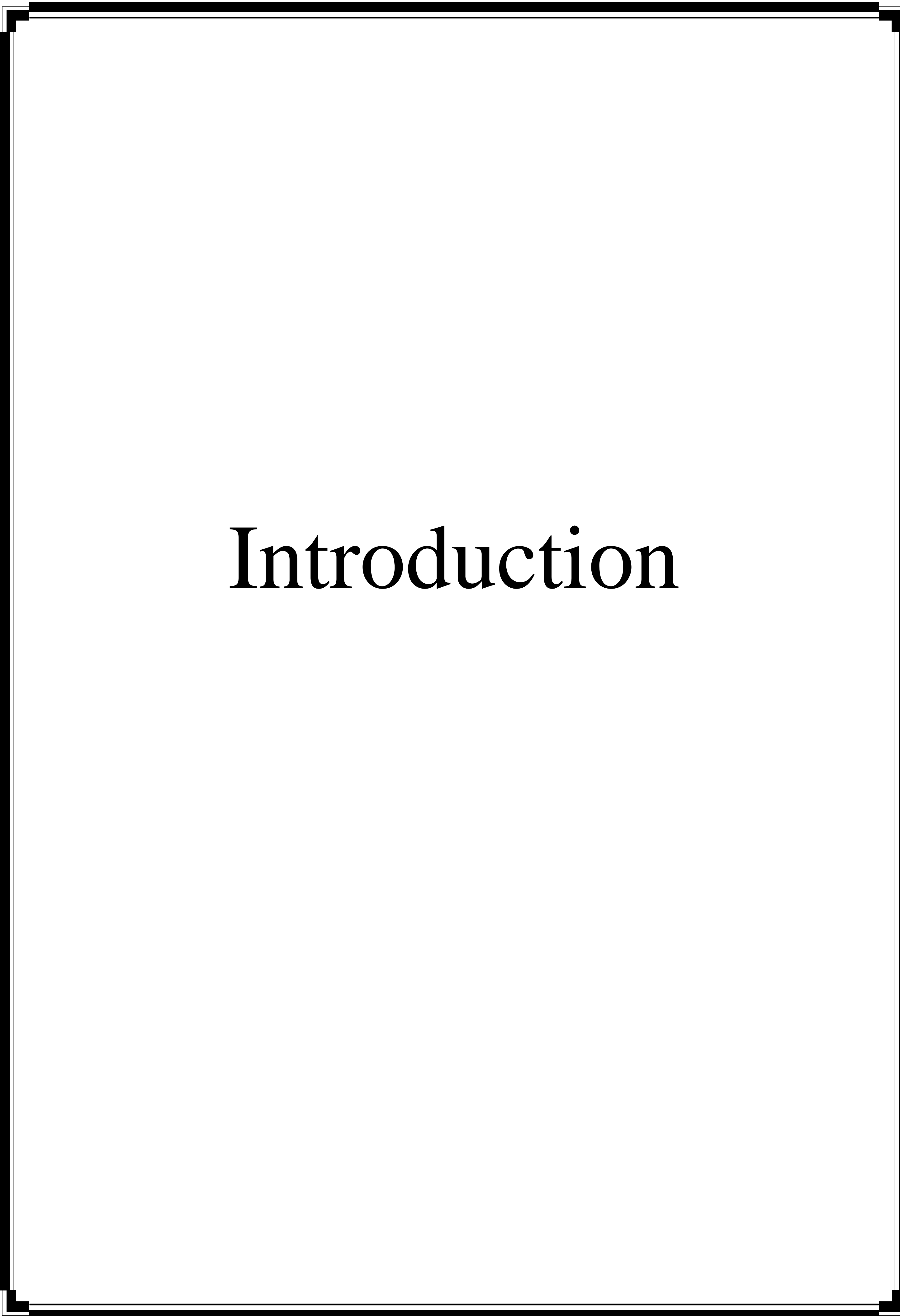
**Annexe 8:** Results of determination of MIC and MBC of plant extracts against *H. pylori* -219-

**Annexe 9:** Results of determination of DZI of probiotics against *H. pylori* ...................... -220-

**Annexe 10:** Results of DZI of medicinal plants with probiotics against *H. pylori* ............ -221-

**Annexe 11:** Photos of invivo study of combined mixtures against *H.pylori*...................... -224-

**Annexe 12:** Composition of culture media.......................................................................... -225-



# Introduction

## Introduction

In Algeria, as in other developing countries, infectious and parasitic diseases constitute a public health problem because of their frequency and seriousness **(Mimoune et *al.,* 2022).** The situation is more worrying because of the appearance of strains antibiotic-resistant microorganisms and the emergence of uncommon infections which compromise treatments using existing drugs **(WHO, 2002).**

*Helicobacter pylori* infection is the underlying cause of noncardia gastric cancer, the second commonest cause of death from cancer in the world, it is also responsible for deaths from peptic ulcer. Gastric cancer and peptic ulcer together cause more than a million deaths per year worldwide, it is therefore a serious public health problem **(Axon, 2014).**

*H. pylori* is usually acquired during childhood and able to establish lifelong chronic infection **(Malaty et *al.,* 2002).** Infected patients are asymptomatic in most cases but infection has been directly linked to chronic gastritis, peptic ulcer, non-ulcer dyspepsia, mucosa-associated lymphoid tissue lymphoma, and gastric cancer **(Crowe, 2019).** On the basis of compelling evidence, the World Health Organization has classified *H. pylori* as a group I carcinogen leading to gastric adeno-carcinoma and recently highlighted the ranking of *H. pylori* in the priority list of research **(Tacconelli, 2018).**

*H. pylori* prevalence varies between countries and their socio-economic levels **(Suerbaum and Michetti, 2002).** It is estimated that the prevalence of this infection is higher in developing countries where it can reach 80% to 90% of the young adult population compared to 40% or less in developed countries **(Heluwaert et *al.,* 2014) .**

A serological study in the 1980s reported a prevalence of 80% in the Algerian population **(Megraud et *al.,* 1989).** Another study carried out in Algeria in 2000 revealed a prevalence of 71% **(Faik, 2000).** Algeria still one of the regions presenting a high prevalence of this infection (**Djennane-Hadibi et *al.,* 2016**).

The eradication of *H. pylori* is essential when it is detected, in particular to avoid the risk of stomach cancer. It eradication treatment using triple therapy (proton pump inhibitor combined with two antibiotics) is currently the reference treatment recommended **(Malfertheiner et *al.,* 2002).** However, conventional treatment with antibiotics is not always effective against *H. pylori* infection due to the resistance of the bacteria to antibiotics, the main factor in treatment failure **(Raymond et *al.,*2010).** Faced to this obstacle, it is essential to seek new effective antibacterial substances with a broad spectrum of action exploring the plants used in traditional medicine **(Omolo et *al.,* 2014).**

The history of Aromatic and Medicinal Plants (AMP) is associated with the evolution of civilizations. In all regions of the world, the history of peoples shows that these plants have always occupied an important place in medicine and in culinary preparations **(Bouzouita et *al.,* 2008).** The last decades have been marked by the particular interest shown in the implementation of value of medicinal plants as sources of bioactive natural substances **(El-Haci, 2012).** Nowadays between 350,000 plants are used in the pharmacopoeia human. 75% of drugs are of plant origin and 25% of them contain at least one active molecule of plant origin **(Salmerón-Manzano et *al.,* 2020).**

Garlic (*Allium sativum L.*), onion (*Allium cepa L.*), fenugreek (*Trigonella Foenum-graecum L.*) and cumin (*Cuminum cyminum L.*) are among the ancient foods grown in Algeria, and known for their virtues and medicinal applications. They are a rich source of phyto-nutrients advised as important elements of the Mediterranean diet **(Lanzotti, 2006; Lazouni et *al.,* 2007).** These herbs are used to treat a range of illnesses related to: digestive disorders (gastric and intestinal), urinary system, cardiovascular diseases and respiratory diseases, etc. **(Eddouks et *al.,* 2007).** Currently, we understand more and more, that the active principles of medicinal plants products are often related to the products of secondary metabolites, which are widely used in therapy, as anti-inflammatory preventive agents, antimicrobial, antiseptics, diuretics, but mainly antioxidant **(Hussein and El-Anssary, 2019).**

Many studies have been carried out to investigate the anti-*H. pylori* activity of plant extracts, partially purified fractions of natural compounds **(Kabangu and Tambwe, 1990).** Numerous plants present a strong anti-*Helicobacter pylori* activity such as *Impatiens balsamina* L. **(Wang et *al.,* 2009)**, *Persea americana*, *Annona cherimola*, *Guaiacum coulteri*, *Moussonia deppeana* **(Castillo-Juárez et *al.,* 2009),** *Myristica fragrans* (seed), *Rosmarinus officinalis* (rosemary leaf ) **(Mahady et *al.,* 2003)** and *Curcuma amada* Roxb., *Mallotus phillipinesis* (Lam) Muell., *Myrisctica fragrans* Houtt., *Psoralea corylifolia* L. **(Zaidi et *al.,* 2009).** Remarkable anti-*H. pylori* activity was reported for garlic **(Sivam, 2001)**; onion **(Ramos et *al.,* 2006)**, fenugreek **(Randhir and Shetty, 2007)** and cumin **(Moghaddam, 2010).**

Further more numerous scientific studies have reported the prophylactic and therapeutic properties of lactic acid bacteria (LAB) **(Parker, 1974; Varcoe et *al.,* 2003; Marelli et *al.,* 2004).** These beneficial microorganisms for the health of humans and animals have been given the name “Probiotics”. This concept was developed especially after the emergence, in

recent decades, of bacteria resistant to antibiotics and the interest aroused by natural inhibition agents for the control of pathogenic germs **(Kailasapathy and Chin, 2000).**

LAB have been used for years against infectious diseases and are widely studied for their ability to protect humans against pathogenic bacteria **(Ryan et *al.,* 2008).** Several studies have shown that LAB have an inhibitory activity against the pathogenic bacterium *H. pylori in vitro* and *in vivo* **(Coconnier et *al.,* 1998; Michetti et *al.,* 1999; Elsalmi et *al.,* 2019).** This power exerted by LAB is usually due to the production of organic acids, hydrogen peroxide, as well as bacteriocins **(Silva et *al.,* 1987; Vandenbergh, 1993; Midolo et *al.,* 1995).** LAB clearly show better tolerance to classic triple therapy and seem to have a preventive effect. Therefore, the administration of probiotics could be exploited as a potential therapeutic agent for the eradication of *H. pylori* **(Hamilton-Miller, 2003).**

Symbiotics are defined as a combination of probiotics (live microorganisms which, when administered in adequate amounts, confer a health benefit to the host) and prebiotics (a food ingredient selectively metabolized by beneficial intestinal bacteria), which may act synergistically **(Kumari et *al.,* 2020).**

To the best of our knowledge, no research so far has been carried out on the synergistic effect of garlic, onion, cumin and fenugreek with probiotics on the growth inhibition of *H. pylori*. For this reason, the aim of this study was to highlighting the *in vitro* and *in vivo* probiotic and/ prebiotics effect of some LAB and plant extracts on *H. pylori.*

The objectives of our work were to evaluate the effect of plant extracts and probiotics against *H. pylori* (*in vitro*), to determine the potential of combination between extract plants and probiotics against *H. pylori* (*in vitro*) and to confirm of the *in vivo* antibacterial effect of fenugreek extract (*Trigonella Foenum-Graecum* L.) and *Bifidobacterium breve* on *H. pylori* colonization.

# Part I:

# Literature Review

# Chapter -I-

## Generalities on *Helicobacter pylori*

## I.1 History

The first well known report of gastric Helicobacters has been credited to an Italian anatomist **Giulio Bizzozero**, as early as in **1893**. In hand drawn illustrations, Bizzozero documented the presence of "spirochetes" with approximately 10 wave lengths within the parietal cells and gastric glands in the stomachs of dogs. However, the first record on the presence of spiral organisms in the human mucosa, adjacent to carcinomas was described by **Krienitz (1906).**

In **1939**, **Doenges** showed 43% of human stomach autopsies harboured spiral organisms and a year later, **Freedberg and Baron (1940)** presented findings of "spirochetes" in about 40% of the resected gastric specimens. These findings were viewed with scepticism as most of the samples of spiral organisms were obtained post mortem and the possibility of contamination could not be disregarded. Moreover, the hypothesis of contamination gained superiority in the early **1950s** when **Palmer** performed a study on more than 1,000 gastric biopsies taken with a blind suction biopsy instrument and found no evidence of spirochetes. This incorrect conclusion drawn during that period could be due to the rigid endoscopes available, which only allowed biopsies to be taken from the fundus and not from the antrum, where *H. pylori* is usually located. The possibility that the appropriate staining solutions were not used could not be ruled out. The interest in this gastric spiral bacterium was re-awakened when **Steer and Colin-Jones (1975)** noted that numerous spiral bacteria were present in 80% of their gastric ulcer specimens. Unfortunately, attempts to culture the organism yielded only growth of *Pseudomonas aeruginosa*. This was later assumed to be the contaminants from the endoscope.

A major breakthrough in locking the link between gastroduodenal diseases and the spiral bacteria was established when two Australian researchers **Robin Warren** and **Barry Marshall (1984)**, after numerous unsuccessful attempts, managed to culture the *Campylobacter*-like organism (CLO) by chance. Furthermore, Barry Marshall himself had fulfilled the Koch's postulate, confirming an association of the bacteria and clinical disease by voluntarily ingesting a culture of *H. pylori* and was subsequently diagnosed with gastritis **(Marshall et *al.*, 1985a).**

The brief history on the discovery of *H. pylori* as illustrated in **Table 1** shows that *H. pylori* has existed all this while, but investigators were not able to detect the bacteria in biopsies or merely considered their findings a result of contamination. The discovery of the

gastric pathogen, *H. pylori*, has indeed led to a revolution in our understanding of gastroduodenal pathology, mainly gastritis and peptic ulcer.

**Table 1**: History of the discovery of *H. pylori* **(Ling, 2004)**

| History of *H. pylori* | | |
|---|---|---|
| **Year** | **References** | **Report** |
| **1893** | Bizzozero | Spiral organisms in dogs |
| **1896** | Salomon | Spiral organisms in dogs and cats |
| **1906** | Krienitz | First description in a human with gastric cancer |
| **1939** | Doenges | Spirochetes in stomach (autopsies) |
| **1940** | Freedburg and Barron | Spirochetes in stomach with ulcers or carcinoma |
| **1954** | Palmer | All bacteria in stomachs believed to be contaminants |
| **1975** | Steer and Colin-Jones | Bacteria in gastric ulcer patients identified as *Pseudomonas aeruginosa* |
| **1983** | Warren and Marshall | First culture of *H. pylori* |

### I.2 Taxonomy and classification

This novel gastric bacterium isolated resembled *Campylobacter* in several aspects, both morphologically and microbiologically. Therefore, the primary isolate was first referred to as *Campylobacter pyloridis* **(Marshall and Warren, 1984).** The specific epithet was later revised to *C. pylori* conforming to the correct Latin genitive of the noun pylorus **(Marshall and Goodwin, 1987).** However, further characterization of the organism indicated that perhaps *C. pylori* was not a true *Campylobacter*. The ultra structure details showed multiple sheathed flagella at one pole of the bacterium, in contrast to the single bipolar unsheathed flagellum typical of *Campylobacter* species **(Goodwin et *al.*, 1985).**

The protein patterns and cellular fatty acid composition of *C. pylori* also differed markedly from those of *Campylobacter* species **(Pearson et *al.,* 1984; Goodwin et *al.,* 1985).** Analysis of the 16S rRNA sequence provided more evidence to exclude *C. pylori* from the *Campylobacte*r genus **(Romaniuk et *al.,* 1987).** Finally, **Goodwin et *al.* (1989)** proposed the new genus name *Helicobacter* and since then, *C. pylori* was renamed *H. pylori*, the first member of the new genus.

*H. pylori* is a helical-shaped bacterium found in an area of the stomach near the pylore, hence its name. It is considered the leader of a new group of bacterium, called Gram-negative Super Family VI bacteria, which includes four genera: *Helicobacter, Campylobacter,*

*Arcobacter and Wolinella*. *H. pylori* is differentiated from other groups primarily by the structure of its ribosomal RNA (16S) but also by the multitude of sheathed flagella (4-6), and by the possession of particular fatty acids and menaquinones that have valuable taxonomic value **(Megraud, 1993).**

The classification of *H.pylori* is represented in the **Table 2**:

**Table 2:** Classification of *H.pylori* **(Garrity et *al.,* 2005)**

| **Domain** | Eubacteria |
|---|---|
| **Kingdom** | Bacteria |
| **Phylum** | Proteobacteria |
| **Class** | Epsilonproteobacteria |
| **Order** | Campylobacterales |
| **Family** | *Helicobacteraceae* |
| **Genus** | *Helicobacter* |
| **Species** | *Helicobacter pylori* |

Since the discovery of *H. pylori* in 1982, many other species of the genus *Helicobacter* have been identified. They colonize the digestive mucosa of humans or animals **(Megraud and Lamouliatte, 2003).** The genus *Helicobacter* currently includes 30 validated species **(Menard et *al.,* 2016).** Depending on the specific niches to which they are associated, the *Helicobacter*s are divided into two groups: the gastric *Helicobacter*s comprising *H. pylori* and *H. felis* in particular and the enterohepatic *Helicobacter*s colonizing the gastro-intestinal and bile ducts **(Fig. 1).**

### I.3 Characteristics of *H. pylori*

#### I.3.1 Morphological and physiological

*H. pylori* is a helical S shaped Gram negative bacterium. It is 2.5-5 µm in length, 0.5-1 µm in width and possesses a tuft of 4 to 6 polar sheathed flagella **(Goodwin et *al.,* 1985).** Each flagellum is 2.5 µm long and about 30 µm in thickness, with a membranous terminal bulb **(Goodwin et *al.,* 1989).** The characteristic corkscrew motility enables the bacterium to burrow into the mucin lining the epithelial mucosa of the stomach **(Goodwin et *al.,* 1985).**

The flagella components consist of the hook protein (Flg E) and two flagellin proteins, FlaA and FlaB. *H. pylori* with disrupted FlgE was non-motile and lacked the filaments, although both flagellin proteins were produced **(O'Toole et *al.,* 1994).** Both flagellin subunits were found to be essential for motility and colonization of the stomach **(Fig. 2) (Josenhans et *al.,* 1995).**

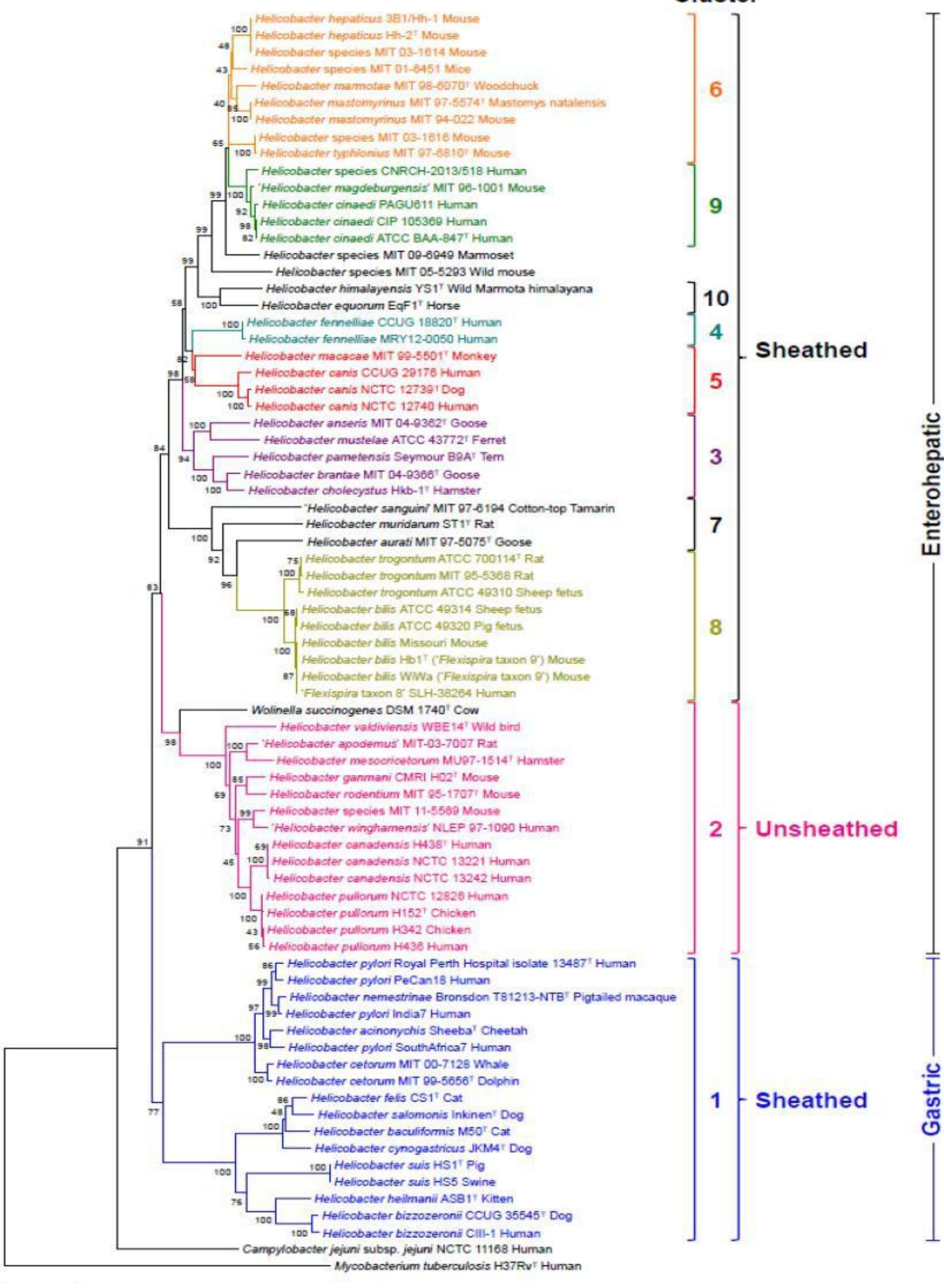



**Figure 1:** Phylogenetic tree representing the different taxons of the genus *Helicobacter*

**(Menard et *al.,* 2016)**

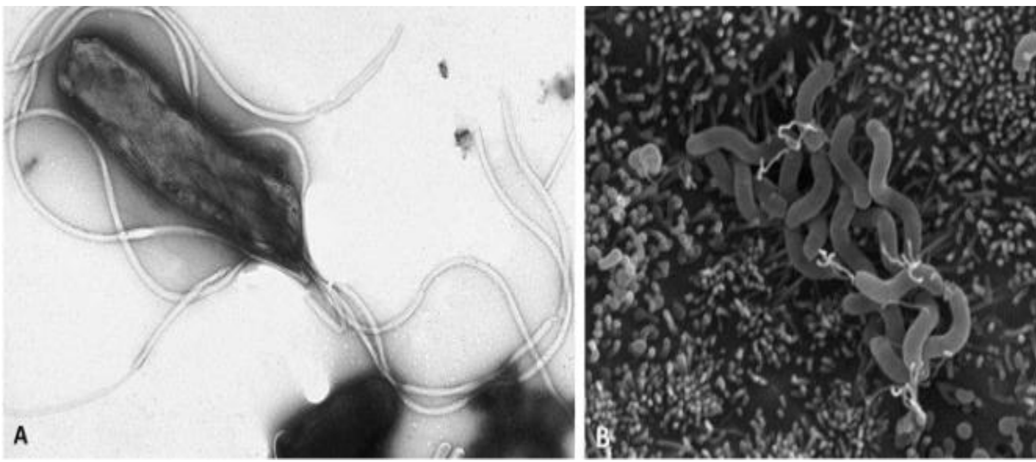



**Figure 2:***Helicobacter pylori* under electron microscopy (Gx 30000) **(Tan et *al.,* 2010)**

A. *H. pylori*; B. *H. pylori* cluster at gastric epithelial cell level

*H. pylori* prefers a microaerophilic environment with 5-10% carbon dioxide environment for *in vitro* culture. A variety of solid media containing 5-10% horse/sheep blood were used to culture the bacteria under microaerophilic atmosphere, *H. pylori* colonies usually appeared after 3-5 days incubation at 37°C **(Hachem et *al.,* 1995).**

This bacterium presents two different morphologic manifestations: spiral and coccoid forms. The role of the spiral form has been shown to be strongly associated with gastroduodenal diseases **(Annibale et *al.,* 2001).** However, the biological significance of the coccoid form, which is non-culturable *in vitro*, has yet to be determined **(Zheng et *al.,* 1999).** Some investigators postulated that the coccoid form may represent a persistent form in which *H. pylori* can exist in the environment as VBNC **(Nilsson *et al.,* 2002)** and could possibly play a role in the transmission cycle and treatment failure **(Cave, 1997).**

### I.3.2 Cultural

The proper specimen for culturing *H. pylori* is biopsy specimens obtained during endoscopy. Since proton pump inhibitors (PPI) may change distribution of mucosal bacteria (**Megraud et *al.*, 1991**), subjects who are scheduled to undergo endoscopy should quit PPIs or antibiotics for two weeks or more. There is a controversy over the number of tissue samples required for the diagnosis of *H. pylori*. A single biopsy at antrum (2 cm from the pylorus) is sensitive but it is not sufficient for a reliable diagnosis **(Megraud and Lehours, 2007).** As *H. pylori* may have patch distribution, the larger number of samples taken increases the sensitivity. Therefore, it is recommended to take two specimens from the antrum as well

as two specimens each from the anterior and posterior corpus **(Megraud and Lehours, 2007).**

Specimens of *H. pylori* is fragile in room temperature and air. It is obligatory to avoid air exposure of the specimens and to place them in saline or transport media such as Stuart's transport medium (4 h maximum) **(Megraud and Lehours, 2007).** If these transport condition cannot be available, it is better to freeze the specimens at −70 °C or in liquid nitrogen in a dry tube and transport them frozen to the laboratory. Storage at 4°C in a medium containing 20% glycerol also led to *H. pylori* recovery in 81% of the biopsy specimens tested **(Han et *al.,* 1995).**

*H. pylori* is not distributed evenly in most cases; more colonies appear in the grinded specimens. In addition, direct plating to solid medium is used as *H. pylori* is difficult to grow in the broth culture. The medium includes an agar base, growth supplements, and selective supplements. Blood or serum components are examples of growth supplements, which promote the growth of *H. pylori*, and the proportion is 5 %, 7 %, or 10 %. Other growth supplements include yolk, charcoal, starch, bovine serum albumin, catalase **(Megraud and Lehours, 2007).** Selective supplements are crucial due to the presence of contaminating bacteria. It consists of antibiotics and antifungals: antibiotics which inhibit the growth of Gram-positive bacteria such as vancomycin or teicoplanin, antibiotics targeting Gram-negative bacteria such as nalidixic acid colistin or trimethoprim, and antifungals such as nystatin or amphotericin B. To increase sensitivity of the test, selective medium or the blood containing nonselective medium is used **(Piccolomini et *al.,* 1997).** Selective medium such as *Pylori* agar and Skirrow agar and non selective medium such as blood agar and Columbia agar are frequently used **(Garza and Perez, 2014).** Incubating the bacteria cultures will be made in microaerobic conditions of 35–37 °C (85 % $N_2$, 10 % $CO_2$, 5 % $O_2$), and the colonies appear within 3–4 days in optimal conditions **(Fig. 3)**. However, 7–10 days of incubation is recommended to make sure that the result is negative **(Van der-Hulst et *al.,* 1996).**

**Figure 3:** Appearance of *H.pylori* colonies **(Davood et *al.,* 2009)**

**I.3.3 Biochemical**

*H. pylori* is inactive in most of the conventional biochemical tests. Carbohydrates are neither oxidized nor fermented. It dproduces catalase and cytochrome oxidase but is most notable for its high level of urease and alkaline phosphatase activity. *H. pylori* is a homogeneous species in its enzymic profile, with the exception of some minor strain differences in aminopeptidase and other presented enzyme activities. Typical strains are positive for alkaline phosphatase, acid phosphatase, leucine arylamidase, naphthol-AS-Bl-phosphohydrolase, esterases C4 (butyrate) and C8 (caprylate), and gamma glutamyl transpeptidase. Strains are usually negative in hippurate hydrolysis, nitrate reduction, indole formation, arylsulphatase activity, growth in the presence of 1 % and 3.5% NaCl, and indoxyl acetate hydrolysis **(Tab. 3)**. Some *H. pylori* has been reported to be negative for catalase and urease production but, in general, the isolation of such strains directly from clinical material is rare. Another important difference between strains is their ability to produce a vacuolating cytoxtoxin in human and animal cell lines **(Owen, 1998).**

**I.3.4 Genetical**

*H. pylori* has a relatively small genome (~ 1.65 Mb) and is only about 1/3 the size of the *E. coli* genome. In comparison, the *H. pylori* genome was shown to possess 17% of species specific genes to the closely related organism *Campylobacter jejuni* **(Pennisi, 1999).**

**Table 3:** Biochemical and cultural characteristics of *H.pylori* **(Goodwin et *al.,* 1989)**

| **Biochemical characteristics of *H. pylori*** | | |
|---|---|---|
| Oxydase | + | -: Negative<br>+: Positive<br>S: Sentitive<br>R: Resistant |
| Catalase | + | |
| Urease | + | |
| Hippurate hydrolysis | - | |
| Nitrate reduction (microaerophilic) | - | |
| $H_2S$ production in triple sugar iron agar | - | |
| Gamma-glutamyl transpeptidase | + | |
| Alkaline phosphatase | + | |
| Motility in brain heart infusion broth | + | |
| Motility from agar plate | - | |
| Growth microaerophilically at 37°C | + | |
| Growth on blood agar containing 3.5% NaCl | - | |
| Growth on 0.5% glycine | + | |
| Growth on 1% glycine | - | |
| Growth on 1% bile | - | |
| Susceptibility to nalidixic acid (30-pg disk) | R | |
| Susceptibility to cephalothin (30-pg disk) | S | |
| Susceptibility to metronidazole (5-pg disk) | S | |

*H. pylori* genomic (chromosomal) DNA is a single circular molecule of small size from 1.4 to 1.73 Mb depending on the strains, but genomic analysis of 30 strains resulted in an average value of 1.71 Mb **(Taylor et *al.,* 1992).** On the other hand, the species of *H. pylori* is distinguished by the more basic value of (G+C) of its DNA, which varies between 36 and 37%, different from that of the genus *Vibrio* (G + C = 38-51%) and the genus *Wolinella* (G + C = 46-49%) **(Sobhani et *al.,* 1991a).** *H. pylori* present more than 30% of specific genes. However, about 1200 genes are common to all strains, and 200 to 400 genes located mainly in plasticity zones or in the cag pathogenicity island (cagPAI) are present in a variable way between the different strains **(Varon and Megraud, 2013).**

*H. pylori* present two genomes that don't have the same number of genes: 1587 genes for the first one and 1491 genes for the second , these genomes posses 16S, 23S and 5S rRNAs, which don't carry antibiotic resistance genes or virulence genes. The genome of *H. pylori* is composed of a circular chromosome 1.66 pB. The sequencing of this genome was carried out in 1999 **(Raymond, 2016).** The genome of *H. pylori* is composed of three parts: a stable part that ensures the homogeneity of the species, a variable part for the adaptation of the bacterium to its environment and a last part has recently been described and would be decisive for the virulence **(Basso et *al.,* 2008).** Many elements of this genome suggest that it is frequently altered by intra-gene alterations or by the acquisition of exogenous DNA. This diversity could influence the pathogenicity of certain strains or play a role in the survival of the bacterium at the host level **(De Reuse and Bereswill, 2007).**

Completed genomes of two unrelated *H. pylori* strains (26695 and J99) as well as the comparison of the two have been made available **(Alm and Trust, 1999).** *H. pylori* 26695, sequenced by The Institute of Genome Research, was isolated in the mid 1980s in the United Kingdom from a patient suffering from gastritis. In contrast, *H. pylori* J99, an isolate from a duodenal ulcer patient in USA was sequenced in a collaborative study between Astra Zeneca R and D Boston (formerly Astra Research Center Boston) and Genome Therapeutics Corporation.

Comparison of the two genomes shows that *H. pylori* J99 is 24.036 bp shorter and contains 57 fewer predicted open reading frames than strain 26695. In addition, the analysis between the genomes of the two strains have also identified that each strain contains a set of genes (~6-7%) that are unique to each strain. Interestingly, almost one half of these genes were clustered in a single hyper variable region, namely the plasticity zone. In both J99 and 26695 genomes, almost 60% of the open reading frames (ORFs) were attributed with

predicted functions **(Alm and Trust, 1999).** Comparison of the two completed sequenced genomes has provided important information regarding the genetic heterogeneity. There are now evidence for some genetic loci such as *cagA* and *vacA* of the Western and Asian strains represented distinct lineages of *H. pylori* strains **(Van Doorn et *al.*, 1999).**

## I.4 Habitat

The stomach is the main habitat of *H. pylori*. There may be extension of the *H. pylori* habitat into the proximal duodenum or distal esophagus, usually in the presence of gastric metaplasia in those sites **(Talley et *al.,* 1988).** *H. pylori* also has been found overlying ectopic gastric epithelium in Meckel's diverticulum, but this is an uncommon circumstance. It genetic sequences have been identified in oral and colonic contents, but it is not clear whether these organisms are transient or residential **(Ackerman et *al.,* 2003).**

## I.5 Epidemiology of *H. pylori* infection

*H. pylori* infects around 50% of the population, it is acquired during childhood, and typically persists in the stomach throughout life unless treated with antibiotics. Overcrowding, poor sanitation, and low economic standards are the risk factors for acquiring *H. pylori* infection, which is inversely correlated with socioeconomic status **(Khalifa et *al.,* 2010).** Because of the improved living standards in developed compared to developing countries, the infection rate is still much higher in developing countries where it can sometimes reach 80-90% **(Fig. 4).** Related to this, the birth cohort effect was seen in developed countries where the *H. pylori* prevalence is much higher in the older generation because of a lower acquisition rate in the younger generation and wide spread use of antibiotics. In a study of Peruvian children, 71% were infected by the age of 6 months (determined by urea-breath test) compared to 1.2% of 6 months-old Swedish children (determined by serological test) **(Delport and Van der-Merwe, 2007).** The increased growth rate of developing countries proposes that the total number of infected individuals in the world will increase even further and consequently, gastric disease related to *H. pylori* infection will also increase **(Khalifa et *al.,* 2010).**

The *H. pylori* infection has emerged as one of the most common chronic bacterial infections worldwide and affects more than half of the world's population, with clinical signs of infection only manifesting in <20% of these individuals **(Percival and Suleman, 2014).** *H. pylori* is thought to be indigenous to the human population and is well adapted to existing in the human stomach for the lifetime of its host unless eradication using appropriate chemotherapeutic agents is successful **(Blaser, 1997)**.

**Figure 4:** Prevalence of *H. pylori* infection worldwide in 2017 **(Michael et *al.,* 2017)**

Life long colonization seems to be due to the ability of some strains of *H. pylori* to both adapt to the host's immunological responses and to also withstand the constantly changing gastric environment **(Salaun et *al.,* 2005).**

The rate of *H. pylori* infection differs among groups as well as within the population. Strains from different geographical areas exhibit phylogeographic features **(Blaser, 2005; Ahmed et *al.,* 2007).** The genomic patters of *H. pylori* have been shown to be extremely diverse, and gastric mucosa may be colonized by strains with small differences in the genomic patterns suggesting subtype variation **(Colding et *al.,* 1999).**

### I.5.1 Prevalence

The prevalence of *H. pylori* infection varies widely by geographic area, age, race, and socioeconomic status. While the infection is on a fast decline in the most of the Western countries, mainly due to the success of therapeutic regimens and improved personal and community hygiene that prevents reinfection, in developing countries, the prevalence rates can reach 90% and is higher among individuals belonging to low socioeconomic status group **(Khalifa et *al.,* 2010)** . It occurs especially due to failure of treatment and emergence of drug resistance (**Blaser, 2005).**

Most studies suggest that males and females are infected at approximately the same rates **(Kawasaki et *al.,* 1998).** In spite of it, a meta-analysis population-based study reported a male predominance of *H. pylori*-related diseases in adults but not in children **(De Martel and Parsonnet, 2006).** The infection probably occurs in the childhood, and children are often infected by a strain with a genetic fingerprint identical to that of their parents **(Covacci et *al.,* 1998**). Besides, local prevalence of *H. pylori* within a country also should be considered, and there are estimates that infection is more common in rural developing areas than in urban

developed ones **(Vale and Vitor, 2010).** Moreover, differences by ethnic and racial groups are evident **(Bardhan et *al.,* 1997).** In addition, the main risk factors of *H. pylori* infection, especially if present during childhood, have been associated with socioeconomic status. **Malaty and Graham (1994)** demonstrated that there is probably an inverse correlation between prevalence and socioeconomic status. It has also been reported that overcrowding, such as living in a crowded environment, sibship size, number of persons or children in the home, number of persons per room, crowding index, and living in an institutionalized population, is a situation consistently related to *H. pylori* positivity **(Peach et *al.,* 1997; Kikuchi et *al.,* 1999).** Finally, it is important to consider that the pathogenetic role of *H. pylori* in gastroduodenal pathologies has been elucidated and confirmed in the past 30 years **(Malfertheiner et *al.,* 2014)** redirecting the scientific and medical understanding of great part of gastrointestinal diseases. The development of effective therapies against *H. pylori* infection has progressed, and its successful eradication leads to healing of chronic active gastritis and reverses inflammation of the mucosa. In spite of it, the challenge nowadays is gastric cancer and the understanding of gastric carcinogenesis, almost always is associated with *H. pylori* long-term infection **(Roesler and Zeitune, 2014).**

### I.5.2 Transmission pathways

Although the natural niche for *H. pylori* is the human stomach, some questions about other possible reservoirs for bacterium have been appearing in the last years. Nevertheless, most part of the questions about the transmission of *H. pylori* remains unclear, and, because of it, the possible modes of transmission are still unknown. Consequently, the routes of transmission of *H. pylori* are supposed to occur via an array of different pathways.

Some important studies have reported and highlighted the importance of *H. pylori* biofilms, the presence of coccoid forms within the biofilm, and resistance, providing insight into the prevalence of coccoid forms in the gastric mucosa. These reports are very important because these can bring a better understanding about the mechanisms behind recalcitrant coccoid states and how they can phenotypically shift into more virulent spiral forms **(Cellini et *al.,* 2008; Cellini, 2014).**

The infection is typically acquired in early childhood and once established commonly persists throughout life unless treated. Person-to-person transmission within the family appears to be the predominant mode of transmission, particularly from mothers to children and among siblings, indicating that intimate contact is important. The route of transmission is

uncertain, but the gastro-oral, oral-oral, and fecal-oral routes are likely possibilities **(Weyermann et *al.,* 2009; Khalifa et *al.,* 2010)**.

The community and environment may play additional roles for *H. pylori* transmission in some settings. Molecular analyses show that the microorganism is also present in various aquatic environments suggesting that human-fecal-contaminated water sources could be a plausible reservoir of the pathogen. The persistence of the environment virulent *H. pylori* strain in a clustered state, such as the biofilm, suggests a long-term survival of the bacterial community outside the host, enabling bacterial transmission with important clinical repercussions **(Hu and Ehrlich, 2008).** In addition, zoonotic transmission by houseflies **(Junqueira et *al.,* 2017)** and some domestic animals such as dogs, cats, and sheep **(Momtaz et *al.,* 2014),** as well as iatrogenic transmission, have been proposed. Besides, there can be factors both from host and bacterium which may modify the acquisition and persistence of *H. pylori* infection **(Peters et *al.,* 2011)**.

Another possibility of *H. pylori* transmission which has been extensively reported is the water. The contamination of drinking water by human feces has been suggested as one of the possible routes of *H. pylori* transmission, and it has been demonstrated that the microorganism is present in the so-called VBNC state in this unsuitable environment, meaning that their role in fecal-oral transmission via contaminated water sources cannot be disregarded **(Mishra et *al.,* 2008; Cellini, 2014).** The first evidences of water transmission route were obtained in studies developed in some Latin American countries- Peru, Colombia, Chile, and Venezuela and since then *H. pylori* has been detected in several water sources, including lakes, rivers, tap water, well water, irrigation water, and sea water, and also in water distribution systems. Consequently, it can be hypothesized that drinking water could be the pathway for returning to humans **(García et *al.,* 2014).** Consequently, it can be suggested that water can serve as an intermediate source in the fecal-oral transmission of *H. pylori*, acting as a reservoir in which this pathogen can survive for long periods **(Roesler, 2019).**

## I.6 Pathogenesis

### I.6.1 Adherence and colonisation

Being a bacterial pathogen, *H. pylori* has to establish itself in the stomach following transmission. Adherence to the gastric epithelium is a crucial step in colonisation, a precursor of pathogenesis of *H. pylori*. All isolates expressed several putative colonization factors, including various adhesins, flagellar motility and urease **(Eaton et *al.*, 1992; Hocker and Hohenberger, 2003).**

*H. pylori* infection is a chronic infection and is considered unlikely that such infection remains with the absence of adhesin-host cell interactions **(Evans and Evans, 2000).** Adhesins are bacterial proteins, glycoconjugates or lipids that are involved in the initial steps of *H. pylori* infection and are important virulence factors. Presently, there is no consensus as to which *H. pylori* adhesins are most important *in vivo*. However, the best characterized adhesin is the blood group antigen-binding adhesion (BabA) which binds to difucosylated Lewisb (Leb) blood group antigens found on the gastric epithelial cells **(Ilver et *al.*, 1998).** Besides being an important adhesin, the presence of gene allelebabA2was shown to be associated with *H. pylori* pathogenesis **(Prinz et *al.*, 2001).** Other adhesins included heat shock protein Hsp 60, *H. pylori* lipopolysaccaride (LPS), mucin binding proteins and neutrophil-activating protein **(Valkonen et *al.*, 1994; Yamaguchi et *al.*, 1997; Namavar et *al.*, 1998).**

*H. pylori* possesses 4-6 sheathed flagella and their presence appear to be essential in *H. pylori* infection. An isogenic non-motile mutant in the flagellar secretion apparatus component filQ resulted in a 30% reduction in adherence to human gastric cancer AGS cells (American Type Culture Collection no.CRL-1739) **(Foynes et *al.*, 1999).** In the *in vivo* study by **Eaton et *al.* (1992),** the non-motile *H. pylori* survived for only 6 days in infected piglets while the motile variant survived for a longer period of time (21 days). The study inferred that motility is necessary for full colonisation of gnotobiotic piglets by *H. pylori*.

The environment of the human stomach is usually within the pH range of 1-3.5. *H. pylori* is able to colonize the gastric epithelium in acidic conditions with the production of urease which metabolized urea to generate a neutral microenvironment, urease was shown to be essential for initial infection of normal mucosa in gnotobiotic piglets (**Eaton and Krakowka, 1994).**

### I.6.2 Virulence factors

Virulence is defined as the ability of a bacterial species to induce disease. Consequently, the outcome of a bacterial infection is highly dependent on the prevalence and status of its virulence factors. The genetic diversity and variability of *H. pylori* is mirrored in the wide range of virulence factors that vary by disease, age, country, and ethnicity (**Boyanova et *al.*, 2011).** To be defined as an *H. pylori* virulence factor, the protein must be correlated with disease both *in vitro* and *in vivo* and with epidemiological disease patterns **(Lu et *al.*, 2005).**

Three main virulence factors of *H. pylori* are the cytotoxin-associated gene pathogenicity island (*cag*PAI), the vacuolating cytotoxin (VacA), and the outer membrane

proteins (OMPs). Many of the OMPs are proposed to be involved in disease-associated mechanisms such as adherence and manipulation of the immune response. VacA and CagA are,together with BabA, associated with the more severe cases of gastric **(Aljeboury et *al.,* 2020; Haamadi et *al.,* 2021).**

**I.6.2.1 Cytotoxin-associated gene pathogenicity island (cagPAI)**

The *cag*PAI is a pathogenicity island in the *H. pylori* genome and encodes numerous genes that, upon cell contact, are expressed and assembled into the needle-like type 4 secretion system (T4SS) **(Rohde et *al.,* 2003).** The T4SS is evolutionarily conserved among many Gram-negatives such as *Agrobacterium*, *Bordetella,* and *Legionella*, but differs in different organisms in terms of what substrates are transferred. *H. pylori* T4SS binds the integrin β1 receptor that is located on the basal membrane and transfers the cytotoxin associated gene A (CagA) which is also encoded by the *cag*PAI **(Kwok et *al.*, 2007; Jiménez-Soto et *al.,* 2009).** Once inside the cell, CagA is phosphorylated on specific EPIYA motifs by host kinases, and phosphorylated CagA goes on to manipulate the cell by interacting with numerous host cell proteins. In addition, injected non-phosphorylated CagA manipulates proliferation and immune response of host cells **(Suzuki et *al.,* 2009).** Cultured epithelial cells respond by forming the characteristic "hummingbird phenotype" that is the effect of both cell scattering and elongation **(Fig. 5) (Tegtmeyer et *al.,* 2011).** CagA is not considered as a virulence factor (although the *cag*PAI is), but it is considered an oncoprotein and is associated with development of gastric adenocarcinoma **(Parsonnet et *al.,* 1991).** *H. pylori* infections of Mongolian gerbils resulted in more gastric adenocarcinomas in a CagA-dependent manner and so did mice that were transgenic for CagA expression **(Franco et *al.,* 2005; Ohnishi et *al.*, 2008).**

**I.6.2.2 Vacuolating cytotoxin VacA**

Vacuolating cytotoxin A (VacA) is a multifunctional secreted cytotoxin. The *vacA* gene is found in all *H. pylori* isolates though there are differences among the alleles. The S1 allele, especially in combination with the m1 allele, is highly associated with the risk of developing peptic ulcers and gastric cancer **(Palframan et *al.,* 2012).** The VacA toxin forms large vacuoles in gastric cells; however such vacuoles are not seen in biopsies. VacA localizes to, and exerts effects on, the mitochondria where it triggers the apoptotic cascade and induces cell death by mitochondrial fission **(Fig. 6) (Jain et *al.,* 2011; Palframan et *al.,* 2012).** In addition, VacA has been found to bind the integrin subunit CD18 on T-cells and suppressing their activities **(Gebert et *al.,* 2003; Sewald et *al.,* 2008).**

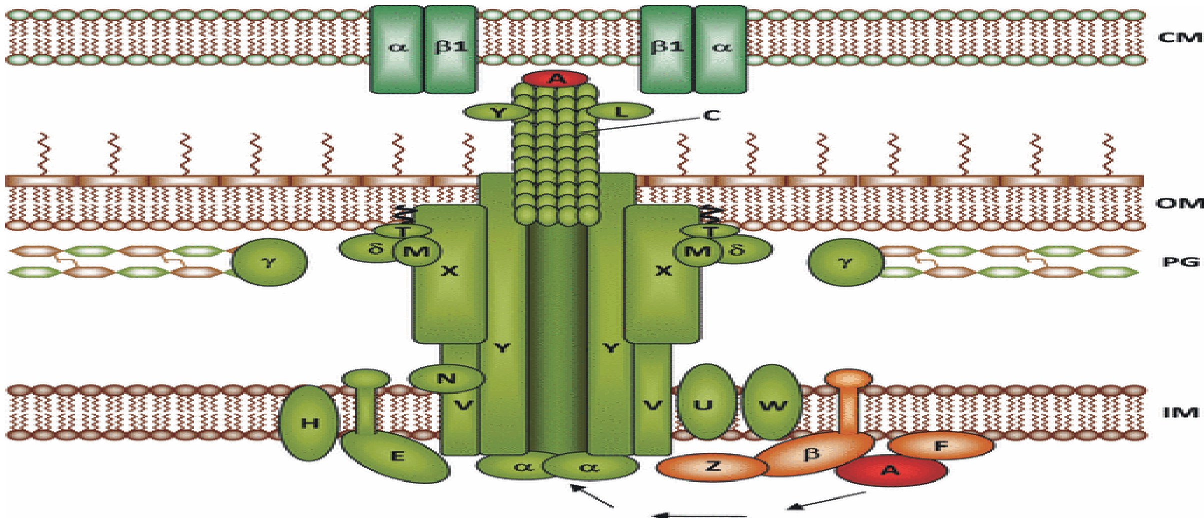



**Figure 5:** Assembly and interaction model of the cag type IV secretory apparatus **(Fischer, 2011)**

Cag proteins are indicated in their most likely locations. Essential apparatus components are depicted as green, translocation factors in orange and effector cagA, red. *cag*L (L), *cag*Y (Y), *cag*T (T), *cag*X (X), *cag*M (M), *cag*N (N), *cag*V (V), *cag*U (U), *cag*W (W), *cag*Z (Z), *cag*H (H), *cag*E (E), inner bacterial membrane (IM), peptidoglycan (PG), outer bacterial membrane (OM), cytoplasmic membrane of target cell(CM)

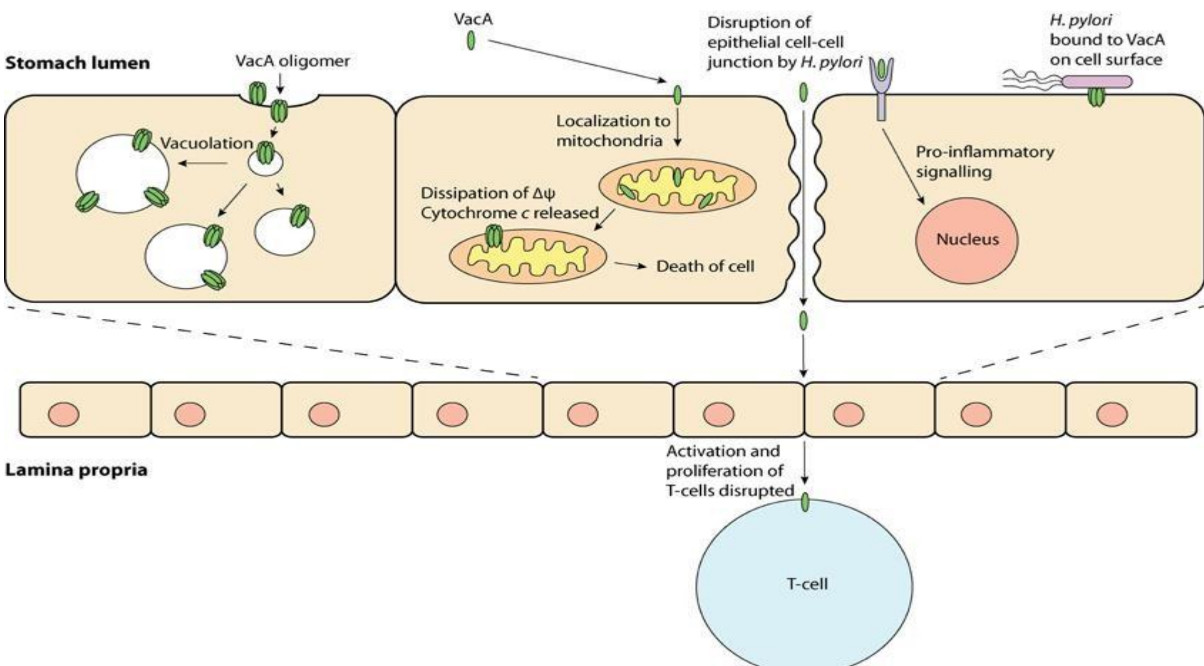



**Figure 6:** Schematic representation of *vacA* pathogenesis **(Palframan et *al.,* 2012)**

### I.6.2.3 Outer membrane proteins

The OMPs of *H. pylori* comprise about 4% of the genome, more than in any other bacterial species. They are divided into five families with the largest group being the Hop family. The Hops are comprised of 21 genes that all encode a conserved N-terminal motif **(Alm et *al.,* 1999).** Related to the Hop family are the 11 Hor genes, which are homologous in sequence to the Hop family but lack the N-terminal motif. Together the Hop and Hor families form the major OMP family **(Alm et *al.,* 2000).** The C-terminal domains of the OMPs are homologous and have alternating hydrophobic and hydrophilic domains that are proposed to form antiparallel amphiphatic ß-sheets that assemble into a ß-barrel in the outer membrane **(Bina et *al.,* 2000).**

Phylogenetic analyses based on the homologous C-terminal domains reveal clustering of certain genes. High nucleotide similarities in the 5' and 3' regions of the genes indicate a preference for recombination between these alleles and this has indeed been show for *babA, babB*, and *babC*, and more recently for *sabA, sabB*, and *hopQ* as well **(Bäckström et *al.,* 2004; Solnick et *al.,* 2004; Talarico et *al.,* 2012).**

Located between the N- and C-terminal domains, and exposed to the extracellular environment, is the hypervariable region that is unique for each OMP and is proposed to determine their functions. This variable region not only varies between the OMPs, but also varies for the same OMP between different strains **(Alm et *al.,* 1999).** Because this region is exposed to the outside of the cell, it is part of the 'face' towards the immune response and is, therefore, under constant selective pressure to avoid recognition. In relation to the diversity seen in the variable region, some genes (*sabA*, *sabB*, *hopZ*, *oipA*, *babB*, and *babC*) are also proposed to have CT repeats that can be regulated by slipped-strand mispairing to undergo phase variation **(Alm et *al.,* 1999; Oh et *al.,* 2006).** By readily turning proteins on and off, the bacterial community is more dynamic and able to prepare for sudden changes that can occur during the course of the infection. Although functions are still not assigned to all OMPs, their cellular localization hints that these proteins are involved in adherence or transport of nutrients. The first five characterized OMPs were classified as porins, and two of the OMPs, BabA (HopS) and SabA (HopP), are defined and characterized as adhesins **(Ilver et *al.,* 1998; Mahdavi et *al.,* 2002).** Many others are proposed to be involved in adhesion such as HopZ, HopQ and AlpAB (HopB and HopC) but no receptors for these proteins have yet been identified **(Loh et *al.,* 2008; Senkovich et *al.,* 2011).** The OMPs are thought to have been acquired as a single gene event that underwent duplication and divergence before the

speciation of *H. pylori* because they are also present in their closest relative, *H. acinonychis*, though the gene repertoire is somewhat dissimilar **(Gressmann et *al.,* 2005).** The evolution of the OMPs has probably been a continuous process because some genes are still found as duplicates (*hopJ/K* and *hopM/N)* indicating a recent duplication event **(Alm et *al.,* 2000).** The geographic variation that can be seen for other virulence factors of *H. pylori* such as CagA and VacA is also seen for the OMPs. **Kawai et *al.* (2011)** have recently published a comparison between 20 complete genomes from East Asia, Europe, West Africa, and United state and found distinct differences in the prevalence of *babC* (HopU), which only seems to be present in the European strains.

### I.6.3 *Helicobacter*-induced gastric pathology in humans

Chronic infection with *H. pylori* is strongly associated with gastric pathology, including chronic active gastritis, peptic ulcer disease, gastric adenocarcinoma and gastric extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue type (MALT lymphoma), of these outcomes, the most significant in terms of mortality is gastric adenocarcinoma. Recent meta-analyses suggest that the relative risk of developing gastric cancer is 2 to 3 times higher for people infected with *H. pylori* than for those without infection **(Danesh, 1999).** Understanding these different pathological conditions is important for understanding how faithfully the available models recapitulate the clinical features of *H. pylori* pathology.

#### I.6.3.1 Superficial gastritis

The commonest outcome of *H. pylori* infection is gastritis. Acute gastritis has rarely been described in humans, but has been reported in the context of experimentalists being exposed to *H. pylori* either accidentally **(Sobala et *al.,* 1991)** or in a deliberate attempt to induce gastric pathology **(Morris and Nicholson, 1987; Morris et *al.,* 1991).** In these cases, the infected individuals reported symptoms and underwent endoscopic assessment with biopsy of the inflamed gastric mucosa. The early stages of disease are marked by the presence of a polymorpho-nuclear leukocyte infiltrate in the gastric mucosa and a transient reduction in gastric acid output. According to **Marshall et *al.* (1985b)** and **Morris and Nicholson (1987),** *H. pylori* eradication therapy was prescribed. This was effective in eradicating *H. pylori* from the gastric mucosa, and led to the complete resolution of symptoms and of gastric histological abnormalities. **Sobala et *al.* (1991)** observed that symptoms and signs resolved spontaneously, and repeat endoscopy demonstrated low levels of *Helicobacter* colonizing the gastric antrum, together with an increase in lymphocytes within the gastric mucosa. These histological changes correlated with IgM and IgG sero conversion for *H. pylori*, which are

typical for chronic, superficial *H. pylori* gastritis. This is the most prevalent *H. pylori*-induced gastric pathology worldwide **(Filipe et *al.,* 1995; Campbell et *al.,* 2001).**

### I.6.3.2 Peptic ulcer disease

Individuals colonized with *H. pylori* present a higher risk to develop peptic ulcer disease (PUD) than those not exposed to this infection **(Li et *al.,* 2010).** However, the reduced incidence of *H. pylori* infection worldwide has coincided with a reduction in PUD **(Groenen et *al.,* 2009).** In contrast to the 1980s, when the association of *H. pylori* and PUD was first established **(Graham, 1998),** individuals presenting with this disease are now less likely to be colonized with *H. pylori*; more often, their condition is linked to non-steroidal anti inflammatory drug use or to low-dose aspirin (**Sung et *al.,* 2009; Musumba et *al.,* 2012).** *H. pylori*-induced peptic ulceration occurs in the context of pre-existing chronic superficial gastritis, but is associated with increased gastric acid secretion and a T-helper 1 (Th1) polarized immune response, compared with individuals with isolated superficial gastritis **(D'Elios et *al.,* 1997; Shimada et *al.,* 2002).** Frequently, individuals with PUD exhibit antral predominant gastritis, which leads to enhanced gastrin secretion. In turn, this stimulates the parietal cells of the gastric corpus to secrete more acid, leading to mucosal ulceration (**McColl et *al.,* 1997**). Eradication of *H. pylori* is reportedly sufficient to suppress excess gastrin secretion, which is an important component of the healing process of *H. pylori*-associated peptic ulcers (**McColl et *al.,* 1991**).

### I.6.3.3 Gastric adenocarcinoma and its precursor lesions

In 2012, gastric cancer was the fifth commonest malignancy worldwide, and the third commonest cause of cancer-related death, with over 720,000 deaths worldwide caused by the disease **(Ferlay et *al.,* 2013).** *H. pylori* colonization is the single biggest risk factor for gastric carcinogenesis and is a risk factor in at least 80% of cases of gastric cancer **(Graham, 2015).** However, as only a very small percentage of people infected with *H. pylori* go on to develop gastric cancer, understanding why those individuals do so is a key aim of future studies in this field. Other risk factors linked to gastric cancer **(Fig. 7)** fall into two main groups **(Burkitt et *al.,* (2017).** The first consists of potentially modifiable exogenous risk factors, such as dietary salt and nitrosamine intake **(Jakszyn et *al.,* 2006; Wang et *al.,* 2009),** *H. pylori* virulence factors **(Yamaoka, 2010),** non-*Helicobacter* gastric microbiota **(Dicksved et *al.,* 2009; Lofgren et *al.,* 2011)** and smoking status **(La Torres et *al.,* 2009).** The second group consists of unalterable host genetic, or intrinsic, risk factors. Amongst these genetic factors are polymorphisms at loci encoding cytokines and their receptors **(Persson et *al.,* 2011),** stromal remodelling proteins, such as matrix metallo proteinases **(Tang et *al.,* 2008),** and prostate

stem cell antigen (PSCA), which in the context of gastric pathology, acts as a tumour suppressor gene **(Garcia-Gonzalez et *al.,* 2015; Ichikawa et *al.,* 2015).** The development of gastric cancer occurs through a stereotypical pathological pathway **(Fig. 7),** which was first proposed well before the identification of *H. pylori* **(Correa et *al.,* 1975).** Over the course of several decades, some individuals with chronic superficial gastritis develop gastric atrophy, characterized by the patchy loss of parietal cells in the gastric corpus mucosa. This decreases gastric acid secretion, leading to higher intraluminal pH, decreased somatostatin secretion and consequent gastrin secretion. In addition to stimulating acid secretion from parietal cells, gastrin also enhances proliferation in the gastric epithelial stem cell zone, leading to an increase in epithelial cell turnover **(Burkitt et *al.,* 2009)**.

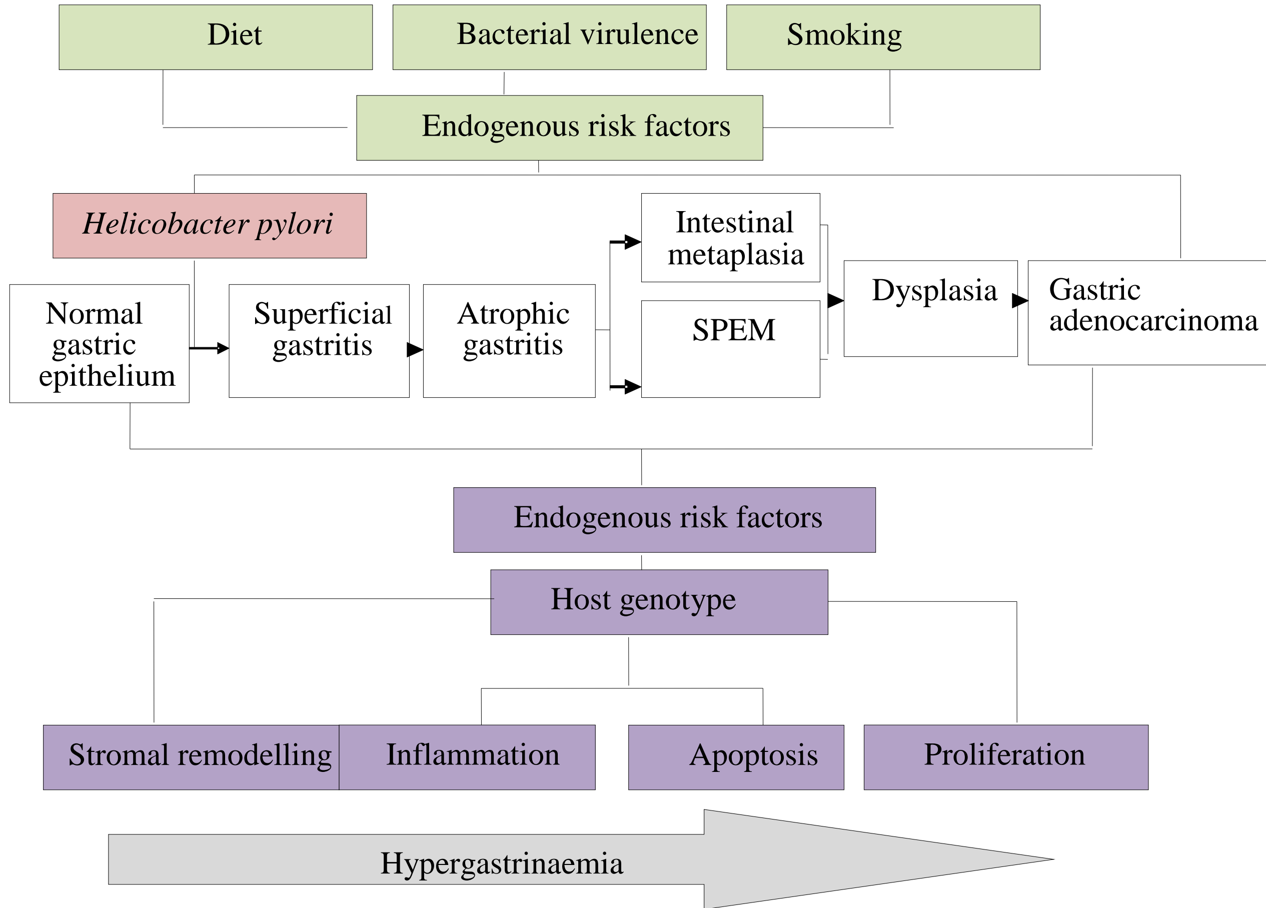



**Figure 7*:*** *H. pylori* infection and progression to gastric cancer. A schematic demonstrating the pathological progression of *H. pylori*- induced gastric pre-neoplasia, and highlighting endogenous risk factors for progression towards gastric cancer **(Graham, 2015).**

SPEM: Spasmolytic polypeptide expressing metaplasia

A proportion of people with established gastric atrophy develop intestinal-type metaplasia of the gastric mucosa over time, where oxyntic glands are replaced by CDX2 (caudal-type homeobox 2)-expressing glandular units, which are morphologically similar to the intestinal crypt. Intestinal metaplasia in the stomach is linked to gastric dysplasia; up to 20% of affected individuals with intestinal metaplasia have concurrent dysplasia **(Den Hoed et *al.,* 2011).** Gastric epithelial dysplasia is associated with an at least 10-fold increased risk of developing gastric cancer, but it has been difficult to represent this risk accurately from population-based studies **(Lauwers et *al.,* 1999)**.

Several studies have assessed the strategy of testing for, and eradicating, *H. pylori* in populations at a high risk of developing gastric cancer. Unfortunately, a recent well-designed meta-analysis confirmed a relatively poor outcome for this strategy. The eradication of *H. pylori* reduces the incidence of gastric cancer in healthy asymptomatic infected Asian individuals **(Ford et *al.,* 2014).** However, when individuals with pre-existing pre-neoplastic gastric pathology (defined as the presence of gastric atrophy, intestinal metaplasia or dysplasia) were considered, there was no evidence that eradication of *H. pylori* decreased the risk of gastric cancer. For this highest risk group, therefore, there are currently no effective therapeutic strategies **(Burkitt et *al.,* 2009)**.

**I.6.3.4 MALT lymphoma**

Gastric extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue (MALT lymphomas) are B-cell lymphomas that develop within the mucosa-associated lymphoid tissue of the stomach. The incidence rate of gastric MALT lymphoma in the USA was estimated to be 3.8 in 1,000,000 individuals between 2001 and 2009, making it a rare outcome of *H. pylori* infection **(Khalil et *al.,* 2014).** In the only published systematic review of this condition, 79% of 1844 reported cases of MALT lymphoma were associated with *H. pylori* infection **(Asenjo and Gisbert, 2007; Gisbert and Calvet, 2011)**.

As with other haematological malignancies, characteristic cytogenetic profiles have been described for MALT lymphoma. Proper regulation of nuclear factor kB (NF-kB) transcriptional activity is required for normal lymphocyte function, and deregulated NF-kB signaling can facilitate lymphomagenesis. The API2-MALT1 fusion oncoprotein created by the recurrent t(11;18)(q21;q21) in mucosa-associated lymphoid tissue (MALT) lymphoma induces proteolytic cleavage of NF-kB–inducing kinase (NIK) at arginine 325. NIK cleavage requires the concerted actions of both fusion partners and generates a C terminal NIK fragment that retains kinase activity and is resistant to proteasomal degradation. The resulting

deregulated NIK activity is associated with constitutive noncanonical NF-kB signaling, enhanced B cell adhesion, and apoptosis resistance **(Rosebeck et *al.,* 2011).**

MALT1 encodes mucosa-associated lymphoid tissue lymphoma translocation protein 1, which is essential for the activation and proliferation of T- and B-lymphocytes, and also plays a fundamental role in NF-κB activation. One of the downstream effects of this fusion protein is enhanced cleavage of NIK (NF-κB-inducing kinase), which is a critical regulator of alternative pathway NF-κB signalling **(Merga et *al.,* 2016).**

## I.7 Diagnosis of *H. pylori* infection

The detection of *H. pylori* infection is a primary requisite for diagnosis of gastroduodenal diseases related to this bacterium. In the paediatric population, duodenal ulcer is strongly associated with *H. pylori* infection and the risk of development of gastric cancer is relatively high if the infection is acquired at a young age **(Blaser et *al.*, 1995; Huang et *al.*, 1999).** In view of these critical issues, accurate diagnosis of *H. pylori* infection is a key step towards proper patient management. Two categories of diagnostic methods for *H. pylori* infection are defined: invasive and non-invasive.

Several factors such as the need to evaluate the sensitivity, specificity, positive and negative predictive value of a given test must be taken into account when selecting for a test. In addition, the age of patients being tested also has to be considered. At present, no single test can be absolutely relied upon to detect *H. pylori* colonization but if feasible, a combination of two tests is recommended **(Laheij et *al.*, 2000).** The European *Helicobacter pylori* Study Group (EHPSG) also recommend that two or more tests can be performed as the gold standards in comparative studies **(EHPSG, 1997).**

### I.7.1 Invasive tests

The invasive methods require gastric biopsy specimens obtained during gastroduodenoscopy. Presently, invasive biopsy tests include staining of histological samples, biopsy urease test, culturing of biopsy specimens and Polymerase chain reaction (PCR).

#### I.7.1.1 Gastric biopsies

The most accurate method for detecting *H. pylori* in tissue is a combination of culture and histologic staining (Warthin Starry stain, Giemsa stains or hematoxylin and eosin stain) of the biopsy specimens obtained during endoscopy. With histologic staining, a histopathologist can document *H. pylori* infection and at the same time assess the atrophic changes in the stomach. However, the reliability of detecting *H. pylori* infection depends on factors such as the site, patching distribution of *H. pylori*, number and size of the biopsy specimens, as well

as the stain used, and also the high level of expertisin staining play an important role on the visualization of the bacteria **(Woo et *al.*, 1996; El-Zimaity and Graham, 1999).**

Despite the fact that culture will provide unequivocal evidence of the presence of *H. pylori,* successful recovery of the bacteria from biopsies will depend on transporting and processing of the samples, selection of media and culture conditions **(Piccolomini et *al.*, 1997).** This method enables the growth of fresh clinical isolates which can be tested for antibiotics susceptibility which can be useful in treatment management. The other advantage is to use the culture obtained for research, especially in molecular epidemiological study. Essentially, obtaining the culture enables fingerprinting of the isolate and typing of the isolate with respect to the virulence genes **(Grove et *al.*, 2001).**

### I.7.1.2 Molecular biology techniques

Polymerase chain reaction (PCR) is particularly useful for molecular epidemiology and for fingerprinting *H. pylori* isolates. This technique has been used to genotype *H. pylori* in paraffin-embedded gastric biopsy specimens **(Scholte et *al.*, 2002),** which may be useful in the post-treatment period, to differentiate recurrence of infection from re-infection with another strain of *H. pylori* **(Hildebrand et *al.*, 2001).** Recently, molecular techniques such as real time PCR and fluorescent in situ hybridisation (FISH) were evaluated for use in detection of *H. pylori* in gastric biopsy samples. The c*agA* and *vacA* genotypes were tested by melting curve using the real-time PCR and compared to the gastritis status and cell proliferation status **(Ruzsovics et *al.*, 2001). Russmann et *al.* (2001)** demonstrated that this bacterial pathogen was detected in 63 biopsy specimens as compared to 67 cultured positive specimens by Fluorescence in situ hybridization (FISH) with rRNA-targeted fluorescence-labeled oligonucleotide probes specific for *H. pylori*.

### I.7.2 Non-Invasive tests

Non-invasive tests obviate the need for endoscopy which can inflict physical stress on the patient. Moreover, invasive tests could yield possible false negative results due to the patchy distribution of the bacteria in the stomach **(Pronovost et *al.*, 1994).** Clinical tests like the Urea breath test and serology test are well-established screening procedures which help to reduce the cost and workload of invasive endoscopy, given the special niche of *H. pylori*, attempts are continuously made to improve the non-invasive diagnostic tests **(Laheij et *al.*, 1998; Vaira et *al.*, 1998).**

### I.7.2.1 Urea breath tests (UBT)

The UBT are easy, straightforward and accurate non-invasive tests for *H. pylori* infection. The test is based on the principle that in the presence of *H. pylori* urease activity, $CO_2$ is liberated from urea and produces ammonia to buffer its acidic environment. Ingestion of C-labelled urea results in the excretion of labelled $CO_2$ which can be detected in the expired breath. C13 and C14 UBT are semi-quantitative tests of active *H. pylori* infection **(Debongnie et *al.*, 1991; Chang et *al.*, 2002).**

C-UBT, containing the nonradioactive isotope, has high diagnostic accuracy in children **(Delvin et *al.*, 1999).** However, this method has its drawbacks: 1. It is difficult to perform in noncompliant children who are unwilling to ingest the C13-urea, 2. Age of children may make exhaled breath collection difficult, 3. Mental or physical disturbances may present further difficulty. This was illustrated in the study by **Imrie et *al.* (2001)** which showed that borderline or false positive results occurred more frequently in children younger than 2 years compared with older children.

### I.7.2.2 Serological tests

*H. pylori* elicits a local mucosal and a systemic antibody response. Serologic testing is based on the detection of anti-*H. pylori* IgG antibody in the patient's serum **(Blecker et *al.*, 1995; Wang et *al.*, 2003).** The simplicity and cost-effectiveness have enabled the serological tests to be widely employed for epidemiological research to assess the prevalence of *H. pylori* infection in various populations **(Brown et *al.*, 2002; Chong et *al.*, 2003).** The commonly employed sero-diagnostic technique is the enzyme-linked immuno-sorbent assay (ELISA). The sensitivity and specificity of ELISA is dependent on the nature of the bacterial antigen preparation. Therefore, considerable research effort has been placed in search of a suitable antigen **(Ho and Marshall, 2000).** Commercial tests, the *Pylori* set and the Helico-G use an acid extract of *H. pylori* antigen. The HM-CAP uses a mixture of high molecular weight *H. pylori* cell associated proteins, consisting mainly of urease and cell wall adhesin **(Evans et *al.*, 1989)**.

The performance of serologic tests in diagnosis of treatment success is hampered by the lingering serological response after successful eradication therapy or spontaneous healing which will lead to false positive results. Furthermore, studies have shown that the cut-off values for children and adults may differ **(Kindermann et *al.,* 2001, Kolho et *al.,* 2002). Crabtree et *al.* (1991)** showed that if the adult cut-off value was used, 50% of the children with *H. pylori* gastritis would have been considered sero-negative. Similarly,

**Sunnerstram et *al.* (1999)** also recommended a lower cut-off value for children than for adults. It is clear that the choice of cut-off value is a mean of adapting the serological test to the patient population analysed. Hence, when children are tested for *H. pylori* antibodies, it is important to choose a method which has already been validated in the paediatric population. Immuno-blotting is highly sensitive and more specific than ELISA. It provides a full serologic profile of the immunogenic proteins that can be obtained from an individual. This method can be used to complement ELISA, especially when ELISA results are doubtful **(Raymond et *al.,* 2000).**

**I.7.2.3 Stool antigen test (HpSA)**

In 1998, the United States Food and Drug Administration (FDA) approved the use of a new enzyme immunoassay for the detection of *H. pylori* antigen in stool for diagnosis and eradication efficacy of *H. pylori* infection in adult population. *H. pylori* stool antigen test (HpSA) has been devided to detect the presence of *H. pylori* antigen by an ELISA method using monoclonal/polyclonal antibodies **(Vaira et *al.*, 1999; Suzuki et *al.*, 2002).** Many studies have reported that HpSA test is useful for the initial diagnosis of *H. pylori* infection **(Vakil et *al.*, 2000).** However, its application in monitoring the efficacy of eradication therapy has been controversial as the assay can detect dead or partially degraded bacteria long after the successful eradication, leading to false positive results **(Kabir, 2001).** Nevertheless, a recent study by **Tanaka et *al.* (2003)** has shown that the HpSA test is a useful method for initial diagnosis and after eradication therapy. They reported a high sensitivity (98.3%) and specificity (95%) prior to treatment. The sensitivity and specificity at the end of eradication therapy were 90% and 97.7%, respectively. Accessibility and non-invasiveness have made this new test an invaluable diagnostic tool in the paediatric population. Data concerning reliability of the test in children has yielded satisfactory results **(Van Doorn et *al.*, 2001; Koletzko et *al.*, 2003).** In the study by **Ni et *al.* (2000)**, involving 53 children, the performance of HpSA was evaluated and compared with 6 other diagnostic tests (Culture, biopsy urease test, histology, PCR, UBT and serology). The diagnostic accuracy of HpSA (sensitivity 92.6%, specificity 100%) was observed to be comparable to the other tests. This non-invasive test was also found to be a useful method for post-treatment eradication testing of infection in children **(Gosciniak et *al.*, 2003).** In addition, the European *H. pylori* Study Group has recommended the stool antigen test and C13-UBT for diagnosis of the infection and eradication assessment in older children **(Malfertheiner et *al.*, 2002).**

### I.7.3 Other non-invasive tests

Some studies have looked into the use of saliva and urine as possible non-invasive means to detect antibody to *H. pylori* infection **(Yamamoto et *al.*, 2000).** It was observed that the evaluation result for salivary assay was not satisfactory **(Luzza *et al.*, 2000).** However, **Yamamoto et *al.* (2000)** recorded a sensitivity of 92% and a specificity of 93.1% with the urine based ELISA kit. Culturing of *H. pylori* from a gastric string which was to be swallowed by the individual has also been considered as a substitute for endoscopy biopsy **(Ferguson et *al.*, 1999; Samuels et *al.*, 2000).** The study of **Samuels et *al.* (2000),** demonstrated that *H. pylori* was isolated from 32/33 patients from a swallowed string coupling with the use of selective bacterial culture media. The string test also showed potential use as the culture obtained can be used for molecular epidemiological studies and the routine determination of antibiotic susceptibility prior to further therapy **(Wang et *al.*, 2003).**

## I.8 Treatment

### I.8.1 First-line therapy

Triple therapy based on a PPI combined with clarithromycin and amoxicillin and/or metronidazole is the established first-line therapy over the past years around the world **(Caselli et *al.,* 2007).** Standard triple therapy started from eradication rates of more than 90%, and has now decreased to 70–80% **(Paoluzi et *al.,* 2010).** In a recent randomized, open-label, non-inferiority, phase 3 trial, the efficacy of standard triple therapy was low with 55% eradication rate compared with the quadruple therapy **(Malfertheine et *al.,* 2011).** Several attempts, such as the extension of treatment duration, have been undertaken to improve the efficacy of the standard PPI triple therapy. However, the increased length of therapy only results in a modest or insignificant rate of treatment success. Only some studies from the USA found a limited advantage of the 14 days regimen compared to the 7 days regimen. A recent meta-analysis including 21 studies from all over the world compared 7 days and 14 days treatments. This meta-analysis yielded relative risks (RRs) for eradication of 1.05 (95% CI 1.01– 1.10) for 7 day compared with 10-days triple therapy and 1.07 (95% CI 1.02–1.12) for 7 days compared with 14 day therapy. The eradication rates were 73 and 78%, respectively. Taken together, the extension of the PPI-clarithromycin based triple therapy to 14 days slightly improves the eradication success and can be considered in specific clinical situations **(Fuccio et *al.,* 2007).** Finally, a recent meta-analysis including 26 studies was presented at the Digestive Disease Week 2010. This study has yielded very similar results, with a RR of 0.79 (95% CI: 0.71–0.89; $p < 0.0001$) for 14-day therapy with an improvement of eradication rates

of approximately 6%.A similar benefit was seen with 10 days of treatment compared to 7 days (RR 0.84, 95% CI 0.73– 0.96, P ¼ 0.01). The improvement of *H. pylori* cure rates for 10 days of triple therapy was 4% **(Flores et *al.,* 2010).** Concerns need to be expressed for decreased compliance and possibly more side-effects, which may occur with the prolonged treatment regimen **(Vakil et *al.,* 2004; Fucciod et *al.,* 2007) .** At the moment, it seems that standard triple therapy which represents the accepted standard therapy for *H. pylori* since the mid 1990s needs to be reconsidered. At this point of time, it remains the choice of treatment when the *H. pylori* infection is known to be susceptible to clarithromycin and local antimicrobial resistance rates are below 15–20%. In areas with high clarithromycin resistance bismuth containing quadruple is superior to standard triple therapy **(Malfertheine et *al.,* 2007).**

The original quadruple therapy based on omeprazole, bismuth subcitrate, metronidazole, and tetracycline (OBMT) achieves high eradication rates compared to standard triple therapy **(Gene et *al.,* 2003; Laine et *al.,* 2003).** In a randomized, open label, non inferiority, phase 3 trial, the efficacy of the quadruple therapy as first-line therapy was proven. In this study, 10 days of treatment with omeprazole and a single three-in-one capsule containing bismuth subcitrate potassium, metronidazole, and tetracycline (quadruple therapy) has been compared with 7 days of omeprazole, amoxicillin, and clarithromycin (standard therapy). The quadruple therapy yielded higher eradication rates compared to standard triple therapy **(Malfertheine et *al.,* 2007).** Eradication rates were 80% in the quadruple therapy group vs. 55% in the standard therapy group. The study further demonstrated that the efficacy of the quadruple therapy does not seem to be affected by either metronidazole or clarithromycin resistance. The safety and tolerability of the quadruple therapy, which is still one of the unjustified concerns against the quadruple therapy, were similar to standard therapy **(Selgrad and Malfertheiner, 2011).**

Thus, it has to be concluded that quadruple therapy needs to be considered as first-line therapy in areas with a high prevalence of clarithromycin-resistant *H. pylori* strains. It should be noted that the galenic formulation of three drugs with antibacterial activity provides more consistent results **(Gene et *al.,* 2003; Laine et *al.,* 2003; Malfertheine et *al.,* 2007),** than the loose combination of these drugs as used in several other studies **(Luther et *al.,* 2010).** In a Chinese study, a new quadruple regimen adding bismuth potassium citrate to the standard triple therapy, including PPI, clarithromycin and amoxicillin has been introduced. This new regimen showed a higher efficiency than PPI triple therapy and the addition of bismuth and

prolongation of the treatment from 7 to 14 days allowed to overcome clarithromycin resistance in 84% of the patients **(Sun et *al.,* 2010).**

The sequential therapy originally introduced and extensively studied in Italy has constantly demonstrated higher eradication rates than the standard triple therapy. Sequential treatment is a nonbismuth quadruple therapy, consisting of a PPI, amoxicillin, clarithromycin and a nitroimidazole (metronidazole or tinidazole). The sequential therapy lasts up for 10 days and is built up in two parts. During the first 5 days of treatment a PPI and amoxicillin is administered followed by another 5 days therapy with clarithromycin and a nitroimidazole (metronidazole or tinidazole). The interesting aspect of the sequential therapy is that this regimen is generally effective in areas of high clarithromycin resistance and/or dual clarithromycin and metronidazole resistance **(Gatta et *al.,* 2009).** Within the first 5 days of therapy, clarithromycin-resistant strains get eradicated by PPI amoxicillin therapy, and in the remaining 5 days the remnant strains are eliminated by the triple therapy. The sequential therapy has now been evaluated in various randomized trials and the therapeutic success was overall confirmed to be superior with respect to the standard triple therapy **(Gatta et *al.,* 2009).** This regimen provides high eradication rates (>90%) in various countries and continents of the world, though not always in controlled trials **(Sirimontaporn et *al.,* 2010) .**

However, it must be noted that it may not work in all geographic areas as recent studies have shown eradication rates of around 80% in areas with high clarithromycin resistance **(Romano et *al.,* 2010; Mahachai et *al.,* 2011).** This may also be related to higher rates of dual resistance in this area. Furthermore, previous studies demonstrated significantly lower eradication rates with the metronidazole-based regimen compared to the tinidazole based regimen **(Vaira et *al.,* 2009).**

Interestingly, the increase of therapy duration from 10 to 14-days confirms that duration prolongation does not improve eradication rates **(Graham and Fischbacht, 2010).** A simplification of the sequential therapy represents the so-called concomitant therapy. Concomitant therapy is a quadruple that contains all four compounds of the sequential and all medications are given together. This regimen is therefore less complex than the sequential therapy and might improve compliance. Interestingly, this therapy regimen was equal to sequential therapy in one study **(Wu et *al.,* 2010).** Concomitant therapy was proven to be effective with eradication rates above 90% **(Okadar et *al.,*1999; Graham and Fischbacht, 2010).** Up to now, the sequential and concomitant therapies are promising therapeutic approaches also as first-line treatment options. But for a general recommendation of the

sequential therapy or its quadruple variation as first-line treatment, studies from multicenter, multi-region randomized trials are eagerly awaited **(Selgrad and Malfertheiner, 2011).**

### I.8.2 Second-line treatment

An important indication for the bismuth-containing quadruple therapy is its use as empirical second-line therapy after failure of PPI, clarithromycin-containing triple therapy. Several studies have proven the efficacy of quadruple therapy as second-line and/or salvage therapy with eradication rates constantly higher than 80% **(Lin et *al.,* 2002).** Levofloxacin therapy is a reasonable second-line therapy. The efficacy of levofloxacin-based triple therapy has been proven in a meta-analysis comparing this regimen with quadruple therapy as salvage therapy **(Gisbert and Morena, 2006).** The overall eradication rate with the levofloxacin regimen was 81 vs. 70% with the quadruple combination, this meta-analysis also revealed fewer side effects and adverse events using levofloxacin-based triple therapy. The increasing *H. pylori* resistance to clarithromycin has prompted authors to investigate the opportunity to incorporate levofloxacin instead of clarithromycin in the standard triple therapy regimen. This suggestion was examined in a recent crossover study, comparing levofloxacin based and clarithromycin-based triple therapies as first-line and second-line treatments for *H. pylori* infection. Clarithromycin-based triple therapy (PPI-AC) achieved a higher eradication rate than levofloxacin- based triple therapy (PPI-AL) as the first-line treatment (87.4% PPI-AC vs. 80.1% PPI-AL). However, in patients who failed with standard triple in first line, PPI-AL was superior as second line when compared to PPI-AC second line after failure with the levofloxacin-containing triple used in first line **(Liou et *al.,* 2010).**

The study clearly demonstrated that antibiotics which are currently used as rescue medications (i.e. levofloxacin) cannot randomly be replaced and then switched to first line. After failure of recommended first-line therapies, levofloxacin-based triple therapy can be recommended. However, rising rates of levofloxacin resistance as described above need to be taken into account and it has to be noted that quinolone resistance is often associated with resistances to metronidazole and clarithromycin. Moxifloxacin-based regimes have been recently proposed as a treatment option for *H. pylori*. Already in 2005, eradication rates of up to 92% in the moxifloxacin- based triple regimens compared to 79% in the clarithromycin based regimens were demonstrated **(Nista et *al.,* 2005)**. A recent study from Korea has reported a steady increase of moxifloxacin resistance from 5.6% in 2004 up to 28.2% in 2008 with the need to optimize dosage and duration of treatment **(Yoon et *al.,* 2009)**. Treatment for 10 days should be preferred over a 7 day course, and there is evidence that a dose of 800 mg per day is superior to the 400 mg standard dose **(Bago et *al.,* 2010; Sacco et *al.,* 2010)**.

Moxifloxacin based triple therapy remains an alternative as second-line treatment (**Kang et *al.,* 2007; Miehlke et *al.*, 2008**). After the failure of second-line therapies, rescue therapy should be guided by antimicrobial resistance testing whenever possible, as recommended in the current European guidelines (**Malfertheine et *al.,* 2007).**

### I.8.3 Alternative therapies

While antibiotics are the main agents used in the therapy of *H. pylori* infection, the development of resistance has limited their application. Also, administration of antibiotics perturbs the microbiota, the microorganisms that colonize the human gastrointestinal tract, and thus causes side effects, such as diarrhea. Because of this, alternative therapies, including the use of phytomedicines and probiotics, have been used for the treatment of *H. pylori* infection.

#### I.8.3.1 Phytomedicines

There is increasing evidence that traditional Chinese medicines (TCMs) are effective in the treatment of various diseases. The efficacy and safety of TCMs for the treatment of *H. pylori* have been reviewed and the average eradication rate was found to be about 72% **(Lin and Huang, 2009)**, suggesting that TCMs may not be a stand-alone therapy for *H. pylori* infection. Nevertheless, the role of TCMs in *H. pylori* treatment remains to be clarified. In addition to TCMs, other phytomedicines that have been used for the treatment of *H. pylori* infection are green tea catechins, garlic extract, cranberry juice, and propolis **(Vítor and Vale, 2011)**. For example, it has been demonstrated that a combination of catechins and sialic acid can effectively prevent *H. pylori* infection in animals and improve the eradication rate **(Yang et *al.,* 2008; Yang et *al.,* 2013)**. As catechins and sialic acid have different anti-bacteria actions, the additive or synergistic effects caused by such a combination may provide a potential strategy for treating *H. pylori* infection in the future. However, since most studies have been carried out *in vitro* or in animals, the efficacy of phytotherapy in humans needs to be verified by suitable clinical trials.

Numerous studies have been carried out to investigate the anti-*H. pylori* activity of plant extracts, partially purified fractions and essential oils. Anti-*H. pylori* activity for the medicinal plant extracts and partially purified fractions is listed in **Table 4**, which has those results categorized as 4 classes according to their minimum inhibitory concentration (MIC): (1) strong activity (MIC: < 10 µg/ml); (2) strong-moderate activity (MIC: 10-100 µg/ml); (3) weak-moderate activity (MIC: 100-1000 µg/ml); and (4) weak activity (MIC: >1000 µg/ml) **(Wang, 2014).** 34 studies including more than 80 plants were collected. Surprisingly **(Tab. 4)**, only a few studies exhibited strong (2.9%, 1/34) and strong-moderate (11.8%, 4/34)

activity. Most studies revealed weak-moderate (50%, 17/34) and weak (32.4%, 11/34) activity against *H. pylori*.

**Table 4:** Anti-*H. pylori* activity of some medicinal plant extracts **(Wang, 2014)**

| **Plant** | **Test sample** | **MIC/MBC** (µg/ml) |
|---|---|---|
| *Impatiens balsamina* L. | Acetone extract<br>Ethanol extract (95%) | MIC: 0.625-2.5 |
| | Ethyl acetate extracts | MBC: 1.25-2.5 |
| *Persea americana, Annona cherimola, Guaiacum coulteri, Moussonia deppeana* | Methanol extract | MIC: 7.5-15.6 |
| *Myristica fragrans (seed), Rosmarinus officinalis* (rosemary leaf ) | Methanol extract | MIC: 12.5-25 |
| *Curcuma amada* Roxb., *Mallotus phillipinesis* (Lam) Muell., *Myrisctica fragrans* Houtt., *Psoralea corylifolia* L. | Ethanol extract (70%) | MIC: 15.6-62.5 |
| *Achillea millefolium, Foeniculum vulgare* (seed), *Passiflora incarnata* (herb), *Origanum majorana* (herb) and a (1:1) combination of *Curcuma longa* (root), ginger rhizome | Methanol extract | MIC: 50 |
| *Carum carvi* (seed), *Elettaria cardamomum* (seed), *Gentiana lutea* (roots), *Juniper communis* (berry), *Lavandula angustifolia* (flowers), *Melissa officinalis* (leaves), *Mentha piperita* (leaves), *Pimpinella anisum* (seed) | Methanol extract | MIC: 100 |
| *Abrus cantoniensis, Saussurea lappa, Eugenia caryophyllata* | Ethanol extract | MIC: 40 |
| *Hippophae rhamnoides, Fritillaria thunbergii, Magnolia officinalis, Schisandra chinensis, Corydalis yanhusuo, Citrus reticulata, Bupleurum chinense, Ligusticum chuanxiong* | Ethanol extract | MIC: 60 |
| *Myroxylon peruiferum* | Methanol extract | MIC: 62.5 |
| *Aristolochia paucinervis* | Rhizome/leave fraction | MIC: 4-128 |
| *Cistus laurifolius, Spartium junceum, Cedrus libani, solstitialis, Momordica charantia, Sambucus ebulus, Hypericum perforatum* | Solvent extract and hexane fraction | MIC: 1.95-250 |
| *Larrea divaricate Cav* (leaves and tender branches) | Aqueous extract | MIC: 40-100 |
| *Acacia nilotica* (L.) Delile, *Calotropis procera* (Aiton) | Methanol/acetone extract | MIC: 8-256 |
| W.T. Aiton, *Fagonia arabica* L., *Adhatoda vasica* Nees, *Casuarina equisetifoli*a L. | | |
| *Zingiber officinale* | Ethanol extract (95%) | MIC: 10-160 |
| *Tephrosia purpurea* (Linn.) Pers. | Methanol extract and fraction | MIC: 25-400 |
| *Terminalia macroptera* (*root*) | Root solvent fraction | MIC: 100-200 |
| Black myrobalan (*Teminalia chebula* Retz) | Water extract | MIC: 125 |
| | | MBC: 150 |
| *Rubus ulmifolius* leaves | Ethyl acetate extract<br>Methanol extract | MIC: 134-270 |
| *Amphipterygium adstringens* | Bark petroleum ether | MIC: 160 |

| | fraction | |
|---|---|---|
| *Lycopodium cernuum* | Hexane fraction | MIC: 16-1000<br>MBC: 125-1000 |
| *Ageratum conyzoides, Scleria striatinux, Lycopodium cernua* | Methanol extract | MIC: 63-1000<br>MBC: 195-15000 |
| *Sclerocarya birrea* | Acetone/aqueous stem bark extract | MIC: 80-2500 |
| 43 plants : Including *Artemisia ludoviciana subsp.mexicana* | Methanol/aqueous extract | MIC: 312-500 |
| *Pteleopsis suberosa* | Stem bark methanol extract | MIC: 313-500 |
| *Ageratum conyzoides, Scleria striatinux, Lycopodium cernua* | Methanol extract | MIC: 32-1000 |
| 17 plants : Including *Cuminum cyminum L., Cynara scolymus L., Origanum vulgare L.* | Ethanol extracts | MIC: 600-10000 |
| *Allium sativum* | Aqueous extract | MIC: 2000-5000 |
| *Menthapiperita*, Peppermint oil, *Origanum vulgare, Pimpinella anisum*, Aniseed oil, *Syzygium aromaticum* | Essential oil | IC$_{50}$: 160-1460 |
| *Chamomila recutita* L., *Ilex paraguariensis* A. St.-Hil. | Ethanol extract (96%) | MIC: < 625-1250 |
| *Allium ascalonicum* Linn. (leaf) | Methanol extract | MIC: 625- 1250 |
| *Sclerocarya birrea* | Stem bark acetone/aqueous extracts | MIC$_{90}$: 60-2500 |
| *Punica granatum, Quercus infectoria* | Ethanol extract | MIC: 160-> 2500 |
| *Mentha piperita*, Peppermint Oil, *Origanum vulgare, Pimpinella anisum*, Aniseed Oil, *Syzygium aromaticum* | Essential oil | IC$_{50}$: 160-1460 |
| 13 plants : Including *Anthemis melanolepis* | Methanol extract (70%) | MIC: 625-5000 |
| 17 plants : Including *Cuminum cyminum* L. | Ethanol extract | MIC: 75-10000 |
| *Plumbago zeylanica* L. | Acetone extract | MIC: 320-10240<br>MBC: 5120-81920 |
| *Anisomeles indica* (L.) O. Kuntze, *Alpinia speciosa* (Wendl.) K. Schum., *Bombax malabaricum* DC., *Paederia scandens* (Lour.) Merr. | Ethanol extract (95%) | MIC: 640-10240 |
| *Allium sativum* | Aqueous extract | MIC: 0.1% (v/v) |
| 13 plants : Including *Cymbopogon citratus* (lemon grass) | Essential oil | MIC: 0.1% (v/v) |

MBC : Minimum bactericidal concentration  MIC : Minimum inhibitory concentration

#### I.8.3.2 Probiotics

Probiotics are living organisms that are administered orally to confer a health benefit on the host. In recent years, their application of probiotics in the treatment of *H. pylori* infection has become an active research field. Several probiotics, including *Saccharomyces boulardii* (*S. boulardii*) and *Lactobacillus* strains, have been combined with antibiotic containing therapies to treat infection. Compared to standard triple therapy, although addition of *S. boulardii* significantly reduced the incidence of antibiotic associated diarrhea, it did not significantly improve the eradication rate of *H. pylori* (**Cremonini et *al.,* 2002; Hurduc et *al.,* 2009**).

Likewise, addition of *Lactobacillus GG* significantly reduced the incidence of diarrhea, but did not improve the eradication rate of triple therapy **(Armuzzi et *al.,* 2001; Cremonini et *al.,* 2002).** Addition of *Lactobacillus acidophilus* was reported to significantly increase treatment outcome of triple therapy **(Canducci et *al.,* 2000)**, but, in another study, addition of the combination of *Lactobacillus acidophilus* and *Bifidobacterium lactis* failed to show an improvement in *H. pylori* eradication . Intriguingly, in contrast to the capsule sachet based probiotic preparations, fermented milk based probiotics have been reported to improve *H. pylori* eradication rates by about 5%-15%, possibly because some of contain additional components (e.g., lactoferrin and glycomacropeptide) that may inhibit *H. pylori* **(Sachdeva and Nagpal, 2009)**.

Probiotics may inhibit *H. pylori* growth by secreting antibacterial substances. Certain lactobacilli synthesize antimicrobial compounds related to the bacteriocin family **(Klaenhamme, 1993; Jack et *al.,* 1995).** Other known substances secreted by these bacteria are the endproducts of lactic acid fermentation, such as lactic and acetic acids, and hydrogen peroxide **(Vandenbergh, 1993).** The production of relatively large amounts of lactate by *Lactobacilli* has been implicated as an inhibitory factor of *H. pylori* **(Aiba et *al.,* 1998; Sgouras et *al.,* 2004).** Lactic acid, in addition to its antimicrobial effect resulting from the lowering of the pH, could inhibit the *H. pylori* urease. However, the inhibitory effects of *Lactobacilli* on *H. pylori* differ from strain to strain. For example, *L. johnsonii* La10 does not inhibit *H. pylori* although it produces as much lactic acid as *L. johnsonii* La1 **(Michetti et *al.,* 1999).**

On the other hand, it has been shown that other strains (*L. acidophilus* LB, *L. casei*, *L. johnsonii* La1, and *L. lactis*) exert an inhibitory effect on *H. pylori* by a lactic acid- and pH-independent mechanism **(Bernet et *al.,* 1997; Coconnier et *al.*, 1998).** The involvement of

proteinaceous compounds in this inhibitory effect has been demonstrated by several authors **(Michetti et *al.,* 1999).** However, the exact nature of antimicrobial substances secreted by these strains remains to be determined. Other probiotic bacteria, such as *Weissella confusa* **(Nam et *al.,* 2002)**, *L. lactis* **(Kim et *al.,* 2003)**, and *Bacillus subtilis* **(Pinchuk et *al.,* 2001)**, were shown to secrete bacteriocins able to inhibit *H. pylori* growth *in vitro*. In the case of *B. subtilis*, these substances were similar to animocumacins, belonging to the isocoumarin group of antibiotics **(Pinchuk et *al.,* 2001)**.

In **Table 5**; some studies including more than 10 probiotics against *H. pylori* were cited.

**Table 5:** Mechanisms of inhibition of *H. pylori* by probiotics *in vitro*
**(Drahoslava et *al.,* 2007)**

| Probiotic | Mechanism of inhibition |
|---|---|
| *L. acidophilus* 4356 | Lactic acid |
| *L. casei* 393 | Lactic acid |
| *L. salivarius* WB1040 | Lactic acid |
| *L. casei* strain Shirota | Heat-labile substance |
| *L. acidophilus* LB | Heat-stable protein |
| *L. lactis* BH5 | Bacteriocin |
| *L. acidophillus* | CRL639 autolysins |
| *W. confusa* PL9001 | Class II bacteriocin |
| *L. johnsonii* La1 | Heat-stable substance |
| *L. acidophilus* | Lactic acid |
| *L. casei* subsp. Rhamnosus | Lactic acid |
| *L. reuteri* TM 105 | Glycolipid-binding proteins |
| *B*. subtilis 3 | Anticoumacin A, B, C |
| *L. casei* strain Shirota | Lactic acid |

# Chapter -II-

## General facts on probiotics

## II.1 History

Although the preservation role of fermented dairy products was widely recognized and appreciated early, scientists first realized in the late 19th century that a wide range of traditional sour milk products had additional benefits in addition to prolonged shelf-life and pleasant sensory properties **(Vasiljevic and Shah, 2008).**The work of numerous scientists, mainly microbiologists, resulted in important developments and expansion of knowledge pertaining to the microbiology of the human body. **Escherich (1885)** was the first to recognize the importance of examining bacteria appearing in normal faeces and the intestinal tract, and consequently understanding the physiology of digestion and the pathology and therapy of intestinal diseases of microbial origin. In 1900, two microbiologists, Tissier and Moro, reported their findings of isolates from the faeces of breast-fed infants. Tissier noted that the anaerobically cultured organism had, in general, staining reactions and morphological appearance similar to those of *Lactobacilli*; however, many of them appeared in bifurcated forms. Thus, he named them *Bacillus bifidus.* Similarly**, Moro (1900)** postulated that the isolate, which he termed *Bacillus acidophilus* due to its unusual acid tolerance, was derived from the mother's breast and normally resided in the neonate's oral cavity and intestinal content. Later, **Tissier (1908)** also showed that *Bacillus bifidus* was the predominant organism in the faeces of breast-fed infants approximately three days postpartum as opposed to bottle-fed neonates, which predominantly contained *B. acidophilus* **(Moro, 1900).** At the same time, Nobel Laureate Ilya Metchnikoff noticed that Bulgarian peasants had an average life-span of 87 years, exceptional for the early 1900s, and that four out of every thousand lived past 100 years of age. One of the major differences in their lifestyle in comparison with the contemporary diet was a large consumption of fermented milk. In his well known auto-intoxication theory **(Metchnikoff, 2004)**, Metchnikoff suggested that a human body was slowly poisoned by toxins present in the body produced by pathogens in the intestine and body's resistance steadily weakened by proliferation of enteric pathogens, all of which were successfully prevented by the consumption of sour milk and lactic acid producing bacteria. His work was based on an organism previously isolated by **Grigoroff (1905),** who cultivated it from ''podkvassa'' used as a starter for production of the Bulgarian ''kisselo mleko'' (''sour milk'' or ''yahourth'') and called it *Lactobacillus bulgaricus*. In the process, Grigoroff also identified another organism, *Streptococcus thermophilus*, which received no attention since it was considered a pathogen at that time. Metchnikoff's experiments led him to believe that *L. bulgaricus* could successfully establish itself in the intestinal tract and prevent multiplication and even decrease the number of putrefactive bacteria. However, the

work of **Herter and Kendall (1910)** showed that this organism failed to establish itself in the gut, although other substantial changes in the gut microflora were observed.

Despite the fact that these findings disputed Metchnikoff's theory, scientists continued to investigate possible benefits of bacteria to the human health. Consequently, certain strains of *Lactobacillus acidophilus* were isolated and found to be capable of colonizing human digestive tract where they exerted appreciable physiological activity. **Rettger and Horton (1914)** and **Rettger and Cheplin (1920)** reported that feeding of milk or lactose to rats or humans led to a transformation of the intestinal microflora resulting in predominance of *acidophilus* and *bifidus* type culture. These findings stimulated commercial interest in products fermented by *L. acidophilus* **(Burke, 1938).** Other researches followed suit with Minoru Shirota in Japan, who recognized the importance of the preventive medicine and modulation of the gastrointestinal microflora. In 1930, he succeeded isolating and culturing a *Lactobacillus* strain capable of surviving the passage through the gastrointestinal tract. The culture identified as *Lactobacillus casei* strain Shirota was successfully used for the production of the fermented dairy product called ''Yakult'', which initiated the foundation of the same company in 1935 **(Yakult, 1998).** In the period between late 1930s and late 1950s, the research in this area lost its pace likely due to extraordinary conditions (depression, war) the world was facing at that time. The rejuvenated interest in the intestinal human microflora was seen in the late 1950s and early 60s that led to the introduction of the probiotic concept.

**II.2 Definition**

The word ''probiotics'' was initially used as an antonym of the word ''antibiotic''. It is derived from Greek words pro and biotos and translated as ''for life'' **(Hamilton-Miller et *al.,* 2003).** The origin of the first use can be traced back to **Kollath (1953)**, who used it to describe the restoration of the health of malnourished patients by different organic and inorganic supplements. A year later, **Vergin (1954)** proposed that the microbial imbalance in the body caused by antibiotic treatment could have been restored by a probiotic rich diet; a suggestion cited by many as the first reference to probiotics as they are defined nowadays.

Similarly, **Kolb (1955)** recognized detrimental effects of antibiotic therapy and proposed the prevention by probiotics. Later on, **Lilly and Stillwell (1965)** defined probiotics as substances produced by one microorganism that promoted the growth of another microorganism. Similar to this approach, **Fujii and Cook (1973)** described probiotics as compounds that either stimulated microbial growth or improved the immune response of the host without inhibiting the growth of the culture *in vitro.*

Another definition offered by **Parker (1974)** resembles more recent description of probiotics. He defined them as organisms and substances, which contribute to intestinal microbial balance. This definition was disputed by many authors since various substances even antibiotics might have been included. Late 1980s and 1990s saw a surge of different definitions of probiotics. Most frequently cited definition is that of **Fuller's (1992)**, who defined them as ''a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance''. However his definition was more applicable to animals than to humans. Other authors followed this line offering their versions. Some of these definitions are listed in **Table 6**. Although all cited authors agreed that probiotics include live microorganisms, **Salminen et *al.* (1999)** offered their view incorporating non-viable bacteria in the definition. Following recommendations of a **FAO/WHO** working group on the evaluation of probiotics in food **(2002)**, the suggested definition describes probiotics as live microorganisms that when administered in adequate amounts confer a health benefit on the host. Consequently, a wide variety of species and genera could be considered potential probiotics; commercially, however, the most important strains are lactic acid bacteria (LAB) **(Holzapfel et *al.,* 1998)**.

### II.3 Taxonomy and classification

The classification of probiotics is strict and organized: it depends on its genus, its species and strain **(WGO, 2008).** The main strains recognized as probiotics in humans are the most often lactic acid bacteria (*Lactobacilli* and *Bifidobacteria*) and yeasts of the genus *Saccharomyces* **(Robin and Rouchy, 2011).**

#### II.3.1 Yeasts

There is a great interest in finding yeast strains with probiotic potential. Different yeast species such as *Debaryomyces hansenii, Torulaspora delbrueckii*, *Kluyveromyces lactis, Kluyveromyces marxianus* and *Kluyveromyces lodderae* **(Kumura et *al.,* 2004)**.Kumura and his collaborators have shown tolerance to passage through the gastrointestinal tract or inhibition of enteropathogens. However, *Saccharomyces boulardii* is the only yeast with clinical effects and the only yeast preparation with proven probiotic efficiency in double-blind studies **(Sazawal et *al.,* 2006).**

#### II.3.2 Lactic Acid Bacteria (LAB)

The classification of LAB genera was based on morphology, mode of glucose fermentation, growth at certain temperatures, and range of sugar utilization. Even though the taxonomy has been revised since then, characters used by Orla-Jensen are still very important in current classification of LAB. They constitute a group of bacteria that have morphological,

metabolic and physiological similarities, and are also relatively closely related phylogenetically.

**Table 6:** Descriptions of probiotics **(Vasiljevic and Shah, 2008)**

| Year | Description |
|---|---|
| 1953 | Probiotics are common in vegetable food as vitamins, aromatic substances, enzymes and possibly other substances connected with vital processes |
| 1954 | Probiotics are opposite of antibiotics |
| 1955 | Deleterious effects of antibiotics can be prevented by probiotic therapy |
| 1965 | A substance secreted by one microorganism which stimulates the growth of another |
| 1971 | Tissue extracts which stimulate microbial growth |
| 1973 | Compounds that build resistance to infection in the host but do not inhibit the growth of microorganisms *in vitro* |
| 1974 | Organisms and substances that contribute to intestinal microbial balance |
| 1992 | Live microbial feed supplement which beneficially affects the host animal by improving microbial balance |
| 1992 | Viable mono- or mixed culture of live microorganisms which, applied to animals or man, have a beneficial effect on the host by improving the properties of the indigenous microflora |
| 1996 | Live microbial culture or cultured dairy product which beneficially influences the health and nutrition of the host |
| 1996 | Living microorganisms which, upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition |
| 1999 | Microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host |
| 2001 | A preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effect in this host |
| 2002 | Live microorganisms that when administered in adequate amount confer a health benefit on the host |

The general description of the bacteria within the group is Gram-positive, non-sporulating, non-respiring cocci or rods, which do, through fermentation of carbohydrates, produce lactic acid as their major end product. The common agreement is that there is a core group consisting of four genera: *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus.* Recent taxonomic revisions have proposed several new genera and the remaining group now comprises the following: *Aerococcus, Alloiococcus, Carnobacterium, Dolosigranulum, Enterococcus, Globicatella, Lactococcus, Oenococcus, Tetragenococcus, Vagococcus, Weissella. Lactobacilli, Carnobacteria* and some *Weissella* are rods while the remaining genera are cocci **(Jin et *al.,* 2009).** For identification of LAB, phenotypic methods have been most commonly used **(Corsetti et *al.,* 2001).** More recently, genetic techniques, such as 16S

rDNA sequencing have been developed which allows a more consistent and accurate identification of individual strains **(Buddhiman et *al.,* 2008).** Determination of short sequences of 16S rDNA is today used as a simple way for species determination of isolates of LAB **(Schleifer and Ludwig, 1995).**

The taxonomy of LAB has been based on the Gram reaction and the production of lactic acid from various fermentable carbohydrates. Their classification into different genera is largely based on morphology, mode of glucose fermentation, growth at different temperatures, and configuration of the lactic acid produced, ability to grow at high salt concentrations, and acid or alkaline tolerance **(Khalid, 2011).** For some of the newly described genera **(Pilar et *al.,* 2008),** additional characteristics such as fatty acid composition and motility are used in classification. The measurements of true phylogenetic relationship with rRNA sequencing have aided the classification of lactic acid bacteria and clarified the phylogeny of the group. Most genera in the group form phylogenetically distinct group, but some, in particular *Lactobacillus* and *Leuconostoc* are very heterogeneous and the phylogenetic cluster do not correlate with the current classification based on phenotypic characters. New tools for classification and identification of LAB are underway **(Sascha and Magdalena, 2010).** The most promising for routine used are nucleic acid probing techniques, partial rRNA gene sequencing using the PCR, and soluble protein patterns. The growth is optimum at pH 5.5-5.8 and the organisms have complex nutritional requirements for amino acids, peptides, nucleotide bases, vitamins, minerals, fatty acids and carbohydrates **(Khalid, 2011).**

LAB are Gram-positive usually non- motile, non-spore-forming rods and cocci. They lack the ability to synthesize cytochromes and porphyrins (components of respiratory chains) and therefore cannot generate ATP by creation of a proton gradient. The LAB can only obtain ATP by fermentation, usually of sugars. Since they do not use $O_2$ in their energy production, LAB happily grow under anaerobic conditions, but they can also grow in oxygen's presence. They are protected from oxygen by products (e.g. $H_2O_2$) because they have peroxidases. These organisms are aero tolerant anaerobes **(Michaela et *al.,* 2009).** Two main sugar fermentation pathways can be distinguished among lactic acid bacteria. Glycolysis (Embden-Meyerhof pathway) results in almost exclusively lactic acid as end product under standard conditions, and the metabolism is referred to as homolactic fermentation **(Derek et *al.,* 2009).**

The 6 phosphogluconate/phosphoketolase pathway results in significant amounts of other end products, such as ethanol, acetate, and $CO_2$ in addition to lactic acid and the

metabolism is referred to as heterolactic fermentation. Various growth conditions may significantly alter the end-product formation by some LAB. These changes can be attributed to an altered pyruvate metabolism and/or the use of external electron acceptors such as oxygen or organic compounds **(Michaela et *al.,* 2009).**.

### II.3.2.1 Physiology and morphology

Orla-Jensen used a few characters as classification basis: morphology (cocci or rods, tetrad formation), mode of glucose fermentation (homo- or heterofermentation), growth at certain "cardinal" temperatures (e.g., 10ºC and 45ºC), and form of lactic acid produced (D, L, or both) **(Kenji et *al.,* 2009).** As well be seen, these characters are still very important in current lactic acid bacteria classification. After the work by Orla-Jensen, the view emerged that the core of lactic acid bacteria comprised four genera: *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus* **(Sangoyomi et *al.,* 2010).** Orla-Jensen noted LAB as a "great natural group" indicating a belief that the bacteria included were phylo-genetically related and separated from other groups. At that time, only phenotype characters could be examined and evaluated phylogenetic markers. Today, we have means to examine, in detail, macro-molecules of the cell, believed to be more accurate in defining relationships and phylogenetic positions. These are, of course, the nucleic acids. Fortunately, nature has provided us with different kind of nucleic acids for different kind of taxonomic studies. Close relations (at species and subspecies level) can be determined with DNA-DNA homology studies **(Todd, 1993).** For determining phylogenetic positions of species and genera, ribosomal RNA (rRNA) is more suitable, since the sequence contains both well-conserved and less- conserved regions. It is now possible to determine the sequence of long stretch of rRNA (~1500 bases of 16S rRNA) from bacteria **(Huili et *al.,* 2011).** Comparisons of these sequences are currently the most powerful and accurate technique for determining phylogenetic relationships of microorganisms **(Philippe et *al.,* 2009).** With this technique, a clearer picture of phylogeny of LAB is emerging, and the ideas of Orla-Jensen can be examined with some accuracy. In addition, rRNA sequencing is becoming an important aid in the classification of LAB, as exemplified by the descriptions of new genera **(De Klerk et *al.,* 1967; Dower et *al.,* 1988).**

The physiology of LAB has been of interest ever since it was recognized that these bacteria involved in the acidification of food and feed products. Increased knowledge of LAB physiology, such as metabolism and nutrient utilization has been one way to achieve more controlled processes. Today, modern genetic techniques are considered to be promising in this regard. However, effort in this direction will not be fruitful unless there is a sound understanding of the physiology of these bacteria. The designation LAB perhaps implies that

these bacteria have a somewhat "simple" metabolism**,** resulting in one or few fermentation end products. This may also be the case in laboratory environment that we often impose to them. However, it is clear that LAB have a very diverse metabolic capacity, which enables them to adapt to a variety of conditions (**Delphine et *al.,* 2011**).

**II.3.2.2 Ecology and habitat**

In general, LAB occur in habitats with a rich nutrition supply. They occur on decomposing plant material and fruits, in dairy products, fermented meat and fish, beets, potatoes, mash, sauerkraut, sourdough, pickled vegetables, silage, beverages, plants, water, juices, sewage and in cavities (mouth, genital, intestinal and respiratory tract) of human and animals. They are part of the healthy microbiota of the human gut. Apart from dental caries, *Lactobacilli* are generally considered apathogenic. *Lb. plantarum* could be associated with endocarditis, septicemia and abscesses. Some species are applied as starter cultures for food fermentation. Because of the acidification they prevent food spoilage and growth of pathogenic microorganisms **(Hammes et *al.* 1995).** Some LAB are employed as probiotics, which are potentially beneficial bacterial cells to the gut ecosystem of humans and other animals **(Tannock, 2005).** *Oenococcus oeni* strains induced strain-specific cytokine patterns measureable immunomodulatory potential **(Foligne´ et *al.,* 2010).** LAB can also be found on grapes, in grape must and wine, as well as beer. Undamaged grapes contain $<10^{3}$ CFU per g and the initial titer in must is low **(Lafon-Lafourcade et *al.,* 1983).** Because of the acidic conditions (pH: 3.0–3.5) grape must provides a suitable natural habitat only for a few microbial groups which are acid tolerant such as LAB, acetic acid bacteria and yeasts. While many microbes are inhibited by ethanol concentrations above (4%), ethanol tolerant species survive in young wine or wine. Besides yeasts, some *Lactobacillus* species (e.g. *Lb. hilgardii*) and *Oenococcus oeni* can grow at higher ethanol concentrations. While only a few LAB species of the genera *Lactobacillus* (Lb.), *Leuconostoc* (Lc.), *Pediococcus* (P.), *Oenococcus* (O.) and *Weissella* (W.) and the acetic acid genera *Acetobacter, Gluconobacter* and *Gluconoacetobacter* can grow in must and wine, more than 90 yeast species have been found. Malolactic fermentation by LAB is occasionally desirable during vinification, but they can also produce several off-flavours in wine. The genera *Carnobacterium, Streptococcus* and *Bifidobacterium* have not been isolated from must and wine, but sometimes also species of the genus *Enterococcus* (*E. faecium*) could be detected in wine **(Perez-Martın et *al.,* 2014).** The **table 7** present different ecological environments of LAB.

**Table 7:** Different ecological environments of LAB

| Genus | Habitat | Reference |
|---|---|---|
| ***Lactococcus*** | Milk and vegetables | **Novel (1993)** |
| ***Streptococcus thermophilus*** | Dairy products: yoghurt, artisanal sourdoughs | **Jeantet et *al.* (2007)** |
| ***Lactobacillus*** | Surface of plants, dairy products, meat, water, mouth, tract bowel and vagina | **Prescott et *al.* (2003)** |
| ***Leuconostoc*** | Fresh vegetables and meat products | **Dellaglio et *al.* (1995)** |
| ***Pediococcus*** | Fermented meat products and milk | **Larpent (1996)** |

### II.3.2.3 Properties

The most important genera of LAB are: *Lactobacillus*, *Lactococcus*, *Enterocococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, and *Bifidobacterium* based on their GC (guanine–cytosine) pair content, Gram-positive bacteria are divided into two major phylogenetic branches. In contrast to other above-mentioned genera, *Bifidobacterium* exhibit a relatively high guanine plus cytosine (G + C) content of 55–67 mol% in the DNA and form part of the so-called Actinomycetes branch. The “true” LAB form part of the so-called *Clostridium* branch, which is characterized by a G + C content of < 55 mol% in the DNA **(Schleifer and Ludwig, 1995).** However, *Bifidobacterium* shares certain physiological and biochemical properties with typical LAB and some common ecological niches such as the gastrointestinal tract. Therefore, for practical and traditional reasons, *Bifidobacterium* are still considered a part of the LAB group (**Stiles and Holzapfel, 1997**). Members of the LAB are usually subdivided into two distinct groups based on their carbohydrate metabolism. The homo-fermentative group consisting of *Lactococcus*, *Pediococcus*, *Enterococcus*, *Streptococcus* and some *Lactobacilli* utilize the Embden Meyerhof–Parnas (glycolytic) pathway to transform a carbon source chiefly into lactic acid. As opposed to homofermentors, heterofermentative bacteria produce equimolar amounts of lactate, $CO_2$ , ethanol or acetate from glucose exploiting phospho-ketolase pathway. Members of this group include *Leuconostoc*, *Weissella* and some *Lactobacilli*. The species belonging to *Enterococcus* genus are frequently found in traditional fermentations and may be included as a component of some mixed starters. However, their deliberate utilization in dairy fermentations still remains controversial, especially since some of the species have been now recognized as opportunistic human pathogens associated with hospital-acquired- and urinary tract infections **(Franz et *al.,* 1999).**

### II.3.2.4 Different genus of LAB and their metabolic properties

#### II.3.2.4.1 *Lactobacillus*

*Lactobacillus* is one of the most important genus involved in food microbiology and human nutrition, owing to their role in food and feed production and preservation, as well as their probiotic properties. In October 2016, this genus contained in total 189 validly described species **(König and Fröhlich, 2017).** In addition, several species consist of well characterized subspecies. *Lactobacillus* species live widespread in fermentable material. *Lactobacilli* contribute to the flavour of fermented food by the production of diacetyl, $H_2S$ and amines. They play a role in the production as well in the spoilage of food (sauerkraut, silage, dairy and meat as well as fish products) and beverages (beer, wine, juices) **(Kandler and Weiss 1986; Hammes et *al.,* 1991).** *Lactobacilli* are straight Gram-positive non-motile or rarely motile rods (e.g. *Lb. mali*), with a form sometimes like *Coccobacilli* **(Fig. 8)**. Chains are commonly formed. The tendency towards chain formation varies between species and even strains. It depends on the growth phase and the pH of the medium. The length and curvature of the rods depend on the composition of the medium and the oxygen tension. Peritrichous flagellation occurs only in a few species, which is lost during growth in artificial media. They are aciduric or acidophilic. The maximum for growth pH is about 7.2 **(König and Fröhlich, 2017).**

*Lactobacilli* are strict fermenters. They can tolerate $O_2$ or live anaerobic. They have complex nutritional requirements for carbohydrates, amino acids, peptides, fatty acids, nucleic acid derivatives, vitamins and minerals. Some species possess a pseudocatalase and some strains can take up porphorinoids and then exhibit catalase, nitrite reductase and cytochrome activities **(Rodas et *al.,* 2006).**

They gain energy by homofermentative or heterofermentative carbohydrate fermentation in the absence or presence of oxygen. An energy source is also the conversion of carbamyl-phosphate to $CO_2$ and $NH_3$ during arginine degradation. They possess flavine-containing oxidases and peroxidases to carry out an oxidation with $O_2$ as the final electron acceptor **(Khalid, 2011).**

The pathways of sugar fermentation are the Embden-Meyerhof pathway converting 1 mol hexose to 2 mol lactic acid (homolactic fermentation) and the phosphoketolase pathway (heterolactic fermentation) resulting in 1 mol lactic acid, ethanol/acetate and $CO_2$ . Pyruvate produced during hexose fermentation may be converted to lactate, but also to other products such as diacetyl or acetic acid, ethanol and formate/$CO_2$ **(Khalid, 2011).**

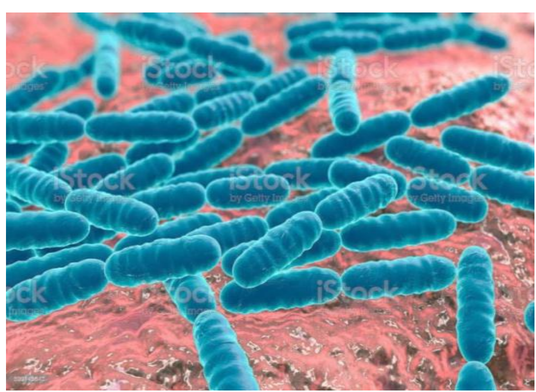


**Figure 8:** Micrographs of strains *Lactobacillus* by scanning electron microscope (G×30000) **(Guiraud, 2003)**

In the presence of oxygen, lactate can be converted to pyruvate and consequently to acetic acid and $CO_2$ or acetate and formate. The conversion of glycerol to 1,3-propanediol with glucose serving as electron donor was observed in *Lb. brevis* isolated from wine **(Schütz and Radler 1984).** The homofermentative species possess anfructosediphosphate aldolase, while the heterofermentative species have a phosphoketolase. The facultative heterofermenters possess an inducible phosphoketolase. Heterofermentative species can also use pentoses as substrate. Some homofermenters use pentoses homofermentatively **(Rodas et *al.,* 2006).** Strains of *Lactobacillus kunkeei* turned out to be fructophilic LAB **(Endo et *al.,* 2012).** Sucrose is also used for the formation of dextrans with the help of dextran sucrase. Fructose can serve as electron acceptor and mannitol is formed by heterofermentative species. Monomeric sugars and saccharides are taken up by permeases or the phosphotransferase system. They are split inside the cell by glycosidases. Galactose-6-phosphate from lactose phosphate is fermented via the tagatose-6-phosphate pathway **(Kandler and Kunath, 1983).** Several organic acids such as citric acid, tartaric acid or malic acid are degraded. Several amino acids are decarboxylated to biogenic amines **(Radler, 1975).**

**II.3.2.4.2 *Bifidobacterium***

*Bifidobacteria* were first isolated and described in1899–1900 by Tissier, who described rod-shaped, non gas-producing, anaerobic microorganisms with bifid-morphology, present in the faeces of breast-fed infants, which he termed *Bacillus bifidus*. *Bifidobacteria* are generally characterized as Gram-positive, non-spore forming, non-motile and catalase negative anaerobes **(Fig. 9) (Sgorbati et *al.,* 1995).**

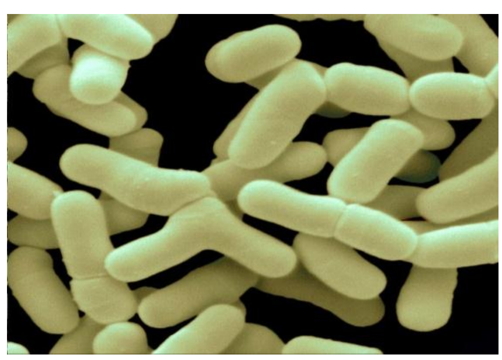


**Figure 9:** Micrographs of strains *Bifidobacterium* by scanning electron microscope (G×30000) **(Wallace et *al.,* 2003)**

They have various shapes including short, curve drods, club-shaped rods and bifurcated Y-shaped rods. Presently, 30 species are included in the genus *Bifidobacterium*, 10 of which are from human sources (dental caries, faeces and vagina), 17 from animal intestinal tracts or rumen, two from waste water and one from fermented milk. *Bifidobacteria* are phylogenetically grouped in the actinomycete branch of Gram positive bacteria **(Sgorbati et *al.,* 1995),** In addition, there are notable differences in physiological and biochemical properties, including cell-wall constituents, they are saccharolytic organisms that produce acetic and lactic acids without generation of $CO_2$, except during degradation of gluconate **(Gomes and Malcata, 1999).** Heterofermentation is initiated by splitting fructose-6-phosphate into one $C_2$ and one $C_4$ moiety. The conversion of the $C_2$ moiety to acetate is paralleled by the formation of heptose-7-phosphate from the $C_4$ moiety concomitant with the formation of a triose moiety derived from an additional molecule of fructose-6-phosphate **(De Vuyst, 2000).** The heptose-7-phosphate is subsequently split into two molecules of acetate and one molecule of pyruvate. The second triosemoiety left from fructose-6 phosphate is converted into lactate. Therefore, the fermentation of two moles of hexose results in three moles of acetate and two moles of lactate **(Oberman and Libudzisz, 1998).** The key enzyme in such glycolytic fermentation, fructose-6-phosphate phosphoketolase, may beused as a taxonomic character in identification of the genus, but does not enable interspecies differentiation. Besides glucose, all *Bifidobacteria* from human origin are also able to utilize galactose, lactose and, usually, fructose as carbon sources. The lactose transport system for *B. bifidum* DSM 20082 was identified recently as a proton symport, based on inhibition of lactose uptake by inhibitors of ATP synthesis and by compounds that interfere with proton and metal ionophores **(Krzewinski et *al.,* 1996).** *Bifidobacterium* spp. are, in some instances, also able to ferment complex carbohydrates; a recent study **(Crociani et *al.,* 1994),** in which 290 strains of 29 species of *Bifidobacteria* from human and animal origin were surveyed for

their ability to ferment complex carbohydrates, has confirmed this potential. The substrates fermented by the largest number of species were D-galactosamine, D-glucosamine, amylose and amylopectin. Porcine gastric mucin was fermented only by *B. bifidum*, whereas *B. infantis* was the only species that could ferment D-glucuronic acid. Strains of *B. longum* fermented arabinogalactan and arabic, ghatti and tragacanth gums. The optimum pH for growth is 6–7, with virtually no growth at pH 4.5–5.0 or below or at pH 8.0–8.5 or above. Optimum growth temperature is 37- 4°C, with maximum growth at 43- 45°C and virtually no growth at 25- 28°C or below **(Crociani et *al.,* 1994).**

**II.3.2.4.3 *Leuconostoc***

*Leuconostoc* thrive on plants and sometimes in milk, milk products, meat, sugar cane and other fermented food products. One species, *Lc. mesenteroides*, has been isolated from must. It is nonhemolytic and nonpathogenic to plants and animals **(Garvie, 1986).** *Leuconostoc*s are heterofermentative cocci producing only D-lactic acid from glucose and are unable to produce ammonia from arginine **(Bjorkroth and Holzapfel, 2006)**. *Leuconostoc*s form spherical or lenticular cells, pairs or chains **(Fig. 10)**. The peptidogly can belongs to type A. Sugars are fermented by the 6-P-gluconate/phosphoketolase pathway with D- lactic acid, ethanol/acetate and $CO_2$ as end products. NAD + or NADP + will serve as coenzyme of the glucose-6-phosphate dehydrogenase. During malolactic fermentation malate is degraded to L -lactate and $CO_2$ . Cells are nonproteolytic. Nitrate is not reduced. Cells grow in a glucose medium as elongated cocci. Cells are found singly or in pairs, and form short to medium length chains. On solid media, cells form short rods. *Leuconostoc*s share many features with the heterofermentative *Lactobacilli* **(Dellaglio et *al.*, 1995).** Dextrans, which are of industrial importance, are produced by leuconoctocs, especially *Lc. mesenteroides*, from sucrose as substrate. *Leuconostoc* species were divided into six different groups according to the fermentation of 19 carbohydrates **(Garvie, 1960)**. Electrophoretic mobilities of enzymes e.g. lactate dehydrogenase (LDHs), cell protein pattern, cellular fatty acids, DNA base composition and DNA homology are applied for differentiation of the species **(Dellaglio et *al.,* 1995).** Citrate metabolisms of *Lc. mesenteroides subsp. mesenteroides* might be plasmid linked **(Cavin et *al.,* 1988).** No other phenotypic features were found to be coded on plasmids, while plasmids of *Lactobacillus* and *Pediococcus* code for sugar utilisation, proteinase, nisin, bacteriocins production, drug resistance, slime formation, arginine hydrolysis and bacteriophage resistance **(Dellaglio et *al.,* 1995).**

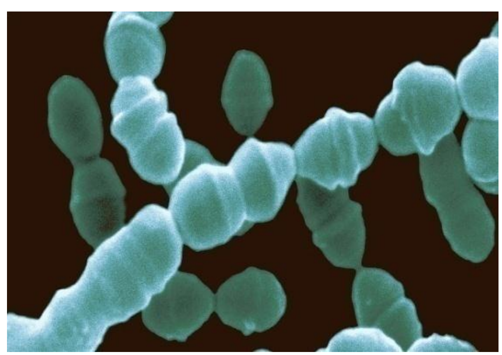


**Figure 10:** Micrographs of strains *Leuconostoc* by scanning electron microscope (G×30000) **(Bukhari et *al.,* 2015)**

**II.3.2.4.4 *Lactococcus***

The genus *Lactococcus* was proposed by Schleifer and colleagues in 1985 to reclassify some species of the genera *Streptococcus* (Lancefield group N lactic *Streptococci*) and *Lactobacillus*. It has been defined on the basis of chemotaxonomic studies confirmed by 16s rRNA sequencing **(Collins et *al.,*1989).** The *Lactococcus* genus includes five species, *L. garvieae* (formerly *E. serolicida*), *L. piscium, L. plantarum, L. raffinolactis* (formerly *S. raffinolactis*) and *L. Lactis*, which is differentiated into subspecies *L. lactis subsp. cremoris, L. lactis subsp*. hordniae (formerly *Lactobacillus hordniae*) and *L. lactis subsp. lactis* (formerly *Lactobacillus xylosus, Streptococcus lactis*) **(Odamaki et *al.,* 2011).** *Lactococci* are Gram positive cocci and belong to the group of LAB **(Fig. 11**). They are homofermentative and exclusively produce L(+) lactic acid. They are not β hemolytic and they are poorly α hemolytic. *Lactococci* are generally found on plants and the skins of animals. *L. plantarum*is mainly isolated from plants, *L. garvieae* from fish, animals and milk, and *L. piscium* from salmon **(Williams et *al.,* 1990).**

The presence of *Lactococci* in raw milk is due to contamination from forage during milking. The two *Lactococci* most commonly found in raw milk, cheese and other dairy products are *L. lactis subsp. lactis and L. lactis subsp. cremoris.* These two subspecies generally reach a high level (>$10^{8}$ CFU $g^{-1}$) as early as the first day of manufacturing and maintain it throughout the ripening period of many raw milk cheeses such as Camembert **(Corroler et *al.,* 1999),** Serra **(Macedo et *al.,* 1996),** Venaco **(Casalta, 2003)** and Pecorino Sardo **(Ledda et *al.,* 1996*).*** *L. raffino lactis* has occasionally been found in raw milk and cheeses **(Lopez-Diaz et *al.,* 2002).** *L. garvieae* may also be isolated from raw milk **(Villani et *al.,* 2001)** and raw milk cheeses: PDO Salers **(Callon et *al.,* 2004),** Egyptian cheeses **(El-**

**Baradei et *al.,* 2005),** Jben cheese (**Ouadghiri et *al.,* 2005),** Italian Toma Piedmontese PDO cheeses **(Fortina et *al.,* 2003).**

**Figure 11**: Micrographs of strains *Lactococcus* by scanning electron microscope (G×30000) **(Menad, 2017)**

A study of 35 European artisanal dairy products indicated *Lactococci* as the most commonly found LAB genus, accounting for 38% of the bacterial isolates identified **(Cogan et *al.,* 1997).** *L. lactis subsp. lactis* and to a lesser extent *L. lactis subsp. cremoris* have long been extensively used in starter cultures or dairy fermentation (i.e. in cheeses, sour cream and butter), composed of single or multiple strains with or without other LAB **(Beresford et *al.,* 2001).** Their main role in dairy fermentation is acidification, mainly by producing L-lactic acid. They contribute to the development of texture by producing exopolysaccharides, or to flavor by producing aromatic compounds (alcohols, ketones, aldehydes) or by citrate, amino acid or fat metabolism **(Smit et *al.,* 2005).** They can also be used for food preservation due to their ability to produce organic acids and bacteriocins, nisin being the best characterized and recognized **(Delves-Broughton et *al.,* 1996).** Their use as probiotics has been also considered **(Ouwehand et *al.,* 1999).** The annotation of the genomes of different subspecies will undoubtedly open up new prospects for identifying new useful functions in the species **(Kok et *al.,* 2005).** It will also be a great help in assessing the safety of *Lactococci* (**Kok et *al.,* 2005).**

**II.3.2.4.5** ***Streptococcus***

The genus *Streptococcus* consists of Gram-positive, non motile, spherical or ovoid cells that are typically arranged in pairs or chains when grown in liquid media **(Fig. 12)**. All species are facultatively anaerobic, some requiring additional $CO_2$ for growth. They are non-sporing, catalase negative, homofermentative and have complex and variable nutritional requirements. They metabolise carbohydrates by fermention resulting mainly in lactic acid but no gas. Their temperature optima are usually around 37°C, but maximum and minimum

temperatures vary somewhat amongest species. Many species are pathogenic to man and animals and some are highly virulent **(Jones 1978; Colman et *al.,* 1990).**

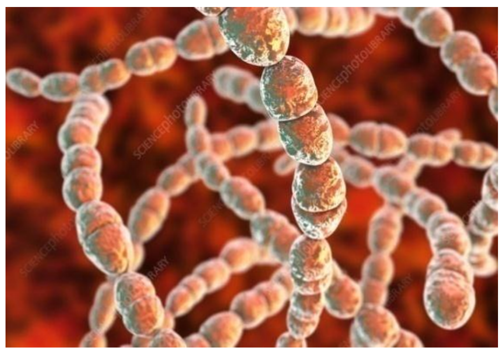


**Figure 12:** Micrographs of strains *Streptococcus thermophilus* by scanning electron microscope (G×30000) **(Le Guerhier, 2013)**

**II.3.2.4.6** ***Pediococcus***

*Pediococci* occur on plant material, fruits and in fermented food. They are non-pathogenic to plants and animals. Cells are spherical and never elongated as it is the case with *Leuconostoc*s and *Oenococci*. The cell size is 0.36–1.43 µm in diameter. Cell division occurs in two directions in a single plane. Short chains by pairs of cells or tetrads are formed **(Fig. 13) (Garvie, 1986).** Tetrad-forming homofermentative LABs in wine are *Pediococci. Pediococci* are non motile and do not form spores or capsules **(Simpson and Tachuchi, 1995).** Glucose is fermented by the Embden–Meyerhof–Parnas pathway to DL or L- lactate. A wide range of carbohydrates is used such as hexoses, pentoses, disaccharides, trisaccharides and polymers such as starch.. The phospho transferase system is used for glucose transport. Species producing DL -lactate possess an L- and D-LDH. Pyruvate can be converted mainly by *P. damnosus* to acetoin/diacetyl. *P. pentosaceus* and *P. damnosus* can degrade malate. They are nonproteolytic and nitrate is not reduced. *Pediococci* are catalase negative. Some strains of *P. pentosaceus* produce pseudocatalase. *Pediococci* do not reduce nitrate. *Pediococci* can have plasmids, which code for production of bacteriocins or fermentation of carbohydrates. *P. pentosaceus* has three different plasmids for the fermentation of raffinose, melibiose and sucrose (**Simpson and Tachuchi, 1995).**

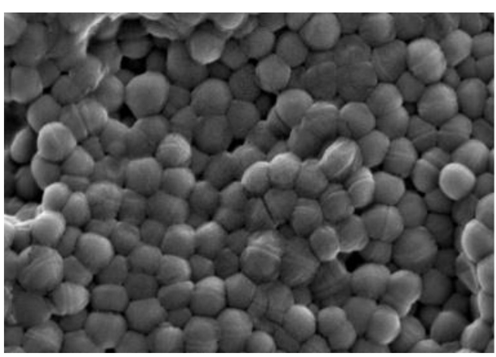


**Figure 13:** Micrographs of strains *Pediococcus* by scanning electron microscope (G×10000) **(Herdian et *al.,* 2018)**

**II.3.2.4.7 *Oenococcus***

*Oenococci* have been isolated from must and wine **(Garvie, 1986).** They form spherical or lenticular cells, pairs or chains. Murein belongs to type A **(Fig. 14) (König and Fröhlich, 2017).** Only NAD + will serve as coenzyme of the glucose-6-phosphate dehydrogenase **(Bjorkroth and Holzapfel 2006). Petri et *al.* (2015)** applied MALDI-TOF-MS and nested SAPD- PCR for the discrimination of *Oenococcus oeni* isolates at the strain level. *Oenococci* have been separated from the genus *Leuconostoc* by 16S rDNA sequence analysis **(Dicks et *al.* 1995).** Only three species *O. oeni* **(Dicks et *al.,* 1995),** *O. kitaharae* **(Endo and Okada 2006)** and *O. alcoholitolerans* **(Badotti et *al.*, 2015)** have been described **(König and Fröhlich, 2017),** and can easily be distinguished. *O. kitaharae* (type strain: DSM 17330 T ) has been isolated from a composting distilled shochu residue. L-Malate is not decarboxylated to L-lactate and $CO_2$ in the presence of fermentable sugars. Cells do not grow below pH 4.5 and in 10% ethanol. Growth is not stimulated by tomato juice. *O. kitaharae* possess several functions in cellular defence (bacteriocins, antimicrobials, restriction- modification systems), which are lacking in *O. oeni* living in must with fewer competitive microbes **(Borneman et *al.,* 2012).** *O. alcoholitolerans* was isolated from an ethanol production plant in Brazil. Distinctive phenotypic characteristics are the ability to metabolise sucrose but not trehalose **(Badotti et *al.,* 2015).** The usage of glucose, cellobiose, trehalose, and mannose was demonstrated **(Jamal et *al.,* 2013).** *O. oeni* can grow at pH 3.0 and 10% ethanol. Many strains of *O. oeni* can even grow at 14% of ethanol **(Bordas et *al.,* 2013).** Heat shock proteins and special membrane lipids are produced under these environmental conditions **(Coucheney et *al.,* 2005).** Changes in the expression level of the geranylgeranyl pyrophosphate synthase gene was detected under ethanol stress **(Cafaro et *al.,* 2014). Vigentini et *al.* (2016)** isolated *O. oeni* strains from wineries of the Aosta Valley developing at 10 C in Petit Rouge wine.

*Oenococci* can be distinguished from less acid tolerant *Leuconostoc* species by using saccharose, lactose and maltose as substrate **(Garvie, 1986).**

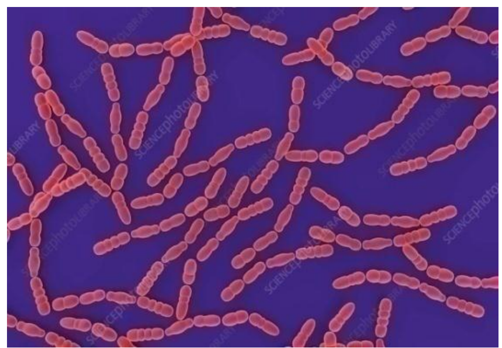


**Figure 14:** Micrographs of strains *Oenococcus* by scanning electron microscope (G×10000) **(Bastard et *al.,* 2016)**

**II.3.2.4.8 *Weissella***

Based on rDNA analysis, *Lc. paramesenteroides* ("Lc. paramesenteroides group") was reclassified as *W. paramesenteroides*. Five heterofermentative *Lactobacilli* (*Lb. confusus, Lb. halotolerans, Lb. kandleri, Lb. minor, Lb. viridescens*) were also assigned to the genus *Weissella* **(Bjorkroth and Holzapfel 2006).** Weissellas are spherical, lenticular or irregular rods **(Fig. 15)**. They are heterofermentative species, which produce D, L -lactic acid, while *W. paramesenteroides* forms D -lactic acid from glucose. They have been isolated from food and meat. Weissellas produce greenish oxidized porphyrins in meat products by $H_2O_2$ accumulation. The genus *Weissella* contained 21 validly described species **(König and Fröhlich, 2017).**

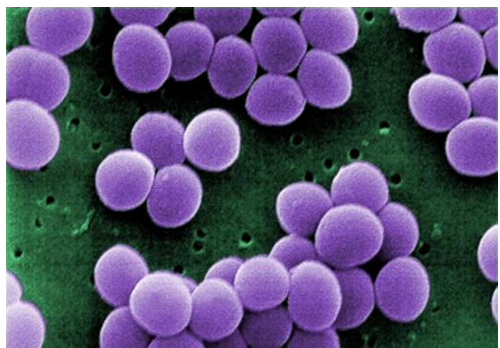


**Figure 15:** Micrographs of strains *Weissella by* scanning electron microscope **(G×30000) (Lakra et *al.,* 2020)**

**II.4 Beneficial effects**

LAB are useful probiotics, their beneficial effects were revealed by a Russian Scientist **Metchnikof (1845–1919)** who proposed that extended longevity of people of Balkan could be attributed to their practice of ingesting fermented milk products **(Hove et *al.,* 1999).** LAB are

useful for human being and animals presenting many bioactivities such as epithelial barrier enhancement, effect on intestinal mucosa adhesion, competitive exclusion to eliminate pathogenic microorganisms, antimicrobial substances production, immunomodulatory activity, probiotics on toll-like receptors (TLRs), effect on nucleotide oligomerization domain-like receptors (NLRs), also on lactose intolerance, prevention of diarrhea (infective diarrhea antibiotic-associated diarrhea, *Clostridium difficile* infected diarrhea, traveler's diarrhea), prevention of inflammatory bowel syndrome, prevention of urogenital infections, gastric ulcer treatment, food allergy, obesity management, hypo-cholesterolemic effect, prevention of diabetes, liver diseases, cancer prevention, increased synthesis of short-chain fatty acids, maintaining of oral health (dental caries and orthodontic treatment) **(Das et *al.,* 2022).**

### II.4.1 Effects on gastrointestinal tract

About 10 billions of bacteria belong to 500 species coexist in human gastrointestinal tract. 20 genera are dominant among these including LAB. these are *Bacteroids, Lactobacillus, Clostridium, Fusobacterium, Bifidobacterium, Eubacterium, Peptococcus, Peptostreptococcus, Escherichia,* and *Veillonella* **(Hove et *al.,* 1999)**. Microbial balance is very important for maintaining the intestinal homeostasis. Live LAB intake through dairy products have myriad beneficial effects on gastrointestinal tract of human beings ranges from correction of lactose malabsorption, alleviation of viral and drug induced diarrhea, post operative pouchitis, irritable bowel syndrome, inflammatory bowel syndrome, antine-oplastic effects on human cell line, maintenance of normal insulin level in blood and also helpful to enhance the absorption of fatty acids through intestine **(Harish and Varghese, 2006)**. LAB produce these beneficial effects by restoration of normal intestinal flora, elimination of intestinal pathogens, reinforcement of intestinal barrier capacity to foreign antigens, stimulation of non specific immunity such as phagocytosis, stimulation of humoral immunity and production of anti-inlammatory products **(Heyman, 2000).**

### II.4.2 Effects on lactose intolerance and malabsorption

Lactose intolerance is the inability to digest lactose into its constituent’s, i.e., glucose and galactose owing to low level of ß-galactosidase enzyme in the brush border of duodenums. It commonly occurs in children. Symptoms of lactose intolerance appear from 30 min to 2 hours after consumption of food that contain lactose in it. Symptoms include, bloating, cramping, latulence, and loose stool **(Strzałkowska et *al.*, 2018).** There are three clinical forms of lactose intolerances, i.e., primary lactose intolerance occurs after weaning, secondary lactose intolerance due to diarrhea, inflammatory bowel disease and HIV infection

and third type is congenital lactose intolerance which has genetic origin. Lactose malabsorption is the condition in which lactose is digested into its constituents but because of deficiency of anatomical and cofactors these constituents are not properly absorbed by the intestine **(Rusynyk and Still, 2001).** Lactose intolerance problem appears to occur less frequently among cattle raising cultures because these cultures usually drink milk but culture groups those do not drink milk have high incidences of lactose intolerance. It has been found that people with lactase deficiency tolerate the lactose in yogurt better than the same amount of lactose in milk this probably due to the assumption that either yogurt supply lactase enzyme or bacteria which produce lactase enzyme **(Masood et *al.,* 2011).**

### II.4.3 Effects on diarrheal diseases

Diarrhea is the common problem of both developed and developing countries but incidences are more common in developing countries due to the poor life style and poor hygiene conditions. People at high risk of diarrhea are small children, elders, persons with intestinal infections and HIV carriers **(Farthing, 2000).** According to a report published by **WHO in 2000,** diarrhea accounts for 4% of total deaths and 5% of disabilities worldwide. Mortality rate is 2.2 millions globally annually and morbidity rate is 4 billion annually. There are several causes of diarrhea but most common among them is fecally contaminated food and water **(WHO, 2000).** There are many forms of diarrhea such as Rota virus induced diarrhea, antibiotic induced diarrhea, bacterial diarrhea travelers and diarrhea fungal diarrhea **(Harish, 2006).** *Lactobacillus GG* strain has been to be very effective against viral and idiopathic diarrhea as identified by Harish and Vargese in their studies **(Harish and Vargese, 2006). Canani et *al.* (2007)** investigated the effects of lactic acid bacteria (*Lactobacillus rhamnosus, Lactobacillus plantrum, Bifidobacterium,* and *Enterococcus faecium SF68*) on children of 6 to 36 months of age with diarrheal complication and found that these were effective in preventing diarrheal complications. *Lactobacillus GG* was found to be more effective antidiarrheal agent *Streptococcus faecium* strain SF68 was effective against diarrhea associated with respiratory tract infection. Due to these beneficial effects of LAB in diarrheal disease especially in children, the use of LAB containing food such as yogurt and fermented milk should be promoted in children.

### II.4.4 Role of LAB in treating ulcer

**Myllyluoma et *al.* (2007)** in their study reported the beneficial effects of LAB in gastric ulcer. They proposed that these effects were due to the destructive actions of LAB on *H. pylori*. If LAB are used in combination of anti-ulcerative therapy then results are astonishingly fast recovery and improved efficacy of therapy. The use of *Lactococcus*

*rhamnosus* as an adjuvant therapy during *H. pylori* eradication has been proved. *Lactococcus rhamnosus* not only is used as adjunct in anti-ulcerative therapy but also reduced ethanol-induced mucosal lesion. Pre-treatment with *Lactococcus rhamnosus* also significantly increases the basal mucosal prostaglandin E2 (PGE2) level, also attenuates the suppressive actions of ethanol on mucus- secreting layer and transmucosal resistance and reduces cellular apoptosis in the gastric mucosa. Hence we can say that *Lactococcus rhamnosus* is an antiulcerative in many ways as reported by researchers **(Lam et *al.,* 2007).**

### II.4.5 Effects of LAB on human immune system

Immunity is defined as the resistance of body against foreign invaders or anybody abnormalities. Immunity is basically of two types: innate and acquired immunities. Farmer is nonspecific type of immunity and latter is specific type of immunity. Innate immunity includes mechanical barriers, antiseptic actions of body fluids, inflammatory response while acquired immunity consists of lymphocytes, specific types of proteins and antibodies to defend the body, these pathways are help full to maintain the body functional **(Arora, 2007).**

Antibodies are the major component of immune system. These may be monoclonal and polyclonal. For exemple when *Staphylococcus aureus* is injected in its inactive state can generate IgA, the antibodies produced in this way are polyclonal and can provide immunity against various antigens. To provide a cheap source of immunization cow milk can be used. This can provide immunity against *Staphylococcus aureus* infections as reported by **Plat-Sinnige et *al.* (2009).** Also *Lactobacillus keiranofaciens* M1 has strong potential to induce production of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-12 in RAW (macrophage cell line) 264.7 cells and murine peritoneal macrophages. In addition to this blocking toll-like receptor (TLR)-2 showed a significant inhibition of IL-6 and TNF-α production. From these findings it is indicated that they have a potential beneficial effect on promotion of cell-mediated immune responses against tumors and also against intracellular pathogenic infections **(Hong et *al.,* 2009).** Specific auto antibodies have physiologic control via antibodies **(Fetissov et *al.,* 2008).**

**Waard et *al.* (2001)** have performed a series of experiments on *L. casei* Shirota. From their experiments they have demonstrated that *L. casei* Shirota has prominent effects on cellular innate immunity via phagocyte activation, this in turn enhances TH1 cell activity. From this they concluded that *L. casei* Shirota has a direct or indirect effects on TH1 cell activity. Stronger role in immunity by LAB is further strengthened by the studies of Sun et al, they concluded that LAB strain is responsible for certain immune responses **(Sun et *al.,* 2005).**

### II.4.6 Antifungal activity

LAB have been found to show antifungal activity. Fungal diseases are difficult to treat. Different strains of LAB have been screened out to identify their potential anti-fungal activity. Among various strains of LAB, *Lactobacillus fermentum* has been marked possessing a strong anti- fungal property, especially against *Candida albicans* and *Candida glabrata*. As LAB possesses anti-mycotic property they can be used as probiotics against various lethal fungal diseases. Fungal infection causes by *Candida glabrata* and *Candida albicans* are common. LAB use as probiotics may address these issues in a better way **(Rönnqvist et *al.*, 2007).** Hydrogen peroxide has deleterious effects on fungi. It is also effective against bacterial infections. Some types of LAB produce hydrogen peroxide. These strains possess antibacterial as well as anti-fungal activity. **Mijac et *al.* (2006)** in their work demonstrated that *Lactobacilli* are the special type of LAB which have this unique property of producing Hydrogen peroxide. These bacteria can be used in a wide range of infections including vulvovaginal candidiasis (VVC) and trichomoniasis (TV). In fact vagina is the main site of infection in females. Different types of fungal infections are common to this area. The use of LAB as probiotics may contribute a lot of ease in therapy of these infections **(Mijac et *al.*, 2006).** *Lactobacillus casei* and *Lactobacillus acidophilus* possess good antifungal properties and are able to protect immuno compromised people from opportunistic infections by *Candida albicans* as described by **Polishchuk et *al.* (1999)** and **Wagner et *al.* (2000).** LAB show antifungal activity by possessing antiadhesive actions against *Candida albicans* as described by **Anokhina et *al.* (2007).** It is obvious that LAB have pronounced property as anti-fungal agent. It is to be investigated that whether these *Lactobacillus* are effective orally or vaginally.

### II.4.7 Role of LAB in preventing colon cancer

**Kim et *al.* (2006)** found that LAB such as *Lactobacillus rhamnosus* ATCC 9595 was useful in preventing colon cancer in human being. They conducted experiments on two cell lines of cancer, i.e., PANC-I (pancreas) and HI-29 (colon). They found that LAB successfully decreased the cancer growth. The anticancer activity of *Lactobacillus rhamnosus* was might be due to the induction of apoptosis by two expolysaccarides of bacteria namely rEPS (released exopolysaccharides) and cbEPS (cell bound exopolysaccharides). rEPS was more effective in preventing cancer than cbEPS. Uncontrolled growth of colon cells may be affected by different strains of LAB. Various types of LAB were investigated by **Baricault et *al.* (1995),** the most important strains with anticarcinogenic property were *Lactobacillus helveticus*, *Bifidobacterium, Lactobacillus acidophilus,* or a mixture of *Streptococcus*

*thermophilus* and *L.bulgaricus*. From these *Lactobacillus helveticus* is the most effective one in inhibiting the uncontrolled growth of colonic cells. Effects of LAB on colon cancer may vary from strain to strain. Some strains may be helpful in minimizing mutagenic threat and other may potentiate this abnormality.

### II.4.8 Inhibition of intestinal pathogens

Probiotic cultures produce a wide range of antibacterial compounds including organic acids (e.g., lactic acid and acetic acid), hydrogen peroxide, bacteriocins, various low molecular mass peptides, and antifungal peptides/proteins, fatty acids, phenyllactic acid, and OH-phenyllactic acid. Lactic and acetic acids are the main organic acids produced during the growth of probiotics and their pH lowering effect in the gastrointestinal tract has a bactericidal or bacteriostatic effect. Low-molecular- mass compounds such as lactic acid have been reported to be inhibitory towards Gram-negative pathogenic bacteria **(Alakomi et *al.*, 2000).** Moreover, a heat-stable, low-molecular-weight antibacterial substance different from lactic acid was present in the cell-free culture supernatant resulting in the inactivation of a wide range of Gram-negative bacteria and inhibition of the adhesion and invasion of Caco-2 cells by *Salmonella enterica Serovar typhimurium* **(Coconnier et *al.,* 2000; Lie et *al.,* 2002).** Also, probiotics like many other LAB can produce various bacteriocins. Bacteriocins are ribosomally synthesized antimicrobial peptides effective against other bacteria, either in the same species (narrow spectrum), or across genera (broad spectrum) with immunity to their own bacteriocins **(Cotter and Ross, 2005).** Recently, **Corr et *al.* (2007)** showed that *L. salivarius* was capable of protecting mice against *Listeria monocytogenes* by direct antagonism mediated by the bacteriocin Abp118. In some instances, the inhibition of gastrointestinal pathogens is multifactorial including all mentioned factors **(Fayol-Messaoudi et *al.,* 2005).** The production of these antimicrobial compounds appeared to be stimulated by the presence of pathogens **(Rossland et *al.,* 2005).** In general, many mechanisms have been suggested by which probiotics prevent the detrimental effect of intestinal pathogens including competition for limited nutrients, inhibition of epithelial and mucosal adherence of pathogens, inhibition of epithelial invasion by pathogens, production of antimicrobial substances and/or the stimulation of mucosal immunity **(Rolfe, 2000).**

*Helicobacter pylori* is an intestinal pathogen, long-term infection by which leads to chronic gastritis, peptic ulcer and increases the risk of gastric malignancies **(Plummer et *al.,* 2004).** Currently *H. pylori* infection is treated by a combined therapy consisting of two antibiotics and a proton pump inhibitor, which, although in many cases appeared very effective, presents a very expensive treatment with many side effects including antibiotic-

associated diarrhoea and likelihood of induction of the antibiotic resistance in intestinal pathogens (**Malfertheiner et *al.,* 2002).** The clinical outcome of *H. pylori* infection depends on several factors including the strain of *H. pylori*, extent of inflammation and cell density **(Ernst and Gold, 2000).** The risk associated with the development of peptic ulcer and gastric cancer is directly proportional to the level of infection (**Tokunaga et *al.,* 2000).** One of the measures, which may help reduce the rate of *H. pylori* infection, is a diet modulation with the inclusion of probiotics **(Khulusi et *al.,* 1995).** Probiotic organisms do not appear to eradicate *H. pylori*, but they are able to reduce the bacterial load and inflammation in animal and human studies. It has been suggested that the suppression effect is strain dependent **(Sgouras et *al.,* 2005).**

**II.5 Mechanism action of LAB**

Major probiotic mechanisms of action include enhancement of the epithelial barrier, increased adhesion to intestinal mucosa, and concomitant inhibition of pathogen adhesion, competitive exclusion of pathogenic microorganisms, production of anti-microorganism substances and modulation of the immune system **(Fig. 16).**

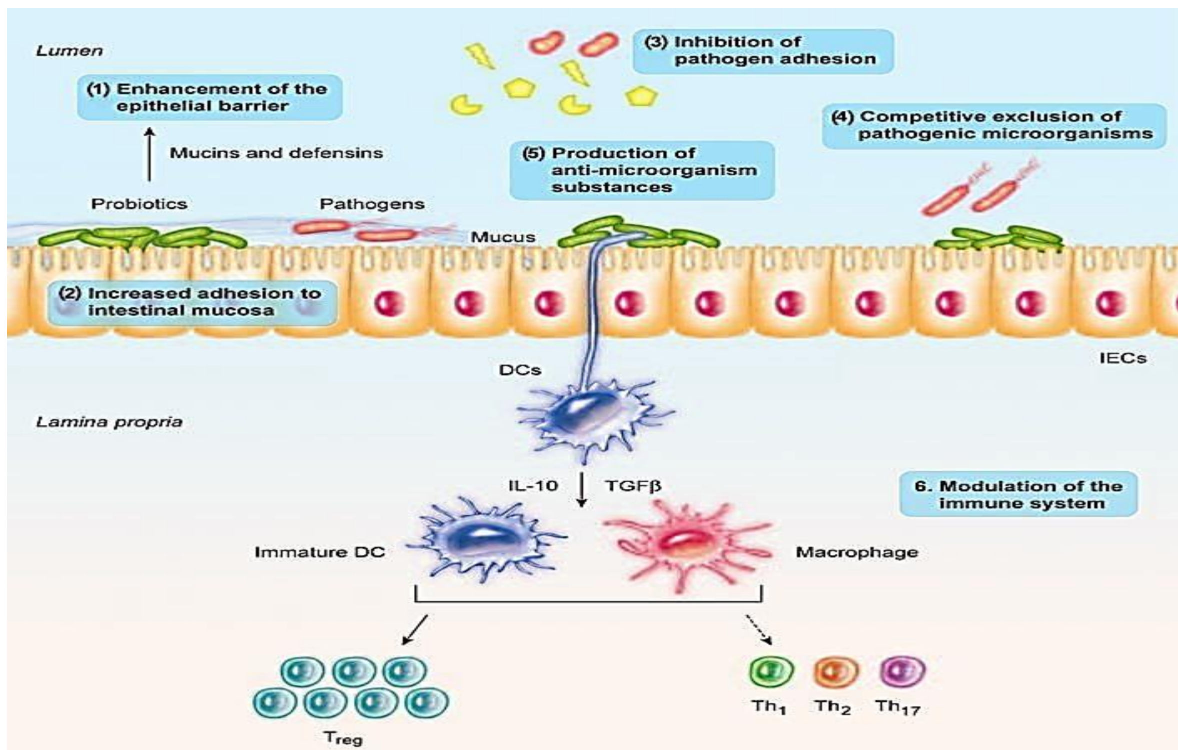



**Figure 16:** Mechanism action of LAB (**De Bermudez-Brito et *al.,* 2012**)

DCs: Epithelial and dendritic cells   IECs: Intestinal epithelial cells

# Chapter -III-

## Medicinal Plants

## III.1 *Allium sativum* .L (Garlic)

### III.1.1 History

Garlic is native to central Asia. It is descended from the species *Allium longicuspis*, which grows wild in central and south western Asia. It has been a culinary and medicinal staple in Asia, Africa and Europe for more than 6,000 years**.** Cultivated garlic was thought to fall into two main types of *Allium sativum*: hardneck garlic (*Allium sativum var. ophioscorodon*), also known as “bolting” or “top-setting,” and softneck garlic (*Allium sativumvar. sativum*), or “non-bolting.” Experts do not agree about whether these types are different subspecies. Hardnecks produce a flowers talk, called a scape, and are most closely related to wild garlic. Softnecks do not have this woody stalk, and they comprise most “super market” garlic varieties. New evidence suggests that a third type, “weakly bolting” garlics, are genetically softnecks that exhibit characteristics of hardnecks under certain environmental conditions. Softneck garlic produces more cloves per bulb and stores longer than hardneck garlic. Softnecks lacka hard stalk, which makes them easy to braid together for storage. However, in cold climates like Alaska, hardneck types are much harder and more flavorful, produce much larger bulbs, and can be quite productive with optimal cultivation **(Aaron, 1997).**

### III.1.2 Origin and distribution

Garlic is a perennial that originally came from central Asia, and is now grown throughout the world **(Wolfgang, 2008).** According to **FAO (2019)**, China is the highest producer of garlic in the world, it is also massively produced in the United States and Mediterranean countries **(Tab. 8)**. Among the latter, Spain is the highest European producer.

### III.1.3 Botanical description

*Allium sativum* L., is a monocotyledonous perennial plant, giving cloves (garlic clove, bulbils) appreciated in the culinary field for their characteristic taste and smell. Their underground part consists of a compound bulb with numerous fibrous rootlets and their bulb extends above the surface into a stem surrounded by green, linear, flat and smooth leaves, measuring 1 to 2.5 cm wide and 30 to 60 cm long. The inflorescences are umbels, and small bulblets are produced in the inflorescences, concerning the flowers are variable in number and sometimes absent and rarely open, they may wither in the egg, they are installed at the end of thin pedicels and consist of a 6-piece perianth about 4-6 mm long, pink or white in color, bell-shaped;6 stamens; and a trilocular superior ovary. Finally the fruit is a small capsule of loculicidal dehiscence **(Fig. 17) (Farnsworth et *al.*, 1992).**

The pods have 12 to 16 bulbs. The latter have a diameter of 5 to 10 mm and are composed of an outer shell, an epidermis containing a chlorophyll-free mesophyll; parenchyma and a base of lower epidermal cells **(WHO, 1999).**

**Table 8:** World garlic production statistics in 2019: the top 10 producers **(FAOSTAT, 2019)**

| Country | Production in 2019 (Tons) |
|---|---|
| China | 23 258 424 |
| India | 2 910 000 |
| Bangladesh | 466 389 |
| Republic of Korea | 387 671 |
| Egypt | 318 800 |
| Spain | 271 350 |
| United States | 237 340 |
| Algeria | 223 311 |
| Azberkistan | 216 272 |
| Ukraine | 215 070 |

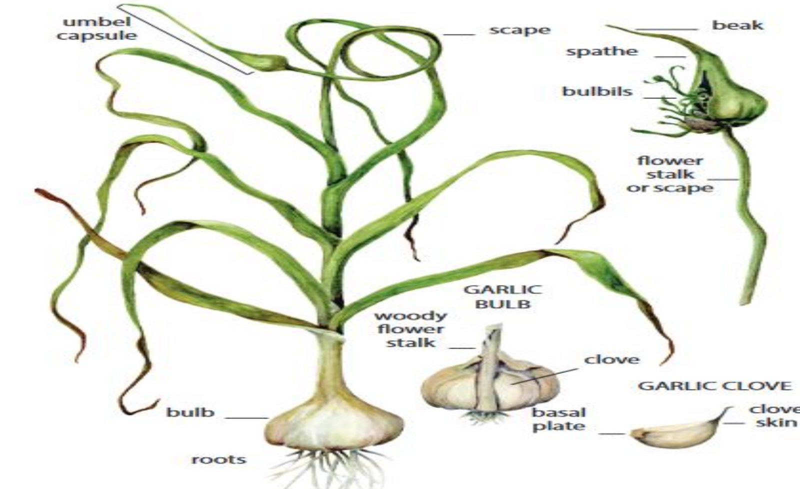



**Figure 17:** *Allium sativum* L. plant **(Dethier, 2010)**

### III.1.4 Taxonomy and classification

Formerly classified in the lily (*Liliaceae*) family, garlic was at one time known as *Allium controversum*, which hints at the problems classifying the herb. Most sources recognize one major hardneck variety, *A. sativum var. ophioscorodon*, and one major softneck variety, *A. sativum var. sativum*. One other little-known variety, *A. sativum var. pekinense* (Peking garlic) also exists **(Ourouadi et *al.,* 2016).** It belongs to the genus *Allium*. Recent taxonomy revisions place garlic in the family of *Alliaceae*, which is made up of approximately 700 Species **(Apawu, 2009).** A great number of species in this genus are perennial plants with underground storage organs consisting of bulbs or rhizome **(Abdul Ghani, 2010).** The most common edible members include chives, (*A. Schoenoprasum* L.),

leek (*A. porrum* L.), andonion (*A. Cepa* L.)**.** It belongs to the species *sativum* and has the scientific name *Allium sativum* L. **(Apawu, 2009).** *Allium sativum* is a diploid species (2n = 2x = 16) in the subgenus *Allium* of the *Alliaceae* (formerly in the *Liliaceae*, and then the *Amaryllidaceae*). The other cultivated plants in this subgenus are leek, usually tetraploid, or elephant garlic, usually hexaploid (both *A. Ampeloprasum* L.). Leek and garlic have flat and folded leaves. Elephant garlic and garlic form a bulb, but leek does not. Elephant garlic bulbs consist of 2 to 6 large cloves and several small cloves, while garlic bulbs usually have more cloves of a relatively consistent size, especially for bolting types. Bolting garlic, leek, and elephant garlic have a solid scape, unlike the hollow scape of the most economically important *Allium*, onion (*A. cepa* L.) **(Simon and Jenderek, 2003).**

The systematic classification of garlic is shown in **Table 9.** It was recently, subject of an ever-controversial amendment, with some scientists classifying the *Allium* in the subfamily *Liliaceae*, or *Amaryllidaceae*, and not in a separate family *Alliaceae* **(Lambinon et *al.*, 2004).**

**Table 9:** Classification of *Allium sativum* **(Lambinon et *al.,* 2004)**

| **Domain** | Eukaryota |
|---|---|
| **Kingdom** | Plantae |
| **Phylum** | Spermatophyta |
| **Subphylum** | Angiospermae |
| **Class** | Liliopsides |
| **Subclass** | Liliidae |
| **Order** | Liliales |
| **Family** | *Alliaceae* |
| **Genus** | *Allium* |
| **Species** | *Allium sativum* |

### III.1.5 Habitat and ecology

Garlic can be grown on variety of soils but thrives better on fertile, well-drained loamy soils. Heavy clay soils may result in misshapen bulbs and make harvesting difficult. Garlic is a frost-hardy plant requiring cool and moist period during growth and relatively dry period during maturity of bulbs **(Singh, 2018).** Bulbing takes place during longer days and at high temperatures. Exposure to low temperature subsequent to bulb formation favors the process. Adequate vegetative growth promotes bulb formation. Bolting does not seem to be influenced by temperature, and some clones never produce flowers **(Purseglove, 1975).** It was also observed that long days and high temperatures encouraged bulb development. **Abdel (1973)** found yield and survival higher at lower temperatures. **Moravec et *al.* (1974)** observed

that during cool growing, seasons the yield differences between the cultivars were more conspicuous, whereas warm wet weather reduced it. These results showed that low temperature is a pre-requisite for higher yields in garlic. As soon as bulbing commences, leaf initiation ceases **(Singh, 2018).** Because of this reason and to get high yield, garlic should be planted early to promote vegetative growth under short photoperiod and cool temperature. In regions having temperate climate, garlic is often planted in late autumn to facilitate top growth at higher temperature in spring. In general, garlic needs irrigation at an interval of 8 days during vegetative growth and 10 to 15 days during maturation **(Singh, 2018).**

### III.1.6 Varieties

Several botanical varieties of garlic are described in the literature, including *A. sativum var. sativum L.*, which rarely or never flowers; *A. sativum var. ophioscorodon* (Link) Doll (the varietal name meaning “serpent garlic”), which regularly flowers; and *A. sativum var.* pekinense (Prokh.) Makino, which rarely flowers, like *A. sativum var. sativum*, but has wider, leaves **(Ourouadi et *al.,* 2016).** Based on genetic DNA analysis there are 10 major garlic varieties or types **(Tab. 10)** within two main categories: hard neck and soft neck. These varieties are the result of random mutations of yesteryears of these two principals categories **(Sethi et *al.,* 2014).**

### III.1.7 Chemical composition

#### III.1.7.1 Physicochemical

In human nutrition, the energy value of garlic is 138.7 kcal/100 g, garlic clove contains water, storage polysaccharides, proteins mainly enzymes (alliinase, peroxidases), free amino acids, and organo-sulphur compounds which are responsible for the characteristic smell and taste of garlic. Certain vitamins (A, B1, B2 and C), phytic acid, β-pistosterol and selenium are also present **(Meredith, 2008).** Garlic comprises higher concentration of sulfur compounds which yield characteristic flavor and taste and are also responsible for beneficial effects **(Medjoudj, 2007).** The essential components of fresh garlic are shown in the **Table 11**. Garlic cloves contain rather large amounts of vital minerals and trace elements like calcium, phosphor, potassium, sodium, magnesium, aluminium, iron, copper, manganese, chromium, molybdene, selenium, germanium and iodide **(Tab.12) (Sendl, 1995).**

In addition to these, about seventeen amino acids that include alanine, arginine, aspartic acid, asparagine, histidine, leucine, methionine, phenylalanine, praline, serine, threonine, tryptophan, and valine have been identified and isolated **(Agarwal,1996).**

**Table 10:** Varieties of garlic **(Sethi et *al.*, 2014)**

| N° | Variety | Height (ft) | Bulbils | Bulbs | Clove skin | Double clove | Typical named selection |
|---|---|---|---|---|---|---|---|
| 01 | Rocambole | 3-4 | Numerous, purple color | Off-white Purple sreak | Brownish and easy to peel | Prone to double clove | German Red, German Brown, Spanish Roja, Russian Red, Killarney Red, Montana Gian |
| 02 | Purple stripe | 3-5 | Numerous, purple color | Purple sreak 8- 12 cloves | Brownish and difficult to peel | Rarely occur | Chesnok Red, Persian Star |
| 03 | Glazed purple stripe | 3-5 | Numerous, intensely purple color | Purple sreak 8- 12 cloves | Brownish and difficult to peel | Rarely occur | Purple Glazer, Red Rezan |
| 04 | Marbled purple stripe | 3-5 | Numerous, purple color | Purple stripes 4-7 cloves | Brownish | Rarely occur | Siberian, Brown Tempest, Krasnodar Red |
| 05 | Porcelaine | 4-6 | Numerous, small, white color | 4-6cloves | Difficult to peel | Rarely occur | Romanian Red, Georgian Crystal, Music, Polish Hardneck, Zemo, Georgian Fire, Northern White, German White, Krasnodar White |
| 06 | Artichoke | / | Purple | Whitish to purple blush 12-20 cloves | Difficult to peel | / | Inchellium Red, California Early, Susanville, California Late, Early Red Italian, Machashi, Red Toch |
| 07 | Asiatic | 3 | Dark purple, larger | 4-8 cloves | Difficult to peel | Present | Asian Tempest, Japanese, Wonha, Sakura, Pyong Vang |
| 08 | Turban | / | Numerous, purple, small | Dark purple, 7-11 cloves | Brownish Easy to peel | Not present | Red Janice, Blossom, Xian, Tzan, Chinese Stripe |
| 09 | Creole (warm climate) | / | Small and white to pink | 8 to 12 cloves | Dark purple, sweet in taste, Difficult to peel | / | Ajo Rojo, Burgundy, Creole Red |
| 10 | Silver skin (warm climate) | / | / | 8-40 | Difficult to peel | / | Silver White, Nookota Rose, Mild French, S and H Silver, Idaho Silver |

**Table 11:** Chemical composition of fresh garlic g/100g **(Lefief, 2012)**

| Components | Value (g) | Vitamins | Value (mg) |
|---|---|---|---|
| Water | 63.7 | B1 | 30 |
| Proteins | 7 | B2 | 1.2 |
| Starch | 22.1 | B3 | 0.65 |
| Fibers | 3 | B5 | 0.60 |
| Carbohydrates | 24.5 | B6 | 0.20 |
| Reducing sugars | 2.4 | C | 0.10 |
| Lipids | 0.5 | E | 0.08 |

**Table 12:** Trace elements of fresh garlic **(Sendi, 1995)**

| Trace element | Amount per 100 g fresh garlic |
|---|---|
| Sodium (Na) | 8.0 mg ± 2 |
| Potassium (K) | 373.0 mg ± 9 |
| Calcium (Ca) | 18.0 mg ± 3 |
| Phosphorus (P) | 64.0 mg ± 3 |
| Iron (Fe) | 2.2 mg ± 5 |
| Magnesium (Mg) | 8.0 mg ± 4 |
| Manganese (Mn) | 1.3 mg ± 2 |
| Zinc (Zn) | 0.9 µg ± 5 |
| Cobalt (Co) | 0.9 µg ± 3 |
| Selenium (Se) | 77.1 µg ± 2 |
| Fluorine (F) | 22.0 µg ± 2 |
| Iodine (I) | 94 µg ± 4 |

### III.1.7.2 Phytochemical

Bulbs of *A. sativum* are reported to contain hundreds of phytochemicals including sulfur-containing compounds such as ajoenes (E-ajoene, Z-ajoene), thiosulfinates (allicin), vinyldithiins (2-vinyl-(4H) -1,3-dithiin, 3-vinyl-(4H)-1,2-dithiin), sulfides (diallyl disulfide (DDS), diallyl trisulfide (DTS)) **(Tab. 13) (Al-Snafi, 2013)**. And phenolic acids such as caffeic acid, p-coumaric acid, ferulic acid, and sinapic; acid pyrocatech acid; coffee acid and p-hydroxybenzoic acid **(Brewer, 2011).** However, **Berginc et *al.*, (2010)** showed that garlic contain also flavonoids such as nobiletin, tangeretin, rutin. **Chekki et *al.* (2014)** reported that the content of flavonoids in garlic was 132 $mg.kg^{-1}$ and **Fratianni et *al.* (2016)** identified some of these flavonoids such as epicatechin (1.178 $mg.kg^{-1}$), rutin (43.43 $mg.kg^{-1}$), luteolin (0.15–22.92 $mg.kg^{-1}$), hyperoside (0.37–20.24 $mg.kg^{-1}$), quercetin (6.55–10.17 $mg.kg^{-1}$), apigenin (3.24$mg.kg^{-1}$) and naringenin (11.75–56.71 $mg.kg^{-1}$).

Alliin, the main cysteine sulfoxide is transformed to allicin by allinase enzyme after cutting of the garlic and breaking down the parenchyma. S-propyl-cysteine sulfoxide (PCSO),

allicin and S-methyl cysteine-sulfoxide (MCSO) are the main odoriferous molecules of freshly milled garlic homogenates. PCSO can produce more than fifty metabolites depend on water content and temperature as well as allinase enzyme that can act on the mixture of MCSO, PCSO, and alliin to produce other molecules, such as allyl methane thiosulfinates, methyl methanethiosulfonate, and further corresponding thiosulfinates (R-S-S R′), by which R and R′ are allyl, propyl, and methyls groups **(Zeng et *al.*, 2017)**. S-alk(en)yl-l-cysteine sulfoxides are the secondary metabolites obtained from cysteine which accumulate in the plants of *Allium* genus **(Souza et *al.,* 2011)**. Garlic formulations consist of several organosulfur compounds, N-acetylcysteine (NAC), S-allyl-cysteine (SAC) **(Asdaq and Inamdar, 2011)**, and S-ally mercapto cysteine (SAMC), which are derived from alliin **(Tran et *al.,* 2018)**. Notably, SAC has antioxidant, anti-inflammatory, regulated redox, pro energetic, antiapoptotic, and signaling capacities **(Liu et *al.,* 2015)**, while SAMC shows an anticancer activity through preventing the cancer cells multiplication **(Cao et *al.,* 2017)**.

Allicin (allyl thiosulfinate), is a sulfenic acid thioester and its pharmacological effect is attributed to its antioxidant activity as well as its interaction with thiol-containing proteins **(Miron et *al.,* 2000)**. In the allicin biosynthesis, cysteine is transformed to alliin that is hydrolyzed by the allinase enzyme **(Borlinghaus et *al.,* 2014)**.This enzyme composed of pyridoxal phosphate (PLP) which splits alliin and produces ammonium, pyruvate, and allyl sulfenic acid that are highly reactive and unstable at room temperature, where two molecules were combined to form allicin **(Miron et *al.,* 2000; Shimon et *al.,* 2007).**

**Table 13:** Structures of some sulfur-containing compounds isolated from garlic
**(El-Saber Batiha et *al.,* 2020)**

| Compounds | Molecular formula | Structure |
|---|---|---|
| **Alliin** | $C_6H_{11}NO_3S$ | |
| **Allicin** | $C_6H_{10}OS_2$ | |
| ***E*-Ajoene** | $C_9H_{14}OS_3$ | |
| ***Z*-Ajoene** | $C_9H_{14}OS_3$ | |
| **2-Vinyl-4H-1,3-dithiin** | $C_6H_8S_2$ | |
| **Diallylsulfide (DAS)** | $C_6H_{10}S$ | |
| **Diallyldisulfide (DDS)** | $C_6H_{10}S_2$ | |
| **Diallyltrisulfide (DTS)** | $C_6H_{10}S_3$ | |
| **Allylmethylsulfide (AMS)** | $C_4H_8S$ | |

A summary of the phenolic acid constituents and the chemical structures of some flavonoid in garlic are presented in **Table 14** and **Table 15.**

**Table 14:** Phenolic acid constituents in fresh garlic **(Kim et *al.*, 2013)**

| Acid derivatives | Selected acids | Content of acids ($mg.kg^{-1}$) |
|---|---|---|
| Hydroxybenzoic acid derivatives | p-Hydroxybenzoic acid | / |
| | Gallic acid | 2.06 ± 0.09 |
| | Vanillic acid | / |
| | Chlorogenic acid | / |
| | Caffeic acid | 7.48 ± 0.23 |
| | p-Coumaric acid | 1.25 ± 0.03 |
| Hydroxycinnamic acid derivatives | Ferulic acid 1. | 1.57 ± 0.02 |
| | m-Coumaric acid | 4.84 ± 0.04 |
| | o-Coumaric acid | 0.66 ± 0.03 |
| | Total hydroxybenzoic acid derivatives | 2.06 ± 0.09 |
| Total hydroxycinnamic acid derivatives | | 15.80 ± 0.08 |
| Total phenolic acids | | 17.86 ± 0.07 |

**Table 15:** Chemical structures of some flavonoid in garlic **(Brunetti et *al.*, 2013)**

| Compounds | Structure |
|---|---|
| **Quercitin** | |
| **Apigenin** | |
| **Luteolin** | |
| **Epicatechin** | |
| **Rutin** | |

### III.1.8 Pharmacological properties

In recent years, natural plant products are gaining popularity in preventing various diseases. Garlic is one of the most extensively researched products for investigating its beneficial effects. Potential health benefits of *Allium* vegetables, in particular garlic has its origin in antiquity. It has acquired a reputation as a formidable prophylactic and therapeutic medicinal agent in the folklore of many cultures, over the centuries. The bioactive

components of garlic are mainly responsible for the healing properties **(Santhosh et *al.,* 2013).** Main pharmacological effects of garlic are attributed to its organosulphur compounds, and allicin is the chief biologically active component of garlic **(Arzanlou and Bohlooli, 2010).** Garlic have been stated to have several biological activities including antioxidant, antidiabetic, antimicrobial, and antiviral activities **(Tab.16) (El-Saber Batiha et *al.,* 2020).**

**Table 16:** Pharmacological activities of garlic **(El-Saber Batiha et *al*., 2020)**

| **Pharmacological Activities** | **Mechanism of Action** |
|---|---|
| Antidiabetic | ❖ Decreasing the insulin secretion from pancreatic cells, increasing liver metabolism, and thus enhancing the short-acting insulin production |
| Antihypertensive | ❖ Inhibiting the angiotensin converting enzyme |
| Hypolipidemic, Hypocholesterolaemic | ❖ Decreasing serum TC, TG, and LDL levels and moderately elevating HDL cholesterol |
| Anti-Atherosclerotic, antithrombotic | ❖ Preventing ADP-activated platelets binding to immobilized fibrinogen and platelet aggregation, inhibiting GPIIb/IIIa receptor and increasing cAM |
| Antioxidant | ❖ Modulation of ROS, increasing glutathione and cellular antioxidant enzymes<br>❖ Controlling ROS generation and preventing mitogen-activated protein kinase (MAPK)<br>❖ Suppressing the enzymatic activity of cytochrome P450-2E1, reducing the generation of reactive oxygen and nitrogen species |
| Antibacterial | ❖ Chemical interaction with enzymes containing thiol |
| Antifungal | ❖ Irreversible ultrastructural changes in the fungal cells, loss of structural integrity and affected the germination ability |
| Antiviral | ❖ Chemical interaction with enzymes containing thiol<br>❖ Enhancing Natural killer-cell (NK-cell) activity that destroys virus infected cells |
| Antiprotozoal | ❖ Preventing the parasite's RNA, DNA and protein synthesis<br>❖ Inhibiting the human glutathione reductase and *Trypanosoma cruzi* trypanothione reductase |
| Anti-inflammatory | ❖ Enhancing the immune cell activity f, inhibiting the SDF1α chemokine and Transendothelial migration of neutrophils<br>❖ Diminishing the expression of the inflammatory cytokines (e.g., NF- κB, IL-1β, and TNF-α), and ROS generation by suppressing CYP 2E1 hepatic enzyme<br>❖ Blocking the NF-κB activity |
| Anti-cancer | ❖ Enhancing p38 expression and cleaved caspase 3<br>❖ Stimulating apoptosis in human leukemic cells, promoting the peroxide production, caspase-3-like, and caspase-8 activities |
| Immunomodulatory | ❖ Suppressing BuChE (Butyrylcholinesterase) and AChE |

| | |
|---|---|
| | (Acetylcholinesterase) |
| Anti-obesity | ❖ Decreasing the fat accumulation in 3T3-L1 adipocytes and dramatically decreases the body weight gain<br>❖ Decreasing the C/EBPα, PPARγ2, and LPL expression and the PPARγ effect in human adipocytes |
| Cardiovascular Protection | ❖ Decreasing the levels of blood total lipids, triglyceride, and cholesterolby reducing the mRNA expression of sterol regulatory element binding protein<br>❖ Reduce cardiac hypertrophy remodeling induced by isoproterenol byincreasing Na+/K+-ATPase protein level<br>❖ Protecting the heart function Activating sirtuin 3-manganesesuperoxide dismutase pathway by<br>❖ Deacetylating manganese superoxidedismutase<br>❖ Protecting heart rate variability, cardiac dysfunction, and mitochondrialdys function<br>❖ Protecting the heart tissue<br>❖ Reducing oxidative stress<br>❖ Protecting against cardiotoxicity |

### III.1.8.1 Antioxidant

**Asdaq and Inamdar (2001)** reported that the frequent garlic intake promotes internal antioxidant activities and reduces oxidative adverse effects either by increasing the endogenous antioxidant synthesis or reducing the production of oxidizers such as oxygen-free radical species (ORS). It is demonstrated that garlic protects against gentamycin as well as acetaminophen induced hepatotoxicity by improving antioxidant status, and regulating oxidative stress **(Wallock-Richards et *al.,* 2014).** As reactive oxygen species (ROS) seems to be at the core of many ailments, it is justified to assume that the antioxidant effect of garlic might be through modulation of ROS, increasing glutathione and cellular antioxidant enzymes **(Shokrzadeh and Ebadi, 2006)**. Moreover, garlic extract was found to increase the activities of some antioxidant enzymes (e.g., superoxide dismutase (SOD)) and decrease glutathione peroxidase (GSH-Px) in hepatic tissues of rats. Notably, several reports indicated that AGE rich in flavonoid, phenol, and different sulfur compounds e.g., SAC shows high radical scavenging activity **(Jang et *al.,* 2017).** Additionally, AGE acted by stimulating the expression of different antioxidant enzymes, namely glutamate-cysteine ligase modifier (GCLM) and heme oxygenase-1 (HO-1) subunit by the nuclear factor erythrobia-2 related factor 2 (Nrf2)-antioxidant response element (ARE) pathway that is responsible for human endothelial cells protection against oxidative stress **(Liu et *al.,* 2018).** Alliin, the major compound isolated from AGE, showing wide-spectrum antioxidant activities by controlling ROS generation and preventing mitogen-activated protein kinase (MAPK). Moreover, it was

reported to prevent ROS production by inhibiting NADPH oxidase 1, and thus, inhibiting the osteoclast fusion caused by receptor activator of nuclear factor-kappa B ligand (RANKL) **(Chen et *al.,* 2016).** Allicin, DDS, and DTS are the main antioxidative compounds that showed an antioxidant effect in lower doses at the physiological level **(Gruhlke et *al.,* 2010).** Saponins extracted from garlic were reported to scavenge intracellular ROS and protect mouse-derived $C_2C_{12}$ myoblasts towards growth inhibition and $H_2O_2$-induced DNA damage **(Shang et *al.,* 2019).** Interestingly, **Abdel-Daim et *al.* (2018)** reported that DAS exhibited potent antioxidant and cytoprotective activities and these activities may be due to suppressing the enzymatic activity of cytochrome P450-2E1 and thereby reducing the generation of reactive oxygen and nitrogen species or by inducing the mRNA expression of Nrf2 and heme-oxygenase enzyme.

**III.1.8.2 Antibacterial**

The antibacterial activity of garlic is attributed to allicin activity that was reported toward a wide variety of microorganisms including antibiotic-resistant, Gram-positive and Gram-negative bacteria such as *Shigella*, *E. coli* **(Ross et *al.,* 2001),** *S. aureus*, *Pseudomonas aeruginosa* **(Kuda et *al.,* 2004),** *Streptococcus mutans*, *S. faecalis*, *S. pyogenes*, *Salmonella enterica*, *Klebsiella aerogenes* **(Cutler and Wilson, 2004)**, *Vibrio, Mycobacteria, Proteus vulgaris,* and *Enterococcus faecalis* **(Wallock-Richards et *al.,* 2014).** Various garlic extracts (aqueous, chloroform, methanolic, and ethanolic) were reported to inhibit the growth of several pathogenic bacteria with varying degrees of susceptibility. For instance, a study revealed that ethanolic garlic extract showed higher inhibitory effect against *E. coli* and *Salmonella typhi* than the aqueous extract that showed little or no inhibition effect **(Mikaili et *al.,* 2013). Meriga et *al.* (2012)** reported that aqueous garlic extract showed antibacterial activity toward Gram-negative (*Klebsiella pneumoniae* and *E. coli*) as well as Gram-positive (*B. subtilis* and *S. aureus*) strains, whereas methanolic garlic extract showed antimicrobial activity against all tested strains except *S. aureus*. Moreover, garlic extracts prevented the growth of enterotoxigenic *E. coli* strains and other pathogenic intestinal bacteria, which are the main cause of diarrhea in humans and animals. Besides the antibacterial activity of garlic, it was reported to prevent the toxins produced by bacterial infection **(Shokrzadeh and Ebadi, 2006).** Allicin showed effectiveness toward methicillin-resistant *S. aureus* (MRSA) **(Wallock-Richards et *al.,* 2014).** Allicin's antimicrobial activity is due to its chemical interaction with enzymes containing thiol (thioredoxin reductase), RNA polymerase, and alcohol dehydrogenase by oxidizing protein cysteine or glutathione residues under physiological conditions. Allicin is a dose-related biocide that can influence essential

metabolism of cysteine proteinase, and thus, kill all eukaryotic cells due to the presence of thiol groups in all living cells **(Gruhlke et *al.,* 2010).** The incidence of stomach cancer is lower in individuals with a high intake of *Allium* vegetables in developed and developing (high risk) countries **(Steinmetz and Potter 1991)**. Because *Allium* vegetables, particularly garlic, have antibiotic activity, **Jonkers et *al.* (1996)** observed that the combination of garlic and omeprazole appeared a synergic effect against *H. pylori.* while **Sivam et *al.* (1997)** investigated the antimicrobial activity of garlic against *H. pylori* using an aqueous extract of a known variety of garlic (Oswego white). The MIC was found to be 40 mg/ml. At this concentration, the control organism *Staphylococcus aureus* was not inhibited by the garlic extract. Thus *H. pylori* is more susceptible to garlic extract. **Cellini et *al.* (1996)** reported a similar study, they tested 16 clinical isolates of *H. pylori* and showed 90% inhibition of the isolates with aqueous garlic extract at 5 mg/ml. The concentration used in that study is the total weight of garlic per milliliter. However, calculations show that the MIC reported in the two studies are comparable. It is plausible that the sensitivity of *H. pylori* to garlic extract at such a low concentration may be related to the reported lower risk of stomach cancer in those with a high *Allium* vegetable intake. The inhibitory concentration of garlic reported in the two studies above is achievable in the stomach by consuming a medium size clove of garlic or equivalent amount of garlic supplements. Thus, this finding may identify a strategy for low-cost intervention for stomach cancer, with few side effects, in populations at high risk, particularly in the case of high resistance to antibiotics **(Sivam, 2001).**

## III.2 *Allium cepa* L. (Onion)

### III.2.1 History

Onion is one of the oldest vegetables known to mankind dating back to 3,500 years. Onion plant is the most frequently portrayed plant in Egyptian tomb paintings. An inscription on the Great Pyramid of Cheops indicates ,100 talents of silver had been spent on onions, garlic, and radishes with which the slave labor were reimbursed, in lieu of money, for their part in building the pyramid in 2500 BC **(Abdel-Maksouda, 2011)**. The Greeks and Romans had a love-hate relationship with this plant, admiring its therapeutic powers and detesting its pungent smell. In order to give his warriors energy and power for combat, Alexander the Great fed them this plant **(Charles, 2013).** The ancient Egyptians loved onion and one of the varieties evoked as a deity and worshipped. The Egyptians ate it raw. Onion was one of the staple foods for the slaves who built the Giant Pyramid **(Abdel-Maksouda, 2011)**. Later the Israelites mourned the loss of Egyptian onions on their way to the Promised Land. The

English name onion is believed to have been derived from the Roman name *unionem* or *unio*, referring to its single bulb. Romans introduced onion to Britain, and Emperor Nero took it for cold, coughs, and sore throats. It was regarded as an aphrodisiac and a symbol of fertility **(Charles, 2013).**

### III.2.2 Origin and distribution

Onion appeared first time in Asia. According to **Vavilov (1951)**, it was originated in Pakistan. **Jones and Mann (1963)**, observed that the area comprising Pakistan, Iran and mountain areas to the North as primary centre of origin of onion. *A. cepa* is a bulbous plant widely cultivated in almost every country of the world. According to the last available **FAO** statistics **(2019)**, in 2016 the top producers of onions were China (23.849.053 tons) and India (19.415.425 tons), followed by Egypt and USA (about 3.000.000 tons), Iran, Turkey, Russian Federation, Pakistan, Bangladesh and Brazil (from 2.345.768 to 1.657.441 tons). Onions produced in European countries accounted for 10.9% of the world production, being Asia (65.5%) the most important producer. Because of its worldwide distribution, a great number of cultivars can be identified: ‘Stardust,’ ‘Snowpack,’ ‘Redlight,’ ‘Hytech’ **(Böttcher et *al.,* 2018), ‘**Tropea,’ ‘Montoro’ **(Tedesco et *al.,* 2015),** ‘Festival,’ ‘Castillo’ **(Marotti and Piccaglia, 2002),** ‘Nazik Red’ and ‘Ailsa Craig’ **(Bennett et *al.,* 2000)**. At present, approximately 13,000 onion accessions are held in gene banks worldwide. A high genetic variability can be observed regarding the morphological features **(Böttcher et *al.,* 2018)**.

### III.2.3 Botanical description

Onion (*Allium cepa* L.) belongs to the family *Alliaceae* or *Amaryllidaceae* which is one of the most important monocotyledonous crops; included to the genus *Allium* and recent estimations accept about 750 species in the genus *Allium*, among which onion, Japanese bunching onion, leeks, and garlic are the most important edible *Allium* crops, and about 60 taxonomic groups at sub-generic, sectional and sub-sectional rank **(Baloch 1994; Rabinowitch and Currah 2002).** Onion from central Asia, the supposed onion ancestor had probably migrated to the Near East, then it was introduced to India and South-East Asia; and into the Mediterranean area and from there to all the Roman Empire **(Grubben and Denton, 2004).** Bulbing onions have cylindrical, hollow leaves and an enlarged bulb that develops at ground level. The roots come off the bottom of the bulb. The flowers are produced in the second growing season (following a required "rest" period) in a rounded umbel (cluster with all flower stems originating from the same point) on a stalk 2-4 ft (0.6-1.2 m) tall. The umbels are about 2 in (5 cm) in diameter and consisting of many small purplish flowers, are quite showy **(Fig. 18) (Ross, 2001).**

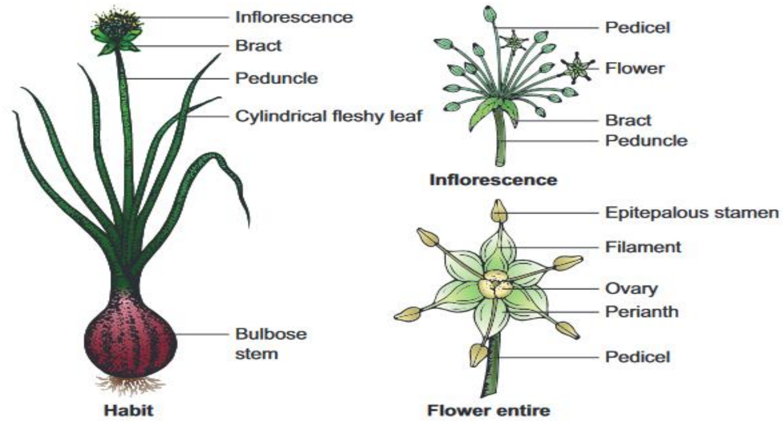



**Figure 18:** *Allium cepa* L. plant **(Ross, 2001)**

Onion is distributed throughout temperate regions of the world including Europe, Asia, North America and Africa **(Khare, 2002).** Onion bulb size and colour varies from variety to variety, cultivation practices, region, and climatic condition **(Nath et *al.,* 2010).**

### III.2.4 Taxonomy and classification

*Allium cepa* L. is considered to be a member of the *Liliaceae* **(Strasburger et *al.,* 1978),** which comprises of 250 genera and 3700 species. Because of their bulbs, tubers and rhizomes, these plants are able to survive under unfavorable conditions (winter or dryness). Plants of the *Liliaceae* show very different habits and contain various classes of chemical compounds **(Hegnauer, 1963).** Therefore, the classification of the *Liliaceae* has been discussed for a long time: **Hurchinson (1959)** signed *Allium cepa* L. to the subfamily *Allioideae*, belonging to the *Amaryllidaceae*. Because of the lack of alkaloids, which are normally typical of *Amaryllidaceae*, the *Allioideae* were also classified as a member of *Liliaceae* **(Hegnauer, 1963)**. Finally, plants of the genus *Allium* were classified in the independant family *Alliaceae* **(Tab. 17) (Dahlgren et *al.,* 1985).**

### III.2.5 Habitat and ecology

Onion can be grown in a wide range of climatic environments, but it thrives best at mild climate without excessive rainfall or extremes of heat and cold. Onion is a cool season crop that has some frost tolerance but is best adapted to a temperature range between 13 and 24°C. Optimum temperatures for early seedling growth are between 23 and 27°C; growth is slowed at temperatures above 30°C. Acclimatized plants are able to tolerate some freezing temperature. Best production is obtained when cool temperature prevails over an extended period of time, permitting considerable foliage and root development before bulb formation starts. After bulb formation begins, high temperature and low relative humidity extending into the harvest and curing period are desirable **(Jilani et *al.,* 2010).**

**Table 17:** Classification of *Allium cepa* **(Dahlgren et *al.,* 1985)**

| **Section** | Spermatophyta |
|---|---|
| **Subsection** | Angiospermae |
| **Class** | Mono cotyledoneae (= Liliarae) |
| **Subclass** | Liliidae |
| **Order** | Liliales (= Liliiflorae) |
| **Family** | *Alliaceae* |
| **Subfamily** | *Allioideae* |
| **Tribe** | *Allieae* |
| **Subrribe** | *Alliinae* |
| **Genus** | *Allium* |
| **Species** | *Allium cepa* |

### III.2.6 Varieties

Onion varieties differ in size, colour of skin, pungency, and maturation. Common onions are normally available in three colour varieties (**Tab. 18**); yellow or brown onions (called red in some European countries) are full flavour red and are the onions of choice for everyday use. With its pungent aroma and strong flavour, it is a good all-round onion. Their varieties are grown in countries like Australia, Nigeria and they vary from cream gold and muray brown **(Mower, 2013).** Other varieties of onion crop also include Red onion; these are sometimes called Spanish onions, which have purplish red skin and white flesh tinged with red. This onion tends to be medium to large size and can have a mild to sweet flavour but after being stored for a short time can become quite pungent. They are often consumed raw, grilled or lightly cooked with other foods or added as colour to salads, these variety of onion plant can be stored three to four months under ideal condition, and their varieties include red shine, red wing and red emperor. Although white onions are considered to be the strongest in flavour after brown onions, their varieties vary in size, skin characteristics and flavour; they include Bianca, Gladalan white and white Spanish **(Okey et *al.*, 2015).**

### III.2.7 Chemical composition

#### III.2.7.1 Physicochemical

Onion is relatively high in food value, generally consisting of carbohydrates, moisture, protein, fat, minerals, fibers, calcium, phosphorus, iron and vitamin C. This composition substantially varies in different types of onion red, white and yellow onion varieties and it also depends on the climatic conditions, cultural practices, bulb stage and its period of storage **(Tripathi, 2006)**. Also the fructo-oligosaccharides, fructose, sucrose and glucose are the principal non-structural sugars in onion bulb tissue. Onions bulbs contain remarkable mineral composition, particularly potassium and phosphorus **(Benite et *al.*, 2011).** The richest

minerals found in the 'Red Amposta' are phosphorus, potassium, soluble proteins, water, sodium, magnesium, calcium, and soluble sugar **(Tab.19) (Abou Azoom et *al*., 2015).**

**Table 18:** Varieties of onion **(Lawande, 2012)**

| Varieties | Characteristics |
|---|---|
| **White** (south port white Globe) | ❖ Medium to large in size<br>❖ Globular in shape with rounded or slightly tapered ends<br>❖ The bulb is encased in a bright white, papery, parchment-like skin that is flaky, dry, and thin<br>❖ Underneath the skin, the white, almost translucent flesh is firm, crisp, and juicy with many layers of thin white rings<br>❖ White onions are crunchy and tender with a pungent, mildly sweet flavor and have a mellow, non-abrasive aftertaste |
| **Red** (red Wethersfield) | ❖ Small to medium in size, averaging 10-15 cm in diameter round to slightly oval in shape<br>❖ The bulb is wrapped in red-purple thin, papery, parchment-like skin that is delicate and easily flakes off when touched underneath the skin, there is a burgundy wine colored sheath that protects the flesh, and the dark purple flesh is firm, crisp, and coarse with many layers of white and purple rings<br>❖ Red onions are crunchy, pungent, sweet, and slightly spicy |
| **Yellow or brown** (yellow Globe) | ❖ Medium to large in size, averaging 5-10 cm in diameter and are globular in shape with rounded to slightly tapered ends<br>❖ The bulb is encased in a light yellow to copper, dry, papery skin<br>❖ The flaky, layered skin surrounds a white to cream-colored translucent flesh<br>❖ The ringed flesh is firm, crisp, and juicy<br>❖ Yellow onions are crunchy with a pungent flavor when raw |

### III.2.7.2 Phytochemical

Onion is an important source of phytochemical contents such as flavonoids. It is especially rich in three important phytochemicals, namely flavonoids, organosulfur compounds, and fructans. Such substances are known for their positive health benefits. Onions are the main suppliers of flavonoids andorganosulfur compounds which are potent antioxidants **(Kandoliya et *al.,* 2015).** It is rich in various organo-sulphur compounds which are responsible for its distinctive flavour, odour, pungency and medicinal properties. In addition to the organic sulphur components it was also a good source of secondary metabolites like flavanoids (flavanols, quercetin and kaempferol), phenols, sterols, saponins, pectic and volatile essential oils. The particular odour of onion is produced when the bulbs are cut or injured due to the enzyme allinase action that convert the trans-S-(1-propenyl) cysteine sulfoxide topropanethial-S-oxide. This pungent flavour is preferred by different people of many countries. In addition to the above constituents it also acts a source of trace mineral that helps in cells response to insulin **(Tripathi, 2006)**.

**Table 19:** Chemical composition of onion bulb **(Tripathi, 2006)**

| Nutrients | Value (g/100g) | Minerals | Value (mg/100g) | Vitamins | Value (mg/100g) |
|---|---|---|---|---|---|
| Water | 78.32-87.31 | Calcium | 18-25 | Vitamin C | 10-70 |
| Food energy | 38-46 (Kcal/100g) | Iron | 0.15-0.24 | Vitamin B1 | 0.04-0.06 |
| Proteins | 1.12-232 | Magnesium | 8-12 | Vitamin B2 | 0.02-0.03 |
| Total Lipids | 0.5-0.9 | Phosphorus | 25-30 | Vitamin B3 | 0.080.09 |
| Ash | 0.3-0.4 | Potassium | 120-175 | Vitamin B5 | 0.12-0.13 |
| Carbohydrates | 7.64-12.13 | Sodium | 2-4 | Vitamin B6 | 0.14-0.15 |
| Total fibers | 1.0-1.8 | Zinc | 0.15-0.2 | Vitamin B9 | 15-22 (µg /100g) |
| Saturated fatty acid | s0.02-0.04 | Copper | 0.03-0.04 | Vitamin B12 | 0 (µg /100g) |
| Monosaturated fatty acids | 0.02-0.04 | Manganese | 0.12-0.14 | Vitamin A | 2-6 (µg /100g) |
| Poly unsaturated fatty acids | 0.05-0.07 | Selenium | 0.4-0.6 (µg/100g) | Vitamin E | 0.01-0.03 (µg /100g) |
| Pyruvic acid | 6.5-14.9 (µmole/ml) | / | / | Vitamin K | 0.03-0.05 (µg /100g) |

Organosulfur constituents of onions are mainly comprised of four diallylsulfides: diallyltetrasulfide (DTTS), diallylmonosulfide (DMS), diallyltrisulfide (DTS) and diallyldisulfide (DDS). Di- and tri-sulfides were the principal compounds extracted by steam distillation of volatile fraction of onion. The primary sulfur-containing components in onions aresalk(en)yl-L-cysteine sulfoxides (ACSOs) such as alliin, they act as biosynthetic intermediates for ACSOs and also act as storage peptides. Volatile compounds including allicin and lipid-soluble sulfur compounds such as diallyl disulfide (DDS) and diallyl sulfide (DAS) originate from different metabolic pathways in the vegetables **(Lancaster and Shaw, 1989)**. The unique smell and taste of onion oil is due to the presence of these compounds **(Lanzotti, 2006)**. Other than the above mentioned constituents, the presence of some steroidal sapogenins and saponins has also been reported in onions. These components play an important role in pharmacological and biological activities and have antibacterial, antithrombotic, anti-inflammatory, antitumor, antifungal and hypocholesterolemic properties **(Fenwick et *al.*, 1985)**.

The presence of volatile S-compounds causes onions to have a pungent flavor. Onions are known as an excellent source of flavonoids which are the part of flavonols family of polyphenols. The flavonoid subclass which includes quercetin is considered a leading and prominent nutritional flavonoid of onions. Other flavonols such as quercetin, isorhamnetin,

and kaempferol were also found in onions **(Fenwick et *al.*, 1985; Dorsch and Wagner, 1991; Dorant et *al.*, 1996)**. The secondary metabolites (phenolics) present in onions have an antioxidant effect and cause aromatic hydroxylated rings **(Nuutila et *al.*, 2003)**. The phenolics are important antioxidant contents of plants **(Colina-Coca et *al.*, 2014).**

The other sources of phytochemicals in onion bulbs are the fructans. The fructans mainly contain fructo-oligosaccharides such as nystose, inulin, fructo-furanosylnystose and kestose. It has been reported that onion samples exhibit the presence of 10 various organic acids; myristic acid (0.004g), palmitic acid (0.034g), stearic acid (0.004g), saturated acid (0.043g), oleic acid (0.013g), linoleic acid (0.013g), linolenic acid (0.004g), monounsaturated acid (0.013g), polyunsaturated acid (0.017g) and glutamic acid (0.258g) **(Mallor et *al.*, 2011).**

A study analyzed onion landraces for their unique composition of phenols. The most abundant phenol found in all landraces was gallic acid, its amount varies from 55.66 to 64.90µg/g DW respectively. Quercetin has an important role among identified phenols. Quercetin is the aglycone component of many other flavonoid glycosides such as quercetrin and rutin. They are present in citrus fruits, buckwheat, and onions **(Farhat et *al.*, 2020).**

**Table 20:** Phytochemical composition of various onion species **(Shabir et *al.*, 2022)**

| **Flavonols and Anthocyanins** | **Cepa (Yellow variety)** (mg/100 g) | **Cepa (Red variety)** (mg/100 g) |
|---|---|---|
| Quercetin 3, 40-diglucoside | 100.40 ± 0.05 | 331.93 ± 0.12 |
| Quercetin 40-monoglucoside | 140.43 ± 0.10 | 298.87 ± 0.13 |
| Myricetin | 8.63 ± 0.01 | 9.31 ± 0.02 |
| Quercetin aglycone | 60.51 ± 0.06 | 70.10 ± 0.08 |
| Isorhamnetin | 2.21 ± 0.01 | 13.73 ± 0.01 |
| Peonidin 30-glucoside | 1.11 ± 0.00 | Nd |
| Peonidin 30-glucoside acetate | Nd | 0.67 ± 0.3 |
| Malvidin 30-glucoside | 0.53 ± 0.00 | 0.24 ± 0.00 |
| Cyanidin 30-glucoside | 7.85 ± 0.11 | 0.11 ± 0.00 |
| Cyanidin 30-glucoside acetate | 0.76 ± 0.00 | 3.44 ± 0.03 |
| Petunidin 30-glucoside | 0.12 ± 0.00 | Nd |
| Petunidin 30-glucoside acetate | Nd | 0.17 ± 0.02 |

Nd: Not determined

### III.2.8 Pharmacological properties

Onion has been used for healing both internally and externally. Internally, onion has been recommended to treat colds, cough, bronchitis, whooping cough, asthma and other respiratory problems. It is believed to help in congestion of the lungs and expands the airways. Onion is also used internally to relieve excess gas and calm an upset stomach. A

mixture of rue and onion is used torid of parasites of the digestive system. Onion is also thought to stimulate appetite. When applied externally, fresh onion juice prevents bacterial and fungal infections **(Tab. 21).** It can be applied to wounds and stings on the skin, to remove warts and to stimulate hair growth. The major benefits of onion on human health are described here in brief **(Tripathi, 2006).**

**Table 21:** Pharmacological activities of onion **(Kumari et *al.,* 2022)**

| Pharmacological Activities | Mechanism of Action |
|---|---|
| Antidiabetic | ❖ *In vitro*: Onion extract demonstrated inhibitory activity (IC50) against sucrase (0.40 mg/ml) >α-glucosidase (1.27 mg/ml) >maltase (2.02 mg/ml) >α-amylase (3.00 mg/ ml)<br>❖ *In vivo*: 0.5 g onion extract/kg body weight significantly decreased the rise in blood glucose levels<br>❖ Onion extracts inhibited α-amylase, 1mg/ml extract Established *invitro* antidiabetic activity |
| Neuroprotective effect | ❖ Onion extract exhibit AChE inhibitory activity (IC50: 37.11 μg/ml)<br>❖ AChE activity was inhibited significantly by onion extract, the 10 g/kg (104%) and 20 g/kg (98%) body weight groups<br>❖ Improved cognitive functioning<br>❖ Inhibitory effect on AChE and BuChE enzymes<br>❖ Thus, it is helpful in the treatment of Alzheimer's and neurological diseases |
| Antioxidant | ❖ Protects cells and tissues from reactive oxygen species (ROS)<br>❖ Stabilize free electrons generated by ROS *in vitro* |
| Antibacterial | ❖ Onion extract were effective against *B. cereus* (MBC 0.6mg/ml)<br>❖ *S. aureus* was sensitive to onion extract<br>❖ Onion extract have antimicrobial activity against *Salmonella typhimurium* and *Staphylococcus aureus* (DZI: Diameter of Zone Inhibition: 9 and 8mm)<br>❖ Onion extract exhibited synergistic antibacterial and anticandidal activity<br>❖ Silver nanoparticles using onion extract exhibited excellent antibacterial activity against all the tested microorganisms in a dose-dependent manner (25–100 μg/ml), the inhibition zones ranged from 14 to 17mm, 13–19 mm, 14–17 mm, 14.5–19.3 mm, 13–17.5 mm and 14.6–18 mm against *Bacillus sp., Staphylococcus aureus, Corynebacterium sp., Escherichia coli, Salmonella* sp., and *Vibrio cholerae,* respectively |
| Antifungal | ❖ Onion bulbs, enhances the potential fungicidal activity<br>❖ The main active antifungal agents fromonion extracts are diallyl trisulphide (DTS), DDS and DAS |
| Antiviral | ❖ The major onion flavonoid, also possesses antiviral activity and enhances the bioavailability of some antiviral drugs<br>❖ Onion have a pronounced anti-HIV activity<br>❖ Onions posses *in vitro* anti-adenoviral activity |
| Antiparasitic | ❖ Onion was successful against *Schistosoma mansoni cryptosporidium parvum* infection in mice in an experiment |

| | |
|---|---|
| | ❖ Effective in the treatment of worm infections |
| Anti-inflammatory | ❖ Reduces the swelling, pain and symptoms associated with severity of inflammation |
| Anti-cancer | ❖ Antigenotoxic activity observed<br>❖ Reduced intracellularROSat1–100 µg/ml concentrations<br>❖ Reduced $H_2O_2$ and hydroxynonenal induced DNA damage in human leukocytes<br>❖ Lipopolysaccharide induced mRNA expression of heme Oxygenase-1($HO_1$) and glutathione S-transferase (GST) detoxification genes were significantly reduced by treatment with onion extract(50–250µg/ml)<br>❖ Exhibited anticancer effect on HeLa cells with IC50 values of 4.8 and 6.3 µM for spiraeoside and quercetin, respectively |
| Anti-obesity | ❖ In the cell model, triglyceride accumulation was reduced after treatment with onion extract (25 µg/ml to 100 µg/ml)<br>❖ In the animal model, the onion extract group showed a significant reduction in body weight and retroperitoneal and mesenteric fat<br>❖ *In vitro:* Onion extract decreased the lipids of 3T3-L1 cells and down regulated the expression of lipogenesis related genes such as peroxisome proliferator activated receptor-γ, fatty acids synthase, and acetyl-CoA carboxylase, thus inhibiting lipid accumulation<br>❖ *Invivo*: The weight of onion extract-fedmice decreased significantly, at the higher dose (144 mg/kg), onion extract significantly decreased serum TG,TC, and LDL C<br>❖ Onion extract reduced accumulation of lipids in liver |
| Cardiovas cular Protection | ❖ Onion extract may improve endothelial function and have a cardioprotective role<br>❖ In mice, arterial thrombosis was delayed in onion extract group Levels of tissue factor, onion extract (100 µl) also reduced thrombin-induced phosphorylation of Jun N terminal kinase and extracellular signal regulated kinase<br>❖ Onion extract increased LDL receptor (LDLR), sterol regulatory element-binding protein (SREBP)- 2, and hydroxyl-3-methylglutaryl coenzyme reductase (HMGCoAR) genes, which were reduced by HFD<br>❖ Onion extract had a cholesterol lowering effect in these rum and liver via fecal excretion |

### III.2.8.1 Antioxidant

Onion due to its high nutritional properties, proved as a potential source of antioxidants that provide protection against harmful free radicals that damages the biological membrane. The effect includes lowering the blood cholesterol level (low density lipoprotein) by inhibiting the lipid peroxidation **(Halliwel, 1992).** In addition, antioxidants present in onion extract are responsible for free radical scavenging and chelation of transition metalions, inhibition of oxidases such as lipoxygenase **(Udayan, 2005).**

#### III.2.8.2 Antimicrobial

Onions have been shown to possess antibacterial and antifungal properties. Volatile oil of onion has been shown to be highly effective against Gram positive bacteria, dermatophytic fungi, growth and aflatoxin production of *Aspergillus* fungi genera **(Bison, 1994).** In addition to inhibitory effects against pathogenic bacteria, onions have been found to promote beneficial microorganisms. Onion has been described as a potent antimicrobial agent to fight against infectious diseases. Many bacteria, fungi, and viruses were found to be susceptible to different solvents extracts of onion. Sulphur compounds have proven to be the principal active antimicrobial agent present in onion **(Rose et *al.,* 2006). Liguori et *al.* (2017 and Vazquez-Armenta et *al.* (2014)** have reconsidered the effect of organosulphur-containing compounds on the growth of microorganisms. The effectiveness of onion extract against bacterial growth of *B. cereus*, *L. monocytogenes*, and *P. aeruginosa* and was as effective as quercetin in inhibiting the growth of *S. aureus* and *M. luteus* **(Santas et *al.,* 2010).** Moreover, **Benkeblia (2004)** observed that essential oil of three types of onion (yellow, green and, red) displayed marked antimicrobial activity against specific pathogens, including *Staphylococcus aureus, Salmonella enteritidis, Aspergillus niger, Penicillium cyclopium*, and *Fusarium oxysporum*. **Azu et *al.* (2007)** found that onion was effective against *P. aeruginosa* isolated from patients suffering from urinary tract infections indicating its potential in the management of such condition.

Epidemiological studies have demonstrated that onion can protect against the *H. pylori* infection and, therefore, to reduce the risk of gastric neoplasia, since *H. pylori* is deeply involved in stomach cancer development **(You et *al.,* 1998).** In addition to organo-sulphur compounds, it has been recently reported that certain quercetin oxidation products found in onion also present antibacterial activity against *H. pylori* **(Ramos et *al.,* 2006).**

## III.3 *Trigonella Foenum-groecum* L. (Fenugreek)

### III.3.1 History

*Trigonella Foenum-graecum* L. originates from the generic name *Trigonella*, which is said to be a combination of the Latin words Foenum graecum, which is Latin for Greek hay or grass, and Trigonella, which refers to the shape of the flower. Fenugreek appears in writing for the first time around 4000 BC. Before its therapeutic benefits were understood, fenugreek was first utilized as a fodder crop in ancient Egypt and Greece. The fresh leaves or sprouted seeds were used as a vegetable in Dynastic Egypt, and the seeds were used to prepare a tonic by first roasting them before boiling them. It was also a key component of kuphi, an

incenseused to embalm the dead. It was found in the tomb of King Tut (1323 BC). It has been cultivated since 1000 BC in Egypt **(Charles, 2013).** The Middle Eastern greeting "May you tread in peace on the soil where it provided new strength, and fearless spirit, and gladiators, fierce and rough, helbah grows" refers to fenugreek, also known as helbah. The Greek physician Dioscorides, who worked as an army physician for Emperor Nero, recommended in his book De Materia Medica that fenugreek powder soaked in wine be used to treat headaches and to relieve the agony of gout. As a remedy for deafness, Pliny recommended the powder/wine concoction (Historia Naturalis, AD 77) **(Charles, 2013).** Fenugreek was grown by Benedictine monks in the ninth century under the patronage of Emperor Charles. Due to its unpleasant smell, the fenugreek seed paste used to treat baldness in Europe during the Middle Ages was referred to as "Greek dung." In the 16th century, it was brought to Britain. All over the Arab world, as well as in Europe, Ethiopia, the Soviet Union, India, and China, it was grown. Fenugreek was highly appreciated by the Arabs. For both culinary and medicinal uses, it was grown in the Mediterranean region, West Asia, and India. It is a little crop in North America and Europe. It is grown as a speciality crop in Australia. During the Sung Dynasty in the eleventh century, it was brought to China. Fenugreek, according to the great English culinary writer Elizabeth David, "is to curry what malt vinegar is to English salads."**(Charles, 2013).**

### III.3.2 Origin and distribution

Fenugreek, is an ancient and annual legume crop mainly grown for multiple uses in many parts of the world. Landraces and species of *Trigonella* have been found on the continents of Asia, Europe, Africa and Australia. Fenugreek was also cultivated in parts of Europe, northern Africa, west and south Asia, North and South America and Australia **(Acharya, 2006).**

Different authors have widely divergent opinions about the probable ancestry of fenugreek. **Vavilov (1927)** has suggested that it is native to the Mediterranean region, while **De Candolle (1964)** and **Fazli and Hardman (1968)** proposed an Asian origin for the crop. **De Candolle (1964), Fazli and Hardman (1968)** and **Acharya (2006)** notice that fenugreek grows wild in Punjab and Kashmir, in the deserts of Mesopotamia and Persia, in Asia Minor and in some countries in Southern Europe such as Greece, Italy and Spain. **De Candolle (1964)** believes that the origin of fenugreek should be Asia rather than Southern Europe, because if a plant of fenugreek nature was indigenous in Southern Europe. It would be far more common and not be missing in the insular floras of Sicily, Ischia and the Balearic Isles **(Petropoulos, 2002).** Many authors maintain that the direct ancestor of

cultivated fenugreek is the wild *T. gladiata* Ste. that differs from *T. Foenum -graecum* in respect of the entire aggregate of characters, of which seed tuberculation and the small size of the pods are only the most striking. It is possible that the species T. *Foenum graecum* evolved from *T. gladiata*, which had possibly given rise to some new extinct forms of *T. Foenum-graecum* **(Petropoulos, 1973; Petropoulos, 2002; Ahmad et *al.*, 2016).**

### III.3.3 Botanical description

Fenugreek is an annual herb, with a well-developed taproot and a spreading, it has a fibrous root system, its stem is green to purple, smooth, and erect up to 140 cm high, it present a light green leaves which are alternate and pinnate, consisting of three ovate leaflets, its inflorescence is a terminal, compound umbel, their flowers are white to whitish-yellow, while the fruit is light green to yellow brown, ovoid-cylindrical, and slightly curved, with 20–30 small, smooth brownish seeds, and the pod shape also gives the name "goat's horn" to the plant **(Fig. 19)** (**Moradi kor et *al.,* 2013).**

### III.3.4 Taxonomy and classification

The exact number of species of Fenugreek has been debated. Taxonomists such as Linnaeus suggested that as many as 260 species of Fenugreek may exist, of which a total of only 18 species of *Trigonella* are currently recognized. Most species, including fenugreek, are diploids with 2n= 16 chromosomes. However, some species of *Trigonella* may contain 18, 28, 30, 32 or 44 chromosomes **(Acharya et *al.,* 2007).**

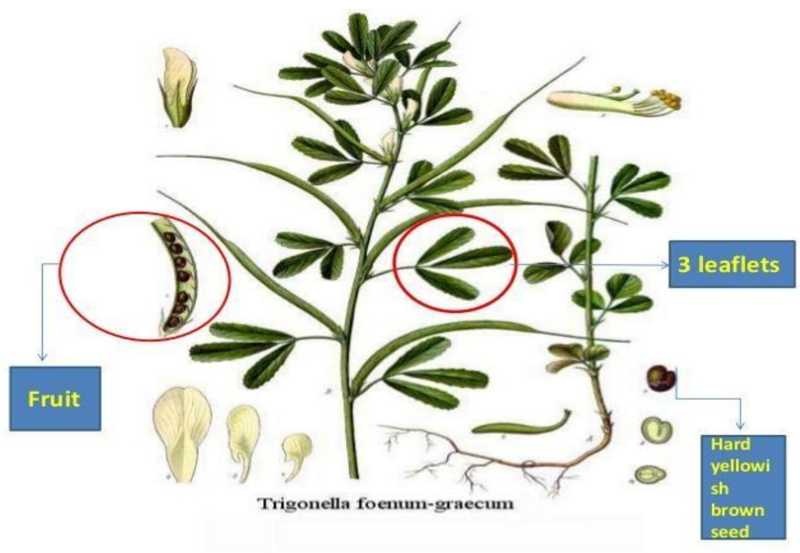



**Figure 19:***Trigonella Foenum-groecum L.* plant **(Gupta, 2006)**

The taxonomical position is presenting in the **Table 22:**

**Table 22:** Classification of *Trigonella Foenum-graecum* L. **(Nathiya et *al.,* 2014)**

| **Kingdom** | Plantae |
|---|---|
| **Super division** | Angiosperms |
| **Division** | Eudicots |
| **Class** | Rosids |
| **Order** | Fabales |
| **Family** | *Fabaceae* |
| **Subfamily** | *Faboideae* |
| **Tribe** | *Trifolieae* |
| **Genus** | *Trigonella* |
| **Species** | *Foenum* |

### III.3.5 Habitat and ecology

Although the main area cultivated with fenugreek is concentrated in some countries of Asia and Africa, however it has been distributed in many countries throughout the world under different environments. This wide distribution of its cultivation in the world is characteristic of its adaptation to variable climatic conditions and growing environments **(Tab. 23) (Petropoulos, 2002). Duke (1986)** reports that fenugreek, ranging from cool temperate steppe to wet through tropical very dry forest life zone, is reported to tolerate an annual precipitation of 3.8–15.3 dm and an annual mean temperature of 7.8 – 27.5˚C. There are indications of the possible benefit of colder nights on the sapogenin content of the seed **(Fazliand Hardman, 1968)**. Depending on the geographical source of the seed its sapogenin content, calculated as diosgenin, varied from 0.8–2.2 percent expressed on a moisture free basis **(Fazliand Hardman, 1968).** The highest sapogenin content was found in an Ethiopian sample and the lowest in a sample from Palestine **(Petropoulos, 2002).**

**Table 23**: Some ecological factors of fenugreek growth **(Mehrafarin et *al.,* 2011)**

| **Ecological factor** | **Descriptions and reports** |
|---|---|
| Climate | ❖ Temperate climate with mild winters and cool summers<br>❖ Moderate or low rainfall<br>❖ Fairly drought resistant and fairly frost sensitive<br>❖ It can tolerate 10° C of frost |
| Altitude | ❖ Up to 1300 - 1400 m<br>❖ In Ethiopia to 3000 m but its main zone of distribution in that's country is between 2150-2400 m |
| Soil | ❖ Well-drained loams and generally slightly alkaline soils are ideal.<br>❖ Lime application in some strongly acid condition may be necessary<br>❖ Heavy and wet soils limit fenugreek growth<br>❖ Optimum pH 8 – 8.5 |

| | ❖ Potash has been used to adjust soil pH to increase nutrients uptake of fenugreek |
|---|---|

### III.3.6 Varieties

Diverse fenugreek genotypes are present in the world, differing in growth habits, morphology, seed quality and crop yield. Since fenugreek is a self-pollinating plant, breeders successfully developed varieties by using breeding techniques. Some of the most important fenugreek varieties diffused throughout the world are summarized in **Table 24.**

### III.3.7 Chemical composition

Fenugreek is a unique functional food crop **(Meghwal and Goswami, 2012).** The chemical constituents of both seed and leaves have made them valuable as food and medicine, in addition to being a rich source of nutrients (macronutrients and micronutrients), it delivers various phytochemicals (non-nutritive plant chemicals that have protective or disease preventive properties) which confer the medicinal values as well as impart the popular spicy flavor. The nutritional value includes several aspects that may be grouped into two categories: organoleptic properties and nutritious contents **(Meghwal and Goswami, 2012).**

**Table 24:** Varieties of fenugreek **(Chaudhary et *al.*, 2018)**

| Fenugreek Variety | Country |
|---|---|
| CO-1, Rajendra Kanti, RMt-1, LamSel 1, Pusa Early Bunching | India |
| UM-9, UM-17, UM-18, UM-23, UM-25, UM-26, UM-27, UM-32, UM-33, UM-36, UM-50, UM-52, UM-58, UM-67, UM-70, UM-75, UM-77, UM-79, UM-83, UM-84, M-105, UM-112, UM-113, UM-114, UM-115, CVT UM-5, CVT UM-17, CVT UM-32, CVT UM-34, CVT UM-35, CVT UM TC 2336, CVT TG 1084, CVT GF 1, CVT CC, CVT NLM, NLM, CO 1, Local check, CT Lam Sel 1 | India |
| RG-07, TG-3, TG-13, TG-18, TG-24, TG-34, UM-5, UM-6, UM-17, UM-20, UM-34, UM-35, UM-38, NI-01, MP-14, IC-99, LamSel 1, Local Bobes, Pusa Earlier, Bangalore-Local | India |
| T-8 | India |
| HM-46 | India |
| IC-74 | India |
| Gharbin-6 | Egypt |
| Ali Lunghe, Ali Corte | Italy |
| Ionia | Greece |
| Gouta | France |
| Fluorescent, Ethiopian | England |
| Barbara, Margaret, Paul | England |
| Fluorescent, Ethiopian, Kenyan, Moroccan | England |

#### III.3.7.1 Physicochemical

**Naidu (2011)** reported that the proximate composition of fenugreek seeds, husk and cotyledons had protein content, fibers, comprising insoluble fibers and soluble fibers. In terms of protein contents, fenugreek has a chemical composition that mimics milk in its ratio between protein and amino acids contents **(Acharya et *al.,* 2004).** Fenugreek endosperm is rich in protein such as globulin, histidine, albumin and lecithin **(Naidu et *al.,* 2011).**

The biochemical benefits of fibers present in fenugreek include: binding to toxins in the food and helps to protect the colon mucus membrane from cancer causing toxins; facilitating insulin secretion as a result of 4-hydroxyisoleucine; helping to lower rate of glucose absorption in the intestines controlling blood sugar levels and water retention capacity for easy bowel movement. Non-starchy polysaccharides increase the bulk of the food and rise movements. Also, non-starchy polysaccharides assist in smooth digestion whereas high fibers of fenugreek helps in relieving constipation ailments **(Naidu et *al.,* 2011).** Fenugreek contains saponins, hemicelluloses, mucilage, tannins and pectin and these compounds help to decrease the level of LDL in blood by inhibiting bile salts re-absorption in the colon. Also, fenugreek husk is a valuable source of dietary fibers and phenolic acids; therefore, it could be an effective source of natural antioxidants and natural ingredients in functional foods **(Naidu et *al.,* 2011).**

Fenugreek is no exception from other vegetable in high contents of vitamins and minerals; it is relatively low in mineral content when compared with the vitamin content. However, it has some of them in good amounts such as phosphorus and sulphur **(Hegazy and Ibrahim, 2009).** High occurrence of calcium, iron and zinc has also been reported in curry made from fenugreek **(Jani et *al.,* 2009).** With reference to micronutrient content, fenugreek is especially rich in choline. Both the seed and leaves contain high levels of vitamin C, nicotinic acid and riboflavin, while the seed is rich in thiamin and folic acid but the leaves contain little of folic acid, β- carotene, a precursor of vitamin A is present in the leaves at relatively high amount (**Tab. 25) (Sharma, 1986).**

#### III.3.7.2 Phytochemical

The main chemical components of fenugreek are total polyphenols, flavonoids, saponin, fixed oils, and some identified alkaloids **(Gupta, 2006**).

**Stem**: Fenugreek contains a number of steroidal sapogenins, also diosgenin was found in the oily embryo and two furastanol glycosides, F-ring opened precursors of diosgenin have been reported, as also hederagenin glycosides. The alkaloid trigonelline, trigocoumarin, trimethylcoumarin, nicotinic acid and about 28% mucilage; 5% of a stronger-smelling, bitter

fixed oil, 22% proteins; a volatile oil; two alkaloids (Trigonelline and choline) are present in stem **(Yadav et *al.,* 2019).**

**Table 25:** Nutrient composition of fenugreek seed
**(Żuk-Gołaszewska and Wierzbowska , 2017)**

| Nutrient | Units | Value per100g |
|---|---|---|
| Water | g | 8.84 |
| Energy | Kcal | 323 |
| Proteins | g | 23.00 |
| Total lipids (fats) | g | 6.41 |
| Carbohydrates | g | 58.35 |
| Total fibers dietary | g | 24.6 |
| Calcium | mg | 176 |
| Vitamin C | mg | 3.0 |
| Vitamin B6 | mg | 0.600 |
| Vitamin B12 | mcg | 0.00 |
| Vitamin A | mg/RAE | 3 |
| Vitamin A | IU | 60 |
| Vitamin D | IU | 0 |
| Total saturated fatty acids | g | 1.460 |

**Leaves**: The leaves contain 7 saponins, known as graecunins. These compounds are glycosides of diosgenin **(Yadav et *al.,* 2019).**

**Seeds:** Fenugreek Seeds are aromatic, bitter, carminative, galactogouge, antibacterial and maybe eaten raw or cooked. Bulk. The chemical components of fenugreek seeds include alkaloids; flavonoids; free amino acids (4-hydroxyisoleucine, arginine, lysine, histidine); saponins; glycosides; mucilage, bitter fixed oil, volatile oils. Bitterness is mainly due to the oil, steroidal saponins and alkaloids **(Helambe et *al.,* 2011).**

Seeds contain 0.1% to 0.9% diosgenin and are extracted on a commercial basis. Plant tissue cultures from seeds grown under optimal conditions have been found to produce as much as 2% diosgenin with smaller amounts of gitongenin and trigogenin. The seeds also contain the saponin fenugrin B. Several coumarin compounds have been identified in fenugreek seeds as well as a number of alkaloids (eg. trigonelline, gentianine, carpaine). A large proportion of the trigonelline is degraded to nicotinic acid and related pyridines during roasting. These degradation products are, in part, responsible for the flavor of the seed. The seeds also yield as much as 8% of a fixed, foul-smelling oil. Three minor steroidal sapogenins also have been found in the seeds: smilagenin, sarsapogenin, and yuccagenin **(Tab. 26) (Helambe et *al.,* 2011).**

Polyphenol compounds rhaponticin and isovitexin were noted as major bioactive compounds in seeds of fenugreek. In addition, fenugreek seed extracts have a number of phenolic constituents similar to beta-D-glucopyranoside, methyl, alpha-d-mannopyranoside, methyl, and diethyl phthalate. Fenugreek also represents a significant source of antioxidants **(Naidu et *al.,* 2011).**The main flavonoids identified in this plant include glycoside, orientin, isoorientin, vitexin, epigenin, and quercetin **(Mirzaei Hari and Venkatesh, 2012).** Trigonelline is the alkaloid of this plant that up to 36% concentration of it has been extracted. Other alkaloids of the seed include gentanin and carpaine choline **(Salehi Surmaghi, 2008).**

**Table 26:** Major phytochemical constituents of fenugreek **(Patil and Jain, 2014)**

| Polyphenolic Compounds | Structure |
|---|---|
| Vitexin | |
| Quercetin | |
| Caffeic acid | |
| Coumarin | |
| Scopoletin | |
| Chlorogenic acid | |
| Vicenin-2 | |

### III.3.8 Pharmacological properties

Fenugreek has a beneficial effect in cleansing the blood and as a diaphoretic. It is able to perspire and help eliminate toxins from the body, because of the pungent smell of fenugreek, which smells great on the skin and sweat under the arm. Fenugreek is also known for its lymphatic cleansing activity, although its vital role is to irrigate cells with nutrients and remove toxic waste, dead cells, and retained proteins from the body **(Al-Habori and Raman, 2002).** On the other hand, blockages in the lymphatic system can mean poor fluid circulation, fluid retention, pain, and loss of energy and disease anywhere in a person's body as it

maintains the condition of mucus in the body, especially the lungs, by helping to get rid of congestion **(Wani and Kumar, 2018).**

**Table 27**: Pharmacological activities of fenugreek **(Chaudhary et *al*. 2018)**

| Pharmacological Activities | Mechanism of Action |
|---|---|
| Antidiabetic | ❖ Decrease of glycaemia, improvement of symptoms in type-2 diabetes patients<br>❖ Diabetes control in type 1 diabetes patients<br>❖ Increase of insulin secretion<br>❖ Increased number of insulin receptors<br>❖ Increase of insulin secretion in animals |
| Antioxidant | ❖ Fenugreek extract has been shown to restore the altered activity of cellular antioxidant enzymes including superoxide dismutase (SOD), glutathione reductase (GR), catalase and glutathione peroxidase (GPx) in tissue such as heart, muscle and brain during diabetes<br>❖ Administration of fenugreek seed-derived soluble dietary fibers (SDF) to type-2 diabetic rats for 28 days enhanced total antioxidant status besides decreasing serum glucose |
| Antibacterial | ❖ Fenugreek extract show an antibacterial effect against *E. coli* (MIC = 125 µg/ml) , *Bacillus* (MIC = 250 µg/ml) and *S. aureus* (MIC = 125 µg/ml) *Pseudomonas syringe* DZI = 3–5 mm<br>❖ Also fenugreek was effective against *E. coli* (DZI = 8.5 mm) , *P. vulgaris* (DZI = 6.7 mm), *P. auriginosa* (DZI = 11 mm), *S. aureus* (DZI = 15.3 mm), *P. vulgaris* (DZI = 0.2 cm), *E. aerogens* (DZI = 0.2 cm) , *B. cereus* (DZI = 0.2 cm), *E. coli* (DZI = 0.3 cm) and *Klebsiella sp*. (DZI = 0.2 cm)<br>❖ Aqeoues extract of fenugreek exhibit an antibacterial activity against *S. aureus* (DZI = 9 mm), *K. pneumonia* (DZI = 10 mm), *B. subtilis* (DZI = 11 mm), *E. coli* (DZI = 5 mm) and *S. typhi* (DZI = 8 mm) |
| Antifungal | ❖ All parts of the fenugreek plant showed antifungal potential against fungal strains including *Botrytis cinerea, Fusarium graminearum, Alternaria sp., Pythium aphanidermatum* and *Rhizoctonia solani* and the magnitude of effect depends upon fungal species and plant parts |
| Anti-inflammatory | ❖ Extract of fenugreek seed inhibited the production of phorbol-12 myristate-13-acetate-induced inflammatory cytokines such as tumor necrosis factor (TNF)- a in cultured THP-1 cells<br>❖ Fenugreek extract of decreased paw edema and decreased levels of IL-1a, IL-1b, IL-2, IL-6 and TNF-a.<br>❖ The extract also decreased the levels of LPO and increased the SOD and GSH levels in cartilage tissue |
| Anti-cancer | ❖ Fenugreek-derived compound protodioscin displayed a growth inhibitory effect against HL- 60 cells by inducing apoptotic changes fenugreek seed extract inhibited 7,12-dimethylbenz(a) anthracene-induced mammary hyperplasia and decreased its incidence in rats<br>❖ Fenugreek extracts showed *in vitro* cytotoxicity against different human cancer cell lines such as IMR-32, a neuroblastoma cell line, and HT29, a cancer cell line |

Fenugreek also acts as a throat cleanser and mucus thinner that also reduces the urge to cough. In addition drinking the water in which fenugreek seeds have been soaked helps in softening, dissolving, and the build up and hardening of clumps of cellular debris. Moreover fenugreek has been used to relieve colds, bronchitis, influenza, asthma, catarrh, constipation, sinusitis, pneumonia, sore throat, laryngitis, hay fever, tuberculosis and emphysema **(Al-Habori and Raman, 2002).**

### III.3.8.1 Antioxidant

**Bukhari et *al.* (2008)** reported that fenugreek seed extract with methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate has a radical scavenging activity. **Bhatia et *al.* (2006)** showed a protective effect of fenugreek, on lipid peroxidation and on enzymatic antioxidants. **Naidu et *al.* (2011)** described that at a concentration of 200 mg, fenugreek seed, extracts of husk and endosperm exhibited 72%, 64%, and 56% antioxidant activities respectively by free-radical scavenging activity. From this study, it was indicated that separation of fenugreek seeds into husk and endosperm could have advantage of process viability with respect to prior selective fractionation of bioactive components for their effective isolation. **Laroubi et *al.* (2007)** studied the prophylaxis effect of fenugreek seeds on renal stone formation in rats. They found that fenugreek can be used in the treatment of patients with calcic urolithiasis.

In a study, it was observed that antioxidant property was checked by reducing power, NBT (nitroblue tetrazolium)assay and $H_2O_2$ scavenging. Fenugreek showed a high reducing power and a highest superoxide and free radical scavenging comparing to other plant extracts **(Joglekar et *al.,* 2012).**

### III.3.8.2 Antimicrobial

The antimicrobial role of fenugreek is recently being shown **Haouala et *al.* (2008)**, determine the action of an aqueous extracts from various plant parts of fenugreek in various solvents including methanol, petroleum ether and ethyl-acetate fractions of the aerial parts against fungal strains such as *Fusarium graminearum*, *Botrytis cinerea*, *Alternaria sp., Rhizoctonia solani* and *Pythium aphanidermatum*, it was found that all parts of the fenugreek plant showed antifungal potential and the magnitude of effect varies with plant parts and species of fungus. It could be suggested that fenugreek is an important source of biologically active compounds useful for developing better and novel antifungal drugs **(Haouala et *al.,* 2008).** In a study, honey samples with highest antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* show maximum pollens from

fenugreek than other plants (**Mercan et *al.,* 2007).** Cysteine-rich peptides, defensins are small with potent antifungal activity. The methanol soluble fraction of fenugreek extract showed nematicidal activity and caused significant mortality of *Meloidogyne javanica* larvae, indicating the potential use against nematodes **(Zia et *al.,* 2001).**

Also germination or sprouting of fenugreek seeds increased their antioxidant profile and antimicrobial activity against *H-pylori* (**Branch, 2013**). Various investigators have also showed effectiveness of fenugreek extracts against *H. pylori* (**Randhir et *al.,* 2004; O'Mahony et *al.,* 2005). Randhir and Shetty (2007)** observed that fenugreek seeds naturally had *H. pylori* inhibition and hence investigated if the inhibition improved with fenugreek .

## III.4 *Cuminum cyminum* L. (Cumin)

### III.4.1 History

Another spice that has a long history of use is cumin. The Greek word kuminon, which is likely descended from the Babylonian kamuna, is where the genus Cuminum gets its name **(Charles, 2013).** Its fruits were employed as seasoning by prehistoric Mesopotamian civilizations in the Euphrates and Tigris valleys, and according to the Ebers Papyrus, cumin was utilized as medicinal in predynastic Egypt approximately 1550 BC. Circa 2000 BC, the Mycenaeans employed cumin to flavor food, and around 1323 BC, the Egyptians used it to embalm royal bodies, including King Tut's. Egyptian rulers' bodies were preserved by being mummified with cumin, anise, and marjoram as early as 5000 BC. Later, the kings were treated with cinnamon and cassia **(Charles, 2013).** It was once connected to cupidity by the ancient Greeks. Marcus Aurelius, the Roman emperor from AD 121 to AD 180, earned the moniker "Cumin" for his avarice. Cumin paste can reportedly whiten the skin, and scholars used it to make their faces appear pale to fool their teachers into thinking they were spending long hours in study in AD 77, according to Pliny, who wrote about it in his Historia Naturalis. But according to Theophrastus, if you want a fair and plentiful harvest, cumin must be cursed and mistreated while being sown **(Charles, 2013).** Ethiopia and the Nile Valley both saw the spread of cumin.In addition, it has long been a favorite spice in India, where it is listed in Ayurvedic writings for both culinary and medicinal uses. Romans introduced it to the western globe, including Britain, while the Spanish eventually brought it to North America. Cumin was a very important spice in Europe, and both the English and the Romans used it to pay taxes. In the Middle Ages, superstition claimed that cumin prevented birds and lovers from

wandering. The bride and groom were said to have a happy life ahead of them if they carried cumin seeds throughout the wedding ritual **(Charles, 2013).**

### III.4.2 Origin and distribution

Cumin is cultivated and grown in many countries including Malta, India, Sicily, Iran, Saudi Arabia, Mexico, and China. The crop is quite easy to grow and adapts well in many climates **(Azeez, 2008).** The crop was initiated to cultivate in Iran and the Mediterranean region. The use of cumin bring up to the second millennium BC as indicated by the Syrian site Tell ed-Der. Spanish and Portuguese colonists were commenced the crop to start in Americas. The cultivation of cumin also spread in Southern England with turned down the frequency of its occurrence **(Chattopadhyay and Maiti 1990).** This spice has became popular in the middle ages, probably due to the renewed interest in ethnic dishes and spicy foods **(Thamaraikannan and Sengottuvel, 2012 ).**

The plant has pantropical distribution with total world production of approximately 300,000 tons. Though it is indigenous to Egypt, the Mediterranean zone, and South Asian countries **(Ebada, 2017; Tabarsa et *al.,* 2020)** but now-a-days it is also grown in India, Pakistan, Uzbekistan, Tajikistan, Iran, Turkey, Morocco, Iraq, Libya, Palestine, Syria, Bulgaria, Cyprus, Chile, Mexico, and China **(Siow and Gan, 2016; Srinivasan, 2018).** India is leading grower and user of *C. cyminum*. In India, Rajasthan (56%) and Gujarat (44%) are the leading cumin producing states **(Al-Snafi, 2016; Agarwal et *al.,* 2017; Belal et *al.,* 2017).**

### III.4.3 Botanical description

Cumin is an aromatic herb of the *Apicaceae* family, and its dried seeds are used as a spice. In India, it is commonly known as cumin orzeera, and is called kummel, comino, zireche sabz, cumino, kemon, zira, and kamun in various other parts of the world. This plant is native to India, Iran, the Mediterranean, and Egypt **(Peter, 2003),** it is a small, slender, or erect glabrous annual herb up to 0.6 m high, withlight brown taproot. its leaves seem to be finely dissected and are alternate, compound of bluish-green hue. while its flowers are bisexual with colors like pink and red growing on the in florescence compound umbel up to 3.5 mm in diameter. concerning the fruit is sometimes brownish or yellow, ovoid-oblong shaped with slightly curved schizocarp. Regarding the seeds are approximately 2–3 mm long and 2 mm thick with a light brown and a yellow hue. They have slight ridge-like lines overlapping as many oil channels **(Fig. 20) (Charles, 2013).**

### III.4.4 Taxonomy and classification

The scientific name of *Cuminum cyminum* L. (cumin) referred to as *Cuminum odorum* Salisb, *Cuminia cyminum* J.F. Gmel, *Cuminum hispanicum* Bunge, *Ligusticum cuminum* (L.) Crantz and belonging to the *Apiaceae* family. The *Apiaceae* family is a collection of typically aromatic plants having hollow stems and the well known members of this family are anise, asafoetida, caraway, carrot, celery, coriander, cumin, dill, fennel, parsley, parsnip, and sea holly **(Gangadharappa, 2017).**

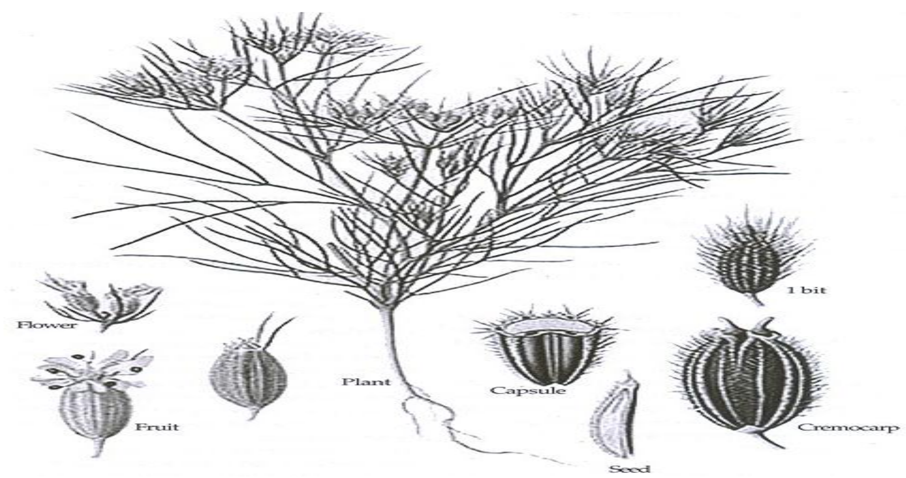



**Figure 20:** *Cuminum cyminum* L. plant **(Krishnamurthy, 2013)**

The taxonomical classification of *Cuminum cyminum* L. is presented in the following table:

**Table 28:** Classification of *Cuminum cyminum* **(Agarwal et *al.,* 2017)**

| **Kingdom** | Plantae |
|---|---|
| **Subkingdom** | Viridiplantae |
| **Infrakingdom** | Streptophyta |
| **Superdivision** | Embryophyta |
| **Division** | Tracheophyta |
| **Subdivision** | Spermatophytina |
| **Class** | Magnoliopsida |
| **Superorder** | Asteranae |
| **Order** | Apiales |
| **Family** | *Apiaceae* |
| **Genus** | *Cuminum* |
| **Species** | *Cuminum cyminum* |

### III.4.5 Habitat and ecology

` Cumin needs dry and cool climate for its growth, with a temperature ranging between 25 to 30°C. It grows excellent on sandy loam to loamy soil with a pH 6.8-8.3 range. The seeds are sown in spring at the time April-May and sowing is performed between middle November and in December, they are transplanted. Generally, irrigation is not required for the crop

grown in black cotton soils, but for light soils, 3-4 irrigations are given; first irrigation is carry out at 2-leaves stage (20-30 days after sowing); the second has to carry out at branching or flowering stage (60-70 days) and the third is performed at seed-filling stage (80-110 days). At the time of flowering beginning, sufficient moisture should be present in the soil **(Ebrahimie et *al.,* 2003).**

### III.4.6 Varieties

There are distinct varieties of cumin seeds that vary in their appearance and taste; Cumin ( *Cuminum cyminum* ), Black Cumin ( *Nigella /* kalonji ) and Bitter Cumin ( Kashmiri Jeera / Shahi jeera) (**Tab. 29**) **(Krishnapura, 2017)**

**Table 29:** Varieties of cumin **(Krishnapura, 2017)**

| Varieties | Characteristics | Photos |
|---|---|---|
| **Cumin (*Cuminum cyminum* )** | **Scientific name:** *Cuminum cyminum*<br>**Genus/family:** *Cuminum* /Apiaceae<br>(a member of Parsley family)<br>**Native of countries growing:** East Mediterranean to South Asia. Now mostly grown in Pakistan, India, Uzbekistan Iran, Turkey, Morocco, Egypt, Syria, Chile, Mexico, and China | |
| **Black Cumin (*Nigella*/kalonji)** | **Scientific name:** *Nigella sativa*<br>**Genus/family:** *Nigella /* Ranunculaceae<br>**Native of countries growing:** South to Southwest Asia. Middle Eastern Mediterranean region, South Europe, Northern India, Pakistan, Syria, Turkey, Iran, and Saudi Arabia | |
| **Bitter Cumin (Kashmiri Jeera / Shahi jeera )** | **Scientific name:** *Cuminum nigrum or Bunium persicum*<br>**Genus/family:** *Cuminum* /Apiaceae<br>**Native of countries growing:** Central Asia to Northern India; Mountainous regions of North India | |

### III.4.7 Chemical composition

#### III.4.7.1 Physicochemical

Cumin seeds present a high nutraceutical potential and are widely used as a natural health booster. They are utilized for various purposes, including vegetable, nutrition, medicine, beverages, fragrances, cosmetics, and other industrial purposes. They possess good amount of vitamins, amino acids, minerals, and so on, which render numerous health benefits. The nutritional composition of cumin seeds is depicted in **Table 30.** Cumin contains

quantitatively significant and nutritionally important composition, which not only imparts the specific characteristics like color, aroma, and taste to this plant but also contribute to the development of phytomedicine for human well being (**Agarwal et *al.,* 2017**)..

**Table 30:** Nutritional factors cumin seeds (**Agarwal et *al*., 2017**)

| Principle | Nutrient value | Principle | Nutrient value |
|---|---|---|---|
| Energy | 375 Kcal | Sodium | 1788 mg |
| Carbohydrates | 44.2 g | Potassium | 68 mg |
| Proteins | 17.8 g | Calcium | 931 mg |
| Total fats | 22.2 g | Copper | 0.8 mg |
| Cholesterol | 0 mg | Iron | 66.3 mg |
| Dietary fibers | 10.5 g | Magnesium | 366 mg |
| Vitamin B9 | 10 µg | Manganese | 3.3 mg |
| Vitamin B3 | 4.5 mg | Phosphorus | 499 mg |
| Vitamin B6 | 0.4 mg | Zinc | 4.8 mg |
| Vitamin B2 | 0.3 mg | Carotene-ß | 762 µg |
| Vitamin B1 | 0.6 mg | Crypto-xanthin-ß | 0 µg |
| Vitamin A | 1270 mg | Lutein-zeaxanthin | 448 µg |
| Vitamin C | 7.7 mg | | |
| Vitamin E | 3.3 mg | | |
| Vitamin K | 5.4 µg | | |

### III.4.7.2 Phytochemical

Cumin seeds are a rich source of alkaloids, coumarins, anthraquinones, flavonoids, glycoside, proteins, resins, saponins, tannins, steroids, dietary fibers, minerals, fats (especially monounsaturated fat), vitamins B (vitamin B1, vitamin B6, vitamin B3, vitamin B2), vitamin A, vitamin C and E, and so forth. Organic acids such as aspartic, citric, malic, tartaric, propionic, ascorbic, oxalic, maleic, and fumaric acids are also found in cumin seeds **(Belal et *al.,* 2017).** The fruits contain estrogenic isoflavonoids, luteolin, and apigenin. The seeds exhibit a good amount of flavonoids (4.15– 5.75%), in which luteolin, apigenin, and quercetin are the dominant flavonoids **(Kang et *al.,* 2019).** Cumin aldehyde is the principal bioactive compound of *C. cyminum* **(Ebada, 2017; Nirmala et *al.,* 2020).** Apart from this, it also contains cymene, cuminic alcohol (cuminol), γ-terpinene, safranal, limonene, eugenol, β-myrcene, α-Phellandrene, β-Phellandrene, α- and β-pinene **(Agarwal et *al.,* 2017).**

The strong aroma of roasted cumin seeds is due to the presence of substituted pyrazines, 2-ethoxy-3-isopropylpyrazine, 2-methoxy-3-sec-butylpyrazine, and 2-methoxy 3 methylpyrazine **(Srinivasan, 2018).** The different bioactives isolated from *C. cyminum* are presented in **Table 31**. It has also been noticed that most of the bioactivities of cumin are

dominantly cumin aldehyde dependent. Though the compound contributes majorly to various ethnomedicinal, pharmacological, and clinical research but side by side the research should also be carried out on different medicinal aspects of other bioactives as well **(Singh et *al.,* 2021).**

**Table 31:** Phytochemical composition of cumin seeds **(Li and Jiang, 2004)**

| Components | Percentage (%) |
|---|---|
| α-Pinene | 0.63 |
| Camphene | 0.01 |
| β-Pinene | 10.22 |
| Sabinene | 0.58 |
| Δ3-Carene | 0.03 |
| Myrcene | 0.83 |
| α-Phellandrene | 1.60 |
| α-Terpinene | 0.11 |
| Limonene | 0.39 |
| β-Phellandrene+1.8-cineole | 0.49 |
| ϒ-Terpinene | 0.11 |
| p-Cymene | 5.51 |
| Terpinene | 17.25 |
| Trans-Sabinenehydrate | 0.09 |
| Cis-Sabinenehydrate | 0.19 |
| Linalool | 0.04 |
| p-Mentha-3-en-7-al | 2.91 |
| β-Caryophllene | 0.45 |
| Terpinen-4-ol | 0.13 |
| (Z)-β-Farnesene | 0.60 |
| α-Terpineol | 0.05 |
| Cuminaldehyde | 27.6 |
| p-Mentha-1,3-diene-7-al | 15.18 |
| p-Mentha-1,4-diene-7-al | 9.48 |
| p-Mentha-1,3-diene-7-ol | 0.31 |
| Cuminalcohol | 0.36 |
| Total | 95.1 |

### III.4.8 Pharmacological properties

The pharmacological activities of cumin are presented on **Table 32.**

#### III.4.8.1 Antioxidant

High antioxidant activity is exhibited by the oil of *C. cyminum* which has been ascribed basically to the occurrence of polyphenolic compounds like flavonoids, linalool, anethole, monoterpene alcohols, and carvacrol **(Gohari and Saeidnia, 2011; Bansal et *al.,* 2014).** It has been reported that aqueous and methanol extracts of several plant sources have well free

radical scavenging activity as compared to the extracts of dichloromethane or ethyl acetate representing the polar nature of the antioxidant biomolecules **(Dua et *al.,* 2012).**

**Table 32:** Pharmacological activities of cumin seeds **(Agarwal et *al.*, 2017)**

| Activity | Action |
|---|---|
| Skin Disorders and boils | ❖ Vitamin-E is good for skin and keeps the skin young and gleaming |
| Antimicrobial | ❖ It inhibits mycelium grow than dtox in production |
| Antidiabetic | ❖ Reduces the blood glucose and inhibits glycosy lated haemoglobin, creatinine, blood urea nitrogen and improved serum insulin and glycogen |
| Anticancer | ❖ Make change to carcinogen metabolism via carcinogen/ xenobiotic metabolizing phase I and phase II enzymes |
| Antioxidant | ❖ Less amount of cumin extract was needed for scavenging the superoxide radicals |
| Antiosteoporotic | ❖ By reduction in urinary calcium excretion and augmentation of calcium content |
| Immunomodulator | ❖ Large quantity of iron, a presence of essential oils and vitamin-C & vitamin-A in cumin boost supour immune system |
| Ophthalmic effects | ❖ By delayed progression and maturation of streptozotocin- induced cataracts |
| Antiasthmatics | ❖ Act as a decongestant |
| Antiepileptic | ❖ Shows the activity against epilepsy induced by pentylenetetrazole (PTZ) |
| Gastrointestinal disorders | ❖ Activates, our salivary glands in our mouth (the mouth-watering flavor),facilitating the primary digestion of the food<br>❖ Antiulcer by enhancing gastric mucin protection and regeneration<br>❖ Provide protection against the patotoxicity |
| Analgesic | ❖ Shows the analgesic activity individually or in combination with *Coriandrum sativum* seed methanolic extract |
| Astringent | ❖ Inhibiting arachidonate induced platelet aggregation |
| Antitussive | ❖ Showed significant reduction of cough number |
| Antifertility | ❖ Anti-implantation effects |
| Antihypertensive | ❖ Improve plasma nitricoxide and decreased the systolic blood pressure |
| Antiinflammatory | ❖ Significantly inhibit them RNA expression sofin duciblenitric oxidesynthase (iNOS), cyclooxygenase (COX-2), interleukin- IL1 and IL-6 |
| Insecticidal effects | ❖ Shows toxicity against *Anopheles gambiae* strain |

The significant amounts of antioxidant compounds of *C. cyminum* oil showed a high antioxidant activity, however, the non volatile extracts also shown a good inhibition properties against the free radicals. It is found that there is better antioxidant action in methanol extracts as compared to the n-hexane extracts. On the other hand antioxidant activities and the total phenolic content also found a good correlation among their non volatile extracts. So, it concludes that there is good antioxidant potential in *C. cyminum.* Finally, This

spice can be utilized to yield flavoring agents as well as novel natural antioxidants that can be used in several food products **(Nadeem and Riaz, 2012).** The oil yield of *C. cyminum* is 2.5 to 4% of the weight of the fruits and the main constituent of essential oil is cuminol **(Sepehri et *al.,* 2014).** Higher antioxidant power is shown by *C. cyminum* and this property made it a virtuous source of natural antioxidant **(Ghasemi et *al.,* 2019).** The methanolic extract of this spice revealed a higher antioxidant activities than the essential oil **(Einafshar et *al.,* 2012).**

### III.4.8.2 Antimicrobial

Cumin alcoholic distillate inhibit the development of *Klebsiella pneumonia* and its therapeutic isolates by capsule expression, improvement of cell morphology, and decreasing urease activity. The main active compound of *C. cyminum* is cumin aldehyde for this property **(Verma, 2016).** The strong antibacterial and larvicidal activity are exhibited by this compound; while *C. cyminum* seed essential oils show an antimicrobial activity against *Escherichia coli, Mycobacterium tuberculosis,* and *Candida albicans* **(Pathak et *al.,* 2011).** Some other minor constituents have been found in *C. cyminum* oil with limonene, α- pinenes, β- pinenes, and cuminol suggested as the active antimicrobial agents and show antimicrobial activity **(Gohari and Saeidnia, 2011).** The anti bacterial property was assessed contradiction of a variety of pathogenic Gram (+) and Gram (-) bacterial strains, against *Streptococcus pyogenes* and *Streptococcus mutants*, there were found biofilm formation defensive properties **(Bansal et *al.,* 2014).** Cumin showed prominent anti-*H. pylori* effects in several studies **(Nakhaei et *al.,* 2006; Nostro et *al.,* 2005).** *In vitro* study showed anti-*H. pylori* effects of cumin at MIC of 691 µg/ml. **Nostro *et al.,* (2005)** and **Nakhaei et *al.,*(2006)** showed that ethanolic extract of the cumin fruit eradicated 90% of the *H. pylori* at 0.075 mg/ml. **O'Mahony *et al* .(2005)** showed that 50 mg/ml of the cumin decoction eradicated 100% *H. pylori* in culture medium **(Alkofahi and Atta 1999).**

# Part II:
# Experimental Work

# Chapter I: Material and methods

# I . Material and methods

## I.1 Objectives, site and duration of study

### I.1.1 Objectives of study

The present study aimed to:

- ✓ Determine the physico-chemical characteristics of four medicinal plants (garlic, onion, fenugreek and cumin).
- ✓ Extract the phenolic compounds from the studied plants.
- ✓ Determine the phytochemical profile (quantitatively and qualitatively).
- ✓ Characterize and identify the phenolic compounds by HPLC method.
- ✓ Estimate the antioxidant properties by measuring radical scavenging activity using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay.
- ✓ Isolate and identify *H. pylori* strains from human gastric biopsy.
- ✓ Evaluate the effect of extract plants and probiotics against *H. pylori.*
- ✓ Evaluate the potential of combination between extract plants and probiotics against *H. pylori.*
- ✓ Highlight the *in vivo* antibacterial effect of fenugreek extract and *Bifidobacterium breve* on *H. pylori* colonization using Wistar rats as an animal model.

### I.1.2 Site and duration of study

This experimental study was carried out during the period from February 2016 to June 2022 at:

- ✓ Biochemistry and Food Technology laboratory and Microbiology laboratory, Faculty of Nature and Life Sciences, University of Tiaret, Algeria.
- ✓ Advanced Technology Application and Research Center, Faculty of Engineering and Architecture, Kilis 7 Aralik University, Kilis, Turkey.
- ✓ Faculty of Pharmacy "Iuliu Hațieganu", University of Medicine and Pharmacy- Cluj-Napoca, Romania.

## I.2 Material

### I.2.1 Plant material

The plant materials used in the present study were garlic (*Allium sativum L.*"var. Cristo") and onion (*Allium cepa L.*"var. Rouge Amposta") bulbs, fenugreek (*Trigonella Foenum-graecum L.*) and cumin seeds (*Cuminum cyminum L.*) **(Fig. 21).** All these plants were identified by Dr. Miara Mohamed Djamel and Dr. Ait Hammou Mohamed botanists at Nature and Life Sciences Faculty, University of Tiaret.

*Allium sativum L.*"var. Cristo" *Allium cepa L.*"var. Rouge Amposta"

*Trigonella Foenum-graecum L.* *Cuminum cyminum L.*

**Figure 21:** Plant materials used in the present study

Garlic and onion were cultivated in organic conditions in the region of Tiaret located on the south-west of Algeria. They were harvesting during the period of August 2017, and their bulbs were separated by hand picking **(Annexe 1).**

Fenugreek seeds (*Trigonella Foenum-graecum L.*) were originated from the region of Mostaganem, Algeria, and cumin seeds (*Cuminum cyminum L.*) were grown in the region of Aleppo, Syria **(Annexe 1).**

Samples of fenugreek and cumin were taken from a local market in Tiaret (Algeria) in March 2017 in order to avoid the dust, the loss of aroma and colour which could occur as a result of their exposure to the direct sun light.

### I.2.2 Probiotic strains

Twelve probiotic strains were used in this study as shown in **Table 33**; six (06) of them were obtained from the Laboratory of Sciences and Technics of Animal Production, Faculty of Agriculture- Abdelhamid Ibn Badis University, Mostaganem- Algeria; one (01) strain was obtained from the Laboratory of Natural and Local Bioresources, University of Hassiba Benbouali, Chlef-Algeria; the remaining strains were purchased as commercial products (France). The probiotic strains were revivied and confirmed using Gram stain, catalase, oxydase and some biochemical tests.

**Table 33:** Source and origin of probiotic strains used in the present study

| Probiotic strains | Source | Origin |
| --- | --- | --- |
| *Lactobacillus acidophilus* | Local dairy product | Laboratory of Sciences and Technics of Animal Production, Mostaganem-Algeria |
| *Lactobacillus fermentum* | Local dairy product | |
| *Lactobacillus plantarum* | Local dairy product | |
| *Lactobacillus casei* | Local dairy product | |
| *Lactococcus lactis* | Local dairy product | |
| *Streptococcus thermophilus* | Local dairy product | |
| *Lactobacillus rhamnosus LA180* | LACTIBIANE | Pharmacy, Lille, France **(Annexe 2)** |
| *Lactobacillus rhamnosus GG* | PROBIOLOG | |
| *Lactobacillus helviticus* | LAXATRANSIT | |
| *Bifidobacterium longum* | BENEFLORA | |
| *Bifidobacterium bifidum* | BENEFLORA | |
| *Bifidobacterium breve* | Local dairy product | Laboratory of Natural and Local Bioresources, University of Hassiba Benbouali, Chlef-Algeria |

### I.2.3 *Helicobacter pylori* strains

Ten fresh gastric biopsy specimens obtained from patients who underwent upper gastrointestinal endoscopy or from symptomatic patients with a positive rapid urease assay at Tiaret Hospital (Youcef Damardji), Algeria were transferred to sterile physiological saline, transported to the laboratory and processed within 4 h after sampling. Biopsy specimens were minced and homogenized in Brain Heart Infusion Broth (0.5 ml) then plated immediately on plates containing Colombia agar supplemented with 5% horse blood. The plates were incubated at 37°C under microaerophilic conditions (15% $CO_2$) using Campy Gas-Pak system for a maximum of 10 days **(Fig. 22).** Suspected isolates were identified as *H. pylori* by conventional methods using Gram stain, urease, catalase and oxydase tests, API Campy (Bio Merieux. Cedex.France) and Polymerase Chain Reaction *(*PCR).

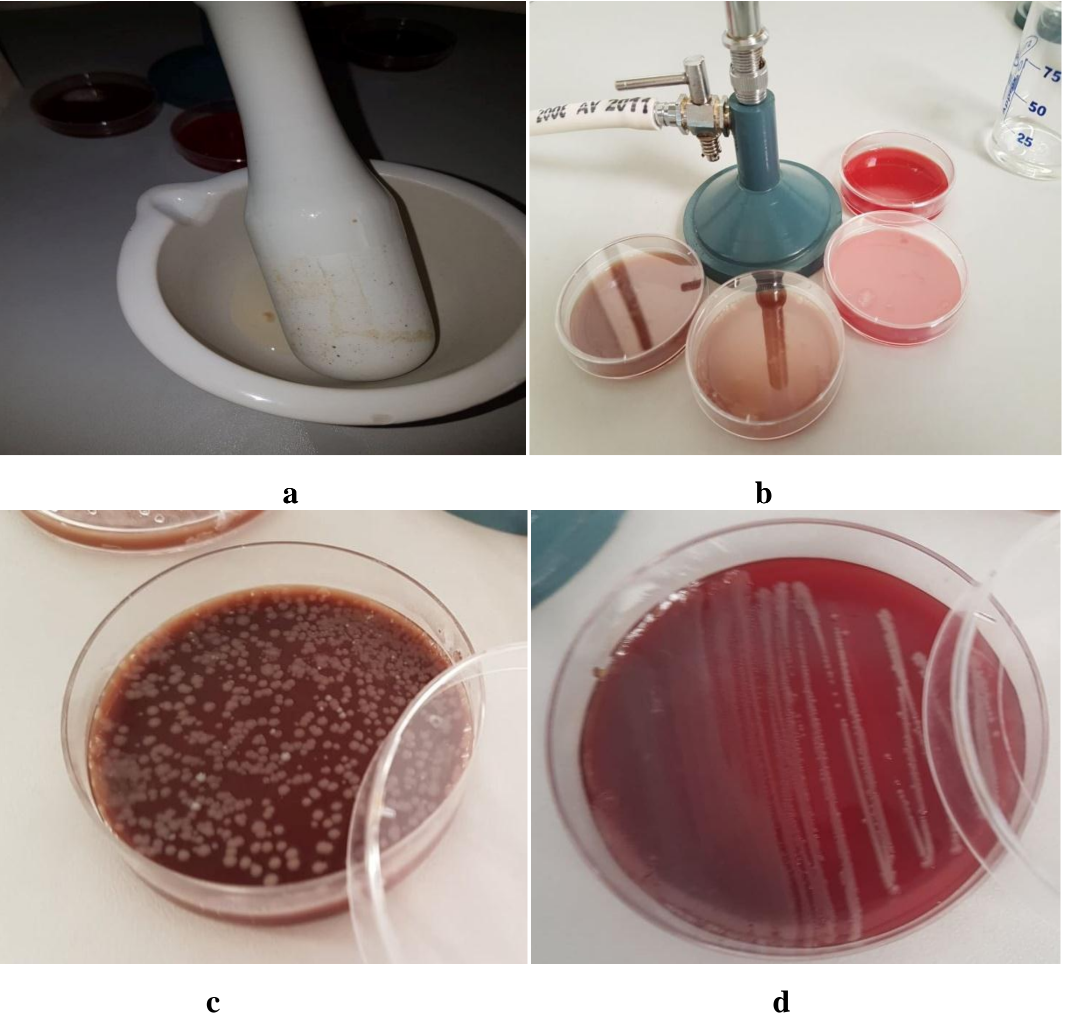



**Figure 22:** Isolation steps of *H. pylori* strains

**a:** Mincing and homogenizing of biopsy; **b**: Plating on colombia agar supplemented with 5% horse blood; **c:** Subculturing and purification; **d**: Subculture of *H. pylori*

### I.2.4 Animals

Sixty healthy male Wistar rats (200 ± 3.81 g) obtained from the Pasteur Institute of Algiers (Algeria) were used to evaluate the combined effect of plant extract and probiotic bacteria against *H. pylori*. The animals were kept in individual cages under appropriate environmental conditions (temperature 22 ± 1°C, 12/12 hours light-dark cycle and relative humidity of 60 ± 10%) for two weeks adaptation period at the Veterinary Sciences Institute, University of Tiaret. They were housed according to relevant Algerian national legislation, were fed a commercial diet and were given water ad libitum, except otherwise stated. Throughout the experiments, all animals received special veterinary care according to the criteria outlined in the internationally accepted principal guidelines of the European Union on Animal Care (CEE Council 86/609, Directive 63/2010 on the protection of animals used for scientific purposes) and under veterinarian supervision and follow-up **(Fig. 23).**

**Figure 23:** Acclimatisation period of animals used in the present study

## I.3 Methods

### I.3.1 Experimental protocol

The experimental protocol summarizing the steps followed in our study is shown in **figure 24.**

### I.3.2 Selection of varieties of fenugreek and cumin

Different varieties of fenugreek (*Trigonella Foenum-graecum L.*) and cumin (*Cuminum cyminum L.*) from Algeria, Egypt, India, Morocco and Syria were purchased from a local market and analyzed for their weight of 1000 seeds and germination rate in order to select the best ones.

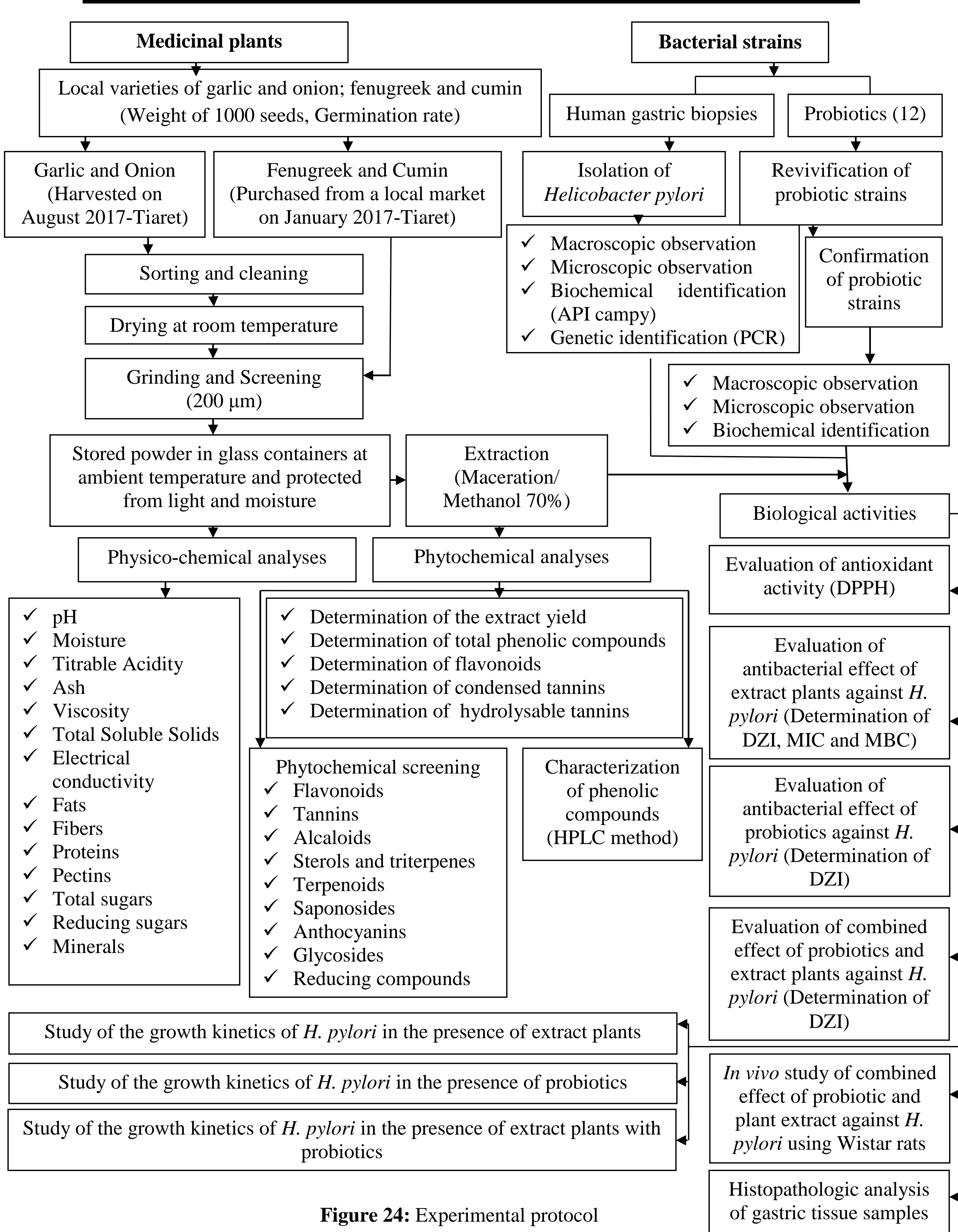



**Figure 24:** Experimental protocol

#### I.3.2.1 Weight of 1000 seeds

The number of seeds taken into by hand count on 100 and 1000 seed weight was measured in (g/mg) and used to estimate the seed rate based on fixed number of seeds and test weight **(Deivasigamani and Sawaminathan, 2018)**

#### I.3.2.2 Germination rate

Seeds were treated with dry heat at 50 °C for 4 days to eliminate residual dormancy that might interfere with germination rate. Two sets of 25 seeds for each cultivar [one from each replication] were placed on Whatman no. 1 filter paper inside a 9 cm Petri dish. The filter paper was moistened with 2.5 ml of distilled water, and the seeds were germinated in the dark at 25 °C and >97% relative humidity (RH) inside a germinator. Seeds showing 2 mm of radicle growth or more were considered germinated. Germination rate was calculated using the following formula and designated as RG index **(Krishnasamy and Seshu, 1989):**

$$\text{Rate of germination } (\mathrm{R}G) = \frac{\text{no. of seeds germinated at 48 h}}{\text{no. of seeds germinated at 168 h}} \times 100$$

### I.3.3 Sample preparation

Algerian variety of fenugreek and Syrian cumin seeds were the best ones, as compared to other varieties with a highest weight and a better germination rate. In this concept, these varieties were chosen for present study. The collected samples of garlic and onion were sorted, peeled, washed and sliced into chips and dried at room temperature, then all plant samples were powdered and screened at 200 µm **(Fig. 25).**

All the steps of preparation of plants powder are presented in **Annexe 3.**

### I.3.4 Physico-chemical analyses

Physico-chemical analyses including pH, moisture, titrable acidity, ash, viscosity, total soluble solids, electrical conductivity, fats, fibers, proteins, pectins, total and reducing sugars and minerals were carried out by the following methods:

✓ **pH**

100 ml of distilled water was added to 10 g of each fresh sample, after 5 min of shaking the pH electrode was immersing in the solution **(AOAC, 2002).**

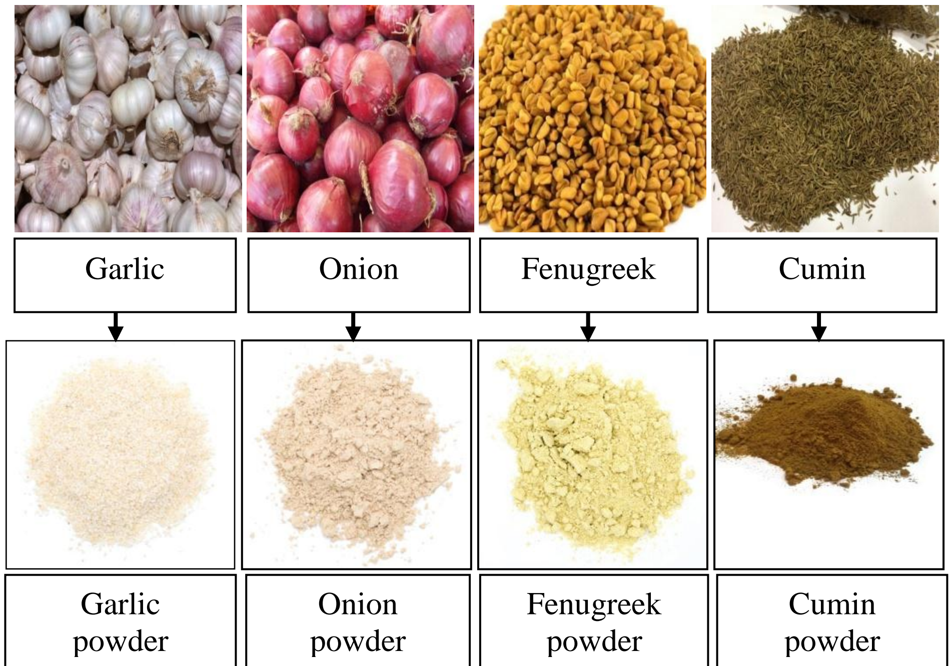



**Figure 25:** Sample plants preparation

✓ **Moisture**

10 g sample was dried in hot air oven at a temperature of 105°C ± 5 until weight was constant **(AOAC, 2000).**

The moisture calculation is made according to the following formula:

$$\mathbf{MC\ (\%) = [W_1\text{-}W_2/W_0] \times 100}$$

M: Moisture content (%);

W0: Weight of the test portion (g);

W1: Crucible weight plus sample before stoving (g);

W2: Weight of crucible plus sample after stoving (g).

✓ **Titratable acidity**

Titratable acidity was determined according to **AOAC (2000).** 5 g of each sample was diluted in 25 ml of distilled water and titrated by NaOH (0.1N) to pH 8.1.

The titratable acidity, expressed in relation to the citric acid content, is calculated by the following formula:

$$\mathbf{TA(\%) = [V \times N \times 10 \times F\ /W] \times 100}$$

TA: Titratable acidity (%);

V: Volume of sodium hydroxide used in the evaluation (ml);

N: Normality of sodium hydroxide;

F: Factor of Citric acid conversion which is equal to 0.0064;

W: Sample weight (g).

✓ **Ash**

10 g of powder sample was weighed into a porcelain crucible and incinerated at 550°C for 6 h in an ashing muffle furnace until ash was obtained. The ash was cooled in desiccator and reweighed **(AOAC, 2000).**

The following formula is used to calculate the ash content:

$$\mathbf{AC\ (\%) = [W_1\text{-}W_2/W_0] \times 100}$$

AC: Ash content (%);

W0: Weight of the test portion (g);

W2: Weight of empty crucible (g);

W1: Sample weight after incineration (g).

✓ **Viscosity**

Viscosity results from the friction of molecules, it results in the greater or lesser resistance of liquids to flow, the absolute viscosity η is usually expressed as centipoise, absolute viscosity is measured by calculating the fall time of a small ball in a column (Hoeppler viscometer) based on Poiseuille's law.

The viscosity was estimated using viscometer at 20 rpm and 25°C:

1. Fill the tube with the sample;

2. Set the temperature at 25°C;

3. When temperature equilibrium is reached, choose a ball for which its flow through the sample in the viscometer tube must be as slow as possible;

4. Then let the ball flow freely and when it reaches the mark on the upper part, start the stopwatch;

5. When the ball reaches the mark located at the bottom of the viscometric tube, note the time of fall of the ball (**NF 12092, 2002).**

The calculation of the viscosity is done according to the following formula:

$$\eta = t\ (D0\text{-}D1).\ K$$

η: Viscosity in centipoise (cP);

t: Fall time of the ball in seconds;

D1: Sample density;

D0: Density of the ball;

K: Gravity calibration constant of the tube (0.10277).

✓ **Total soluble solids**

Total soluble solids (TSS) were directly recorded by digital refractometer and the results were expressed as percent soluble solids (°Brix) (**AOAC, 2002).**

✓ **Electrical conductivity**

Electrical conductivity expresses the ability of the aqueous solution to conduct an electric current. The conductivity meter electrode was immersed in a 20% solid solution. The result of EC was display directly on the conductivity meter (S/m : Siemens per meter) (**AOAC, 2002).**

✓ **Crude fats**

The crude fats were determined using Soxhlet extraction for 6 hrs, using n-hexane as a solvent:

1. Weigh 10g of each sample powder and put them into a cartridge and close it with a piece of cotton; then place it in the "Soxhlet" extractor.

2. Dry and weigh the flask, add 100 ml of petroleum ether.

3. Fill the extractor with a volume of 50 ml of the same solvent.

4. After four hours of extraction, remove the balloon and place it in a rotavapor to recover the solvent.

5. To determine lipid levels, place the flask in the oven to eliminate traces of the solvent and weigh it **(AOAC, 2000)**.

The lipid level is calculated according to the following formula:

$$LF\ (\%) = [W_2\text{-}W_1/W_0] \times 100$$

LF: Level Fat (%);

W0: Weight of the test portion (g) ;

W1: Weight of the empty balloon (g);

W2: Ball weight plus fat (g).

✓ **Crude fibers**

1 g of powdered sample was digested with $H_2SO_4$ (1.25%) followed by NaOH (1.25%) solution. After filtration and washing with distilled water and acetone, remaining residues were weighed and putted in muffle furnace at a temperature of 550-650°C till grey or white ash was obtained **(AOAC, 2000).**

The fiber content is calculated according to the following formula:

$$\mathbf{CF\ (\%) = [W_1 - W_2 / W_0] \times 100}$$

CF: Crude fiber content (%);

W0: Test sample (g);

W1: Crucible weight before steaming (g);

W2: Crucible weight after stoving (g).

✓ **Crude proteins**

The powdered samples of garlic and red onion were tested for crude protein content according to the Kjeldahl's method as described in **AOAC (2000).** Briefly, 2 g of each sample were digested with $H_2SO_4$ by using digestion mixture (catalyst). The digested material was diluted up to 250 ml in volumetric flask. 10 ml of NaOH 40% as well as 10 ml of digested sample was taken in distillation apparatus where liberated ammonia was collected in beaker containing 4% boric acid solution using methyl red as an indicator. The percentage of nitrogen in the samples was assessed by titrating distillate against 0.1N $H_2SO_4$ solution. Crude protein content was calculated by multiplying nitrogen percent (N %) with factor (6.25).

✓ **Pectins**

The extraction of the pectins was estimated by a treatment of samples with high temperature using hydrochloric acid ( 1/30 N) as described by **Multon (1991).** Pectins were separated from the residue by centrifugation and precipitation with 80% alcohol, the obtained precipitate was filtered to remove soluble impurities, then dried and weighed.

The pectin content is calculated by the following formula:

$$PC (\%) = [W\times 200\times 0.9235] / [50\times a]$$

P: Pectin content (%);

W: Weight of precipitate (g);

200: Filtrate volume (ml);

0.9235: Coefficient of transformation of calcium pectate into pectin;

a: Weight of the filtrate (g);

50: Volume of filtrate taken for precipitation (ml).

✓ **Total sugars**

Totals sugars were determined using a colorimetric tests according to **Dubois (1956),** using phenol and concentrated sulfuric acid. In brief, 1 ml of sugar solution was added to 1 ml of phenol 5% and 5 ml of concentrated sulfuric acid, then shaked and placed for 10 to 20 min in a water bath at 25 to30°C. The absorbance was measured at 490 nm. The amount of sugars was determined by reference to a standard curve established with glucose.

✓ **Reducing sugars**

1ml of the sugar solution was removed and 1ml of DNSA reagent was added after 5 min of heating in a water at 100°C, the absorbance reading was made at 540 nm, the results were expressed in relation to a standard curve using glucose as reference **(Miller, 1972).**

✓ **Mineral content**

The plant samples were analyzed for their macronutrients (P, Ca, K, Mg and S), micronutrients (Fe, Cu, B and Zn) and heavy metals (Cd, Co, Cr, Mo, Ni and Pb) by using ICP-AES (**Sekeroglu *et al.,* 2012**). Briefly, 0.2 g of samples were put into burning cup and 5 ml $HNO_3$ 65 % and 2 ml $H_2O_2$ 30 % were added. After burning in a HP-500 CEM MARS 5 microwave at 200 ºC, the solution was cooled at room temperature for 45 min, filtrated by Whatman 42 filter paper. The extracts were cooled by high-deionized water in a 20 ml polyethylene bottles and kept at 4ºC for ICP-AES analyses.

### I.3.5 Phytochemical tests

The extracts were made using the maceration process, with the same extraction procedures and conditions as reported by **Gezici and Sekeroglu (2019).** 100 ml of methanol

70 % was added to 10 g of each sample, the solutions were shaked for 24 h at room temperature, the mixtures were then filtered using Whatman paper N°01 and evaporated using rotary evaporator HEIDOLPH (60 W). The dried extract was stored at 4°C for further analyses. Extraction yield (w/w %) was calculated as the ratio of the weight of the extract to the weight of the crude herb powder.

**I.3.5.1 Determination of total phenolic content**

According to the procedure defined by **Singleton and Rossi (1965),** the method of Folin-Ciocalteu reagent has been used to estimate the total phenolic content. 0.5 ml of varying concentrations of each used extract and 2.5 ml of Folin-Ciocalteu (1/10 dilution in water) were mixed with 1ml of sodium carbonate (20%). This mixture was incubated in the dark at room temperature for 30 min. The absorbance of the solution was measured at 765 nm using UV-Vis spectrophotometer HITACHI (Ratio Beam U-V 5100). A calibration curve was established using gallic acid as standard (0-1 mg/ml).The results were expressed as milligram of Gallic Acid Equivalent (GAE) per 100 g of Dry Matter.

**I.3.5.2 Determination of total flavonoids content**

The total flavonoids content of both extracts was determined using the aluminium chloride method as described by **Zou et *al.* (2004).** 1.5 ml of various concentrations of both extracts was mixed with 75µl of aluminium chloride solution and 0.5 ml of sodium acetate solution, the mixture was completed with distilled water until a volume of 2.5 ml. After an incubation period of 30 min at room temperature in the dark, the absorbance of the solution was measured at 415 nm using UV-Vis spectrophotometer. ). The results were expressed as milligram of Quercitin Equivalent (QE) per 100 g of DM, according to a calibration curve using Quercitin as standard (0-1 mg/ml).

**I.3.5.3 Determination of condensed tannins content**

The analysis of condensed tannins was carried out according to **Price *et al.* (1978).** 1ml of each extract was mixed with 2.5 ml of 4% methanol vanillin solution and 2.5 ml of $H_2SO_4$. After 15 min, the absorbance was measured at 500 nm. Condensed tannin contents were expressed as milligram of Catechin Equivalent (CE) per 100 g of DM based on a calibration curve using Catechin as standard (0-1 mg/ml).

### I.3.5.4 Determination of hydrolysable tannins content

Hydrolysable tannins were estimated using method of **Waterman (1987).** 500 µl of the extract was added to 3.5 ml of the ferric chloride solution. The contents were then quickly mixed and the absorbance read at 660 nm, 15 secs after the addition of the extract solution. A calibration curve was established using Tannic acid as standard (0-1 mg/ml). Hydrolysable tannins content were expressed as milligram of Tannic Acid Equivalent (TAE) per 100 g of DM.

### I.3.5.5 Phytochemical screening

Qualitative tests were realized to detect the presence of some secondary metabolites in plants extracts according to **Trease and Evans (1989) and Sofowora (1993) (Tab. 34).**

### I.3.6 Determination of phenolic content by High Performance Liquid Chromatography (HPLC) analysis

The analytical method used is high-performance liquid chromatography (HPLC), the identification of substances was performed according to their polarity in the solvents, the model of HPLC used for analytical control was: Shimadzu Nexera-I HPLC with auto sampler and quaternary pump. Each extract was dissolved in methanol in a ratio of 1 part extract to 5 parts solvent. The extracts were analyzed as such by injection into HPLC. The operating conditions are as follows: Column: silica gel-C18 type Fortis C18, 150 x 2.1 mm x 3 µm, Eluent: A = water, B = 0.1% formic acid, aqueous solution with pH = 2.5, and C = acetonitrile, Flow rate: 1 ml / min, Injected volume: 5 µl, Detector: DAD, spectrophotometric 220-400 nm, with chromatograms recorded at 254, 326 and 360 nm. The evaluation was based on a comparison of retention times and absorption maxima in the UV-Vis spectra. The resulting chromatographic profile is compared to standards (standard pure of phytochemical molecules) injected into the same operating conditions as that of the sample. Retention time (Rt) of each component is determined by the integrator giving a peak on the chromatogram **(Vlase et *al.*, 2014).**

### I.3.7 Antioxidant activity

The antioxidant activity of extracts was measured with the DPPH method describing by **Shimada et *al.* (1992).** A solution of DPPH (0.1 mM) was freshly prepared by dissolving 4 mg DPPH in 100 ml methanol. Mother solution (1 mg/ml) was prepared and followed by serial dilution in order to obtain all increasing concentration needed (0.1, 0.2, 0.3, 0.4, 0.5 mg/ml), from each extract 1 ml of each prepared diluted extract was added to 1 ml of DPPH

(0.1 mM). The solutions were then incubated for 30 min at room temperature in the dark, and the absorbance was measured at 570 nm. The antioxidant activity was calculated according to the following formula:

% inhibition= [(A control - A sample) / A control] × 100, where A control is the absorbance of DPPH solution without extract and A sample is the absorbance of sample with DPPH solution. The half-maximal inhibitory concentration (IC50) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%.

**Table 34:** Phytochemical screening of garlic and red onion

| **Metabolites** | **Added reagent** | **Expected result** |
|---|---|---|
| Flavonoïds | KOH (50%) | Yellow color |
| Tannins | $FeCl_3$ (1%) | Blue coloration |
| Alkaloids | HCL 2%+ Wagner reagent | Brown precipitate |
| Sterols and triterpenes | Acetic anhydride + $H_2SO_4$ (98%) | Red color (surface) + greenish fluorescence |
| Terpenoids | Chloroform + $H_2SO_4$ | Reddish brown coloration |
| Saponosides | Distilled water | Formation of foam |
| Anthocyanins | Chlorhydric alcohol+ isoamyl alcohol | Reddish brown coloration |
| Cardiac glycosides | Glacial acetic acid + $FeCl_3$(5%)+ $H_2SO_4$ (98%) | Brown ring |
| Reducing compounds | Fehlings (A+B) | Brownish-red precipitate |

### I.3.8 Antibacterial part

The identification of suspected isolates of *H. pylori* strains is based on the determination of morphological, biochemical and genetical characters, and their sensitivity or resistance to different antibiotics.

#### I.3.8.1 Macroscopic examination

The type of colonies and their size are studied from the cultures obtained on the fresh blood agar **(Larpent and Larpent, 1985).**

#### I.3.8.2 Microscopic examination

Microscopic examination is based on the microscopic observation of bacterial cultures in order to highlight the grouping, shape and mobility **(Delarras, 2007).** Gram staining can divide bacteria into two classes: Gram-negative and Gram-positive **(Harley et *al.,* 2010).**

#### I.3.8.3 Biochemical identification

##### I.3.8.3.1 Catalase

Catalase has the property of breaking down hydrogen peroxide ($H_2O_2$) into oxygen and water molecules. Place a drop of 10 volume hydrogen peroxide on the blood agar culture seeded with our strains. The release of gas bubbles indicates the presence of the catalase **(Marchal et *al.,* 1982).**

##### I.3.8.3.2 Urease

The degradation of urea by bacteria possessing urease is accompanied by a alkalinization which causes the color indicator to turn purple red. From a pure culture on blood agar medium, we prepared a dense suspension in 0.5 ml of urea-Indole medium. The preparation is then incubated at 37°C under micro-aerobic conditions for 24 h. The positive result is a change in the indicator from yellow to purplish red or pink red (**Marshall et *al.,* 1987).**

##### I.3.8.3.3 Oxidase

Cytochrome oxidase binds molecular oxygen to cytochrome reduced. The search for this enzyme is made using discs marketed impregnated with N-dimethylparapherine diamine oxalate, oxidized by cytochrome C, transforms into a dark purple compound. The disc (BioMérieux) was placed on the culture, and soaked with a drop of water sterile distilled. In the presence of oxidase (in the case of oxidase positive bacteria), the dark purple color appears immediately or within seconds, then darkens **(Delarras, 2007).**

#### I.3.8.3.4 API Campy

For more precision in the biochemical study, we used API Campy strips for *H. pylori* according to the following technics **(API *Campylobacter*, BioMerieux):**

**A. Preparation of the strip**

- ✓ Remove the strip from its packaging;
- ✓ Separate the strip into two parts, along the center fold;
- ✓ Prepare two incubation boxes (tray and lid);
- ✓ Distribute about 3 ml of distilled water or demineralized water into the honeycombed wells of the trays;
- ✓ Place the strips in the incubation boxes;
- ✓ Discard the desiccant.

**B. Preparation of the inoculum**

- ✓ Open an ampule of API NaCl 0.85% Medium (3 ml);
- ✓ Using a sterile swab, harvest all the culture from the previously prepared subculture plate;
- ✓ Prepare a suspension with a turbidity equivalent to 6 McFarland.

**C. Inoculation of the strip**

- ✓ Distribute approximately 80-100 µl of the previous suspension into each tube (URE to PAL of the first part of the strip and test $H_2S$ of the second part of the strip), avoiding the formation of bubbles (tilt the strip slightly forward);
- ✓ Fill the tube portion of the $H_2S$ test;
- ✓ Overlay the cupule of the URE test with mineral oil;
- ✓ Close the incubation box of the first part of the strip;
- ✓ Incubate for 24 hours (± 2 hours) at 36°C ± 2°C in aerobic conditions;
- ✓ Open an ampule of API AUX Medium and transfer approximately 150 µl of the previous bacterial suspension into the ampule (transfer all the remaining bacterial suspension for slow-growing strains);
- ✓ Distribute this new suspension into the tubes and cupules (GLU to ERO of the second part of the strip), avoiding the formation of a convex or concave meniscus;
- ✓ Close the incubation box of the second part of the strip;
- ✓ Incubate for 24 hours (± 2 hours) at 36°C ± 2°C in microaerophilic conditions (or anaerobic conditions when required by certain strains).

**D. Reading and interpretation**

- ✓ Reading the strip;

- ✓ Add reagents :NIT test : 1 drop of NIT 1 and NIT 2**;** HIP test : 3 drops of NIN**;** GGT, PyrA, ArgA, AspA, PAL tests : 1 drop of FB;
- ✓ Wait 5 minutes, then read the reactions by referring to the Reading Table **(Annexe 4);**
- ✓ If the SUT test is positive, read all the assimilation or growth inhibition tests. If it is negative, reincubate the second part of the strip for 24 hours. Cupules showing bacterial growth, even if it is weak, should be considered positive. The SUT test is a positive growth control. If it is negative after 48 hours, the other assimilation or growth inhibition tests are generally negative and are considered as such by the database;
- ✓ Record the reactions on the result sheet;
- ✓ The ERO test determines the susceptibility of the strain to Erythromycin. It can only be used for a therapeutic prediction if the SUT test (positive growth control) is positive : growth (opaque) = resistance, no growth (transparent) = sensitivity;
- ✓ The catalase reaction should also be recorded on the result sheet (21st test of the strip).

#### I.3.8.4 Antibiogram

The antibiogram is ensured by two technics: technic of diffusion of discs on agar and E test. It makes it possible to study the sensitivity and resistance of strains to antibiotics **(Megraud, 1994).**

**A. Standardization of bacterial suspensions**

Three to five well isolated and identical colonies were taken from a young culture (18 to 20h) on fresh blood agar, and added to 9 ml of sterile physiological water. The standardization of the suspension is carried out using a spectrophotometer set to a wavelength of 625 nm, its opacity must be equivalent to 0.5 Mc Farland or an OD of 0.08 to 0.13, which corresponds to a concentration of $10^7$ to $10^8$ CFU/ml; the inoculum suspension is diluted 1/10 in water physiological to have a concentration of $10^6$ CFU/ml **(Andrewes, 2001).**

**B. Diffusion technique of discs on agar**

This technique is based on disc diffusion on a Muller-Hinton media in using several antibiotics: Amoxicillin (AMC) (25 µg), Gentamycin (GE) (10 µg), Erythromycin (E) (30 µg), Tetracycline (TE) (30 µg), Oxacillin (OX) (5 µg), Doxycyclin (DO) (30 µg), Ciprofloxacin (CIP) (5 µg), Nanaomycin (NNM) (30 µg), Fusidic Acid (FA) (10 µg), Metroniazol (MTZ) (16 µg). The boxes are seeded by flooding with a bacterial suspension containing $10^6$ CFU/ml, on which the discs of the antibiotics are deposited. Incubation takes place at 37°C for 72 hours under microaerobiosis. The results was expressed by measuring the diameters of the zone of inhibition that has appeared (DZI) **(Megraud, 1994).** Antibiogram

results indicate then if the bacterium is sensitive, intermediate or resistant to the antibiotic according to concordance scale of **Madec et *al.* (2018**).

**C. E Test**

This is a technique of diffusion in an agar medium making it possible to measure of the minimum inhibitory concentration of an antibiotic (MIC).This technique introduced in the 1990s quickly proved to be very useful in the routine practice of laboratory. The E test strip includes an antibiotic gradient increasing from one end to the other. The antibiotic diffuses by forming a significant gradient, the zone of inhibition has the shape of an ellipse and the reading is then direct on the strip at the place where the growth of bacteria stops. After inoculation of the bacterial suspension ($10^6$ CFU/ml) by swab, place the E test strip for antibiotics: Amoxicillin (AC) on the Muller-Hinton agar using sterile forceps placing the MIC scale facing opening the box. Ensure good contact between the strip and the agar by pressing on the band starting from the base. Incubation is done at 37°C for 72 h in micro-aerophilic atmosphere **(Joly-Guillou, 2006).**

**I.3.8.5 Genetical identification using PCR**

**I.3.8.5.1 Preparation of samples for PCR amplification**

Genomic DNAs were extracted from all strains by the guanidnium thiocyanate method. The extracted DNAs were dissolved in water. and solutions containing 25mg of DNA per ml were prepared and used throughout the study. Primers used in this identification are: 93275:AAGCTTTTAGGGGTGTTAGGGGTTT3';93276:AAGCTTACTTTCTAACACTA3' which target the ure C gene, are derived from the sequenced urease genes (EMBL accession no. X57132 and GenBank accession no. M60398). The ure C gene was amplified in a volume of 25 µl containing 50 ng of DNA. 200 mM of each dNTP (Promega), 0.4 µM of each primer (ure C. Eurogentec), 1 U of GoTaq® G2 Hot Start Taq Polymerase (Promega), 5% DMSO (dimethyl sulfoxide. 472301 SIGMA-ALDRICH®), 4 mM of $MgCl_2$ (Promega), 5 µl of 5X Green GoTaq Reaction Buffer **(Lage et *al.*,1995).**

**I.3.8.5.2 Detection and analysis of amplified DNA products**

PCR products were cleared by 1% agarose gel electrophoresis with Gel Red TM Nucleic Acid Gel Stain, 10.000X (Biotium). The sample was considered *H. pylori* positive when a 294 bp ure C fragment was amplified and visualized on gel. Deionized water devoid of DNAse was used as a negative control **(Lage et *al.*,1995).**

### I.3.8.6 Evaluation of anti- *H. pylori* effect of plant extracts

#### I.3.8.6.1 Determination of DZI of plant extracts using disc diffusion method

The disc diffusion test was used for primary screening of susceptibility of *H. pylori* to the plant extracts. Bacterial suspensions adjusted to McFarland turbidity standard 0.5 ($1.5 \times 10^8$ CFU/mL) were inoculated on plates containing Muller-Hinton agar with 5% of horse blood . Filter paper discs (6 mm diameter) impregnated with 60 µL of different concentrations of each plant extract ( 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 250, 500 and 1000 µL) were placed on the inoculated agar surfaces. Methanol at a concentration of 80% was used as a negative control. The plates were observed for 5 to 7 days at 37°C under microaerophilic conditions. The antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by the plant extracts **(Nostro et *al.*, 2005).** The strain with a diameter D < 8mm, 9mm ≥ D ≤ 14mm, 15mm ≥ D ≤19mm, D> 20mm was considered as resistant, sensitive, very sensitive, extremely sensitive, respectively **(Duraffourd et *al.*, 1990).**

#### I.3.8.6.2 Determination of MIC of plant extracts with agar dilution method

The extracts presenting an inhibition zone ≥9 mm in diameter were chosen to assay the MIC with the agar dilution method using Mueller Hinton agar with 5% of horse blood. Methanol (80 %) was used in the assay as a negative control. Concentrations of each extract were prepared in methanol 80 % and 1 ml of each solution was incorporated in 20 ml of appropriate melted agar and poured in a Petri dish. The final concentrations of the extracts in the medium ranged from 90 to 1000 µg/ml. Agar plates were inoculated with 1 ml of bacteria suspension ($1.5 \times 10^8$ CFU/ml). The plates were incubated for 5–7 days at 37 °C under microaerophilic conditions. The MIC was defined as the lowest concentration of plant extract inhibiting the visible growth **(NCCLS Guidelines, 1998).**

#### I.3.8.6.3 Determination of MIC and MBC of plant extracts with broth dilution method

Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) were measured using tube dilution methods. Fresh bacterial suspensions were prepared in Mueller Hinton broth and adjusted to McFarland turbidity standard 0.5. Serial concentrations were prepared from crude plant extracts within the range of 90 to 1000 µg/ml, then, 1ml of each extract was added to tubes containing 8 ml of Mueller Hinton broth. Finally, 1 ml of a 1:1000 dilution of bacteria adjusted was added to obtain a 10 ml final volume . Controls of bacteria without extract were also prepared, under similar conditions. The tubes were incubated for 2-5 days at 37°C , before recording the MIC. A volume of 0.1ml of each

suspension was spread onto Columbia agar plates containing 5% horse blood. After incubation in microaerophilic conditions at 37 °C for 72 h, the colonies formed were subsequently computed. The MBC was defined as the lowest concentrations of the plant extract inducing complete inhibition of colony formation of the test bacteria at the latter cultivation **(Yuan-Chuen Wang et *al*., 2004)**

#### I.3.8.6.4 Evaluation of growth kinetics of *H. pylori* in presence of plant extracts

The ability of several plant extracts to suppress *H. pylori* growth was tested by adding 1 ml of each plant extract on the sixth hour of development, while the optical density was subsequently measured every 2h to follow the development of *H. pylori* in the presence of the extract **(Barefoot and Kaenhammer, 1983).**

### I.3.8.7 Evaluation of anti- *H. pylori* effect of probiotics (well diffusion assay)

#### I.3.8.7.1 Preparation of cell-free supernatant (CFS) of probiotics

Cell-free culture supernatants of probiotics were prepared as described by **Kim et *al.*(2009)**. The culture supernatant was collected from a 24 hrs culture by centrifugation at 4.000 rpm/ 10 min. The resulting supernatants were filter- sterilized (pore size 0.22 mm).*H. pylori* cultures were plated on fresh Mueller Hinton agar plates containing 5% horse blood ($1.5 \times 10^8$ CFU/ml), and wells were drilled into the agar by using sterile Pasteur pipettes, 60 µl of fresh probiotic strains cell-free culture supernatants were introduced in the agar wells. Plates were incubated for 48 to 72 h under microaerophilic conditions at 37°C, and the DZI around the wells were measured. The antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by each probiotic.

#### I.3.8.7.2 Evaluation of growth kinetics of *H. pylori* in presence of probiotics

A pre-culture tube of the *H. pylori* strain was inoculated in Mueller Hinton broth and incubated at 37ºC for 18h from which three standardized (0.5 MacFarland) culture tubes were prepared and the measurement of the bacterial growth of the pathogen strain was carried out by measuring the optical density every 2 hrs. After 6 h, 1 ml of each supernatant (normal / organic acid free / $H_2O_2$ free prepared as described below), was added to the culture tubes, the optical density was then measured every 2hrs up to 24hrs and the OD = f (t) curve was drawn **(Barefoot and Kaenhammer, 1983).**

- ✓ **Influence of organic acids (lactic and acetic acids)**

Lactic acid bacteria can produce inhibitor molecules of *H. pylori* such as organic acids, bacteriocins and hydrogen peroxide. To ensure the presence of these molecules, we

took an18 h culture of different strains of probiotics which was transferred to 50 ml of modified MRS broth and incubated at 37°C for 18 h. After incubation, the tubes were centrifuged at 4.000 rpm/ 10 min in order to recover the supernatant **(Barefoot and Kaenhammer, 1983).**

✓ **Influence of bacteriocins**

In order to study the effect of bacteriocins on the growth of *H. pylori*. The effect of organic acids in particular lactic and acetic acids was eliminated, the supernatant has been neutralized (pH = 7) by adding a 0.1 N NaOH solution **(Barefoot and Kaenhammer, 1983).**

✓ **Influence of hydrogen peroxide**

The effect of $H_2O_2$ was inhibited by incubating the supernatant fluid with catalase enzyme solutions prepared to a final concentration of 1 mg $mL^{-1}$ in phosphate buffer saline (pH 7.0) .

The inhibitory effect of the agent was tested by eliminating the possible effect of organic acids by adjustment of the cells-free supernatant's pH to 7 and that of hydrogen peroxide in presence of catalase.

**I.3.8.8 Combined effect of medicinal plants with probiotics on *H. pylori***

**I.3.8.8.1 Determination of DZI of combined mixtures using disc diffusion method**

Fresh Mueller Hinton agar plates containing 5% horse blood were inoculated with 1.5 $\times 10^8$ CFU/ml of *H. pylori*; filter paper discs (6 mm diameter) impregnated with 30 µL of fresh supernatants of probiotics +30 µL of plant extracts were placed on the inoculated agar surfaces (each probiotic has been combined with all four extracts). Plates were incubated for 48 to 72 h under microaerophilic conditions at 37°C, and the DZI around discs were measured.

**I.3.8.8.2 Evaluation of growth kinetics of *H. pylori* in presence of combined solutions**

Different mixtures of probiotics with plant extracts were examined for inhibition of *H. pylori* growth. The measurement of *H. pylori* growth was carried out by measuring the optical density every 2 hrs after adding 500 µl of probiotic supernatant +500 µL of plant extract on the 6th hour of growth (each probiotic has been combined with all four extracts).

### I.3.9 *In vivo* study

#### I.3.9.1 Protocol

The inhibition of *H. pylori* growth by probiotics and plant extracts was investigated using Wistar rats model. Depending on the results, we have chosen the strongest probiotic and extract (*B. breve* and fenugreek extract) based on their anti-*H. pylori* effect in order to complete the *in vivo* study. The rats were divided into nine groups; 6 groups (G1-G6) infected for 2 weeks then treated for the 2 following weeks and 3 preventive groups (G7-G9) infected and treated orally at the same time once daily for 2 weeks . More precisely, the groups were: Group 1 G1 (NC. n = 6); negative control *H. pylori*-infected $1.5 \times 10^8$ CFU without treatment, G2 (PC. n = 6); positive control *H. pylori*-infected and treated using second line therapy clarithromycin with metronidazole in combination with amoxicillin and omeprazole, G3 (TFE1 n = 6): *H. pylori* infected and treated with fenugreek extract (TFE) 150 µg/kg G4 (TFE2. n = 6):*H. pylori*-infected and treated with fenugreek extract 300 µg/kg, G5 (TBB. n = 6):*H. pylori*-infected and treated with *B. breve* (TBB) $1.2 \times 10^9$ CFU. G6 (TFE1 + TBB. n = 6): *H. pylori*-infected and treated with fenugreek extract 150 µg/kg and *B. breve* $1.2 \times 10^9$ CFU, G7 (HP+TFE, n = 6):*H. pylori* infected and treated with fenugreek extract 150 µg/kg, G8 (HP+TBB. n = 6):*H. pylori* infected and treated with *B. breve* $1.2 \times 10^9$ CFU and G9 (HP+TFE+TBB. n = 6): *H. pylori* infected and treated with fenugreek extract 150 µg/kg and *B. breve* $1.2 \times 10^9$ CFU. At the end of the experiment, the rats were euthanized with diethyl ether and a full necropsy was performed **(Fig. 26).**

#### I.3.9.2 Histopathologic analysis of gastric tissue samples

After removal, the stomach of each animal was opened through the longer curvature with sterile surgical instruments. Urease activity was determined in one half, and the other half was fixed in 10% buffered formalin, trimmed to include all areas of the stomach, then processed by standard methods **(Suvana et *al.*, 2018)** and embedded in paraffin. From each block, two 5 µm sections were made, one being stained by hematoxylin and eosin (H&E) and the other by a Giemsa stain for *H. pylori* detection. All cases were examined in a blind manner by a veterinary pathologist, according to criteria established by **Lee et *al.* (1997).** Histopathological examination done for stomach specimens were ranked according to the intensity of *H. pylori* colonization as follows: severe infection (3), moderate infection (2), mild infection (1) and free from infection (0) **(Dixon et *al.*, 1994).** Gastritis was defined by the presence of lymphocytic or neutrophilic infiltration.

**Figure 26:** Differents groups of *In vivo* experimentation

In Giemsa-stained sections, examining the different anatomical regions of the *H. pylori* infected and treated rats, *H. pylori* bacteria colonizing the glands were graded on a scale of 0 to 4 as follows: 0, absence of bacteria; 1, bacteria isolated and randomly distributed; 2, reduced number of bacteria; 3, large number of bacteria and 4, very high number of bacteria **(Fig. 27).**

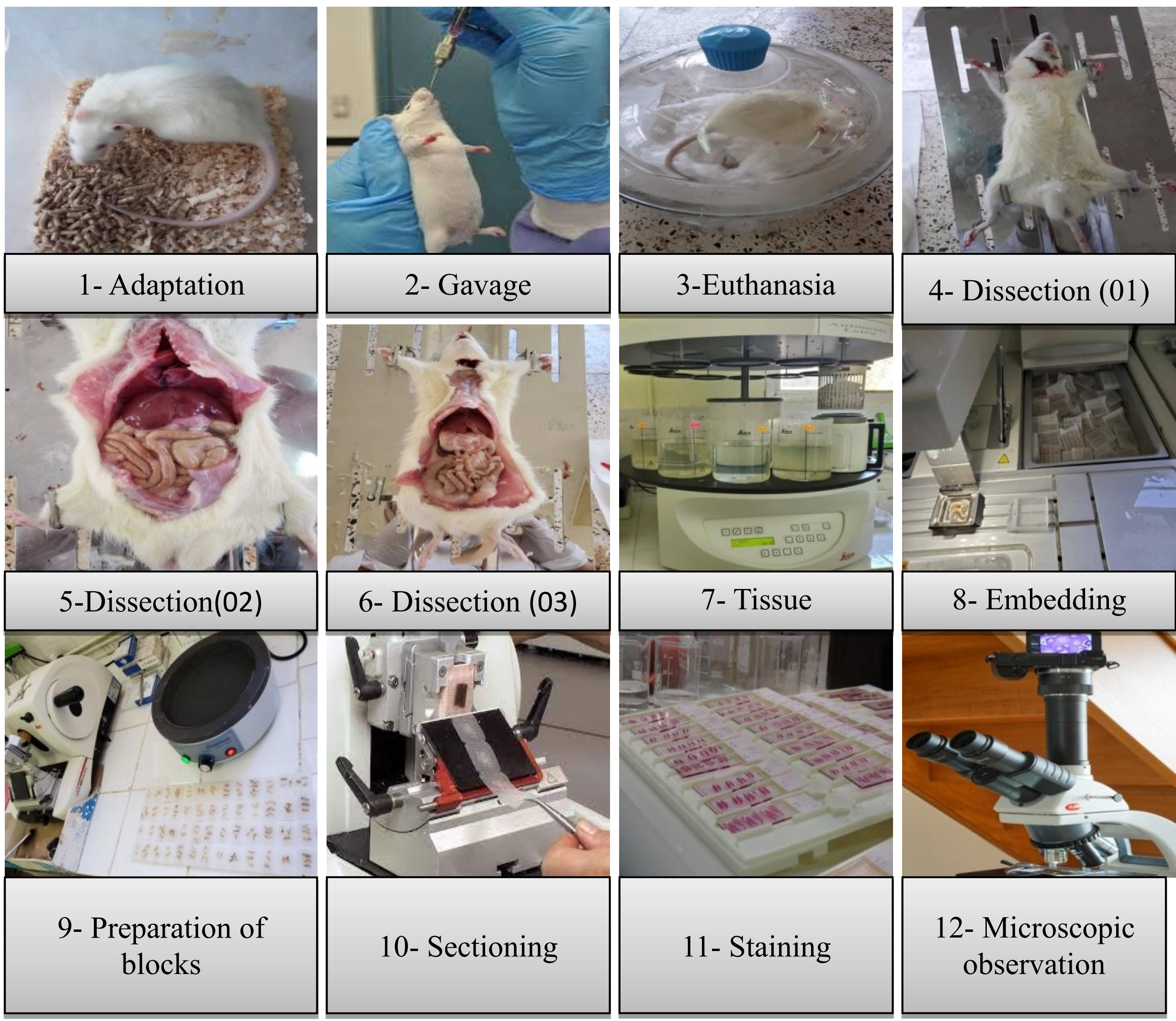



**Figure 27:** *In vivo* protocol steps

### I.3.10 Statistical analysis

- ✓ The data from physicochemical composition were subjected to statistical analysis using the R software. Differences between plants were compared at $P < 0.05$ with ANOVA in order to find the statistically significant differences. The assays were realized out in triplicate and the results were expressed as mean values and standard deviation.
- ✓ The data from phytochemical composition and antioxidant activity were analyzed with a statistical software program (SPSS version 20). Differences between plants were compared at $P < 0.05$ with One-Way ANOVA followed by Tukey's post hoc test in order to find the statistically significant differences. The assays were carried out with three repetitions and the results were expressed as mean values and standard deviation.
- ✓ The data of anti-*H. pylori* effect were collected and analyzed statistically by MS Excel 2007 and presented as mean ± SD of three replicates.

# Chapter II:

# Results and discussion

## II Results and discussion

### II.1 Selection of varieties

As shown in **Table 35** , there are significant differences between the weight of 1000 seeds(g) and germination rate of the different varieties.

**Table 35:**Weight of 1000 seeds and germination rate of different varieties of cumin and fenugreek seeds

| Variety | | Algeria | Egypt | India | Morocco | Syria |
|---|---|---|---|---|---|---|
| **Fenugreek** | Weight of 1000 seeds(g) | 16.8±0.25 | 11.6±0.2 | 10.2±0.03 | 10±0.2 | 10±0.000 |
| | Germination rate(%) | 70±0.000 | 40±0.066 | 30±0.25 | 20±0.75 | 20±0.045 |
| **Cumin** | Weight of 1000 seeds (g) | 10.1±0.033 | 09.8±0.1 | 13.6±0.04 | 10.2±0.00 | 13.9±0.111 |
| | Germination rate (%) | 40±0.05 | 20±0.3 | 60±0.05 | 40±0.05 | 70±0.025 |

The choose of varieties depended on the results of weight of 1000 seeds (g) and germination rate(%) of different varieties of fenugreek and cumin, the results showed that the Algerian variety of fenugreek was the best one comparing to the other varieties with a weight of 16.8±0.25g and germination rate of 70±0.000%, while the Syrian variety of cumin presented the higher weight with an amount of 13.9±0.111g and germination rate with percentage of 70±0.025%.

### II.2 Physicochemical analysis

The results of physicochemial analysis of plant samples exhibited a great diversity in terms of the parameters including moisture, ash, viscosity, TSS, and electrical conductivity, crude fibers, pectin contents, protein, total sugars and reducing sugars. Significant differences ($p>0.05$) were observed between the parameters as presented in **Table 36**.

**Table 36:** Results of physicochemical analysis of plant samples

| Parameters | Results | | | |
|---|---|---|---|---|
| | Garlic | Onion | Fenugreek | Cumin |
| pH | 6.64$^{a}$±0.0025 | 6.08$^{a}$±0.00 | 5.6$^{a}$±0.0075 | 6.5$^{a}$±0.0075 |
| Titrable acidity (%) | 0.67$^{a}$±0.00 | 1.005$^{a}$±0.00 | 3$^{a}$ ±0.00 | 2.8$^{a}$±0.00 |
| Moisture (%) | 65.92$^{a}$±0.00 | 91.12$^{a}$±0.005 | 3$^{a}$ ±0.0005 | 5.6$^{a}$±0.00 |
| Ash (%) | 1.38$^{a}$±0.005 | 0.29$^{a}$ ±0.00 | 3$^{a}$±0.00 | 7$^{a}$±0.00 |
| TSS (°Brix) | 9.16$^{b}$ ±0.0066 | 6.50$^{b}$ ±0.00 | 2.8$^{b}$±0.82 | 5.5$^{b}$±0.00 |
| CE (mvs) | 6.05$^{a}$ ±0.0075 | 5.22$^{a}$±0.0075 | 18.1$^{a}$±0.005 | 42.8$^{a}$±0.00 |
| Viscosity (m/pa/s) | 1.62$^{b}$±0.00 | 1.53$^{b}$ ±0.00 | 2.8$^{b}$±0.0003 | 2.4$^{b}$±0.0009 |
| Proteins (%) | 18.52$^{b}$ ±0.00 | 14.10$^{b}$±0.0033 | 26.8$^{b}$±0.063 | 23,1$^{b}$±0.25 |
| Fats (%) | 1.09$^{b}$±0.006 | 0.94$^{b}$ ±0.006 | 8.8$^{b}$±0.34 | 21$^{b}$±0.00 |
| Fibers (%) | 0.75$^{b}$±0.003 | 11.46$^{b}$ ±0.0033 | 5.1$^{b}$±0.00 | 7.9$^{b}$±0.00 |
| Pectins (%) | 0.30$^{b}$ ±0.0066 | 4.40$^{b}$±0.00 | 1.9$^{b}$±0.00 | 2.8$^{b}$±0.0033 |
| Total sugars (%) | 18.03$^{b}$ ±0.0033 | 14.14$^{b}$±0.0033 | 6.7$^{b}$ ±0.006 | 5.3$^{b}$±0.00 |
| Reducing sugars (%) | 7.88$^{b}$ ±0.0066 | 12.84$^{b}$ ±0.00 | 0.5$^{b}$±0.00 | 1$^{b}$±0.0033 |

**a, b** correspond to the homogeneous groups *obtained* by the post-hoc *Tukey* test for each parameter.

The result of pH obtained in garlic was approximately similar in comparison with the results of **Gimenez et *al.* (1988)** and **Cavallito et Bailey (1944)** which were 6 and 6.5, and higher than the result of **Yin et Cheng (2003)** which was 5.7. While our pH value of onion was nearly similar to the previous data published of **Shenoy et *al.* (2009)** which was 6.5. pH values found in **Capel abad (2014), Dalloccat-Berno et *al.* (2014)** and **Petropoulos et *al.* (2015)** studies were significantly higher with values of 5.96±0.23, 5.50 and 5.2±0.2 respectively. However pH in fenugreek was lower in comparison with the results of **Ahmad Dilshad (2017)** which were in the range of 6.8 and 6.9, however our pH value of cumin was significantly lower to the earlier research of **Al-Snafi (2016)** which was 7.3 and higher than

the result of **Monojit et *al.* (2017)** which was 3. The pH determined for the two spices taken into consideration is in the range of 6-7, which shown slight acidic character. Otherwise differences on pH can be due to the diversity of the variety, the growing conditions, the degree of ripening and climate **(Ariyama et *al.*,2006).**

Concerning titratable acidity, no significant difference between garlic and onion was observed (p=0.134). **Caruso et *al.* (2014), Rodriguez *et al.* (1998), Rodriguez Galdon et *al.* (2008)** and **Zambrano et *al.* (1994)** results present a higher value which were in the range of 1.97 to 2.23. **Petropoulos et *al.* (2015)** result was higher 0.038±0.0. In the other hand, **Tabaestani et *al.* (2013)** found that cumin posses a lower value of titratable acidity in confrontation with our result which was 0.7±0.09. The differences of pH and titratable acidity could be mostly due to the lower water content as well as to different growing conditions **(Ariyama et *al.*, 2006).**

The percentage of moisture content in garlic was comparable to those of **Rasul suleria et *al.*, (2015)**, and **Marina et *al.*, (2014)** which were 65 and 64.58% respectively and significantly higher than the results of **Kallel et *al.*, (2014), Otunola et *al.*, (2010)** which were 3.52±0.03 and 4.55±0.1 respectively. In the other hand, our result was lower than those of **Yin et Cheng (2003)** and **Sampath et *al.*, (2010)** with a value of 77.5±2.3 and 84.09% respectively. However, onion revealed very high percentage of humidity compared to the studies of **Petropoulos et *al.*, (2015),** and **Shovon et *al.*, (2013)** which were in the range of 88.90±0.54-83.99% ±0.05, and 82.99±0.05-82.77%±0.07 respectively. While the percentage of moisture content in fenugreek was similar to those of **Abdelmoneim et *al.* (2008)** which was 4% and significantly higher than the result of **Udayasekhara et *al.* (1996)** which was 2.4%. However, cumin revealed very low percentage of moisture compared to the result of **Al-Snafi (2016)** which was 8%.

A very highly significant difference between garlic and onion was observed (p=3.06e-09). It has been established through various research that food with low moisture content has longer shelf-life with limited deterioration in quality due to microbial activities **(Nwinuka et *al.*, 2005)** which mean that garlic can be stored for long period comparing to onion. The variations in moisture content reported by various investigators could be attributed to the differences in the environmental conditions, the time of harvesting and the storage conditions **Sulieman (1995).**

**Odebanmi et *al.,* (2009)** and **Abdou et *al.,* (1972)** showed a similar amount of ash in garlic compared to our results,1.33%±0.04, 1.3% respectively, and lower than those given by **Rasul suleria et *al.* (2015) and Marina et *al.* (2014),** with a value of 2.3 and 2.46% respectively. Also, **Kallel et *al.* (2014)** research showed a very higher ash contents with value of 16.56%±0.18. Concerning onion, it was observed that it possessed a high content of ash compared to **Shovon et *al.* (2013)** results which were (0.20g±0.08/100g for Bangladesh onion) and (0.248g±0.1/100g for Indian onion) while the present result was lower than those presented by **Petropoulos et *al.* (2015)** and **Capel abad (2014)** which were in the range of 0.40%±0.01-0.58%±0.01 and 4.43%±0.05 respectively.

**Awais et *al.* (2015)** showed a similar amount of ash in fenugreek with our results; 3.4%, and lower than those of **Abdelhamid et *al.* (1984) ,** with a value of 7.6%. Concerning cumin, it was observed that it presents a high content of ash in contrast to **Al-Snafi (2016)** result which was 6.5, while the present result was similar to those presented by **Monojit et *al.* (2017)** which was 7.5% and lower than the maximum limits indicated by the Egyptian Specification Standards [ES: 1930/2008] and by the International Standards Organisation [ISO 9301/2003 ] which were 8.5% and 12% respectively. Low ash is usually an indication of low inorganic mineral content, the highly significant difference (p=3.4e-08) indicates that garlic is richer in inorganic minerals than onion **(Oloyede, 2005).** The variation in the ash content could be due to the soil conditions **(Sulieman, 1995).**

Total soluble solids contents in garlic was higher than onion, concerning onion our result was lower than those of **Petropoulos et *al.,* (2015)** which was 9.95 °Brix ± 0.9, also than the range found by **Caruso et *al.* (2014), Lee et *al.* (2009), Yoo et *al.* (2006)** which were from 7.7 to 8.1°Brix. Although **Capel abad (2014)** and **Dalloccat-Berno et *al.* (2014)** results were significantly higher with an amount of 10.95±0.25 and 11°Brix, respectively.

Also TSS contents in cumin was higher than fenugreek, regarding fenugreek our result was lower than those of **Abdel–Nabey and Damir (1990),** which was 3.5 °Brix, while **Tabaestani et *al.* (2013),** found that cumin TSS contents was higher with 7.7°Brix. No significant difference was detected on TSS between fenugreek and cumin (p=0.000). However, the significant difference on TSS between garlic and onion (p=8.92e-05) may be due to the presence of a linear negative correlation between growth temperature and bulb soluble solids contents (**Coolong and Randle, 2003c).**

Electrical conductivity of garlic was higher than onion, **Fred (1902)** found a higher value 0.0525 mvs for onion, and cumin 35.1 mvs. He concluded that specific conductivity of onion leaves juice is shown to be more than twice compared to juice from bulb. The results of specific conductivity of bulbs onion juice made up from the crude ash indicate that the ash alone was not the cause of the conductivity of the juice, but that the organic compounds were concerned, which confirmed the significant difference (p=1.45e-07).

For the viscosity, there is a significant difference between these plants (p= 0.0475). **Juszczak and Fortuna (2003)** experiments show that the values of viscosity depend strongly on soluble solids content, the viscosity changed with higher soluble solids content.

The crude proteins level of garlic was approximately comparable to those of **Nwinaka et *al.* (2005)** and **Otunola et *al.* (2010)** with a percentage of 17.35 and 15.33% respectively, while the present result was significantly higher than those of **Kallel et *al.,* (2014) and Marina et *al.,*(2014)** with values of 8.43%±0.41, 7.87%±0.82. **Gorinstein et *al.* (2008) and Rasul suleria et *al.* (2015)** mentioned that their results were lowest with an amount of 1-2%, 2%, however the research of **Yin and Cheng (2003)** showed a higher level of proteins in garlic with 21.3±1.2%. Previous studies of **Petropoulos et *al.* (2015)** and **Lim (2015),** revealed that onion had a protein content higher than the present result with an amount of (0.62%±0.03), (1.1%), respectively.

Regarding fenugreek was approximately comparable to those of **Mullaicharam et *al.* (2013)** with a value of 25.9%, however our result was significantly higher than those of **Fahad and Al–Jasser (2012),** with values of 12.9%. While, the found protein content amount in cumin seeds were higher compared to those reported in literature of **Al-Snafi (2016)** and **Monojit et *al.*(2017),** which was 18.4%±0.16.

The high crude protein content of garlic comparing to onion (p=0.00873) may be due to the presence of active proteinous metabolites. Proteins are essential component of the diet needed for the survival of animals and human, they serve as source of nitrogen in the body system along with the amino acids, good skin, increase in growth and ability to replace the worn-out cells are the quality of protein in the body **(Okolo *et al*., 2012).** As conclusion, the difference on crude protein content between plants may be due to different cultural practices, soil and environmental conditions **(Sulieman, 1995).**

Concerning crude fats in garlic, our result was higher than those of **Kallel et *al.* (2014)** and **Marina et *al.* (2014)** with a percentage of 0.86% ±0.04, 0.52%±0.01 respectively.

**Rahman (2003)** and **Gorinstein et *al.* (2008)** evaluated the chemical composition of garlic and concluded that crude fats contents were significantly lower than the presented result with an amount of 0.1%, 0.2% respectively, while fats contents in garlic found by **Yin et Cheng (2003)** were higher with percentage of 2.6% ±0.4, in the present data the level of fats was approximately similar to the studies of **Petropoulos et *al.* (2015)** and **Shovon et *al.* (2013)** with an amount of (0.07%±0.01), and (0.4g±0.06 for Bangladesh onion) and (0.721%±0.05 for Indian onion) respectively.

**Sulieman (1995)** evaluated the chemical composition of fenugreek and concluded that crude fats contents were similar to the presented result with an amount of 8.1%, Also our result was higher than those of **Abdelmoneim et *al.* (2008)** with a percentage of 4%. While, fats contents in cumin found by **Muhammad Sultan et *al.* (2009)** were higher with percentage of 31.2%, in the present data the level of fats was approximately similar to the studies of **Mengmei et *al.* (2015)** with an amount of 22.7%.

Statistically, no significant difference was observed between garlic and onion on fats contents (p=0.0634), they are not considered as significant source for fatty acids in human diet considering both its low content and the small amounts of average daily consumption. The amount of fats can be affected by many factors including genotype, stage of maturity, growing and climate conditions, harvest time and even post-harvest conditions **(Chun et *al.*,2006).** The percentage of total lipids of plants differs according to the location and conditions of cultivation **(Abdelmoneim et *al.*, 2008)**.

Many studies have been carried out to estimate the amount of fibers present in garlic. **Odebanmi et *al.* (2009)** found a similar result to our result with an amount of 0.73%±0.19. **Marina et *al.* (2014) and Rasul suleria et *al.* (2015)** present a higher percentage of fibers 2.3%±0.08 and 1.5%. Fiber contents in onion were significantly higher in comparison with other studies of **Capel abad (2014) and Shovon et *al.*,(2013)** with an amount of (2.16%±0.53) and (1.659%±0.8 for Indian onion) and (2.646%±0.3 for Bangladech one), while pectins contents in onion were lower (4.40%±0.00) than **Lutomsky (1983)** results (10-15%). **Haram (1991)** present a higher percentage of fibers in fenugreek 13%. while pectins contents in fenugreek were lower than **Anita et *al.* (2006)** result which was 3%. Fibers contents in cumin were significantly lower in comparison with other studies of **Peter (2001)** with an amount of 30%, however pectins percentage was higher than **Mengmei et *al.* (2015)** result which was 1.7%.

Crude fiber play an important role in maintenance of normal peristaltic movement of the intestinal tract hence, it helps in digestion of food. Very highly significant difference between garlic and onion was observed (p=7.56 e-07), that's why the higher fiber content in onion makes it suitable for recommendation for patients who have problems with food digestion **(Okolo et *al.,* 2012).** The reduced levels of crude fiber obtained for garlic present no threat since is not usually consumed in isolation but as adjuncts or additives to other foods. Hence, the low fiber contents serves as a boost to the total dietary fibre of the dishes in which it is used, contrary to onion **(Otunola *et al.,* 2010).** There is evidence that crude fibers has a number of beneficial effects related to its indigestibility in the small intestine **(Aremu et *al.*, 2006).**

Sugars analysis expressed that the percentage of total sugars in garlic was significantly higher than that showed by **Garnier (1961)** which was 1.2%, on the other hand it was lower than the results presented by **Rasul suleria et *al.* (2015)** with an amount of 28%,While reducing sugars in garlic were higher than the result of **Garnier et *al.* (1961)** which was 1.2%. Concerning onion, significant differences in total sugars content were also observed compared to previous studies of **Petropoulos et *al.* (2015) and Charles (2013),** which were (3.41%±0.12) and (6.36%), respectively. Reducing sugars contents in onion were similar (12.84%±0.00) to those found by **Bajaj et *al.* (1980)** which were (12%). **Petropoulos et *al.* (2015)** and **Lee et *al.* (2009)** revealed the presence of glucose and fructose in onion with an amount of (0.97%±0.04) and (0.36%±0.01).

The percentage of total sugars in fenugreek was significantly higher than that showed by **Elmahdy and Elsebaiy (1982)** which was 4.2%. On the other hand, it was lower than the results presented by **Anita et *al.* (2006)** with an amount of 8.8%, While reducing sugars in fenugreek were similar to the result of **Rajini et *al.* (2016)** which was 0.5% and lower than **Anita et *al.* (2006)** result; 0.8%. Concerning cumin, significant differences in total sugars content were also observed compared to previous studies of **Kumar et *al.* (2015)** which were 2.4%. Reducing sugars contents in cumin were similar to those found by **Kumar et *al.* (2015)** which were 1.2%.

According to **Mallor et *al.* (2011)**, total and reducing sugars play an important role for onion sweetness. The present data showed a very high significant difference in total and reducing sugars between the two plants with (p=0.00122) and (p=1.23e-05) respectively. The nutritional composition of plants depends on climatic conditions, geographic origin of seeds and cultural practices **(Demarne , 1985).**

### II.3 Minerals analysis

The macronutrients, micronutrients and heavy metal contents of cumin and fenugreek seeds were given in **Table 37**. Analysis of the mineral contents showed no significant differences between plant samples.

**Table 37:** Results of minerals analysis of plant samples

| Minerals (mg/kg) \ Plants | | Garlic | Onion | Fenugreek | Cumin |
|---|---|---|---|---|---|
| Macronutrients | Ca | 683±54 | 4874±235 | 1445±68 | 8077±89 |
| | K | 16538±811 | 21012±787 | 10605±555 | 14647±501 |
| | Mg | 633±55 | 1142±71 | 1229±88 | 2610±111 |
| | P | 3765±211 | 2840±145 | 5143±366 | 3817±321 |
| | S | 8115±425 | 7528±369 | 2648±135 | 3423±211 |
| Micronutrients | B | 6.5±0.04 | 19.1±0.11 | 11.8±0.06 | 22.1±0.14 |
| | Cu | 4.73±0.2 | 9.13±0.5 | 9.9±0.4 | 10±0.5 |
| | Fe | 55±3 | 368±19 | 91±6 | 133±8 |
| | Zn | 22.4±1.2 | 32.2±1.8 | 30.9±1.5 | 37.8±1.8 |
| Heavy metals | Cd | 0.040±0.003 | 0.097±0.002 | 0.03±0.002 | 0.1±0.008 |
| | Co | 0.210±0.001 | 0.230±0.001 | 0.2±0.004 | 0.2±0.000 |
| | Cr | 0.814±0.040 | 1.191±0.080 | 0.2±0.007 | 1±0.009 |
| | Mo | 0.227±0.080 | 0.093±0.002 | 2±0.900 | 0.3±0.009 |
| | Ni | 0.87±0.04 | 1.01±0.01 | 1.3±0.90 | 1.5±0.11 |
| | Pb | 2.71±0.10 | 3.76±0.06 | 0.4±0.00 | 1.4±0.10 |

According to the present data, minerals and heavy metals profile of garlic showed that it contains potassium as a major mineral in a maximum quantity followed by sulphur, phosphorus, calcium, magnesium, iron, zinc and boron, for the heavy metals the higher percentage was their of copper followed by lead, nickel, chromium, molybdenum, cobalt and cadmium. Extensive research has been carried out to determine the amount of mineral

elements in garlic, and results of **Marina et *al.* (2014)** were lower than our results with a value of calcium (26.30±0.14 mg/100g), phosphorous (10.19±0.26 mg/100g), iron (5.29±0.08 mg/100g), sodium (4.10±0.14 mg/100g) and magnesium (4.10±0.14 mg/100g), while zinc, copper and manganese were in lowest quantity (0.34 ± 0.17 mg/100g) (0.001 ± 0.00 mg/100g) and (0.001 ± 0.00 mg/100g) respectively. Another research group **Ujowundu et *al.* (2011)** found that garlic minerals were very lower with an amount of Cu (0.373 mg/100g), Fe (3.48 mg/100g), Ca (1.904 mg/100g), Se (0.02 mg/100g), and Mg (4.334 mg/100g), while zinc was not detected.

Mineral composition of onion differed significantly with previous findings having higher content in K, Mg, P, S and Ca, the present study showed that onion contains potassium as major mineral followed by sulphur, calcium, phosphorus, magnesium, iron, zinc, boron, copper , lead, chromium, nickel , cobalt, cadmium, molybdenum respectively. **Caruso et *al.* (2014)** reported a higher value compared to our results, potassium was (1895.5-2643.7 mg/100g) being the most abundant element in red onion followed by phosphates (595-685 mg/100g), calcium (178.1-251.4 mg/100g), sodium (180.4-240.2 mg/100g), sulphates (144.6-196.3 mg/100g), magnesium (76.2-87.9 mg/100g), nitrates (19.4-34.6 mg/100g), chlorides (3.14-3.78 mg/100g), iron (6.3-6.9 mg/100g), Zinc (4.5-4.6mg/100g) and copper (1.2-1.3 mg/100g) respectively.

Concerning mineral and heavy metals profile of fenugreek, it contains potassium as a major mineral in a maximum quantity followed by sulphur, phosphorus, calcium, magnesium, iron, zinc and boron, for the heavy metals the higher percentage was their of copper followed by lead, nickel, chromium, molybdenum, cobalt and cadmium. Extensive research has been carried out to determine the amount of mineral elements in fenugreek, and results of **Magboul (1986)** were higher than our results with a value of calcium (158 mg/100g), phosphorous (415mg/100g), iron (22.5 mg/100g), sodium (493 mg/100g), magnesium (1550 mg/100g), potassium (1306 mg/100g), copper (331 mg/100g) and zinc (9.9 mg/100g). The levels of Cu, Fe, Mn and Zn were higher than the levels given by **Ozkutlu et *al.* (2007)** (9±0.6mg/kg), (36±3.6mg/kg), (8±1mg/kg) and (19±0.9mg/kg) respectively except Cd which was higher than our result (0.1±1.6 mg/kg). Fenugreek seeds are good source of minerals that helped in a number of physiological functions of body and maintains health status **(Im and Maliakel, 2008).** Although they are required in very low quantities because some trace elements heavy metals including iron, copper, zinc and manganese are essential micronutrients with one or more structural or functional roles for living organisms **(WHO, 1999).** The present study

showed that cumin contains potassium as major mineral followed by calcium, phosphorus, sulphur, magnesium, iron, zinc, boron, copper, lead, chromium, nickel, cobalt, cadmium, molybdenum respectively. **Al-Snafi (2016)** reported a very lower value compared to our results, potassium (35.8mg/100g) was being the most abundant element in cumin followed by calcium (18.6 mg/100g), phosphates (10 mg/100g), magnesium (7.3 mg/100g), sodium (3.4 mg/100g), iron (1.3mg/100g), manganese (0.1mg/100g), copper (0.1mg/100g), selenium (0.1mg/100g) and Zinc (0.1mg/100g). The contents of Cu, Fe, Mn and Zn in cumin reported by **Ozkutlu et *al.* (2007)** were lower with an amount of (8±0.3 mg/kg), (129±2.1 mg/kg), (14±0.8mg/kg) and (22±0.5 mg/kg) respectively except Cd which was higher (77±1.3 mg/kg). **National Research Council (1989)** indicated that the plants collected from rural areas or grown in less industrialized regions had lower contents of heavy metals than those growing in industrialized regions.

Statistically no significant difference was established concerning mineral profile of garlic and onion ($p > 0.05$), the mineral elements contained in these spices are very important in human nutrition. Sodium, calcium magnesium, and potassium play an important role in the regulation of blood pressure. These elements in particular were reported to have important inter relationships in the control of arterial resistance **(Altura and Altura, 1999).** Zinc and chromium are well known trace elements in diabetes as cofactors for insulin while calcium, magnesium and phosphorus are also essential for bone and teeth formation **(Okwu, 2005).**

The non-detection of Lead (Pb) and Cadmium (Cd) is of great advantage to consumers of these spices as these elements have been reported to be highly toxic even at low concentrations **(Oloyede, 2005).** On the other hand, there is no significant difference between fenugreek and cumin in term of all parameters ($P>0.05$) except cobalt ($P= 0.345$), Significant differences might be due to the great heterogeneity in the species studied, plant parts used and growing regions **(Ozkutlu et *al.*, 2007).**

### II.4 Phytochemical analysis

The results of extraction yield, total phenolic, total flavonoids, condensed and hydrolysable tannins content of plant extracts were summarized in **Table 38**.

The extraction yield (mass of extract/mass of dry matter) was used as an indicator of the effects of the extraction conditions. According to the findings, the extract yield of garlic using maceration method and methanol 70% as solvent was higher (62.87±0.50 %) than red onion (57.38±0.56%), fenugreek (17.66 %) and cumin (14.29 %).

**Table 38:** Results of extraction yield, total phenolic, total flavonoids, condensed and hydrolysable tannins content of plant extracts

| Analysis | Garlic extract | Red onion extract | Fenugreek extract | Cumin extract |
|---|---|---|---|---|
| **Extract yield (%)** | $62.51^{b}\pm0.50$ | $57.35^{a}\pm0.56^{***}$ | $17.66^{b}\pm0.033$ | $14.29^{a}\pm0.002$ |
| **TPC (mg GAE /100g DM)** | $45^{a}\pm1.00$ | $86^{b}\pm1.00^{***}$ | $115.3^{b}\pm0.01$ | $91.6^{a}\pm0.001$ |
| **TFC (mg QE /100g DM)** | $34.66^{a}\pm0.57$ | $43.33^{b}\pm0.57^{***}$ | $80.98^{b}\pm0.066$ | $66.04^{a}\pm0.15$ |
| **CTC (mg CE /100g DM)** | $6.8^{b}\pm0.34$ | $4.4^{a}\pm0.52^{**}$ | $2.2^{b}\pm0.01$ | $1.8^{a}\pm0.033$ |
| **HTC (mg TAE /100g DM)** | $0.05^{a}\pm0.01$ | $0.22^{b}\pm0.04^{**}$ | $1^{b}\pm0.045$ | $0.205^{a}\pm0.001$ |

**TPC:** Total Phenolic Content**; TFC:** Total Flavonoids Content; **CTC:** Condensed Tannins Content**; HTC:** Hydrolysable Tannins Content**; DM:** Dry Matter**; ***** Significant at 0.001 or 0.1% ; ****** Significant at 0.01 or 1%; **a, b** correspond to the homogeneous groups obtained by the post-hoc *Tukey* test for each parameter

In present study, the results showed that cumin extract expresses the higher phenolic content (115.3±0.01 mg GAE/100g DM) than fenugreek extract (91.6±0.001 mg GAE/100g DM), red onion extract (86±1.00 GAE /100g DM) and garlic extract (45±1.00mg GAE /100g DM).In addition for total flavonoid, the highest content was given by fenugreek extract (80.98±0.066 mg QE/100 g DM), as compared to cumin extract (66.04±0.15mg QE/100g DM), red onion extract (43.33 ±0.57mg QE/100 g DM) and garlic extract (34.66 ±0.57mg QE /100g DM).

In contrast to red onion (4.4 ±0.52 mg CE /100g DM), garlic has a higher value of condensed tannins (6.8 ±0.34mg CE /100g DM) and fenugreek showed the higher value of condensed tannins (2.2±0.01 mg CE/100g DM) in comparison with cumin (1.8±0.033mg CE/100g DM).For hydrolysable tannins, results were 0.05 ±0.01mg TAE /100g, 0.22 ±0.04mg TAE /100g, 1±0.045 mg TAE /100g and 0.205±0.001mg TAE /100g DM for garlic, onion, fenugreek and cumin respectively.

Our percentage yield of garlic extract was higher than previous studies findings **(Park and Chin, 2010), (Ali and mohsen sabri , 2014)** and **(Bhanot and Shri, 2010)** which were 2.46%, 6% and 7% respectively. According to **Kallel et *al.* (2014),** aqueous garlic extract has a higher percentage of extract yield (26.5%) than ethanolic and methanolic garlic extracts, which were 4% and 7% respectively.

Although, **Park and Chin (2010)** reported a percentage yield of 52.38% for red onion extract, however **Bhanot and Shri (2010)** reported a much lower percentage yield of 6.8%.

The result of percentage yield of fenugreek extract was approximately similar to **Abdouli et *al.* (2014)** result (17.09±1.43%) and higher in comparison with the result of **Sakhira et *al.* (2016)** which was 4.95 %. Although, the percentage yield of cumin extract was higher to the earlier research of **Megha et *al.* (2019)** with an amount of 15.93%, and extremely higher than result of **Elghorab et *al.* (2010)** which was 4.08±0.17%. **Thippeswamy and Naidu (2005)** observed that the high yield extract was given with methanol solvent.

Statistically, there was a significant difference between garlic and red onion (p=0.000), and between fenugreek and cumin (p=0.000). However, this difference can be due to variety diversity, growing conditions, ripening degree and climate **(Kaoru et *al.,* 2006).** Also, the particle size and shape of samples in extraction process are important factors that affect the yield extraction, another factor that may have affected differences in yield between garlic and red onion is sample pre-treatment **(Ali and Mohsen Sabri , 2014).** The highest extraction yield with aqueous solutions can be attributed to the addition of water, which increases the polarity of the solvents **(Kim et *al.,* 2004).**

The chemical composition of phytochemicals, the extraction method used, the particle size of the sample, the solvent used, as well as the presence of interfering substances affect the extraction yield **(Do et *al.,* 2014).**

This latter depends on the solvent with different polarities, extraction time, pH, temperature, and composition of the sample solvent and composition of sample are known as the most significant parameters, under the same extraction time and temperature **(Silva et *al.,* 2014).**

Similarly, this difference may be due to greater solubility in methanol than in other solvents of extractable bioactive components such as carbohydrates and proteins. The difference in extract yields may be due to the difference in solvent polarities used, which also plays a key role in increasing phytochemical compound solubility **(Silva et *al.,* 2014; Naima et *al.,* 2015).** Variations in the structure of phytochemical molecules also determine their solubility in solvents with different polarities **(Felhi et *al.,* 2017).**

The total phenolic content of garlic was approximately comparable to that found in many studies **(Chekki et *al.,* 2014)** and **(Jastrzebski et *al.,* 2007);** with 43.6 mg GAE /100g

and 49.3 mg GAE /100g respectively, while the present result was significantly higher than that reported by **(Nuutila et *al.,* 2003)** and **(Sarafa et *al.,* 2016)** with values of 11.5 mg GAE /100g and 0.42±0.02 mg GAE /100g respectively. However, the results found in the studies of **Lenkova et *al.*(2016), Park et *al.* (2009), Chekki et *al.* (2014)** and **Kallel et *al.* (2014)** were significantly higher with 105.1±18.09 mg GAE /100g, 562.6±1.93 mg GAE /100g, 500-4360 mg GAE /100g and 2283±1.69 mg GAE /100g respectively.

**Nuutila et *al.* (2003)** found that Giant onion had a total phenolic content of 84.5 mg GAE /100g, which was close to the current result. **Petropoulos et *al.* (2015)** result was lower in the range of 8.05-10.8 mg GAE /100g. Although several studies have been carried out to estimate the amount of total phenolic contents present in red onion; **Sarafa et *al.*(2016), Lu et *al.* (2011), Cheng et *al.* (2013), Skerget et *al.* (2009)** and **Singh et *al.*(2009)** found a higher result than our result, with amounts of 103±0.00 mg GAE /100g, 428 mg GAE /100g, 571±0.20 mg GAE /100g, 6362±2.03 mg GAE /100g and 38470±5.0 mg GAE /100g respectively. The high total phenolic content of red onion compared to garlic (p= 0.000) may be due to differences in the method of sample extraction (e.g. solvent used), wherever, these contradictory results are most likely due to differences in the methodology and the experimental conditions used in the different studies **(Nuutila et *al.*, 2003).**

Total phenolic compounds contents of fenugreek were significantly lower than those of **Kaviarasan et *al.*(2004), Sakhira et *al.*(2017), Abdouli et *al.*(2012), Idries Muhsan (2014)** and **Taha et *al.*(2004),** with values of 480 mg GAE /100g, 589±0.02 mg GAE /100g, 1260 mg GAE /100g, 2300 mg GAE /100g and 5430 mg GAE /100g respectively. However result of **Benziane et *al.* (2019)** study about aqueous extract of fenugreek was extremely lower than the present result 18.9 mg GAE /100g. While many studies have been carried out to estimate the amount of total phenolic contents present in cumin found a higher results than our result **Shan et *al.* (2005), Bettaeib et *al.* (2011), Liangliang et *al.* (2014), Thippeswamy and Naidu (2005), Bettaeib et *al.* (2012), Bettaeib et *al.* (2010), Aljuhaimi and Ghafoor (2013), Munuswamy and Ramachandiran (2014)** and **Elghorab (2010)** with an amount of 230 mg GAE /100g, 333-431 mg GAE /100g, 685mg GAE /100g, 900 mg GAE /100g, 1832±0.23 mg GAE /100g, 1920 mg GAE /100g, 2466 mg GAE /100g, 2950±0.58 mg GAE /100g and 3530 mg GAE /100g respectively.

In general, red onion had higher phenolic content than garlic; variations found between these two plants may be due to differences in their genetic composition and growing

conditions, which have a strong influence on the levels of phenolic compounds **(Soto et *al.,* 2016).**

The difference in phenolic composition between fenugreek and cumin (p= 0.000) could be due to method of extraction, plant organ, type of cultivar, time of harvest, conditions of storage, and genetic or geographical origin **(Lawrence, 2002).**

The season and sunlight duration are also known to affect the plant metabolism since some compounds may be accumulated at a particular time to respond to environmental changes **(Koenen, 2001).**

Several studies have also shown that solvent polarity contributes to substantially different phenolic compound extraction capacities in plants **(Parida et *al.,* 2004; Galvez et *al.,* 2005).**

The higher phenolic acid levels in methanolic extracts could be due to extraction of both non polar and semi polar soluble phenolic acids **(Thippeswamy and Naidu, 2005).**

Total flavonoids analysis revealed that garlic contains significantly more total flavonoids content than that reported by **Soto et *al.* (2016)** which was in the range of 7±0.007 - 11±0.02 mg QE/100 g. On the other hand, it was approximately similar to the findings of **Chekki et *al.* (2014)** and **Shuxia chen et *al.* (2013),** which were in the range of 0.42-59.5 mg QE/100 g and 7.5-67.5 mg QE/100 g, respectively. **Kallel et *al.* (2014), Sarafa et *al.* (2016)** and **Moumen et *al.* (2016)** found an increased amount; 60 mg QE/100 g, 113±0.01 mg QE/100 g, and 1521±0.93 mg QE/100 g respectively.

Significant variations in total flavonoids content were also found in red onion compared to previous studies of **Soto et *al.* (2016)** and **Abuga (2014)** with values of 8±0.008-18±0.033 mg QE/100 g, and 10±0.69 mg QE/100 g, respectively. Other researchers, **Cheng et *al.* (2013), Sarafa et *al.* (2016), Skerget et *al.* (2009)** and **Singh et *al.* (2009)** found higher contents ; 165.8±0.41 mg QE/100 g, 366±0.01 mg QE/100 g, 1376±0.41 mg QE/100 g and16520±3.2 mg QE/100 g, respectively.

Total flavonoids analysis expressed that the amount of total flavonoids contents in fenugreek were higher than the amounts of **Abdouli et *al.* (2012), Abdouli et *al.* (2014)** and **Bukhari et *al.* (2008)** which were 0.77mg QE/100 g, 31.8 mg QE/100 g and 20.8-65.3mg QE /100g respectively, however, **Sakhira et *al.* (2017), Rahmani et *al*. (2018) and Yaser et *al.* (2013)** revealed a raised amount: 136 to 274 mg QE /100g, 145 mg QE /100 g , 377.8 mg QE/100 g and 4990 mg/100g respectively. Regarding cumin, significant differences in total

flavonoids contents were remarked relatively to previous study of **Rebey et *al.* (2011)** with a value of 56 mg QE /100g for Tunisian cumin seeds and 88 mg QE /100g for Indian cumin seeds; While our amount was significantly higher than that showed by **Munuswamy and Ramachandiran (2014)** (15.1mg QE/100 g). However other researchers, **Zhang et *al.* (2014); Rebey et *al.* (2012)** and **Deepshikha Gupta (2013)** found a higher contents 102 mg QE/100 g ,560 mg QE/100 g and 4656 mg QE/100 g respectively.

TPC and TFC variability in garlic can be due to a numerous cultivar characteristics, but clove size must be taken into account because it has an indirect effect on the final concentration of phenolic compounds **(Lu et *al.,* 2011).** Different garlic cultivars had different phenolic contents, according to previous study **(Chen et *al.,* 2013).** The present data revealed a highly significant difference in total flavonoids between the two plants (p=0.000), which can be explained by several factors, including experimental parameters and natural qualitative and quantitative variability in the raw material **(Chen et *al.,* 2013).**

The significant differences between plants (p= 0.000) in the term of flavonoids were explained by several factors such as genotypic and environmental differences within species **(Srinivasan, 2005),** choice of parts tested **(Kumar, 1997),** time of taking samples **(Gao et *al.*,2000)** and determination methods **(Pizzale et *al.,*2002).**

The presence of condensed tannins in garlic agreed with the report of **Nwinuka et *al.* (2005)** and **Sarafa et *al.* (2016)** with a significant differences; 0.01±0.0mg CE/100g, 0.82±0.01mg CE/100g respectively. **Moumen et *al.* (2016)** observed that garlic methanolic extract showed the highest amount of condensed tannins 3.01±0.39 mg CE/100g compared to aqueous and ethanolic extract; 1.35±0.5mg CE/100g and 0.69±0.2 mg CE/100g respectively.

Furthermore, a lower condensed tannins content was recorded in red onion in comparison with garlic (p=0.003), the present result was similar to **Abuga (2014)** result; 4.99±0.06 mg CE/100g , higher to **Nwinuka et *al.* (2005)** result; 0.01±0.01 mg CE/100g and lower to **Sarafa et *al.* (2016)** result; 9.82±0.02 mg CE/100g.

The presence of condensed tannins in fenugreek accord with the results of **Abdouli et *al.*(2014), Abdouli et *al.* (2012) and Almaamari et *al.* (2016)** with a significant differences 2.3 mg CE/100g, 0.29 mg CE/100g, and 0.78 mg CE/100g**. Rahmani et *al*. (2018)** found a higher value (73±0.013-105.1±0.030 mg CE/100mg), although, **Benziane et *al.,* (2019)** observed that fenugreek aqueous extract showed a highest amount of condensed tannins 8.69 mg CE/100g. The level of the present results were lower to that (380 mg/100g DM) reported

in Iranian fenugreek seed genotype (**Naseri et *al.* (2013)** and hugely lower than (2000 mg/100g) reported in Yemen genotype **(Yaser et *al.* 2013).** Measured levels in fenugreek seeds were far below the threshold level (5000 mg/100g DM) mentioned by **Muller-Harvey, (2006).** Besides, a lower condensed tannins content was recorded in cumin in comparison with fenugreek, the present result was lower to **Rebey et *al.* (2010)** and Zhang et *al.* (2014) results 200mg CE/100g, 4228 mg CE/100g, and extremely lower than **Rebey et *al.* (2011)** with an amount of 6571 mg CE /100g for Tunisian cumin seeds and 6137 mg CE /100g for Indian cumin seeds. Cumin is recongnized to contain large amount of tannins **(Uma Pradeep et *al.,* 1993).**

The differences in the term of condensed tannins may be attributed to genetic and climatic factors rather than storage time, processing and extraction methods **(Sarafa et *al.,* 2016).** Condensed tannins are water-soluble phenolic metabolites commonly found in almost all plants parts **(Kunyanga et *al.,* 2014).**

For hydrolysable tannins contents, there was a significant difference between these two plants ($p=0.002$), these findings suggest that the level of hydrolysable tannins is greatly influenced by tissue type, solvents (different polarities), and extraction conditions **(Saleha, 2019).** The difference in findings ($p= 0.000$) may be due to extraction methods and solvents used **(Uma Pradeep et *al.,* 1993),** to different cultivars, growing conditions, maturity stage at harvest, storage conditions and sample preparation method **(Mashkor, 2014).** In contrary to hydrolysable tannins contents, there is no substantial difference between these two plants.

**II.5 Phytochemical screening**

The results of the qualitative assay of samples were shown in **Table 39** and **Annexe 5**. They revealed the presence of flavonoids, tannins, terpenoids in garlic, as well as anthocyanins and cardiac glycosides in red onion, fenugreek and cumin extracts with moderate difference. On the other hand, reducing compounds, alkaloids, sterols, triterpenes and saponosides were absent in the tested extracts.

The Results of the phytochemical screening of methanolic extracts of the samples did not concur with **Gazuwa et *al.* (2013)** data, who reported the absence of tannins, saponins and phenolics in red onion and garlic. The presence of flavonoids and tannins in garlic and red onion agreed with the report of **Nwinuka et *al.* (2005)** but contradicted **Green et *al.* (1997)** result.

**Table 39:** Results of phytochemical screening of plant extracts

| Extract / Analysis | Garlic | Red onion | Fenugreek | Cumin |
|---|---|---|---|---|
| **Flavonoids** | + | ++ | ++ | +++ |
| **Tannins** | + | +++ | ++ | +++ |
| **Alkaloids** | - | - | - | - |
| **Sterols and triterpenes** | - | - | - | - |
| **Terpenoids** | ++ | +++ | ++ | +++ |
| **Saponosides** | - | - | - | - |
| **Anthocyanins** | - | ++ | + | ++ |
| **Cardiac glycosides** | - | + | ++ | +++ |
| **Reducing compounds** | - | - | - | - |

(-): absent ; (+): low presence; (++): medium presence; (+++); high presence

The results of the phytochemical screening of methanolic extracts of fenugreek did not exclusively agree with the report of **Asmena et *al.* (2009)** which shows the absence of flavonoids, tannins and cardiac glycosides, even the presence of alkaloids steroids and carbohydrate. However, **Sumaya et *al.* (2012)** assent with our study, and found many secondary metabolites in fenugreek extract as like as flavonoids, tannins, phenols, carbohydrate, glycosides, anthocyanin and terpenoids. Further, **Rodolfo et *al.* (2006)** and **Rahmani et *al.* (2015)** studies showed the presence of flavonoids, steroids alkaloides, and saponins in fenugreek extract. **Gorinstein et *al.* (2009),** also reported the presence of terpenoids, tannins and absence of anthocyanin.

A recent study of **Megha et *al.* (2019)** concerning qualitative analysis of cumin registered a moderate presence of alkaloids, flavonoids, steroids, carbohydrate, phenol and terpenoids, whereas tannins, saponins, protein, glycosides and cardiac glycosides were reported to be absent in this plant extract. Furthermore, **Himanshu et *al.* (2014)** reported the richness of cumin extract with alkaloids, glycosides, flavonoids, tannins, terpenoids and phenolic compounds which concur with our results.

This implied that the studied spices are potential sources of phytochemicals, many of which have been confirmed to have medicinal activity as well as physiological activity **(De**

**and James, 2002).** However, the presence of these vital chemical substances supported the observation of **Pandey (1980)** that plants have some vital chemical substances (alkaloids, carbon compounds, glycosides, tannins and others).

Qualitative and quantitative analysis of important individual phenolics in the spices may help to reveal the structure-activity relationships of antioxidant phenolics and to explain the relationships between total antioxidant activity and total phenolic contents **(Shan et *al.*, 2005).**

**II.6 Chromatographic analysis (HPLC)**

The molecular separation of garlic, red onion, fenugreek and cumin methanolic extracts was achieved by HPLC at three wavelengths: 254nm, 326nm and 360nm. The findings obtained are visible in the peaks and retention time of chromatograms of each molecule. The results obtained are shown in the chromatograms with peaks and retention time of each molecule **(Fig. 28–31).**

HPLC results revealed the presence of five components in red onion extract **(Fig. 28)**, one component in garlic extract **(Fig. 29),** fifteen compounds in cumin extract **(Fig. 30)** and eight compounds in fenugreek **(Fig. 31)**,The identification of molecules found in the samples is based on comparing their retention times (Rt) with that of pure standards under the same experimental conditions (**Tab. 40**).

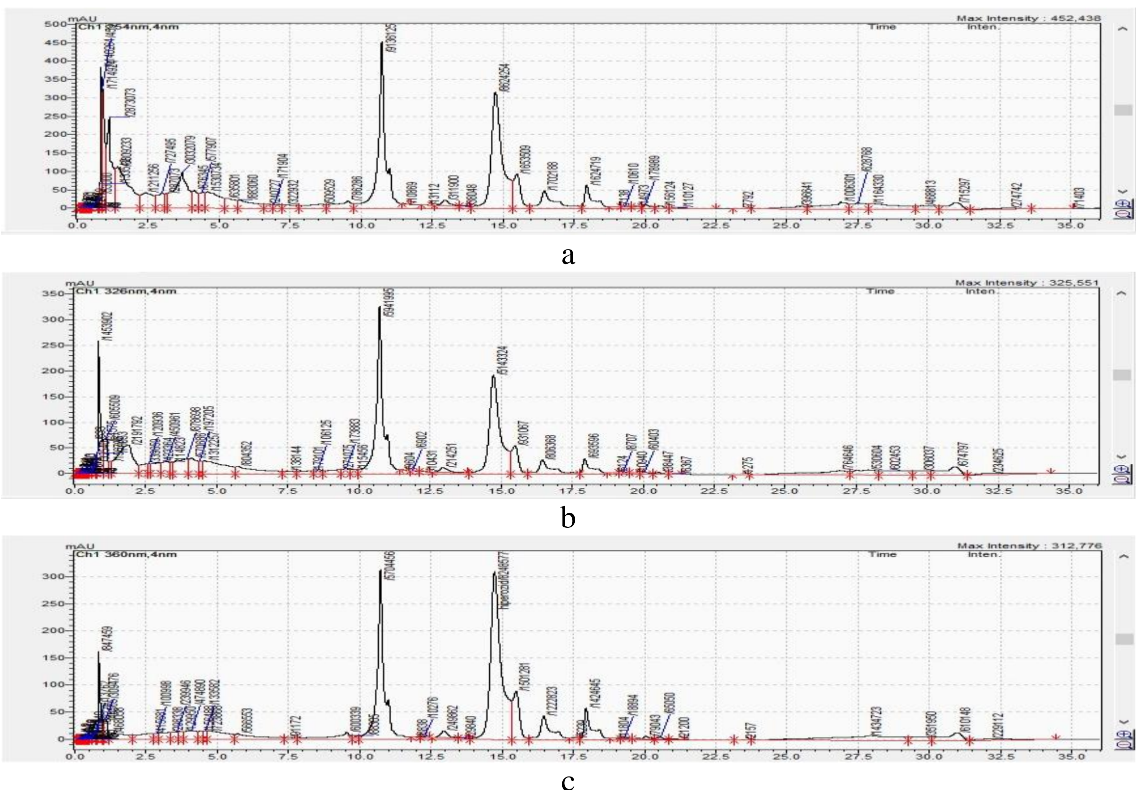
Chromatographic analysis of the samples identified five phytochemical molecules for red onion extract namely: gallic acid, quercitin, rutin, hyperoside and karempferol and one molecule for garlic extract which is gallic acid. On the other hand eight phytochemical compounds could be identified in cumin extract, namely: caffeic acid, isoquercetin, vanillic acid, myricetin 3-0, rutinoside, syringaresinol, citrusine, rosmarinic acid, p-coumaric acid. Seven compounds of fenugreek extract are: gallic acid, sinapic acid, caffeic acid, asterogenic acid, pyrogallol, hyperoside and ferulic acid. The rest of the compounds that appeared on the chromatograms could not be identified.

The polyphenols separated from the red onion extract at retention times of 3.137 min and 3.687 min are of the tannin class, probably Gallic acid derivatives, according to the spectra and absorption maxima.

Flavonoids are isolated from the same extract at retention times of over 10 min, with the ones from 14.734; 16.451; and 17.967 min being probably Quercetol derivatives with maximum absorption at over 350 nm. Among the majority flavonoids in the red onion extract,

the flavonoid from the minute 14.734 represents 48.7%. The flavonoid from minute 10.728 represents 26.5 %, with the rest being in the proportion of less than 10%. There are not many polyphenols in the garlic extract. The only observable component of minute 5.904 is in very low concentration.

Under the same experimental conditions, a comparison of the retention times (Rt) of molecules found in the samples with those of pure standards identified five compounds in the methanolic extracts of red onion (Gallic acid, Quercitin, Rutin, Hyperoside, and Karempferol), as well as one compound in garlic (Gallic acid) and two other compounds that could not be identified.




a

b

c

**Figure 28:** HPLC chromatogram of red onion dry extract at 254 nm, 326 nm and 360 nm (a: 254 nm, b: 326 nm, c: 360 nm)

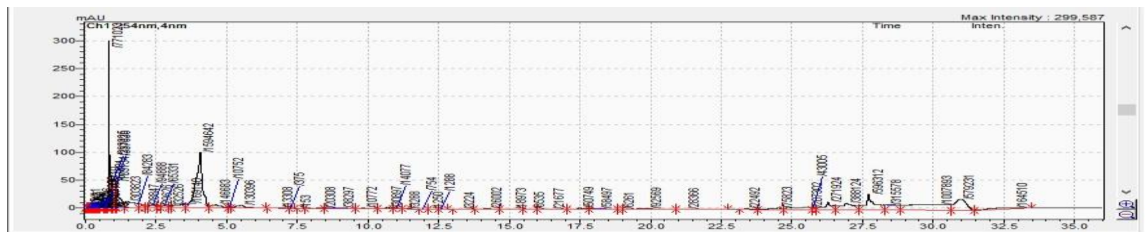
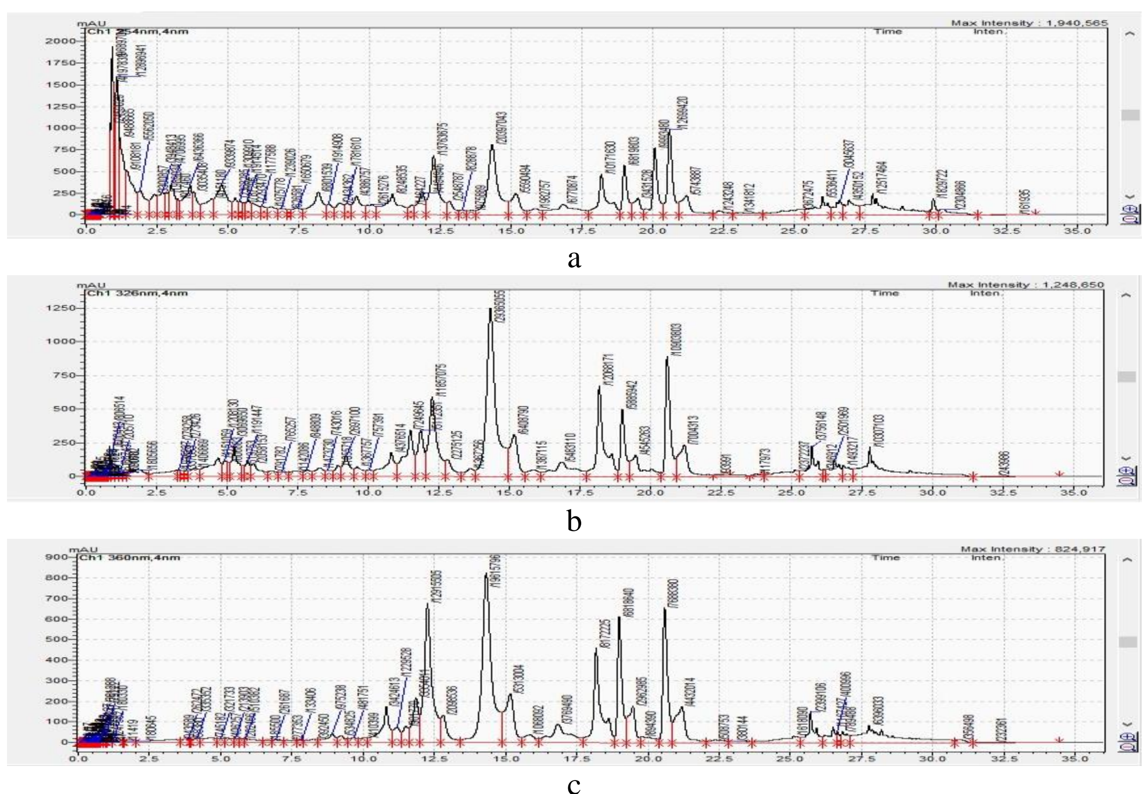



**Figure 29:** HPLC chromatogram of garlic dry extract at 254 nm

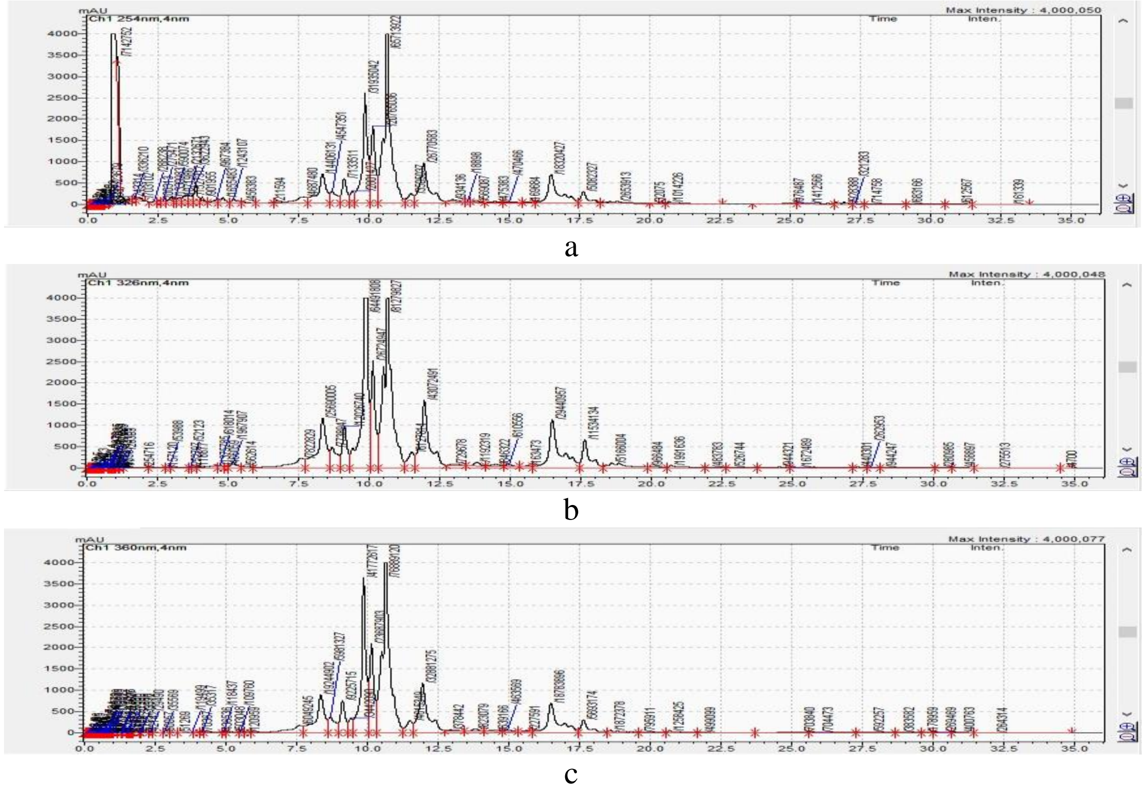



a

b

c

**Figure 30:** HPLC chromatogram of cumin dry extract at 254 nm at 326 nm and 360 nm (a: 254 nm, b: 326 nm, c: 360 nm)




a

b

c

**Figure 31:** HPLC chromatogram of fenugreek dry extract at 254 nm, 326 nm and 360 nm (a: 254 nm, b: 326 nm, c: 360 nm)

In the cumin extract, it is observed the separation of flavonoids at retention times between 10 and 20 min, but which are not derived from quercetin, having absorption maxima between 330 and 345 nm. At minute 20.030, a polyphenol appears that seems to be of the tannin class, probably a complex or condensed tannin. Tannins also appear at 4.864 min and 7.881 min, respectively. At retention times of more than 20 min, polyphenols appear that seems to be of the coumarin class. Cumin extract appears to be the richest in polyphenols.

Among the flavonoids, those from 14.322 min and 12.280 min respectively, that are the majority, representing 30.5% and 20.8%, respectively, of the total of the most important polyphenols. The rest are under 11%.

**Table 40:** Polyphenolic compounds of plant extracts analyzed by HPLC

| Extract | Compound | Retention time (min) |
|---|---|---|
| Red onion | Gallic acid | 3.137 |
| | Unknown | 3.687 |
| | Quercitin | 10.728 |
| | Rutin | 14.734 |
| | Hyperoside | 15.490 |
| | Unknown | 16.451 |
| | Karempferol | 17.967 |
| Garlic | Gallic acid | 5.904 |
| Cumin | / | 4.684 |
| | / | 7.881 |
| | / | 10.812 |
| | Caffeic acid | 12.280 |
| | / | 14.322 |
| | Isoquercetine | 15.165 |
| | / | 15. 857 |
| | Vanillic acid | 15.835 |
| | / | 18.179 |
| | Syringaresinol | 19.007 |
| | / | 19.487 |
| | Myricetine 3-0 pentoside | 20.030 |
| | Citrusine | 20.602 |
| | Rosmarinic acid | 21.197 |
| | P-Coumaric acid | 25.719 |
| Fenugreek | Gallic acid | 3.115 |
| | Sinapic acid | 7.577 |
| | Caffeic acid | 9.838 |
| | Asterogenic acid | 10.146 |
| | / | 10.657 |
| | Pyrogallol | 11.957 |
| | Hyperoside | 16.480 |
| | Ferulic acid | 17.647 |

/: Unidentified compound

In the case of fenugreek extract at 3.115 min a tannin is separated, probably a gallic acid derivative, then at minutes 7.577, 10.146, 10.657 and 11.957 flavonoids with maximum absorption between 330 and 340 nm, and at over 15 minutes polyphenols that seems to be from coumarin class. At minute 9,838, a polyphenol with a spectrum specific to caffeic acid derivatives appears. Among the majority flavonoids, the predominant quantity is the one from minute 10.657, this being in proportion of 47.3%, and the component separated at 11.957 min in proportion of 26.9%.

Previous study concerning characterization of secondary metabolites in red onion observed the presence of Quercetin , Protocatechuic acid , Spiraeoside, Tyrosine, Vanillic acid and Hydroxybenzoic acid **(Lachman et *al.,* 1997).** Afterwards, **Lachman et *al.* (2002)** found that phytochemical characterization of different cultivars of onion (red, yellow and white) revealed the presence of six phenolic compounds with Spiraeoside, Rutin and Quercetin as major constituents, as well as three other unidentified compounds. Different onion varieties (Nirvana, DPS 1032, Yellow 2025, King-Midas, and SBO 133) are one of the highly rich sources of main flavonols, Quercetin **(Sellappan and Akoh, 2002).** In contrast to other vegetables, onions have a 5–10 times higher overall Quercetin content (347 mg/kg). The most common flavonol, Quercetin, is present in both bound and free forms **(Leighton et *al.,* 1992).**

Quercetin-monoglycosides spiraeoside (4´-O-β-D-glucoside), 3-O-β-D-glucoside, 3´-O-β-D-glucoside, and 7-O-β-D-glucoside are very highly manifested **(Ioku et *al.*, 2001).** There are also kaempferol-glycosides present at minor amounts 3,4´-O-β-D-diglucoside, 7,4´-O-β-D-diglucoside, 3-O-sophoroside-7-O-β-D-glucuronide, 4´-O-β-Dglucoside. Another type of flavonols – isorhamnetin – is present only in yellow and red cultivars of onion in both free and bound form in glycosides as: 3,4´-O-β-D-diglucoside, 4´-O-β-D-glucoside and 3-O-β-D-glucoside **(Park and Lee, 1996).**

Eleven major phenol compounds were identified in peel and skin of some onion cultivars (Donna, Barito and Hy Park): Quercetin-3,7,4-triglucoside, p-hydroxybenzoic acid, Quercetin-7,4-diglucoside ,Vanillic acid, Quercetin-3,4-diglucoside, Quercetin-3-O glucoside, Kaempferol-3-O-glucoside, Isorhamnetin-3-O-glucoside, Quercetin-4-O-glucoside, Quercetin and Kaempferol **(Burri et *al.,* 2017).**

Many studies on the phenolic profile of different onion cultivars reported that the only phenolic compound found in detectable quantities was Quercetin **(Hertog et *al.,* 1992; Miean**

**and Mohamed (2001); Sultana and Anwar, 2008; and Zill-e-Huma et *al.*, 2011).** Quercetin and Gallic acid were the two phenol compounds found in the hydrolyzed extract of garlic and onion **(Soto et *al.*, 2016).** Our results obtained for garlic cultivars were close to those of **Sultana and Anwar (2008),** who found no detectable amounts of Quercetin and Kaempferol.

Numerous study about characterization of secondary metabolites in cumin observed the presence of coumaric acid, luteolin, syringic acid, cinnamic acid for Tunisian cumin seeds and trans-2 dihydrocinnamic acid and flavone for Indian cumin seeds **Rebey et *al.* (2011)**. Eventually, **Rebey et *al.* (2012)** found that phytochemical characterization of cumin revealed the presence of eighteen phenolic compounds including gallic acid, caffeic acid, dihydroxyphenolic acid, dihydroxybenzoic acid, chlorogenic acid, syningic acid, vanillic acid, coumaric acid, ferrulic acid, rosmarinic acid, cinnamic acid, flavonoid, leutolin, catechin, coumarin, apigenin, amentoflavone and flavone. Also, **Shan et *al.* (2005)** demonstrated that bioactive compounds present in cumin were phenolic acids, flavonoids, coumarins, caffeic acid, kaempferol and others compounds which weren't determined. **Ani et *al.* (2006)** could identified some phenolic compounds in cumin seeds such as gallic, caffeic, ellagic protocatechuic, ferulic acids and also flavonols such as quercetin and kaempferol.

Concerning phenolic profile of fenugreek, some major components were previously found in fenugreek ethanolic extract such as catechin, epicatechin, gallic acid, caffeic acid, coumaric acid, cinnamic acid, vannilic acid **(Sakhira et *al.*, 2017)** and others in the methanolic extract gallic acid ,chlorogenic acid ,p-coumeric acid ,ferulic acid, sinapic acid and quercetin **(Pasha et *al.*, 2017).**

Four phytochemical compounds were found in fenugreek aqueous maceration extract namely: genistein, kaempferol, vanillin and myrecitin, while three compounds were identified in aqueous decoction extract namely: kaempferol, rutin, and vanillin **(Benziane et *al.*, 2019).**

Also, **Swati et *al.* (2014)** exhibit that characterization of phenolic compounds present in fenugreek extract by HPLC could identify seven contents: vitexin, isovitexin, kaempferol dirhamnoside, kaempferol rhamnoside, quercitin, leuteolin and apigenin. Other research observed that flavonoid glycosides and kaempferol were the two major phenolic compounds found in the aqueous extract of fenugreek **(Benayad et *al* ., 2014).**

The significant difference in the phenolic profile obtained by HPLC between plant extracts confirmed the previous results of total phenolic and flavonoids content. These

differences may be due to many factors including genotype, maturity stage, growing and climate conditions, harvest period and even post-harvest conditions **(Chun et *al.,* 2006).** Furthermore, the results of the characterization by HPLC depend on column's separating strength, flow velocity, and mobile phase composition **(Johnson et *al.,* 2011).**

### II.7 Antioxidant activity

The results of the antioxidant activity of plants extracts carried out by DPPH radical scavenging activities were summarized in **Table 41.** They showed that methanolic extract of red onion had the strongest radical-scavenging effect compared to garlic methanolic extract.

**Table 41:** Results of evaluation of antioxidant activity of plant extracts

| Plant extract | Extract concentration (µg/ml) | Inhibition (%) | IC 50 (µg/ml) |
|---|---|---|---|
| **Garlic** | 1000 | 54.85 | $919.87^{b}\pm4.43$ *** |
| | 500 | 25.31 | |
| | 250 | 14.76 | |
| | 125 | 02.10 | |
| **Red onion** | 1000 | 80.79 | $420.9^{a}\pm5.00$ |
| | 500 | 68.31 | |
| | 250 | 40.19 | |
| | 125 | 24.35 | |
| **Fenugreek** | 1000 | 82.57 | $343.75\ ^{b}\pm4.73$ *** |
| | 500 | 80.59 | |
| | 250 | 39.20 | |
| | 125 | 35.84 | |
| **Cumin** | 1000 | 68.51 | $588.55\ ^{a}\pm5.55$ |
| | 500 | 55.04 | |
| | 250 | 24.15 | |
| | 125 | 22.17 | |

*** Significant at 0.001 or 0.1% ,** Significant at 0.01 or 1%, **a, b** correspond to the homogeneous groups obtained by the post-hoc *Tukey* test for each parameter

The results of our study showed that free radical scavenging activity of garlic was lower than red onion. In terms of IC50, red onion had the lowest value (420.9±0.01 µg /ml), followed by garlic (919.87±0.01 µg/ml). However, these findings clearly show that red onion has more capacity to scavenge the free radicals compared to garlic (p=0.000).

Our study showed that free radical scavenging activity of cumin was lower than fenugreek. Regarding IC50, the lowest value was observed by fenugreek (343.75 ±0.01 µg/ml), followed by cumin (588.55 ±0.01 µg/ml). While, the results establish that fenugreek has more ability to scavenge the free radicals as compared to cumin.

**Che et *al.* (2011)** found that garlic extract has a similar IC50 to the current result with an amount of 0.95±0.01 mg/ml, however other researchers revealed a lower radical scavenging activity **(Nuutila et *al.,* 2003**:IC50 1000 mg/ml), **(Moumen et *al.,* 2016**: IC50 8.36mg/ml ), **(Lenkova et *al.,* 2016**: 17.17%±0.634) and **(Fredotović et *al.*, 2017**: IC50 82.64 mg/ml ), while **Kallel et *al.* (2014)** study showed a higher radical scavenging activity of garlic: (IC50 0.64 mg/ml). Regarding red onion, previous studies showed that its radical scavenging activity was higher **(Nuutila et *al.,* 2003**: IC50 67 mg/ml) and **(Fredotović et *al.,* 2017**: IC50 77.13mg/ml ).

Similarly, according to **Benkeblia (2005),** garlic has higher free radical scavenging activity than red onion. Similar research conducted in other plants and fruits have shown that high radical scavenging activities are commonly associated with high TPC. For instance, **Lim et *al.* (2006)** reported that high phenolic content in extracts led to high radical scavenging activity. Several other studies have shown that phenolic compounds contribute to high radical scavenging activity. **Mohd et *al.* (2006)** suggested that free radical scavenging activity is not due to the phenolics only.

In contrast to our results, **Miller et *al.* (2000)** found that garlic has a six-fold higher antioxidant activity than onion. The difference is probably at least partially due to the different methods used. **Miller et *al.* (2000)** extracted the fresh vegetables using 50% methanol whereas, in our study, 70 % methanol was used for extraction. The high antioxidant activity of *Alliums* and especially high DPPH radical scavenger of garlic was reported by numerous investigators **(Velioglu et *al.,* 1998; Yin and Cheng, 1998).** However, DPPH radical scavenger activity depended on both phenolics and sufur compounds of *Alliums*. On the other hand, **Nuutila et *al.* (2003)** reported that the lowest antioxidant activity was detected in garlic. According to **Benkeblia (2005)** garlic extract reacted faster than other extracts and was the most effective DPPH radical scavenger, followed by purple, red and yellow onion extracts, while green onion extract showed the lowest DPPH radical scavenger.

**Sakhira et *al.* (2017)** observed that fenugreek extract own a similar IC50 our result with a concentration of 285,59±2.01 µg/ml with a higher radical scavenging activity, however other researchers present a lower radical scavenging activities such as **Mashkor (2014)** : 65-68% and **Rababah et *al.* (2004)** : 10%). Regarding cumin, previous study showed that radical scavenging activity of cumin was similar to our result like **Thippeswamy and Naidu (2005):** IC50 520 µg/ml, while others present a higher activities such as **Rebey et *al.* (2011):** 6.24 µg/ml for Tunisian cumin seeds and 15.14 µg/mL for Indian cumin seeds, **Rebey et *al.***

**(2012):** 20.17 µg/ml and **Zhang et *al.* (2014):** 102.42 µg/mL. **Aljuhaimi and Ghafoor (2013)** and **Hinneburg et *al.* (2006)** studies gave a lower activities with IC50: 825-1124 µg/mL and 2000 µg/ml respectively.

Previous study has suggested that garlic contains phenol, flavonoid, and various sulfur compounds such as disulfide (hydrophobic), and S-ally-(L)-cysteine (SAC, hydrophilic), this latter has high radical scavenging activities **(Colin-Gonzalez et *al.,* 2012).** The number of phenolic compounds and flavonoids has positive correlation with DPPH radical scavenging activities, which is due to hydrogen and electron donation from hydroxyl groups of these compounds compounds **(Rice-Evans et *al.,* 1996).**

In addition, the major differences in antioxidant activity between these two plants (p= 0.000) are primarily due to the difference in the polarity of the solvents used and therefore to the different effects of extractability on the antioxidant compounds **(Djeridane et *al.,* 2006; Maisuthisakul et *al.,* 2007).**

It is widely agreed that the antioxidant potentail of phenolic compounds is often linked to the chemical composition of individual compounds, depending on a variety of factors, including geographical variation **(Xi et *al.,* 2014),** harvest time **(Fang et *al.,* 2011),** environmental and agronomic conditions **(Liu et *al.,* 2016),** plant botanical components **(Bessada et *al.,* 2016),** and methods of extraction **(Kurihara et *al.,* 2003).**

The literature presents ample evidence for the biological and biomedical activities of cumin including its use as a treatment of a variety of diseases, such as chronic diarrhoea and dyspepsia, acute gastritis, diabetes, and cancer which have generally been ascribed to its bioactive constituents such as phenols and flavonoids. Although, several animal studies and the clinical data show that the use of fenugreek seeds can be useful in lowering cholesterol and blood glucose level. It has been found that compounds present in fenugreek extracts increases bile secretion and a reduction in blood cholesterol, also administration of extracts from fenugreek seeds has a beneficial effect on blood glucose level as it was confirmed by many studies performed in animals and in humans, it seems that such activity of compounds contained in fenugreek seeds is beneficial for people struggling with concomitant diseases in the metabolic syndrome. In this context, the present study allowed to identify bioactive compounds which are economically important as drugs (pharmaceuticals) in medical field **(Mnif and Aifa , 2015).**

## II.8 Results of isolation and identification of *H. pylori*

### II.8.1 Results of macroscopic and microscopic observation, biochemical identification and antibiogram

After 5 days of incubation at 37°C in a microaerobic atmosphere, the results showed the appearance of small colonies 1 to 2 mm in diameter **(Fig.32 a).** The colonies are grayish or transparent in colour, shiny, round and have a regular outline. Our results are similar to those found by **Medouakh et *al.* (2006)**, which confirms the morphological characters of *H. pylori*.

Microscopic observation in the fresh state demonstrated that *H. pylori* is a small curved, mobile bacillus **(Fig.32 b).**These results are identical to those found by **Rad et *al.* (2007).**

The Gram staining performed from the colonies that have appeared revealed the presence of Gram-negative bacteria **(Fig.32 c). Medouakh et *al.* (2006)** obtained the same results which confirms that it is *H. pylori*.

The results of the biochemical tests confirmed that the strain has positive urease, catalase and oxidase and therefore significant enzymatic activity **(Fig.32 d-f).** The results of the biochemical tests obtained are also found by **Medouakh et *al.* (2006),** which confirms that it is *H. pylori.* Also the identification was confirmed by the search for other biochemical characters using API 20 Campy **(Fig.32 g)**.

The results of the antibiogram and E test obtained are illustrated in **figure 32 (h-i)** and **table 42.**

The study of the sensitivity of *H. pylori* to antibiotics confirmed excellent results compared to the activity of the most antibiotics used. The antibiogram results obtained for *H. pylori* **(Tab. 42)** showed that it was sensitive to Gentamycin, Erythromycin, Doxycyclin and Metronidazol. On the other hand, it was resistant to Oxacillin, Amoxicillin, Ciprofloxacin and Fusidic acid. The E test, which is a technique of diffusion in an agar medium making it possible to determine the sensitivity and resistance of bacteria to antibiotics and measure the MIC of an antibiotic showed that *H. pylori* strain was resistant to Amoxicillin. The studies of **Rochard (2000),** indicated that *H. pylori* was sensitive to the following antibiotics: Erythromycin, Gentamycin, Tetracycline and Amoxicillin. Also *H. pylori* was sensitive to Tetracycline and Amoxicillin (**Megraud and Lehours, 2007).** The results obtained by **Tabak and Bensoltane (2012)** observed that *H. pylori* is sensitive to Penicillin, Gentamycin, Amoxicillin, Erythromycin, Chloramphenicol, and Tetracycline.

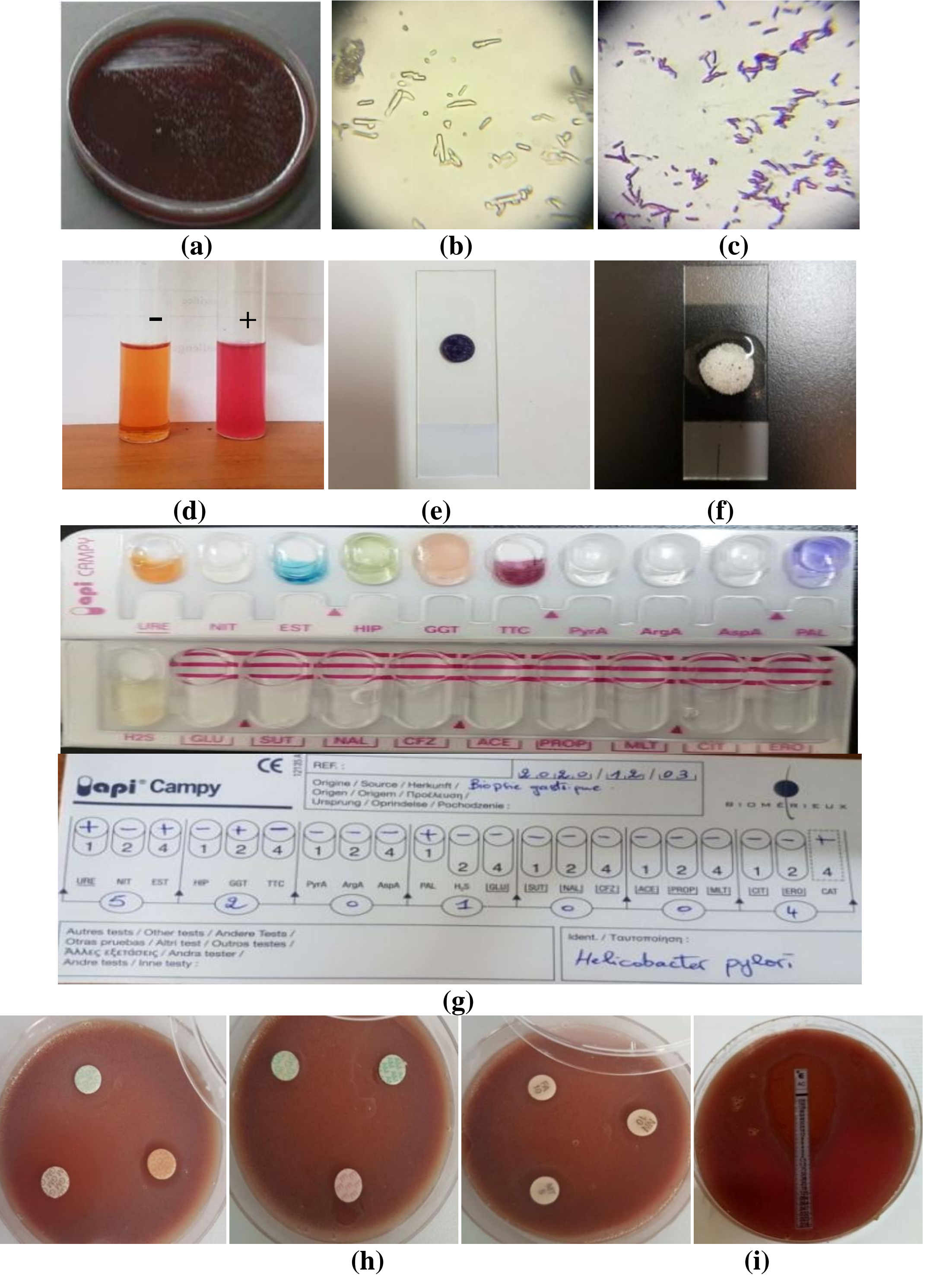



**Figure 32:** Results of isolation and identification of *H. pylori*

(a): Macroscopic observation, (b): Microscopic observation, (c): Gram staining, (d): Urease test, (e): Oxidase test, (f): Catalase test, (g): Results of API Campy, (h): Antibiogram, (i): E Test.

According to **Van Zwet et *al.* (1998),** rare cases of strains resistant to amoxicillin have been reported. They are linked to point mutations in the gene encoding PLP (proteolipid protein) causing a blockage of antibiotic transport. The study of **Liu et *al.* (2008)** showed that some strains can develop efflux pumps, especially with Clarithromycin, Metranidazole and Tetracycline. So it is important for any infection to test the susceptibility of *H. pylori* strain against those antibiotics to justify their uses in the therapeutic field **(Bardhan et *al.,* 2000).**

**Table 42:** Results of Antibiogram test against *H. pylori*

| Antibiotic | DZI (mm) |
|---|---|
| Gentamycin | 13 S |
| Oxacillin | 08 R |
| Doxycyclin | 12 S |
| Erythromycin | 11 S |
| Amoxicillin | 08 R |
| Ciprofloxacin | 06 R |
| Nanaomycin | 09 S |
| Fusidic Acid | 07 R |
| Metronidazol | 12 S |
| E Test (CMI) (µg/ml) | 1.5 |
| S: Sentitive, R: Resistant | |

### II.8.2 Results of identification of *H. pylori* by PCR

Real-time PCR assay for the identification of *H. pylori* isolated strain showed amplification curves for the targeted gene (ure C)**.** A 294 bp fragment was obtained in all *H. pylori* isolates after amplification of the ure C gene **(Fig. 33).**

*Helicobacter pylori* isolated from biopsy samples of gastric ulcer patients was identified by different tests as described above. The detection of the ure C gene of *H. pylori* strain was further confirmed by PCR amplification of ure C gene specific to strain of *H. pylori*. Our results of PCR were similar to previous study of **Lage et *al.* (1995)** that showed the amplification of curves for the targeted gene (ure C).

E1: Sample 1.

T+: Positive control (294 bp);

T-: Negative control (water);

M: Size marker (100-3000bp) (Promega. Madison.Wis.USA).

99% *H. pylori* HUP-B14

**Figure 33:** PCR-amplified products of the Ure C gene of *H. pylori* visualized by Gel Red TM at 1.5% of agarose gels analyzed by electrophoresis

Complete genome (HP) Sequence ID: CP003486.1 >TA3_P.UR --16..270 of sequence CAAACCATCGCCGGTTTTAGCGTAATCGCTAAAAATGATATGCCCGCTTTGCTCG CCTCCAAAATTGGCTTTATTCAATTGCATGCATTCGCTCACAAACTTATCCCCAAT CGCGCAATGCTTCAATTCTAAATCTTGGGATTTTAAGTATTCTTTAAGGGCTAAAT TACTCATGTTTGTAGCGACAATTGCTTGAGAAGAAAGGGCGTTTTTAGATTTTTGA TAAACCCCTAACACCCCTAAAAGCTTCACCCG.

### II.8.3 Results of revivication and confirmation of probiotics

The results of macroscopic and microscopic examination of probiotics are shown in **Figures 34 and 35.**

The macroscopic study of the cultures makes it possible to describe the appearance of the colonies obtained on MRS media (shape, size, color) and to find the criteria relating to the colonies of *Lactobacilli, Bifidobacterium* and *Streptococcus* **(Fig. 34).**

- ✓ *Lactobacillus* are small, about 1mm in diameter, whitish to creamy in color, circular and regular in shape with a smooth surface.
- ✓ *Bifidobacterium* have colony with milky white color or slightly creamy, rounded form with the diameter of 0.1-0.5 mm.
- ✓ *Streptococcus thermophilus* colonies are round with cremated white color and small sizes.

Microscopic observation after Gram staining revealrd the presence of Gram-positive bacteria, bacillary, rod and cocci in shape, more or less long, isolated, in pairs, or in chains typical of *Lactobacilli, Bifidobacterium* and *Streptococcus* **(Fig. 35).** The results of physiological and biochemical tests allowed the classification of isolates in the genus *Lactobacillus, Lactococcus, Bifidobacterium* and *Streptococcus*.

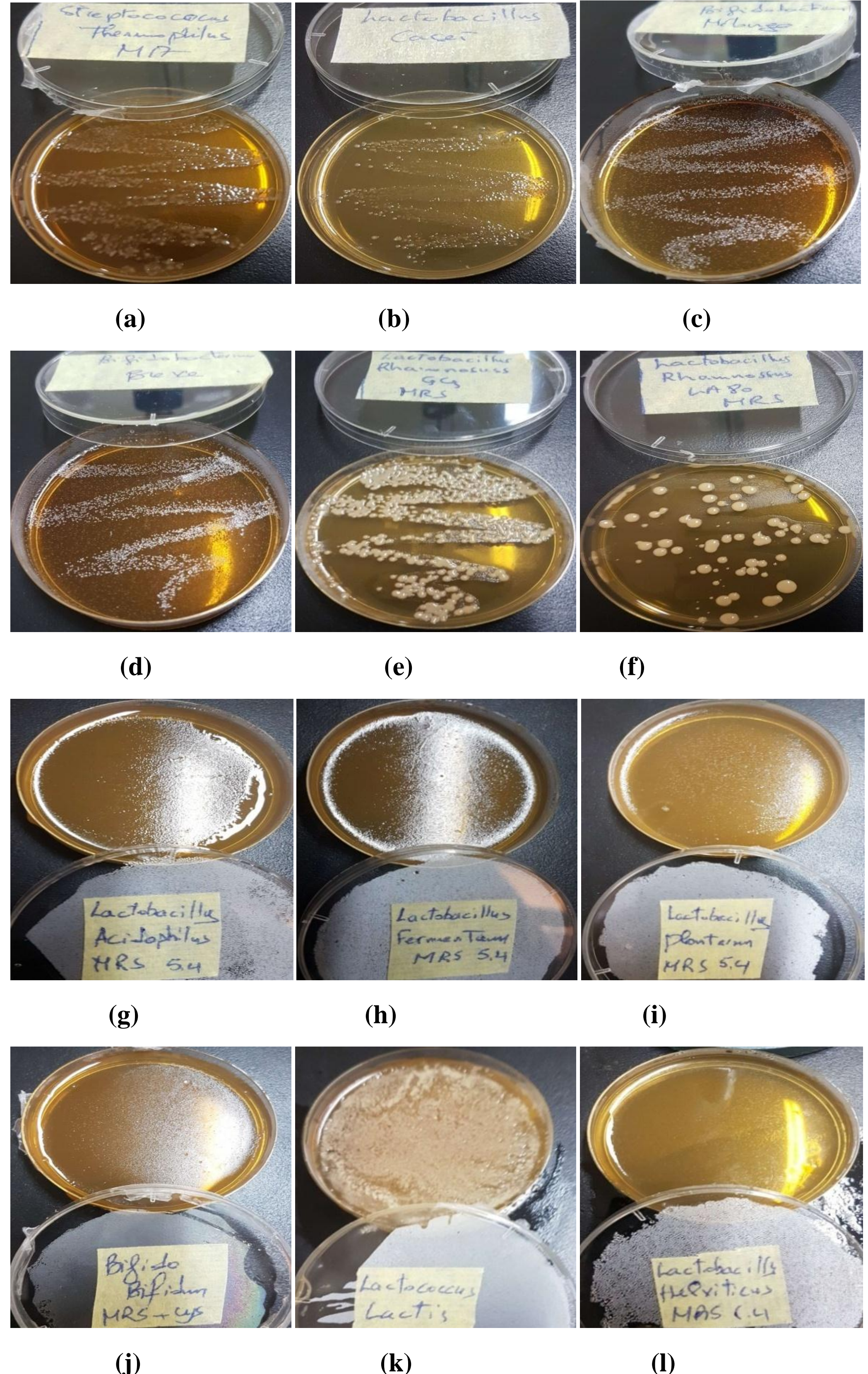



**Figure 34:** Results of macroscopic examination of probiotics

**(a):** *S. thermophilus,* **(b):** *L. casei,* **(c):** *B. longum,* **(d):** *B. breve*, **(e):** *L. rhamnosus GG*, **(f):** *L. rhamnosus LA80,* **(g):** *L. acidophilus*, **(h):** *L. fermentum*, **(i):** *L. plantarum*, **(j):** *B. bifidum*, **(k):** *L. lactis*, **(l):** *L. helviticus.*

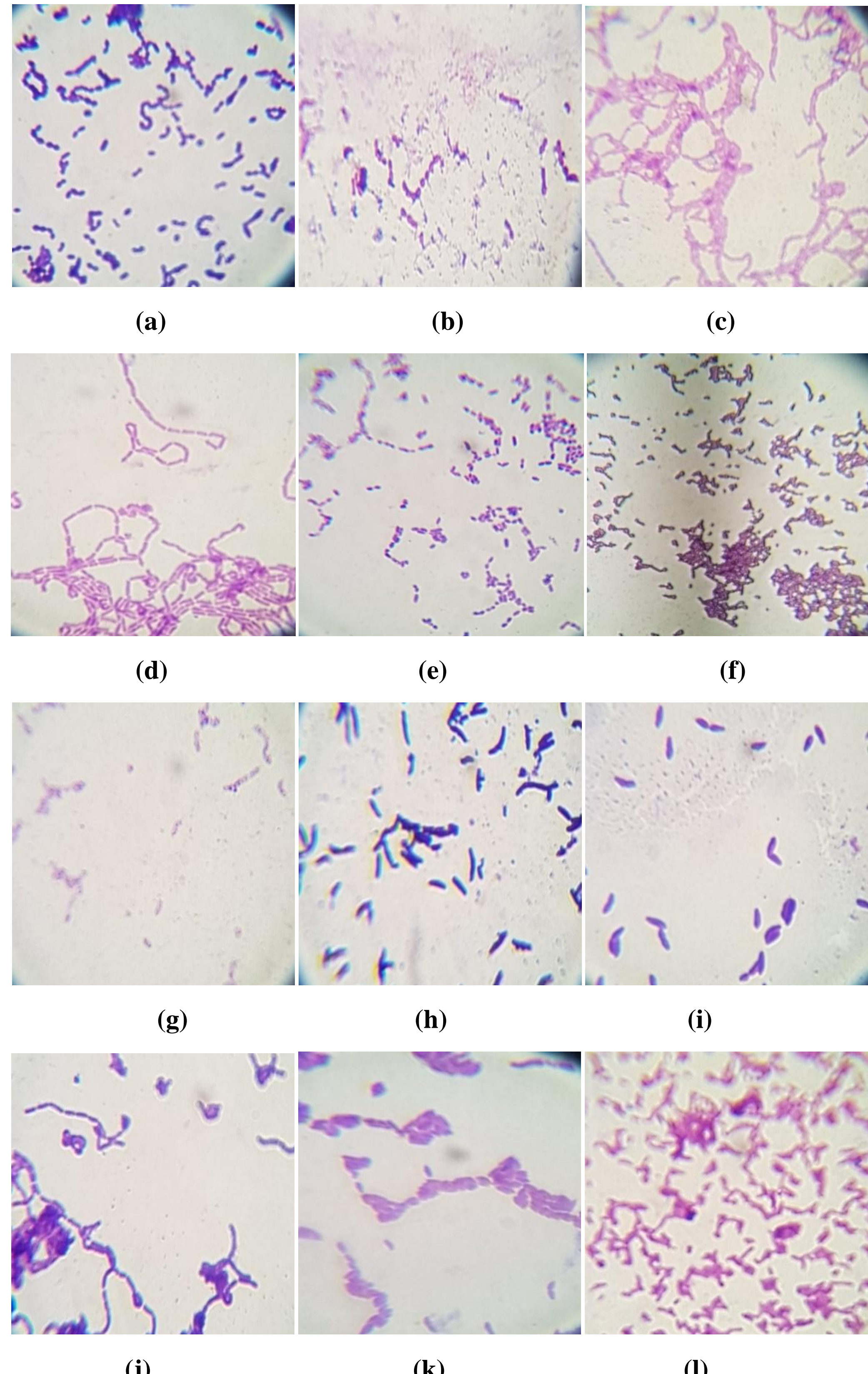



**Figure 35:** Results of microscopic observation (Gram straining) of probiotics

**(a):** *S. thermophilus,* **(b):** *L. casei,* **(c):** *B. longum,* **(d):** *B. breve*, **(e):** *L. rhamnosus GG*, **(f):** *L. rhamnosus LA80,* **(g):** *L. acidophilus*, **(h):***L. fermentum*, **(i):** *L. plantarum,* **(j):***B.bifidum*, **(k):** *L. lactis*, **(l):** *L. helviticus.*

The results of biochemical confirmation tests of probiotics are shown in the following table:

**Table 43:** Results of biochemical confirmation tests of probiotics

| **Strains** / **Tests** | Gram | Catalase | oxydase | ADH | Glucose fermentation test |
|---|---|---|---|---|---|
| ***B. breve*** | + | - | - | - | Heterolactic |
| ***B. bifidum*** | + | - | - | - | Heterolactic |
| ***B. longum*** | + | - | - | - | Heterolactic |
| ***L. rhamnosus LA80*** | + | - | - | - | Homolactic |
| ***L. rhamnosus GG*** | + | - | - | - | Homolactic |
| ***L. helviticus*** | + | - | - | + | Heterolactic |
| ***L. lactis*** | + | - | - | - | Heterolactic |
| ***S. thermophilus*** | + | - | - | + | Heterolactic |
| ***L. plantarum*** | + | - | - | + | Homolactic |
| ***L. acidophilus*** | + | - | - | + | Homolactic |
| ***L. fermentum*** | + | - | - | + | Homolactic |
| ***L. casei*** | + | - | - | + | Homolactic |

+: Positif; -: Negatif

According to the results obtained from the macroscopic and microscopic examination and the various biochemical tests carried out on the probiotic strains and based on the literature, the identification of these strains has been well confirmed **(Lievin 2000; Wasilewska and Bielecka 2003 ; Hadadji et *al.* 2005).**

### II.8.4 Results of evaluation of anti- *H. pylori* effect of plant extracts

#### II.8.4.1 Results of determination of DZI of plant extracts using disc diffusion method

The inhibitory effect of the methanolic extracts on the growth of *H. pylori* was shown in **Table 44** The results indicated a concentration dependent increase of the DZI against *H. pylori* for all plant extracts. The DZI started from 6 mm for the different plant extracts at 10 µg initial concentration. High DZI were recorded for the cumin and fenugreek extracts at 90 µg concentrations and above with slight primacy of the fenugreek extract (16.00±0.00) at 1000 µg. According to **Duraffourd et *al.* (1990)** scale, *H. pylori* was sensitive to garlic and onion extract and very sensitive to cumin and fenugreek extract at concentration of 1000 µg.

**Table 44:** Results of determination of DZI (mm) of plant extracts against *H. pylori*

| Extract / Concentrations (µg) | Garlic | Onion | Cumin | Fenugreek |
|---|---|---|---|---|
| **10** | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 |
| **20** | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 |
| **30** | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 |
| **40** | 6.67±0.58 | 6.67±0.58 | 6.67±0.58 | 6.67±0.58 |
| **50** | 7.00±0.00 | 7.00±0.00 | 7.00±0.00 | 7.00±0.00 |
| **60** | 7.33±0.58 | 7.33±0.58 | 7.33±0.58 | 7.33±0.58 |
| **70** | 7.67±0.58 | 8.00±0.00 | 8.00±0.00 | 8.33±0.58 |
| **80** | 8.00±0.00 | 8.33±0.58 | 8.67±0.58 | 9.00±0.00 |
| **90** | 8.33±0.58 | 8.67±0.58 | 9.33±0.58 | 9.67±0.58 |
| **100** | 9.00±0.00 | 7.79±0.58 | 10.33±0.58 | 10.67±0.58 |
| **150** | 9.67±0.58 | 10.67±0.58 | 11.00±1.00 | 11.33±1.15 |
| **250** | 10.33±0.58 | 11.33±0.58 | 12.33±0.58 | 12.67±0.58 |
| **500** | 11.67±0.58 | 12.67±0.58 | 14.33±0.58 | 14.67±0.58 |
| **1000** | 13.33±0.58 | 14.67±0.58 | 15.67±0.58 | 16.00±0.00 |

### II.8.4.2 Determination of MIC and MBC of plant extracts

The results of plant extracts induced MIC and MBC against *H. pylori* were presented in **Table 45.** The highest MIC and MBC against *H. pylori* were obtained with the fenugreek extract at 100 and 150 µg/ml, respectively. The other extracts rendered as follows: cumin extract 150 µg/ml (MIC)and 250 µg/ml (MBC), while for the onion and garlic extracts, the concentrations needed to be much higher to attain the same MIC and MIBs. This suggested that fenugreek extract provided the best antibacterial effect at the lowest concentrations among all extracts evaluated in the present study.

**Table 45:** Results of determination of MIC and MBC of plant extracts against *H. pylori*

| Plant extract | MIC (µg/ml) | MBC (µg/ml) |
|---|---|---|
| Garlic | 500 | 1000 |
| Onion | 250 | 500 |
| Cumin | 150 | 250 |
| Fenugreek | 100 | 150 |

### II.8.4.3 Results of evaluation of growth kinetics of *H. pylori* in the presence of plant extracts

The inhibition kinetics evaluation showed that plants extracts had an antibacterial activity against *H. pylori* **(Fig. 36-37).** Almost similar growth kinetics were recorded for all plant extracts with relatively overlapping curves up to 6 hrs when adding plants extracts, followed by a marked decrease of *H. pylori* growth recorded with the fenugreek extract compared to other extracts.

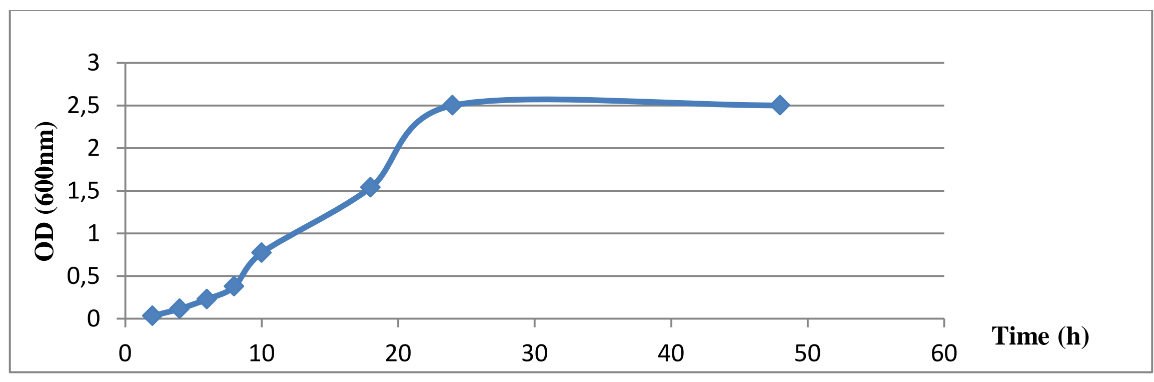



**Figure 36:** Results of evaluation of growth kinetics of *H. pylori* without plant extracts

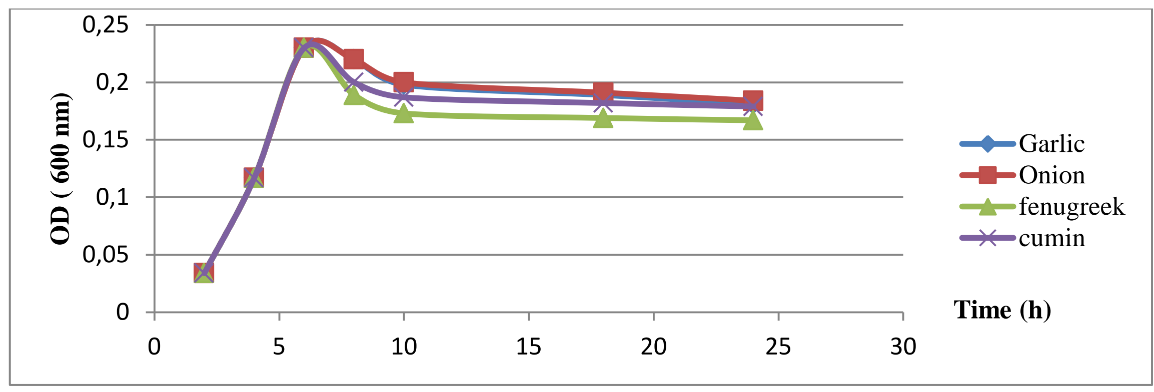



**Figure 37:** Results of evaluation of growth kinetics of *H. pylori* in the presence of plant extracts

The evaluation of antibacterial effect of methanolic extracts from tested plant materials against *H. pylori* clinical isolates strain using a disc diffusion test and by evaluating the MIC on solid media and MBC on broth media indicated an inhibitory activity in high percentages. The results presented in this study emphasize the significant antibacterial effect of methanol extracts especially for fenugreek extract which was higher than that of cumin, onion and garlic extracts respectively; all tested plants showed substantial, but broadly different anti-*H. pylori* effects with an MBC ranging from 150 to1000 µg/ml.

**Jung et *al.* (2003)** tested the anti-*H. pylori* activity of garlic methanolic extract with disc diffusion method and founded a higher results with DZI of 30 mm. Also **Adeniyi et *al.* (2006)** noted that methanolic garlic extract exhibited a high effect on *H. pylori* with DZI of 16-19 mm in comparison with our results. While **Tabak et *al.* (2022)** founded a lower value 8 mm. On the other hand, **Cañizares et *al.* (2002)** tested ethanolic and aqueous garlic extracts on the inhibition of growth of *H. pylori* and found $DZI_s$ of 45 mm and 12 mm for the ethanolic and aqueous extract respectively.

Concerning onion, **Tabak et *al.* (2022)** evaluated the anti-*H. pylori* activity of onion methanolic extract and observed that DZI value was 11 mm, this result was lower to the present result, in contrast, **Ramos et *al.* (2006)** result about aqueous onion extract was similar presenting a DZI of 15 mm.

**Randhir and Shetty (2007)** studied the inhibition of *H. pylori* using fenugreek aquouse extract, finding result was lower than ours with a DZI of 13 mm.

Regarding cumin**, Nostro et *al.* (2005)** examined the activity of ethanolic and aqueous extracts of cumin against *H. pylori* against 45 clinical isolated of *H. pylori*. The growth

inhibition was determined by the disc diffusion method, the resulting DZI was 9 and 14 for aqueous and ethanolic extract respectively. Also **Moghaddam (2010)** confirmed the antibacterial effect of cumin extract against *H. pylori.*

Similar results were obtained by other investigators for anti-*H. pylori* activities of the studied plants (determination of MCI and MCB). Thus, **O'Gara et *al.* (2000)** and **Cellini (1996)** showed that garlic exhibited a potential anti- *H. pylori* effect with MIC ranging from 250 to 500 µg/ml and a MBC ranging from 250 to 500 µg/ml**.** The *in vitro* anti-*H. pylori* activity of extracts and compounds obtained from garlic has been extensively documented **(Cellini et *al.*, 1996 ; Mahady et *al.,* 2001**). An aqueous garlic extract had an MIC of 40 µg/ml against *H. pylori*, and for other garlic compounds (allicin, ajoenes, vinyldithiins, thiosulphinates), the MIC values were approximately 10 to 25 µg/ml **(Sivan et *al.,* 1997; Mahady et *al.,* 2001).** Similarly, the other plants studied in this research represented subject to investigations of other groups. Onion extracts presented a good anti-*H. pylori* activity according to **Yordanov et *al.* (2017**), while cumin showed a MIC of 691µg/ml against *H. pylori* **(Nakhaei et *al.*, 2006),** and finally, fenugreek was observed to moderately (68%) inhibit the growth of *H. pylori* according to **Manjegowda and Dharmesh (2012).**

The bactericidal activity of plant preparations against *H. pylori* significantly depends on the type of the extract and its components, concentration, and the exposure time, as well as the density of the tested bacterial strains **(Ngan et *al.*, 2021).**

The results of phytochemicals analysis showed that all four plants extracts contained components such as phenolic compounds and flavanoids with known biological activity, which could serve as a valuable therapeutic index. The anti-*H. pylori* properties of these extracts may be due to the presence of above mentioned phytochemicals. Further studies are needed to isolate the active components and individually define their activities against *H. pylori*.

### II.8.5 Results of evaluation of anti- *H. pylori* effect of probiotics

#### II.8.5.1 Determination of DZI of probiotics using well diffusion assay

The antibacterial activity of 12 probiotics strains was evaluated *in vitro* by well diffusion method against *H. pylori*. **Table 46** summarizes the microbial growth inhibition of *H. pylori* in the presence of probiotics supernatants. While most probiotic strains (*Lactobacillus* genus) showed mean DZI ranging from10 to 10.67 mm (*L. rhamnosus LA80, L. rhamnosus GG, L. helviticus, L. lactis, S. thermophilus*...etc.), a very high DZI (20.33 ±0.58mm) was recorded for *B. breve* strain.

**Table 46:** Results of determination of DZI of probiotics against *H. pylori*

| Probiotic strains | DZI(mm) |
|---|---|
| *B. breve* | 20.33 ±0.58 |
| *B. bifidum* | 12.67 ±0.58 |
| *B. longum* | 11.33 ±0.58 |
| *L. rhamnosus LA80* | 10.67 ±0.58 |
| *L. rhamnosus GG* | 10.67 ±0.58 |
| *L. helviticus* | 10.00 ±0.00 |
| *L. lactis* | 10.67 ±0.58 |
| *S. thermophilus* | 10.33 ±0.58 |
| *L. plantarum* | 10.67 ±0.58 |
| *L. acidophilus* | 10.67 ±0.58 |
| *L. fermentum* | 10.33 ±0.58 |
| *L. casei* | 10.00 ±0.00 |

### II.8.5.2 Results of evaluation of growth kinetics of *H. pylori* in the presence of probiotics

The results of evaluation of growth kinetics of *H. pylori* showed a slight decrease in the presence of probiotics **(Fig. 38-40).** Important inhibition effect was recorded when the cell free supernatant obtained from a culture of probiotics was used, indicating that a diffusible molecule (organic acids) should be present in this supernatant. However, a slightly lower inhibitory effect was obtained using bacteriocin-free and $H_2O_2$-free (neutralized) supernatants.

✓ **Influence of organic acids (lactic and acetic acids)**

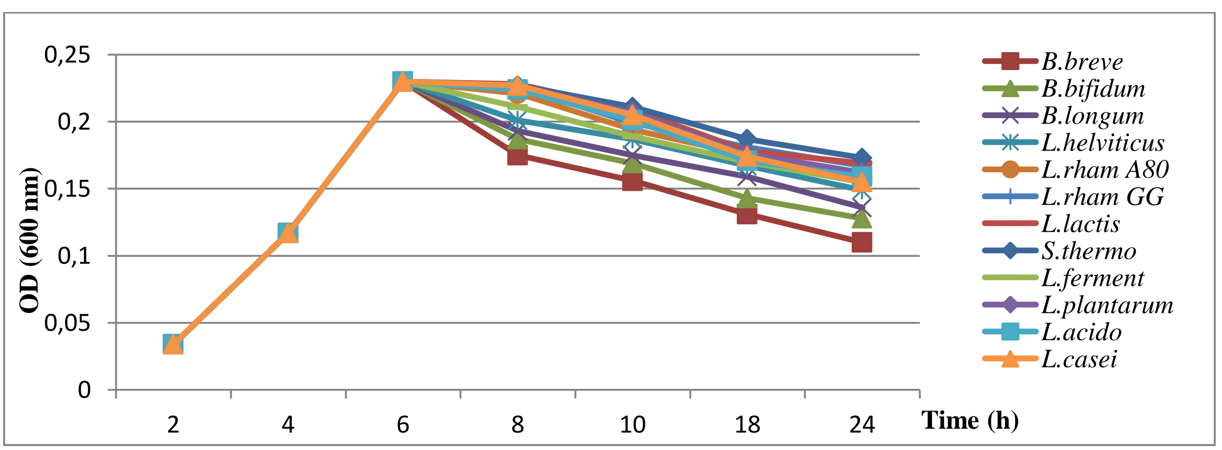



**Figure 38:** Results of evaluation of growth kinetics of *H. pylori* in the presence of supernatant of probiotics

Among the tested probiotics, *B. breve* was responsible for an important growth decrease of *H. pylori* that could be explained by the high production of organic acids.

✓ **Influence of bacteriocins**

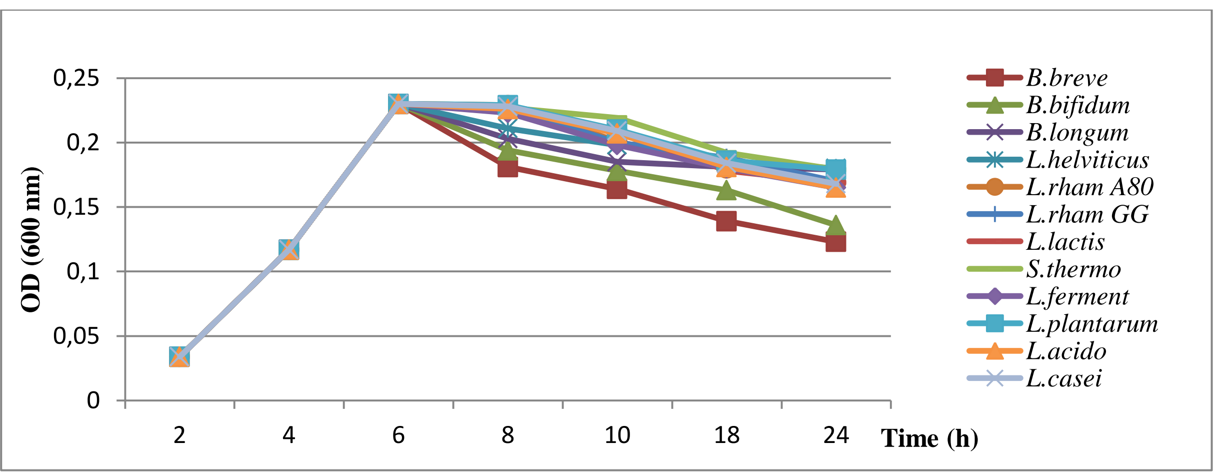



**Figure 39:** Results of evaluation of growth kinetics of *H. pylori* in the presence of neutralized supernatant of probiotics

Looking at the inhibition by organic acids, a highly reduced growth rate of *H. pylori* was induced by *B. breve*, suggesting its ability to synthesize more bacteriocins than the other tested probiotics.

✓ **Influence of hydrogen peroxide ($H_2O_2$)**

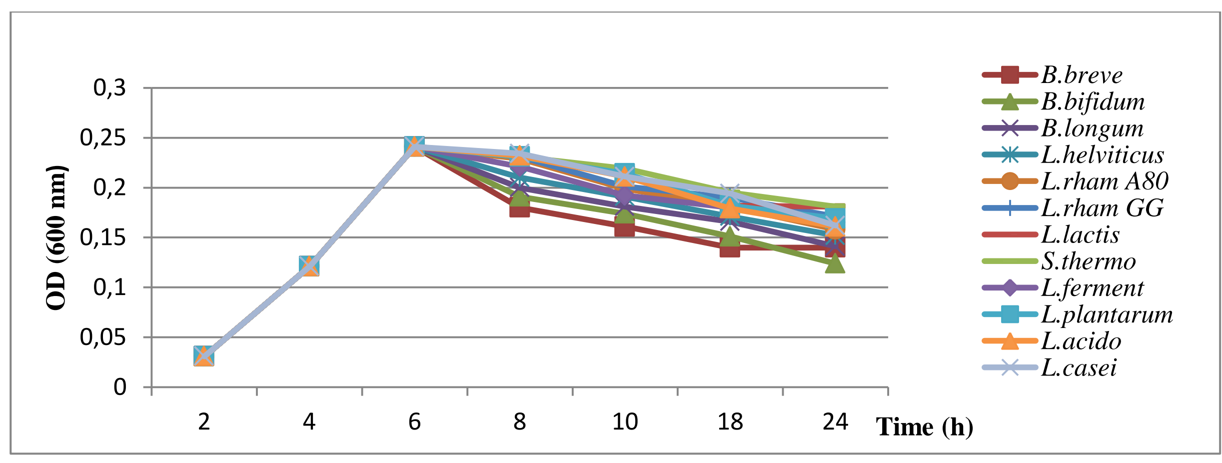



**Figure 40:**Results of evaluation of growth kinetics of *H. pylori* in the presence of supernatant of probiotics + catalase

No significant changes were observed with the suppression of $H_2O_2$ activity for all tested bacteria suggesting that it have no effect on *H. pylori* growth.

Out of the tested probiotic strains, *B. breve* showed the highest antibacterial activity against *H. pylori*. Moreover, the antibacterial activity of the other *Bifidobacterium* strains, *B. bifidum* and *B. longum* was also higher than that of the reference strains (*L. rhamnosus GG, L. rhamnosus LA80* and *L. helviticus*) as well as of isolated probiotic strains (*L. fermentum, L. plantarum, L.casei, L. acidophilus, L. lactis and S. thermophilus*) **(Tab. 46).** Further studies on the inhibition kinetics showed that *B. breve* present the highest antibacterial activity against *H. pylori* when compared to other probiotic strains **(Fig. 38 ).** Regarding the results of growth kinetics of *H. pylori* in the presence of probiotics, when investigating the influence of different variables (organic acids, bacteriocins and hydrogen peroxide), it was clear that the inhibition effect was due to the presence of organic acids such as lactic and acetic acids as well as bacteriocins **(Fig. 38-40).** Thus, in accordance with the bacterial death criteria of **Pearson et *al.*(2008)**, the effect of *L. fermentum* UCO-979C strain at 24 h can be considered lethal for *H. pylori*. Further, after repeating this assay at shorter times and using the same Pearson criteria for interpretation of the results, the death of *H. pylori* after 6 h of treatment with *B. breve* was noticed. Nevertheless, other researchers found different results when evaluating the anti- *H. pylori* effect of some probiotics. **Bae et *al.* (2000)** investigated the inhibitory effects of different *Bifidobacterium* spp. on the growth of *H. pylori,* and indicated that a significant suppression of *H. pylori* growth occurred especially in the presence of *B. breve*. **García et *al.* (2016)** establish that *L. fermentum, L. casei* and *L. rhamnosus* induced an inhibition ranging from 2 to5 mm. **Boyanova et *al.* (2009)** when looking at the anti- *H. pylori* effect of some *Lactobacillus* strains indicated that three strains suppressed the growth of >86% of *H. pylori* strains at low pH values and two strains suppressed the growth of >53% of the test strains at neutral pH values. Further studies of **Boyanova et *al.* (2017)** revealed that the DZI of some *Lactobacillus* strains were higher, from13 to16 mm, while **Chen et *al.* (2019)** found that *L. rhamnosus* and *L. acidophilus* exhibited an inhibitory effect against *H. pylori* reference strain 26695 demonstrated by a DZIs of 12.3 and 11.3 mm, respectively. However, **Paucar-Carrión et *al.* (2022)** when analysing the anti-*H. pylori* activity of *L. fermentum*, concluded that the activity was mild with DZI between 1 mm and 2mm.

Among probiotics, *Bifidobacterium* is one of the favourites, generally used for the prevention of gastrointestinal infection, and it is commonly incorporated in fermented dairy products or food supplements. *Bifidobacterium* exerts an *in vitro* anti-*H. pylori* effect and inhibits adhesion to the mucosa by competitive exclusion **(Chenoll et *al.*, 2011).** Several studies have demonstrated a direct relationship between the addition of potential probiotic strains and the *in vitro* inhibition of *H. pylori* growth. Such strains were *Lactobacillus*

*acidophilus* **(Lorca et *al.,* 2001),** *Lactobacillus casei* Shirota strain **(Sgouras et *al.,* 2004)**, which, among others, have an antagonistic effect on *H. pylori.* Since most organic acids produced by the *Bifidobacterium* spp. inhibited the growth of *H. pylori*, while the *H. pylori* growth was not inhibited by organic acids produced in some other *Bifidobacteria*-cultured media, it was suggest that those *Bifidobacterium* strains may produce antibiotic-like compounds (bacteriocins) (**Bae et *al.*, 2000**). The results of the present study confirm the findings of **Zacharof and Lovitt (2012)** which imply effects of organic acids and other bioactive substances of the *Bifidobacterium* and *Lactobacillus* strains as well as a higher activity of bacteriocins at lower pH values. The activity of the neutralized and catalase-treated supernatants was approximately similar with regard to *H. pylori* growth inhibition effect. Bacteriocin activity depended on numerous factors of the test strains, such as the bacterial cell envelope/membrane composition, reduction in bacteriocin binding/insertion, bacteriocin sequestering and degradation or efflux pumping **(Drider et *al.*, 2016).**

### II.8.6 Results of combined effect of medicinal plants with probiotics on *H. pylori*

#### II.8.6.1 Determination of DZI of combined mixtures ( plant extracts with probiotics) using disc diffusion method

The results of determination of DZI of medicinal plants combined with probiotics against *H. pylori* were shown in **Table 47.** The combined effect of most probiotics with plant extracts is quite similar except for *B. longum, B. bifidum* and *B. breve* showing highly distinct DZI values. The highest DZI values were obtained with all *B. breve* combinations especially with fenugreek extract giving 28.67 ±0.58 mm DZI against the *H. pylori* strain.

In this study, we confirmed in *in vitro* experiments the inhibitory activity of *B. breve* complex mixture containing fenugreek extracts on *H. pylori*. The fenugreek extract inhibited the growth of *H. pylori* in a dose- dependent manner (100 µg/ml). In addition, the inhibitory effect on *H. pylori* of *B. breve* and fenugreek extract when applied as a complex mixture, rather than individual components, was confirmed to be superior.

*Helicobacter pylori* was inhibited by all combined mixtures of extracts and probiotics with varying results, while fenugreek */ B. breve*, cumin */ B. breve*, garlic */ B. breve* and onion / *B. breve* combinations exhibited relevant anti-*H.pylori* activities with DZI of 28.67 ±0.58 26.67 ±0.58, 24.67 ±0.58 and 22.67 ±0.58mm respectively. Preliminary studies on the effect of probiotics against *H. pylori* revealed that inhibition may be due to lactic acids and bacteriocins. However, the presence of phenolic compounds such as gallic acid, caffeic acid, quercetin, and vanillic acid in plant extracts may also have an influence.

**Table 47:** Results of determination of DZI (mm) of medicinal plants with probiotics against *H. pylori*

| Probiotics \ Extracts | Garlic | Onion | Cumin | Fenugreek |
|---|---|---|---|---|
| ***B.breve*** | 22.67 ±0.58 | 24.67 ±0.58 | 26.67 ±0.58 | 28.67 ±0.58 |
| ***B. bifidum*** | 14.33 ±0.58 | 15.67 ±0.58 | 16.33 ±0.58 | 17.67 ±0.58 |
| ***B. longum*** | 13.67 ±0.58 | 14.33 ±0.58 | 15.33 ±0.58 | 16.33 ±0.58 |
| ***L. rhamnosus LA80*** | 11.67 ±0.58 | 12.67 ±0.58 | 13.67 ±0.58 | 14.33 ±0.58 |
| ***L. rhamnosu GG*** | 11.67 ±0.58 | 12.00 ±0.00 | 12.33 ±0.58 | 13.00 ±0.00 |
| ***L. helveticus*** | 11.00 ±0.00 | 11.67 ±0.58 | 12.00 ±0.00 | 12.33 ±0.58 |
| ***L. lactis*** | 12.00 ±0.00 | 12.33 ±0.58 | 12.67 ±0.58 | 13.67 ±0.58 |
| ***S. thermophilus*** | 11.67 ±0.58 | 12.33 ±0.58 | 13.33 ±0.58 | 13.67 ±0.58 |
| ***L. plantarum*** | 11.33 ±0.58 | 12.33 ±0.58 | 13.00 ±0.00 | 13.67 ±0.58 |
| ***L. acidophilus*** | 11.00 ±0.00 | 11.67 ±0.58 | 12.00 ±0.00 | 12.67 ±0.58 |
| ***L. fermentum*** | 11.33 ±0.58 | 12.33 ±0.58 | 13.00 ±0.00 | 13.33 ±0.58 |
| ***L. casei*** | 11.33 ±0.58 | 12.33 ±0.58 | 13.33 ±0.58 | 13.67 ±0.58 |

### II.8.6.2 Evaluation of growth kinetics of *H. pylori* in presence of combined mixtures (plant extracts with probiotics)

The results of evaluation of *H. pylori* growth kinetics showed a remarkable decrease in the presence of probiotics combined with plant extracts **(Fig.41-44).** All probiotics have induced an important decrease in the growth of *H. pylori* in combination with plant extracts. However, the most notable decrease of growth was recorded with the fenugreek extract when combined to *B. breve* **(Fig. 44).**

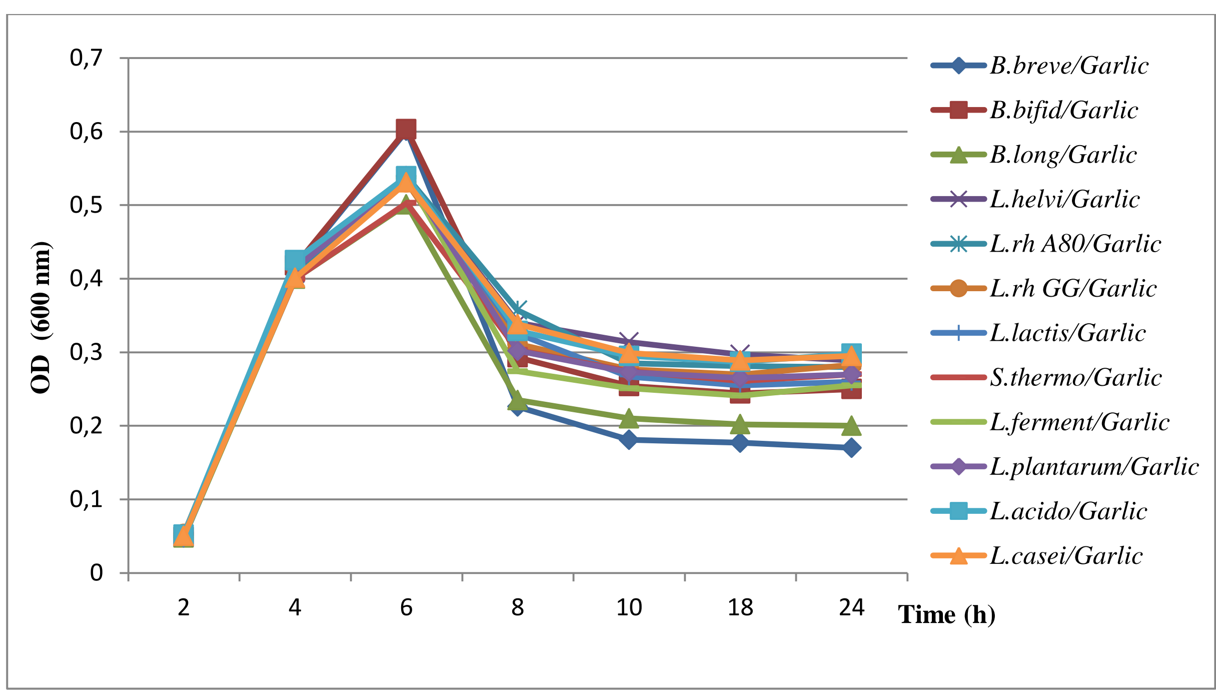



**Figure 41:** Results of evaluation of growth kinetics of *H. pylori* in the presence of probiotics supernatant + garlic extract

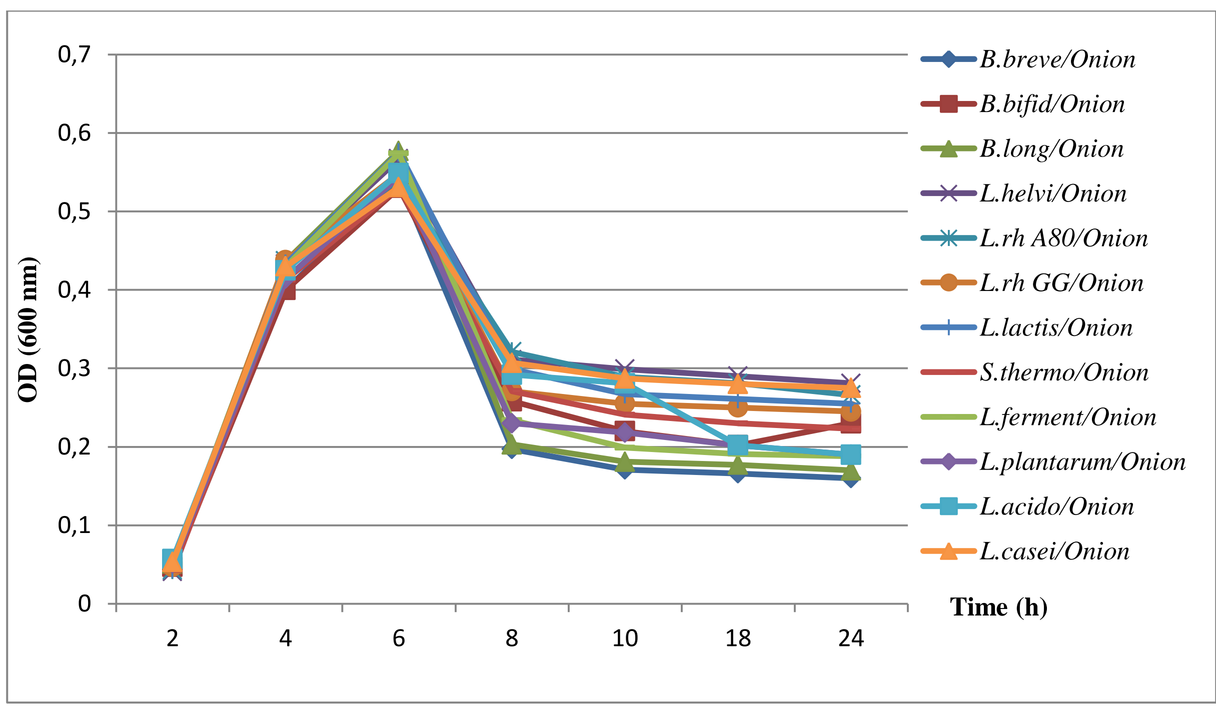



**Figure 42:** Results of evaluation of growth kinetics of *H. pylori* in the presence of probiotics supernatant + onion extract

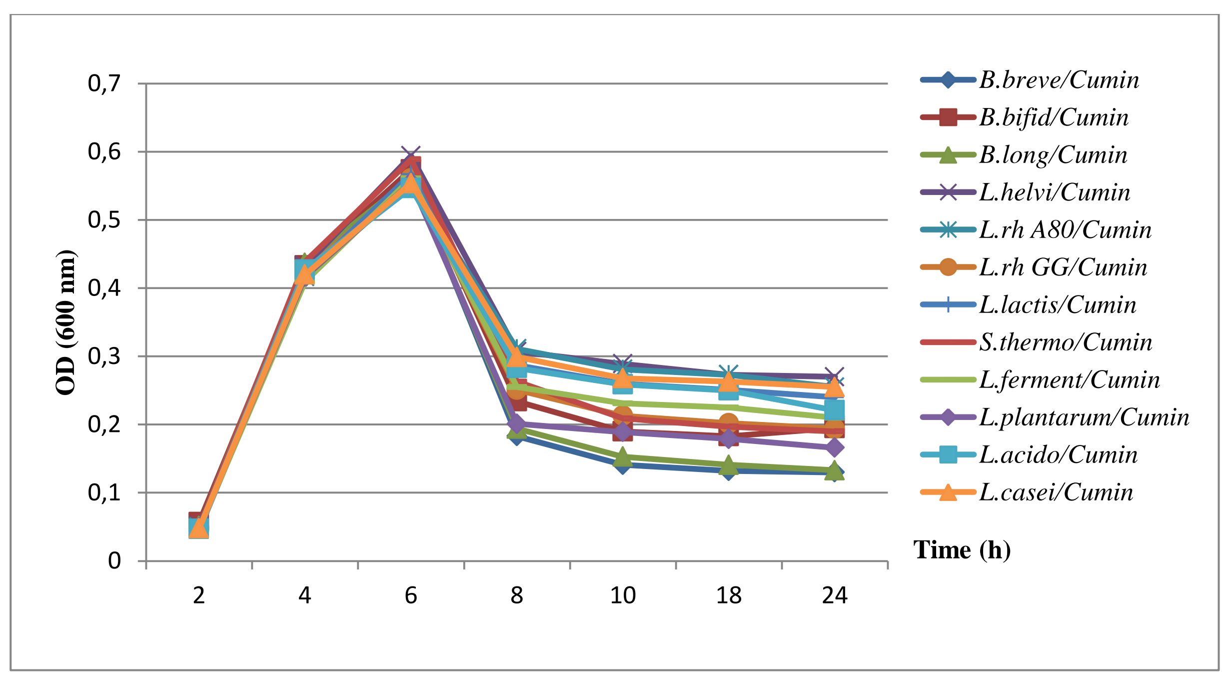



**Figure 43:** Results of evaluation of growth kinetics of *H. pylori* in the presence of probiotics supernatant + cumin extract

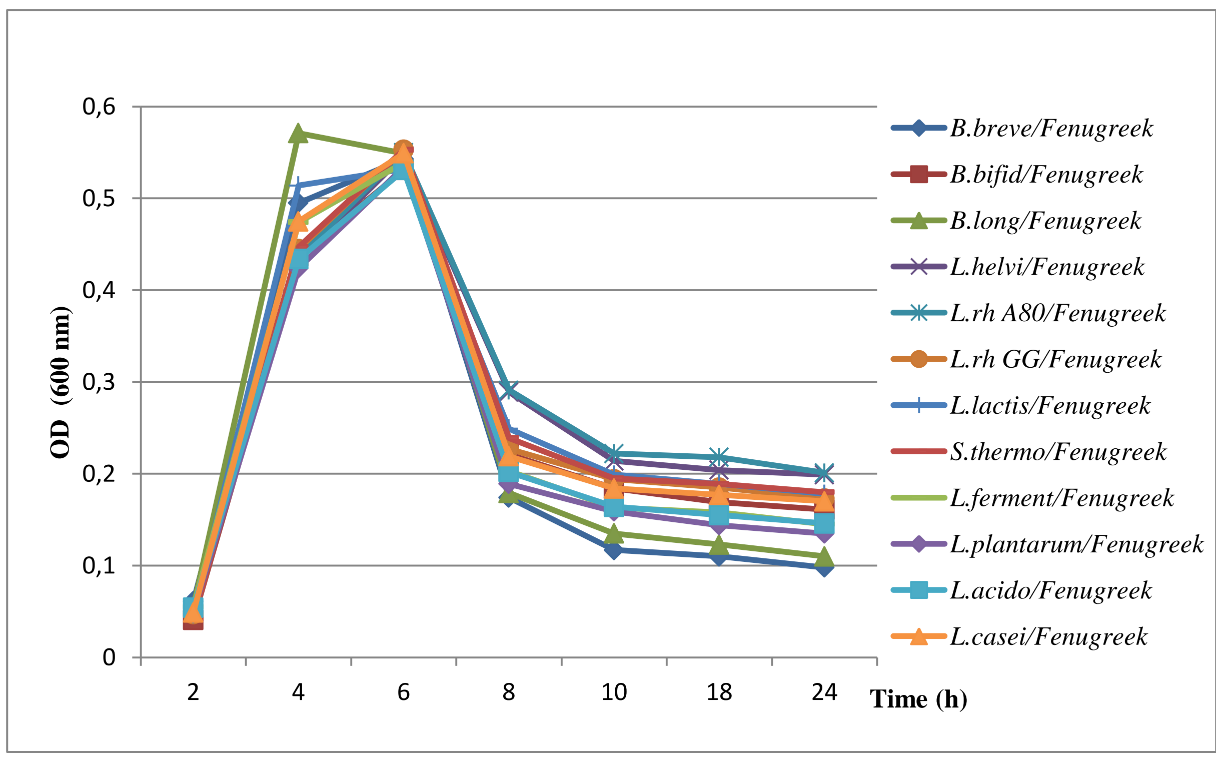



**Figure 44:** Results of evaluation of growth kinetics of *H. pylori* in the presence of probiotics supernatant + fenugreek extract

Few studies investigate the antimicrobial effects of combinations between probiotics and plants extracts. **Lee et *al.* (2020)** demonstrated that the treatment with a complex mixture of *L. paracasei* HP7, including the extract of *Perrila frutescens* and *Glycyrrhiza glabra* could inhibit the growth of *H. pylori* and is thus a promising treatment for patients with gastric symptoms, such as gastritis caused by *H. pylori* infection. **Behrad et *al.* (2009)** recorded that mixture of cinnamon extract, *L. acidophilus LA-5* and NCFM*, Bifidobacterium* Bb-12*, L.casei* LC-10*,* and *Streptococcus thermophilus* Th-4 exhibited the strongest inhibitory effect on *H. pylori* growth *in vitro* (13.5mm).

**Kanani (2018)** studied the eradication of *H. pylori* using a yogurt prepared with a combination between nettle extract (*Urtica dioica*) and *Bifidobacterium* (BF). The main challenge is to transfer these two compounds into an environment containing *H. pylori* such as the stomach. The interaction between nettle extract and BF against *H. pylori* was investigated measuring DZI. The results revealed that nettle extract with *Bifidobacterium* exhibited greater DZI (60 mm) and were more effective in preventing the growth of *H. pylori* due to nettle extract which enhanced BF activity causing the production of more antimicrobial metabolites.

In the study of **Yoon et *al.* (2019),** the beneficial effect of fermented milk containing *L. paracasei* HP7 and herbal extract (*Glycyrrhiza glabra*) was evaluated in patients with *H. pylori* infection. The combination of fermented milk containing *L. paracasei* and *G. glabra* reduced *H. pylori* density and improved histological inflammation, even though it failed to eradicate *H pylori.*

Other studies contradict the synergic effect between plant extracts and probiotics. **Kang et *al.* (2021),** investigated the anti-*H. pylori* effects of *Lactobacillus plantarum* (pH3A), monolaurin, grapefruit seed extract, and their synergies *in vitro* and *in vivo*. Monolaurin and grapefruit seed extract suppressed *H. pylori* growth at an MIC of 62.5 ppm. Also *L. plantarum* pH3A significantly inhibited *H. pylori* growth. In the *in vivo* study, *H. pylori* colonization of the mouse stomach was significantly reduced by *L. plantarum* treatment, but the addition of monolaurin or grape fruit seed extract did not contribute to these anti-*H. pylori* activities. Therefore, the *L. plantarum* strain can potentially be applied as an alternative anti-*H. pylori* therapy, but evidence of its synergy with monolaurin or grapefruit seed extract *in vivo* is still lacking. **Sadeghi et *al.* (2017)** examined the synergistic effect of broccoli sprout extract with probiotics on *H. pylori* growth inhibition and the findings showed a synergistic effect of the mixture on bacterial inhibition and suggest the use broccoli sprout extract and probiotic bacteria in a yogurt form that is effective in the therapy of *H. pylori* infection.

### II.9 Results of *invivo* study

During the acclimation and the experimental periods, most of the rats did not show any treatment-dependent clinical signs, except those in group 9 (HP+TFE+TBB) where severe weight loss that could be attributed to the number of gavage (three consecutive gavages per day) was observed. No gross lesions were observed during the necropsy at the end of the experimental period. The histological results obtained by administering the combination between *B. breve* and fenugreek extract in order to evaluate their effect on *H. pylori*-induced stomach inflammation *in vivo* were shown in **Figure 45.**

The histopathological examination of gastric mucosa showed a moderate inflammatory infiltration in the *antrum* and *fundus* of animals in the non-treated groups infected with *H. pylori* and severe colonization of antral mucosa with *H. pylori* bacteria by use of Giemsa stain. Gastric samples of *H. pylori*-infected animals treated with second line therapy showed reduced number of inflammatory cellsin the *lamina propria*, however, no changes of colonizing bacteria were observed in the Giemsa-stained samples. Tissue samples of rats infected with *H. pylori* and treated with TFE2 and TBB showed significant decrease of infiltrating inflammatory cells and colonizing bacteria scores (1-0 and 1-1) for inflammation and colonization, respectively. However, the group of rats receiving the lowest dose of fenugreek extract (TFE1, 150 mg/kg), showed slight decrease in inflammatory infiltration and colonization of gastric mucosa (score 2-2) compared to the higher dose group animals.

All animals in preventive groups showed normal gastric histology with significant reduction of bacterial colonization (score 1). However, the gastric mucosa of treated animals exhibited mild inflammation due to infiltration by lymphocytes from previous *H. pylori* infections. Animals in the *H. pylori* infected group treated with fenugreek extract associated to *B. breve* showed normal gastric histology with total absence of *H. pylori* characteristic forms of colonialization in Giemsa-stained samples.

Data from the current study suggest that administration of TFE, TBB or their associations to *H. pylori* infected rats can moderately reduce the number of *H. pylori* colonizing the gastric mucosa with a important effect on associated gastritis. However, prophylactic administration of TFE, TBB or their associations could prevent gastric inflammation and considerably reduce or completely eliminate *H. pylori* colonization.

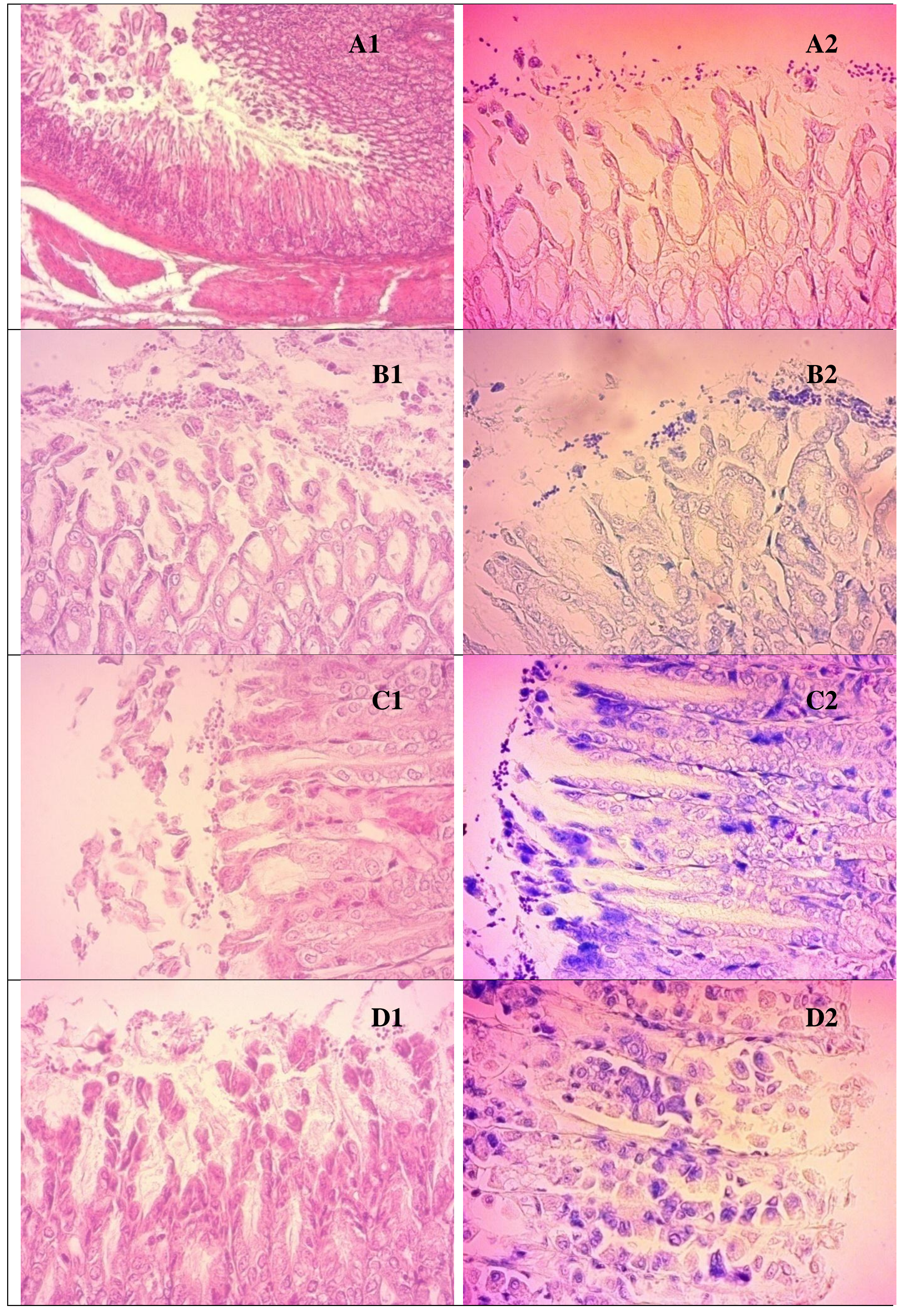

A1
A2
B1
B2
C1
C2
D1
D2

**Figure 45 :** Histopathologic evaluation of antral gastric samples from *H. pylori* infected rat groups, A non-treated showing moderate inflammatory infiltration in the lamina propria (A1: H&E, x100); note the severe colonization of antral mucosa with *H. pylori* bacteria (A2: Giemsa stain, x400); B, gastric samples of animals treated with second line therapy showed reduced number of inflammatory cells in the lamina propria (B1: H&E, x400), no changes of colonizing bacteria were observed (B2: Giemsa stain, x400); C, treatment with TFE2 and TBB have significantly reduced the severity of inflammatory infiltration (C1: H&E, x400) with slight reduction of bacterial colonization (C2: Giemsa stain, x400); D, tissue from animals in all preventive groups showed normal gastric histology (D1: H&E, x400), with significant reduction of bacterial colonization (D2: Giemsa stain, x400).

*In-vivo* anti-*H. pylori* activity of the fenugreek extract and/or *B. breve* has been confirmed by histopathological and urease tests. These compounds have suggestively reduced the number of animals with a positive urease test and the number of detected microorganisms in histological sections stained by Giemsa, exceeding even the standard medications, thus preventing the presence of these organisms in the gastric mucosa.

None of the animals in the control and treated groups exhibited atrophy or metaplasia of the gastric mucosa. Similarly, important differences in the antral and fundic mucosa in respect with colonization by *H. pylori* and the associated gastritis were observed between the treated and control groups, as also reported by **Sgouras et *al.* (2004).** It has been suggested in previous *in-vivo* studies in rats that the fenugreek extract forms a protective barrier over the gastric epithelial lining due to the presence of galactomannans, *H. pylori* possibly interacting with epithelial cells through secretory molecules (vitexin-7-O-glucoside, vicenin-1, luteolin and orientin) or as a result of adherence **(Figer et *al.*, 2017).**

The significant reduction in the severity of gastritis observed in animals treated with the extract indicated a possible role as an anti-inflammatory and anti-secretory agent **(Pandian et *al.*, 2002; Figer et *al.*, 2017).** However, this gastro-protective action was not attributed exclusively to alteration of gastric pH or acidity since the response of the extract was consistent in both *in vivo* and *in vitro* studies **(Figer et *al.*, 2017).** Also, it has been suggested that the *in vivo* activity of the extract may not result solely from the topical action but may also take place by a systemic component **(Tan et *al.*, 2010).**

Several probiotics showed beneficial effects in animal models of *H. pylori* infection. In mice infection models, a combination of probiotics *L. acidophilus* R0052 + *L. rhamnosus* R0011and *L. casei* Shirota strain + *L. johnsonii* strain La1 were found to reduce the effects of

*H. pylori* infection by decreasing *H. pylori* colonization and alleviating *H. pylori*-induced inflammation of the gastric mucosa **(Johnson et *al.*, 2004; Sgouras et *al.*, 2004).**

There are numerous possible mechanisms by which probiotic bacteria can prevent *H. pylori* adhesion **(Lesbros et *al.*, 2007).** *Lactobacilli* such as *L. johnsonii* La1 **(Michetti et *al.*, 1999)** or *L. acidophilus* LB **(Coconnier et *al.*, 1998)** may exert anti-adhesion activity by secreting antimicrobial substances, strains such as *L. reuteri* can inhibit *H. pylori* growth by competing with adhesion sites. Non-specific rather than specific receptor site blockage is the most likely mechanism **(Bernet et *al.*, 1994).**

*In vivo* studies demonstrated that previous colonization with probiotics prevents or reduces *H. pylori* infection in experimental animals **(Johnson et *al.*, 2004).** Consequently, regardless of the mechanisms of inhibition of the *H. pylori* adherence to epithelial cells, probiotics could prevent *H. pylori* colonization by inhibiting bacterial adhesion to gastric epithelial cells **(Nasr et *al.*, 2017).**

Conclusion
and Perspectives

## Conclusion

Algeria, by its biogeographical position, offers great ecosystem diversity, therefore it is one of the Mediterranean countries whose populations have for a very long time been engaged in traditional medical services and have acquired know-how in this field through employment of medicinal plants. Garlic (*Allium sativum L.*), onion (*Allium cepa L.*), fenugreek (*Trigonella Foenum-graecum L.*) and cumin (*Cuminum cyminum L.*) are ones of the most promising medicinal herbs known from ancient times having nutritional value and therapeutic effects. In the present study, alternatives therapies for *H. pylori* responsible for gastro-duodenal diseases using these plants was investigated.

The present research aim was to analyze the physico-chemical and phyto-chemical profil, to characterize the phenolic compounds, to evaluate the antioxidant activities of previously mentioned plants, and to evaluate the anti-*H. pylori* properties of medicinal plants, probiotics and combinations between them (*in vitro* and *in vivo*). This work has highlighted the synergistic effect between prebiotics (medicinal plants) and probiotics (Lactic Acid Bacteria) on *H. pylori*.

The obtained results showed that the Algerian variety of fenugreek and Syrian variety cumin seeds gave the highest weight with a value of 16.8, 13 g respectively and the better germination rate with a percentage of 70%. The pH and titratable acidity of studied plants ranged from 5.63 to 6.64 and 0.67 to 3.04% respectively. The moisture and ash content varied from 3.08 to 91.12% and 0.29 to 7.02 % respectively. Total soluble solids, electrical conductivity, viscosity varied from 2.75 to 5.5 °Brix, 18.19 to 42.75 mvs and 2.4 to 2.8 m/pa/s respectively. The analysis showed that fenugreek and cumin contained a high amount of proteins which was between 23.10 and 26.8% respectively. On the other hand, fats ranged from 8.81 to 21.02%. While crude fiber, pectin varied from 5.1 to 7.9% and 1.94 to 2.84% respectively. Finally, total and reducing sugars varied from 5.27 to 6.74%, and 0.47 to 1.07% respectively.

According to the present data, the results of mineral and heavy metals profile of the studied plants reavealed that they contain potassium as a major mineral followed by sulphur, phosphorus, calcium, magnesium, iron, zinc and boron. For the heavy metals, the higher percentage was giving by copper followed by lead, nickel, chromium, molybdenum, cobalt and cadmium.

The best extract yield was recorded by the maceration method with a concentration of 10g/100ml of dry matter, however methanol 80% remained the best solvent of extraction for all the plants with the values of 62.87%, 57.38% and 14.29%, 17.66% for garlic, onion, fenugreek and cumin respectively. The determination of total phenolic compounds in methanol extract of garlic and onion using Folin Ciocalteu reagent gave an amount of 45 mg GAE /100g; 86mg GAE /100g DM respectively. Also fenugreek and cumin methanolic extracts seem richer on phenolic compounds with an amount of 115.3 mg GAE / 100g and 91.6 mg GAE / 100g DM respectively. Flavonoids contents in garlic, onion, fenugreek and cumin were 34.62 mg QE /100 g, 43.1 mg QE /100g, 80.98 mg QE /100 g and 66.04 mg QE /100 g DM respectively. Condensed tannins analysis gave a value of 6.8±0.33 mg CE /100g, 4.4±0.001 mg CE /100g,2.2±0.01 mg CE/100g and 1.8±0.033mg CE/100g DM for garlic, onion, fenugreek and cumin respectively. For hydrolysable tannins, results were 0.05 ±0.01mg TAE /100g, 0.22 ±0.04mg TAE /100g, 1±0.045 mg TAE /100g and 0.205±0.001mg TAE /100g DM for garlic, onion, fenugreek and cumin respectively.

The phytochemical screening of above extracts revealed the presence of flavonoids, tannins, anthocyanins, steroids, terpenoids, cardiac glycosides and mucilage.

Chromatographic analysis of the samples showed that five phytochemical molecules were identified in onion methanolic extract (Gallic acid, Quercitin, Rutin, Hyperoside and Karempferol) and one molecule in garlic methanolic extract (Gallic acid). Morever, eight phytochemical molecules were identified in cumin methanolic extract (caffeic acid, isoquercetine, vanillic acid, myricetine 3-0 rutinoside, syringaresinol, citrusine, rosmarinic acid, and p-coumaric acid ) and seven molecules in fenugreek methanolic extract (gallic acid, sinapic acid, caffeic acid, asterogenic acid, pyrogallol, hyperoside and ferulic acid).

The antioxidant activity of the methanol extract of our plants was measured by the method of Free radical scavenging method (DPPH) which gave IC 50 values; 1000 µg / ml, 400 µg / ml, 250 µg / ml and 350 µg / ml for garlic, onion, fenugreek and cumin respectively.

The anti-*H. pylori* activities results indicated that DZI started from 6 mm for the different plant extracts at 10 µg as initial concentration, while the highest DZI were recorded for the cumin and fenugreek extracts at 1000 µg (16.00 mm).

The highest MIC and MBC against *H. pylori* were obtained with the fenugreek extract at 100 and 150 µg/ml, respectively.

The inhibition kinetics evaluation showed that similar growth kinetics were recorded for all plant extracts with relatively overlapping curves up when adding plants extracts, followed by a marked decrease of *H. pylori* growth recorded with the fenugreek extract compared to other extracts.

The anti-*H. pylori* activity of probiotics strains from the genus of *Lactobacillus* exhibited that DZI range from 10 to 10.67 mm (*L. rhamnosus LA80, L. rhamnosus GG, L. helviticus, L. lactis, S. thermophilus*), however the highest DZI was recorded by *B.breve* strain (20.33 ±0.58mm).

Important inhibition effect was recorded by probiotics when the cell free supernatant obtained from their culture was used, indicating that a diffusible molecule (organic acids) should be present in this supernatant. However, a slightly lower inhibitory effect was obtained using bacteriocin-free and $H_2O_2$-free (neutralized) supernatants.

*Helicobacter pylori* was inhibited by all combined mixtures of extracts and probiotics with varying results, while fenugreek */ B. breve* , cumin */ B. breve*, garlic */ B. breve* and onion */ B. breve* combinations exhibited the higher anti-*H. pylori* activities with DZI of 26, 29, 23 and 25 mm respectively. Preliminary studies on the effect of probiotics against *H. pylori* revealed that inhibition may due to lactic acids and bacteriocins. However, it may due to the presence of phenolic compounds present in our extract plants such as gallic acid, caffeic acid, quercetine, and vanillic acid.

*In-vivo* anti-*H. pylori* activity of the fenugreek extract and *B. breve* has been confirmed by histopathological and urease tests. These combination have suggestively reduced the number of animals with a positive urease test and the number of detected microorganisms in histological sections stained by Giemsa, exceeding even the standard medications, thus preventing the presence of these organisms in the gastric mucosa.

In addition, the *B. breve* and fenugreek extract complex mixture significantly reduced the stomach inflammation in *H. pylori* infected rats. These results suggest that this complex mixture maybe an alternative to treating *H. pylori* infection and preventing from diseases caused by this bacterium.

## Perspectives

✓ Isolation of further *H. pylori* strains from patients originated from other regions;

✓ Testing other *H. pylori* strains responsible for various gastro-duodenal diseases (ulcer and cancer stomach);

✓ Evaluating additional extracts obtained with different extraction methods and solvents;

✓ Testing further medicinal plants, probiotics and more combinations on *H. pylori;*

✓ Extraction of essential oils from different medicinal plants and evaluation of their anti-*H. pylori* activities;

✓ Identification of the target bioactive molecules (produced by medicinal plants) responsible for the anti-*H. pylori* effect;

✓ Identification of bacteriocins produced by probiotics;

✓ Determination of mechanism of action (of prebiotics and probiotics) on *H. pylori;*

✓ Combining numerous plant extracts with probiotics and evaluation of their *invitro* and *invivo* anti-*H. pylori* at different concentrations;

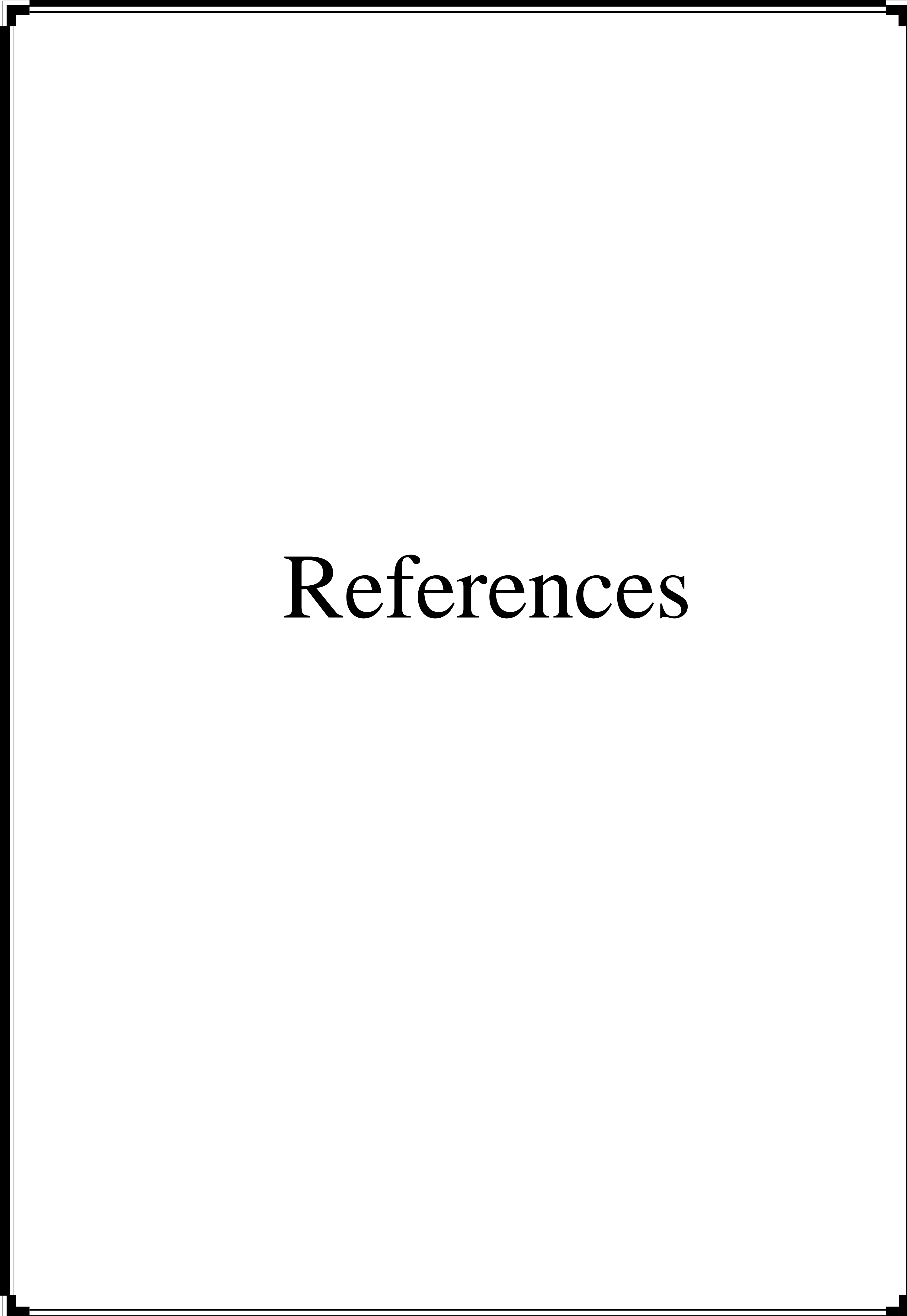
✓ Evaluation of *invivo* anti-*H. pylori* of combinations using other animals model;

✓ Carrying out long-term *in vivo* studies on the curative and preventive effect of probiotics and prebiotics and their combinations on *H. pylori;*

✓ Comparing the *invivo* anti-*H. pylori* effect of probiotics and prebiotics combinations with conventional therapies (triple therapy and quadruple therapy);

✓ Improvement of dairy products such as fermented milk (yogurt), butter, cheese using combinations between probiotics and medicinal plants;

✓ Testing these dairy products on patients suffering from gastro-duodenal diseases originated from different regions.



# References

1. Aaron, C. (1997). The Great Garlic Book: A Guide with recipes. Ed. Ten Speed Press, USA. PP160.
2. Abdel Hamid, M.R., Alalla, R.K., Moue, Z.N. (1984). Chemical studies on Egyptian fenugreek seed, *Annals of Agricultural Sciences*, 29(1): 43–60.
3. Abdel Moneim, E., Sulieman, Heba E., Ahmed Awad, M., Abdelrahim, A. (2008). The chemical composition of fenugreek (*Trigonella foenum graceum* L.) and the antimicrobial properties of its seed oil, *Journal of Engineering and Applied Science*, 3(2):52–71.
4. Abdel Nabey, A.A., Damir, A.A. (1990). Changes in some nutrients of fenugreek (*Trigonella Foenum graecum* L.) seeds during water boiling. *Plant Foods for Human Nutrition,* 40 (26): 72–74.
5. Abdel, A. Z. E. (1973). Experimental investigations on the yield and quality of garlic. *Acta Horticulturae*, 33:43-49
6. Abdel-Daim, M.M., Shaheen, H.M., Abushouk, A.I., Toraih, E.A., Fawzy, M.S., Alansari, W.S., Aleya, L., Bungau, S. (2018). Thymoquinone and diallyl sulfide protect against fipronil-induced oxidative injury in rats. *Environmental Science and Pollution Research,* 25: 23909–23916.
7. Abdel-Maksoud, G., El-Amin, A.R. (2011). A review on the materials used during the mummification processes in ancient Egypt. *Mediterranean Archaeology and Archaeometry*, 11(2): 129-150.
8. Abdou, I.A., Abou-Zeid, A.A., El-Sherbeeny, M.R., Abou-El-Gheat, Z.H. (1972). Antimicrobial activities of *Allium sativum*, *Allium cepa*, *Raphanus sativus*, *Capsicum frutescens*, *Eruca sativa*, *Allium kurrat* on bacteria. *Plant Foods for Human Nutrition*, 22(1): 29 -35.
9. Abdouli, H. Hadj Ayed, M., Elham, M., Nabila, B., Remedios Alvir Morencos, M. (2012). Proximate composition, and total phenols, tannins, flavonoids and saponins, and *in vitro* ruminal fermentation activity of fenugreek cut at three maturity stages. *Livestock Research for Rural Development*, 24(1): 1-11.
10. Abdouli, H., Missaoui, H., Jellali, S., Tibaoui, G., Tayachi, L. (2014). Comparison of two fenugreek seed genotypes: bitterness value, secondary metabolites contents and biological activities. *Journal of New Sciences*,7:19-27.
11. Abdul Ghani, J.M. (2010). Determination of alliin and allicin in different types garlic using high performance liquid chromatography. *Journal of University of Anbar for Pure Science,* 4(2) : 1-8.
12. Abou Azoom, A.A., Hamdi, W., Zhani, K., Hannachi, C. (2015). Evaluation of mineral element, sugars and proteins compositions in bulbs of eight onion (*Allium cepa* L.) varieties cultivated in Tunisia. *International Research Journal of Engineering and Technology*, 2: 35-39.
13. Abuga, I. (2014).The phytochemicals of onion as affected by inorganic fertilizer international. *On Line Journal of Biological Sciences*, 01(05): 30-40.
14. Acharya, S.N., Blade, S., Mir, Z., Moyer, J.R. (2007). Tristar fenugreek. *Canadian Journal of Plant Science*, 87(4): 901-903.
15. Acharya, S.N., Thomas, J.E., Basu, S.K. (2006). Fenugreek: an old world crop for the new world. *Biodiversity*, 7(3-4): 27-30.
16. Acharya, S., Srichamoren, A.S., Basu Ooraikul, B., Basu, T. (2004) Improvement in the nutraceutical properties of fenugreek (*Trigonella Foenum-graecum* L.). *Songklanakarin* Journal of *Science* and *Technology*, 28(1): 1-9.
17. Ackerman, Z., Peston, D., Cohen, P. (2003). Role of *Helicobacter pylori* infection in complications from Meckel's diverticulum. *Digestive Diseases and Sciences*, 48:1068 –1072.
18. Adeniyi, B.A., Oluwole, F.S., Anyiam, F.M. (2006). Antimicrobial and antiulcer activities of methanol extract of *Allium sativum* on *Helicobacter pylori*. *Journal of Biological Sciences*, 6(3): 521-526.
19. Agarwal, K.C. (1996). Therapeutic actions of garlic constituents. *Medicinal Research Reviews*, 16(1): 111-124.
20. Agarwal, U., Pathak, D.P., Kapoor, G., Bhutani, R., Roper, R., Gupta, V. Kant, R. (2017). Review on *Cuminum cyminum* nature's magical seeds. *Journal of Chemical and Pharmaceutical Research*, 9(9): 180-187.
21. Ahmad, D. (2017), Physicochemical properties of chemurgic fenugreek (*Trigonella foenum–graecum*) herb of different origin. *Der Pharma Chemica*, 9 (1): 102–106.
22. Ahmad, T.A., El-Sayed, B.A., El-Sayed, L.H. (2016). Development of immunization trials against *Eimeria* spp. *Trials in Vaccinology*, 5: 38–47.
23. Ahmadi Jouybari, T., Aghaei, A., Ataee, M., Navabi, J., Anvari, B., Jouybari, HA., Majnooni, MB. (2021). Synergistic effect of cumin (*Cuminum cyminum* L.) decoction alongside with three-drug and four-drug treatment protocols on *Helicobacter pylori* eradication. *Journal of Reports in Pharmaceutical Sciences*. 10:66-70
24. Ahmed Khaja, S., Aleem Ahmed Khan, I., Ahmed Santosh, K., Tiwari, A., Habeeb, J.D., Ahi Zakia Abid, N., Ahmed Chitoor, M.H. (2007). Impact of house hold hygiene and water source on the prevalence and transmission of *Helicobacter pylori*: A South Indian perspective. *Singapore Medical Journal,* 48:543-549.

25. Aiba, Y., Suzuki, N., Kabir, A.M., Takagi, A., Koga, Y. (1998). Lactic acid-mediated suppression of *Helicobacter pylori* by the oral administration of *Lactobacillus salivarius* as a probiotic in a gnotobiotic murine model. *American Journal of Gastroenterology*, 93: 2097-2101.
26. Akter, S., Netzel, M.E., Tinggi, U., Osborne, S.A., Fletcher, M.T., Sultanbawa, Y. (2019). Antioxidant rich extracts of *Terminalia ferdinandiana* inhibit the growth of food borne bacteria. *Foods*,8 (8)*:* 2-18.
27. Al Juhaimi, F., Ghafoor, K. (2013). Extraction optimization and *in vitro* antioxidant properties of phenolic compounds from cumin *(Cuminum cyminum* L.) seed. *International Food Research Journal*, 20:1669-1675.
28. Alakomi, H.L., Skyttä, E., Saarela, M., Mattila-S holm, T., Latva-Kala, K., Helander, I.M. (2000). Lactic acid permeabilizes Gram-negative bacteria by disrupting the outer membrane. *Applied and Environmental Microbiology*, 66(5): 2001-2005.
29. Al-Habori, M., Raman, A. (2002). Pharmacological properties. In: Fenugreek, (Eds. Petropoulos, G.A.), CRC Press. USA, PP. 178-198.
30. Ali, R., Mohsen Saberi, N. (2014). Physicochemical characteristics of garlic (*Allium sativum* L.) oil: effect of extraction procedure. *International Journal of Food Science and Nutrition*, 3(6:1): 1-5.
31. Aljeboury, G.H., Risan, M.H., Algafari, R.N. (2020). Role of VAC a and CAG a genes in detection and identification of *Helicobacter pylori*. *Indian Journal of Public Health*, 11(02): 2287-2290.
32. Alkofahi, A., Atta, A.H. (1999). Pharmacological screening of the anti-ulcerogenic effects of some Jordanian medicinal plants in rats. *Journal of Ethnopharmacology*, 67(3), 341-345.
33. Alm, R.A., Ling, L.S.L., Moir, D.T., King, B.L., Brown, E.D., Doig, P.C., Smith, D.R., Noonan, B., Guild, B.C., Dejonge, B.L., Carmel, G. (1999). Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. *Nature*, 397(6715):176-180.
34. Alm, R.A., Trust, T.J. (1999). Analysis of the genetic diversity of *Helicobacter pylori*: the tale of two genomes. *Journal of Molecular Medicine,*77: 834-846.
35. Al-Maamari, I., Khan, M., Ali, A., Al-Sadi, A., Waly, M., Al-Saady, N. (2016). Diversity in phytochemical composition of omani fenugreek (*Trigonella foenum graecum* L.) accessions. *Pakistan Journal of Agricultural,* 53(4):1-12.
36. Al-Snafi, A. (2013). Pharmacological effects of *Allium* species grown in Iraq. An overview. *International Journal of Pharmaceutical and Health Care,* 1: 132–147.
37. Al-Snafi, A. (2016). The pharmacological activities of *Cuminum cyminum*-A review. *IOSR Journal of Pharmacy*, 6(6): 46-65.
38. Altura, B.M., Altura, B.T (1999). Cardiovascular risk factors and magnesium: relationship to atherosclerosis, ischemic heart disease and hypertension. *Indian Journal of Experimental Biology,* 37(2): 109-116.
39. Andrews, J.M. (2001). The development of the BSAC standardized method of disc diffusion testing. *Journal of Antimicrobial Chemotherapy*, 48(1): 29-42.
40. Ani, V., Varadaraj, M., Naidu, A. (2006). Antioxidant and antibacterial activities of polyphenolic compounds from bitter Cumin (*Cuminum nigrum* L.). *European Food Research andTechnology,* 224:109-115.
41. Anita, K., Malkit N., Rajbir S. (2006), Proximate composition, available carbohydrates, dietary fibers and anti nutritional factors of selected traditional medicinal plants, *Journal of Human Ecology,* 19(3): 195–199.
42. Annibale, B., Negrini, R., Caruana, P., Lahner, E., Grossi, C., Bordi, C., Fave, G.D. (2001).Two-thirds of atrophic body gastritis patients have evidence of *Helicobacter pylori* infection. *Helicobacter*, 6: 225-233.
43. Anokhina, I.V., Kravtsov, EG., Protsenko, A.V., Yashina, N.V., Yermolaev, A.V., Chesnokova, V.L., Dalin, M.V. (2007). Bactericidal activity of culture fluid components of *Lactobacillus fermentum* strain 90 TS-4 (21) clone 3, and their capacity to modulate adhesion of *Candida Albicans* yeast-like fungi to vaginal epithelial cells. *Bulletin* of *Experimental Biology and Medicine*,143(3): 359–362.
44. AOAC. (1995). Official methods of analysis. Association of Official Analytical Chemists. 16$^{th}$ Ed. Washington DC, USA.
45. AOAC. (2000), Official method of analysis. Association of Official Analytical Chemists,17$^{th}$ Ed. Maryland. U.S.A.
46. AOAC. (2002). Official Methods of Analysis. Association of Official Analytical Chemists. 17$^{th}$ Ed. Gaithersburg, U.S.A.
47. Apawu, A.K. (2009). Reversed-phase HPLC determination of alliin in diverse varieties of fresh garlic and commercial garlic products. PhD Thesis, Chemistry, East Tennessee State University, USA, PP 72.
48. Aremu, M., Olaleke, O., Olorunfemi, A., Emmanuel, T. (2006). A Comparative study on the chemical and amino acid composition of some Nigerian under–utilized legume flours, *Pakistan Journal of Nutrition*, 5: 34–38.
49. Ariyama, K., Nishida, T., Noda, T., Kadokura, M., Yasui, A. (2006). Effects of fertilization, crop year, variety, and provenance factors on mineral concentrations in onions. *Journal of Agricultural and Food Chemistry,* 54(9): 3341-3350.

50. Armuzzi, A., Cremonini, F., Bartolozzi, F., Canducci, F., Candelli,M., Ojetti, V., Cammarota, G., Anti, M., De Lorenzo, A., Pola, P,Gasbarrini, G., Gasbarrini, A. (2001). The effect of oral administration of *Lactobacillus* GG on antibiotic-associated gastrointestinal side-effects during *Helicobacter pylori* eradication therapy. *Alimentary Pharmacology Therapeutics,* 15: 163-169.
51. Arora D.R. (2007). Text book of Microbiology. 2nd Ed. CBS Publishers, New Delhi, PP 107–115.
52. Arzanlou, M., Bohlooli, S. (2010). Introducing of green garlic plant as a new source of allicin. *Food Chemistry*, 120(1): 179-183.
53. Asdaq, S.M.B., Inamdar, M.N. (2011). Pharmacodynamic and pharmacokinetic interactions of propranolol with garlic (*Allium sativum*) in rats. *Evidence Based Complementary and Alternative Medicine,*2011: 1-11.
54. Asenjo, L.M. Gisbert, J.P. (2007). Prevalence of *Helicobacter pylori* infection in gastric MALT lymphoma: a systematic review. *Revista Espanola de Enfermedades Digestivas,*99: 398-404.
55. Asmena, M., Alauddin, M., Rahman, M.A., Ahmed, K. (2009). Antihyperglycemic effect of *Trigonella foenum graecum* (fenugreek) seed extract in alloxan-induced diabetic rats and its use in diabetes mellitus: a brief qualitative phytochemical and acute toxicity test on the extract. *African Journal of Traditional*, *Complementary and Alternative Medicines*, 6(3):255-261.
56. Awais, A., Salem, S.A., Kaiser, M., Muhammad, A. (2015), Fenugreek a multipurpose crop: Potentialities and improvements, *Saudi Journal of Biological Sciences*, 23:1–11.
57. Axon, A. (2014). *Helicobacter pylori* and public health. *Helicobacter*, 19: 68-73.
58. Ayala-Hernandez, I., Goff, H.D. Corredig, M. (2008). Interactions between milk proteins and exopolysaccharides produced by *Lactococcus lactis* observed by scanning electron microscopy. *Journal of Dairy Science*, 91(7): 2583-2590.
59. Azeez, S. (2008). Cumin. In: Chemistry of spices. (Eds. Villupanoor A. P., Bhageerathy C.T., John Z.), CABI, Wallingford, UK. PP 211-226.
60. Azu, N.C., Onyeagba, R.A. (2007). Antimicrobial properties of extracts of *Allium cepa* (Onions) and *Zingiber officinale* (Ginger) on *Escherichia coli, Salmonella typhi* and *Bacillus subtilis*. *The Internet Journal of Tropical Medicine*, 3(2): 1-10.
61. Bäckström, A., Lundberg, C., Kersulyte, D., Berg, D.E., Borén, T., Arnqvist, A. (2004). Metastability of *Helicobacter pylori* bab adhesin genes and dynamics in Lewis b antigen binding. *Proceedings of the National Academy of Sciences*, 101(48): 16923-16928.
62. Badotti, F., Moreira, A.P., Tonon, L.A., de Lucena, B.T., Gomes, F., de C, Kruger, R., Thompson, C.C., de Morais, MA. Jr, Rosa, CA., Thompson, FL. (2014). *Oenococcus alcoholitolerans* sp. Nov., a lactic acid bacteria isolated from cachaca and ethanol fermentation processes. *Antonie Van Leeuwenhoek*, 106:1259–12 67.
63. Bae, E.A., Kim, D.H., Han, M.J. (2000). Anti-*Helicobacter pylori* activity of *Bifidobacterium* spp. *Journal of Microbiology and Biotechnology*, 10(4): 532-534.
64. Bago, J., Majstorović, K., Belošić-Halle, Ž., Kućišec, N., Bakula, V., Tomić, M., Bago, P., Troskot, R. (2010). Antimicrobial resistance of *H. pylori* to the outcome of 10-days vs. 7-days Moxifloxacin based therapy for the eradication: a randomized controlled trial. *Annals of Clinical Microbiology and Antimicrobials*, 9(1):1-6.
65. Bajaj, K.L., Kaur, G., Singh, J., Gill, S.P.S. (1980). Chemical evaluation of some important varieties of onion (*Allium cepa L.*). *Plant Foods* for *Human Nutrition,* 30 (2) :117–122.
66. Baloch, A.F. (1994). Vegetable crops. Horticulture. Ed. National Book Foundation, Islamabad, Pakistan. PP 489-537.
67. Bansal, A., Bansal, V., Singh, R. (2014). Cumin : A spice or a drug ?. *World Journal of Pharmaceutical Sciences*, 2(5): 507–515.
68. Bardhan, K.D., Dillon, J., Axon, A.T., Cooper, B.T., Tildesley, G., Wyatt, J.I., Braun, W. (2000). Triple therapy for *Helicobacter pylori* eradication: a comparison of pantoprazole once versus twice daily. *Alimentary Pharmacology and Therapeutics*, 14(1): 59-68.
69. Bardhan, K.D., Dillon, J., Axon, A.T., Cooper, B.T., Tildesley, G., Wyatt, J.I., Braun, W. (2000). Triple therapy for *Helicobacter pylori* eradication: a comparison of pantoprazole once versus twice daily. *Alimentary Pharmacology and Therapeutics*, 14(1): 59-68.
70. Barefoot, S.F., Klaenhammer, T.R., (1983). Detection and activity of lacticin B, a bacteriocin produced by *Lactobacillus acidiphilus*. *Applied and Environmental Microbiology*, 45(6) : 1808-1815.
71. Baricault, L., Denariaz, G., Houri, J.J., Bouley, C., Sapin, C., Trugnan, G. (1995). Use of HT-29, a cultured human colon cancer Cell line, to study the efect of fermented milks on colon cancer Cell growth and diferentiation. *Carcinogenesis*, 16(2): 245–252.
72. Basso, D., Zambon, C.F., Letley, D.P., Stranges, A., Marchet, A., Rhead, J.L., Schiavon, S., Guariso, G., Ceroti, M., Nitti, D., Rugge, M., (2008). Clinical relevance of *Helicobacter pylori* cagA and vacA gene polymorphisms. *Gastroenterology*, 135(1), 91-99.

73. Bastard, A., Coelho, C., Briandet, R., Canette, A., Gougeon, R., Alexandre, H., Guzzo, J. Weidmann, S., (2016). Effect of biofilm formation by *Oenococcus oeni* on malolactic fermentation and the release of aromatic compounds in wine. *Frontiers in Microbiology*, *7*: 1-14.
74. Behrad, S., Yusof, Y., Goh, K., Baba, H., Ahmad, S.(2009).Manipulation of probiotics fermentation of yogurt by Cinnamon and Licorice: effects on yogurt formation and Inhibition of *Helicobacter Pylori* growth *in vitro*. *World Academy of Science, Engineering and Technology*, 60: 590-594.
75. Belal, A.A., Ahmed, F.B., Ali, L.I. (2017). Antibacterial activity of *Cuminum cyminum* L. oil on six types of bacteria. *American Journal of Bio Science*, *5*(4): 70-73.
76. Benayad, Z., Gómez-Cordovés, C., Es-Safi, N.E. (2014). Characterization of flavonoid glycosides from fenugreek *(Trigonella foenum-graecum*) crude seeds by HPLC–DAD–ESI/MS analysis. *International Journal of Molecular* Sciences, 15 (11): 20668-20685.
77. Benites Vílchez, J., Díaz García, R., López Vivar, J., Gajardo Solari, S., Kusch Fuschlocher, F., Rojas Arredondo, M. (2011). Actividad antioxidante y antibacteriana de seis cáscaras de frutos del oasis de Pica. *Biofarbo*, 19 (1):1-7.
78. Benkeblia, N. (2005).Free-radical scavenging capacity and antioxidant properties of some selected onions (*Allium cepa* L.) and garlic (*Allium sativum* L.) extracts. *Brazilian* Archives of *Biology* and *Technology,*48(5):753-759.
79. Benkeblia, N. (2005).Free-radical scavenging capacity and antioxidant properties of some selected onions (*Allium cepa* L.) and garlic (*Allium sativum* L.) extracts. *Brazilian Archives of Biology* and *Technology,*48(5):753-759.
80. Bennett, M.D., Johnston, S., Hodnett, G.L., Price, H.J. (2000). *Allium cepa* L. cultivars from four continents compared by flow cytometry show nuclear DNA constancy. *Annals of Botany,* 85(3): 351-357.
81. Benziane, M.N.A., Acem, K., Aggad, H., Abdali, M. (2019). Phytochemistry, HPLC profile and antioxidant activity of aqueous extracts of fenugreek seeds grown in arid zones of Algeria. *Acta Scientifica Naturalis*, 6(2): 71-87.
82. Beresford, Tom P., Briandet R,. (2001). Recent advances in cheese microbiology. *International Dairy Journal,* 11(4-7): 259-274.
83. Berginc, K., Milisav, I., Kristl, A. (2010). Garlic flavonoids and organosulfur compounds: impact on the hepatic pharmacokinetics of saquinavir and darunavir. *Drug Metabolism and Pharmacokinetics*, 25(6): 521-530.
84. Bermudez-Brito, M., Plaza-Díaz, J., Muñoz-Quezada, S., Gómez-Llorente, C., Gil, A. (2012). Probiotic mechanisms of action. *Annals of Nutrition and Metabolism*, 61(2): 160-174.
85. Bernet, M.F., Brassart, D., Neeser, J.R., Servin, A.L. (1994). *Lactobacillus acidophilus* LA 1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. *Gut*, 35(4): 483-489.
86. Bessada, S.M., Barreira, J.C., Barros, L., Ferreira, I.C., Oliveira, M.B.P. (2016). Phenolic profile and antioxidant activity of *Coleostephus myconis* (L.) An underexploited and highly disseminated species. *Industrial Crops and Products,*89:45-51.
87. Bhanot ,A., Shri, R. (2010).A comparative profile of methanol extracts of *Allium cepa* and *Allium sativum* in diabetic neuropathy in mice. *Pharmacognosy Research*, 2(6): 374-384.
88. Bhatia, K., Kaur, M., Atif, F., Ali, M., Rehman, H., Rahman, S. (2006). Aqueous extract of *Trigonella Foenum graecum* L. ameliorates additive urotoxicity of buthionine sulfoximine and cyclophosphamide in mice. *Food and Chemical Toxicology*, 44: 1744–1750.
89. Bina, J., Bains, M. Hancock, R.E., (2000). Functional expression in *Escherichia coli* and membrane topology of porin HopE, a member of a large family of conserved proteins in *Helicobacter pylori*. *Journal of Bacteriology*, 182 (9): 2370–2375.
90. Bison, P.S., Verma, K. (1994). In: Hand Book of microbiology. Ed. CBS Publications, Delhi. PP 199.
91. Bizzozero, G.(1893). Ueber die schlauchförmigen Drüsen des Magendarmkanals und die Beziehungenihres Epitheles zu dem Oberflächenepithel der Schleimhaut". *Archiv für Mikroskopische Anatomie*,42: 82–152.
92. Björkroth, J., Holzapfel, W. (2006). Genera *Leuconostoc, Oenococcus* and *Weissella. The prokaryotes*, 4: 267-319.
93. Blaser, M.J. (1997). Ecology of *Helicobacter pylori* in the human stomach. *The Journal of Clinical Investigation*, 100:759-762.
94. Blaser, M.J. (2005). An endangered species in the stomach. *Scientific American*, 292:38-45.
95. Blaser, M.J., Chyou, P.H., Nomura, A. (1995). Age at establishment of *Helicobacter pylori* infection and gastric carcinoma, gastric ulcer, and duodenal ulcer risk. *Cancer Research,* 55: 562-565.
96. Blecker, U., Lanciers, S., Hauser, B., De Pont, S.M., Vandenplas, Y., (1995). The contribution of specific immunoglobulin M antibodies to the diagnosis of *Helicobacter pylori* infection in children. *European Journal of Gastroenterology and Hepatology*, 7(10): 979-983.
97. Block, E. (1985). The chemistry of garlic and aims. *Scientific American,* 252: 114-119.

98. Bordas, M., Araque, I., Alegret, J.O., El Khoury, M., Lucas, P., Rozès, N., Reguant, C., Bordons, A. (2013). Isolation, selection, and characterization of highly ethanol-tolerant strains of *Oenococcus oeni* from south Catalonia. *International Microbiology*, 16 (2):113-123.
99. Borlinghaus, J., Albrecht, F., Gruhlke, M.C., Nwachukwu, I.D., Slusarenko, A.J. (2014). Allicin: Chemistry and biological properties. *Molecules*,19:12591–12618.
100. Borneman, A.R., Desany, B.A., Riches, D., Affourtit, J.P., Forgan, A.H., Pretorius, I.S., Egholm, M., Chambers, P.J. (2012). The genome sequence of the wine yeast VIN7 reveals an allotriploid hybrid genome with *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii* origins. *FEMS Yeast Research*, 12(1): 88-96.
101. Böttcher, C., Krähmer, A., Stürtz, M., Widder, S., Schulz, H. (2017). Comprehensive metabolite profiling of onion bulbs (*Allium cepa*) using liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry. *Metabolomics*, 13(4): 1-15.
102. Bouzouita, N., Kachouri, F., Ben Halima, M., Chaabouni, M.M. (2008). Composition chimique et activité antioxydante, antimicrobienne et insecticide de l'huile essentielle de *Juniperus phoenicea*. *Journal de la Société Chimique de Tunisie,*10 :119-125.
103. Boyanova, L., Gergova, G., Markovska, R., Yordanov, D., Mitov, I. (2017). Bacteriocin-like inhibitory activities of seven *Lactobacillus delbrueckii subsp. bulgaricus* strains against antibiotic susceptible and resistant *Helicobacter pylori* strains. *Letters in Applied Microbiology,* 65 (6): 469-474.
104. Boyanova, L., Stephanova-Kondratenko, M., Mitov, I. (2009). Anti-*Helicobacter pylori* activity of *Lactobacillus delbrueckii subsp. bulgaricus* strains: preliminary report. *Letters in Applied Microbiology.* 48(5): 579–584.
105. Branch, S. (2013). Fenugreek (*Trigonella foenum-graecum* L.) as a valuable medicinal plant. *International Journal of Advanced Biological and Biomedical Research*, 1: 922-931.
106. Brewer, M.S. (2011). Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Comprehensive Reviews in Food Science and Food Safety*, 10: 221 – 247.
107. Brown, L.M., Thomas, T.L., Ma, J.L., Chang, Y.S., You, W.C., Liu, W.D., Zhang, L., Pee, D., Gail, M.H. (2002). *Helicobacter pylori* infection in rural China: demographic, lifestyle and environmental factors. *International Journal of Epidemiology*, 31(3): 638-645.
108. Brummer, Y., Cui, W., Wang, Q. (2003), Extraction, purification and physicochemical characterization of fenugreek gum, *Food Hydrocolloids*, 17: 229– 236.
109. Brunetti, C., Di Ferdinando, M., Fini, A., Pollastri, S., Tattini, M. (2013). Flavonoids as antioxidants and developmental regulators: relative significance in plants and humans. *International Journal of Molecular Sciences*, 14(2): 3540-3555.
110. Buddhiman, T., Jyoti, P.T., Ulrich, S., Charles, M.A.P.F., Michael, G., Wilhelm, H.H. (2008). Phenotypic and genotypic identification of lactic acid bacteria isolated from ethnic fermented bamboo Tender shoots of North East India. *International Journal of Food Microbiology,* 121(1): 35-40.
111. Bukhari, M.M., Salem, E.K., Osman, A., Hegazi, S.E.F. (2015). Investigations of the influence of dextran on sugar cane quality and sugar cane processing in Kenana sugar factory. *Journal of Chemical and Pharmaceutical Research*, 7(4): 381-392.
112. Bukhari, S., Bhanger, M.D., Memon, S. (2008). Antioxidative activity of extracts from fenugreek seeds (*Trigonella foenum-graecum*). *Pakistan Journal of Analytical and Environmental Chemistry*, 9(2) :78-83.
113. Burke, A.D. (1938). Practical manufacture of cultured milks and kindred products. In: Practical manufacture of cultured milks and kindred products. The Olsen Pub. Co. Milwaukee, Wisconsin, USA. PP194.
114. Burkitt, M.D., Duckworth, C. A., Williams, J. M., Pritchard, D. M. (2017). *Helicobacter pylori*-induced gastric pathology: insights from *in vivo* and *ex vivo* models. *Disease Models and Mechanisms*, 10 (2): 89 104.
115. Burkitt, M.D., Varro, A., Pritchard, D.M. (2009). Importance of gastrin in the pathogenesis and treatment of gastric tumors. *World Journal of Gastroenterology,* 15: 1-16.
116. Burri, S., Ekholm, A., Håkansson, Å., Tornberg, E., Rumpunen, K. (2017). Antioxidant capacity and major phenol compounds of horticultural plant materials not usually used. *Journal of Functional Foods*, 38(1): 119–127.
117. Cafaro, C., Bonomo, M.G., Salzano, G. (2014): Adaptive changes in geranylgeranyl pyrophosphate synthase gene expression level under ethanol stress conditions in *Oenococcus oeni*. *Journal of Applied Microbiology,* 116(1): 71-80.
118. Callon, C., Millet, L., Montel, M.C. (2004). Diversity of lactic acid bacteria isolated from AOC Salers cheese. *Journal of Dairy Research*, 71(2): 231-244.
119. Campbell, D.I., Warren, B.F., Thomas, J.E., Figura, N., Telford, J.L., Sullivan, P.B. (2001). The African enigma: low prevalence of gastric atrophy, high prevalence of chronic inflammation in West African adults and children. *Helicobacter,* 6: 263-267.

120. Canani, R.B., Cirillo, P., Terrin, G., Cesarano, L., Spagnuolo, M.I., De Vincenzo, A., Albano, F., Passariello, A., De Marco, G., Manguso, F., Guarino, A. (2007). Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations. *British Medical Journal,* 335(7615): 1-16.
121. Canducci, F., Armuzzi, A., Cremonini, F., Cammarota, G., Bar-tolozzi, F., Pola, P., Gasbarrini, G, Gasbarrini, A. (2000). A lyophilizedand inactivated culture of *Lactobacillus acidophilus* increases *Helicobacter pylori* eradication rates. *Alimentary Pharmacology Therapeutics*, 14: 1625-1629.
122. Cañizares, P., Gracia, I., Gómez, L.A., de Argila, C.M., de Rafael, L., García, A. (2002). Optimization of *Allium sativum* solvent extraction for the inhibition of *in vitro* growth of *Helicobacter pylori*. *Biotechnology Progress*, 18 (6): 1227-1232.
123. Cao, X., Cao, L., Ding, L., Bian, J.S. (2017). A new hope for adevastating disease: Hydrogen sulfidein Parkinson's disease. *Molecular Neurobiology,* 55:3789 3799.
124. Capel Abad, S. (2014). Estudi de l'efecte genotípic i ambiental en característiques químiques i sensorials dels calçots (*Allium cepa* L.). Bachelor's thesis, Universitat politecnica de Catalunya, Barcelona. p 59.
125. Caruso, G., Contia, S.,Villari, G., Borrellia, C., Melchionna, G., Minutolo, M., Russo, G., Amalfitano, C. (2014). Effects of transplanting time and plant density on yield, quality and antioxidant content of onion (*Allium cepa* L.) in Southern Italy. *Scientia Horticulturae*, 166 (13):111-120.
126. Casalta, E. (2003). Bases scientifiques de la qualité du Venaco, fromage traditionnel au lait cru: mise au point de ferments sélectionnés spécifiques, PhD Thesis, Microbiology, University of Bourgogne, Dijon. PP 134.
127. Caselli, M., Zullo, A., Maconi, G., Parente, F., Alvisi, V., Casetti, T., Sorrentino, D., Gasbarrini, G. (2007). Cervia II Working Group Report 2006: guidelines on diagnosis and treatment of *Helicobacter pylori* infection in Italy. *Digestive and Liver Disease*, 39(8):782-789.
128. Castillo-Juárez, I., González, V., Jaime-Aguilar, H., Martínez, G., Linares, E., Bye, R., Romero, I. (2009). Anti-*Helicobacter pylori* activity of plants used in Mexican traditional medicine for gastrointestinal disorders. *Journal of Ethnopharmacology*, 122: 402-405.
129. Cavallito, C., Bailey, J.H. (1944). Allicin, the antibacterial principle of *Allium sativum.* Isolation, physical properties and antibacterial action. *Journal of the American Chemical Society,* 66 (11):1950-1951.
130. Cave, D.R. (1997). How is *Helicobacter pylori* transmitted?. *Gastroenterology*,113 (6): S9-S14.
131. Cavin, J.E, Schmitt, P., Arias, A., Lin, J., Divies, C. (1988) Plasmid profiles in *Leuconostoc* species. *Microbiology Alimentary Nutrition*, 6: 55-62.
132. Cellini, L. (2014). *Helicobacter pylori*: a chameleon-like approach to life. *World Journal of Gastroenterology*, 20(19), 5575-5582.
133. Cellini, L., Di Campli, E., Masuli, M., Di Bartolomeo, S. (1996). Inhibition of *Helicobacter pylori* by garlic extract. *FEMS Immunology and Medical Microbiology*, 13: 273–277.
134. Cellini, L., Grande, R., Di Campli, E., Traini, T., Di Giulio, M., Nicola Lannutti, S., Lattanzio, R. (2008). Dynamic colonization of *Helicobacter pylori* in human gastric mucosa. *Scandinavian Journal of Gastroenterology*, 43(2): 178-185.
135. Chang, M. C., Chang, Y. T., Sun, C. T., Wu, M. S., Wang, H. P., Lin, J. T. (2002). Quantitative correlation of *Helicobacter pylori* stool antigen (HpSA) test with 13C-urea breath test (13C-UBT) by the updated Sydney grading system of gastritis. *Hepato-Gastroenterology*, 49(44): 576-579.
136. Charles, D.J. (2013). Antioxidant Properties of Spices. Ed. Herbs and Other Sources. Springer Science and Business Media, New York. PP. 295-303.
137. Chattopadhyay, S.B., Maiti, S. (1990). Diseases of betalvine and spices. Indian Council of Agricultural Research. Krishi Anusandhan Bhawan, New Delhi. Pp122-129.
138. Chaudhary, S., Chaudhary, P. S., Chikara, S. K., Sharma, M. C. Iriti, M. (2018). Review on fenugreek (*Trigonella foenum-graecum* L.) and its important secondary metabolite diosgenin. *Notulae Botanicae Horti Agrobotanici*, 46(1): 22-31.
139. Che, O., Siti, F., Idid, Z.S., Koya, S.M., Rehan, M.A., Kamarudin, K. R.(2011).Antioxidant study of garlic and red onion: A comparative study. *Pertanika Journal of Tropical Agricultural Science,* 34: 253-261.
140. Chekki, R., Snoussi, A., Hamrouni, I., Bouzouita, N. (2014). Chemical composition, antibacterial and antioxidant activities of Tunisian garlic (*Allium sativum)* essential oil and ethanol extract. *Mediterranean Journal of Chemistry*, 3: 947-956.
141. Chen, S., Zhou ,J., Chen, Q., Chang, Y., Du, J., Meng, H. (2013). Analysis of the genetic diversity of garlic (*Allium sativum* L.) germ plasm by SRAP. *Biochemical* Systematics and *Ecology,*50: 139-146.
142. Chen, Y., Sun, J., Dou, C., Li, N., Kang, F., Wang, Y., Cao, Z., Yang, X., Dong, S. (2016). Alliin attenuated RANKL-induced osteoclastogenesis by scavenging reactive oxygen species through inhibiting Nox1. *International Journal of Molecular Sciences,* 17(9):1-13.

143.Chen, Y.H., Tsai, W.H., Wu, H.Y., Chen, C.Y., Yeh, W.L., Chen, Y.H., Hsu, H.Y., Chen, W.W., Chen, Y.W., Chang, W.W., Lin, T.L. (2019). Probiotic *Lactobacillus spp*. act against *Helicobacter pylori* induced inflammation. *Journal of Clinical Medicine*, 8(1): 90-106.

144.Cheng, A., Chen, X., Jin, Q., Wang, W., Shi, J., Liu, Y. (2013). Comparison of phenolic content and anti-oxidant capacity of red and yellow onions. *Czech Journal of Food Sciences*, 31: 501–508.

145.Chenoll, E., Casinos, B., Bataller, E., Astals, P., Echevarría, J., Iglesias, J.R., Balbarie, P., Ramón, D., Genovés, S. (2011). Novel probiotic *Bifidobacterium bifidum* CECT 7366 strain active against the pathogenic bacterium *Helicobacter pylori*. *Applied and Environmental Microbiology Journal*, 77: 1335–1343.

146.Chong, S.K., Lou, Q., Zollinger, T.W., Rabinowitz, S., Jibaly, R., Tolia, V., Elitsur, Y., Gold, B.D., Rosenberg, A., Johnson, A., Elkayam, O. (2003). The seroprevalence of *Helicobacter pylori* in a referral population of children in the United States. *The American Journal of Gastroenterology*, 98 (10): 2162-2168.

147.Chun J., Lee J., Ye L., Exler J., Eitenmiller R. (2006).Tocopherol and tocotrienol contents of raw and processed fruits and vegetables in the United States diet. *Journal of Food Composition and Analysis*, 19:196-204.

148.Chun, O.K., Kim, D., Smith, N., Schroeder, D., Han, J.T., Lee, C.Y. (2005). Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *Journal* of the *Science of Food* and *Agriculture*, 85:1715-1724.

149.Coconnier, M.H., Lievin, V., Hemery, E., Servin, A.L. (1998). Antagonistic activity against *Helicobacter* infection *in vitro* and *in vivo* by the human *Lactobacillus acidophilus* strain LB. *Applied and Environmental Microbiology*, 64 (11): 4573-4580.

150.Coconnier, M.H., Liévin, V., Lorrot, M., Servin, A.L. (2000). Antagonistic activity of *Lactobacillus acidophilus* LB against intracellular *Salmonella enterica serovar Typhimurium* infecting human enterocyte-like Caco-2/TC-7 cells. *Applied and Environmental Microbiology*, 66(3): 1152-1157.

151.Coconnier, M.H., Lievin, V., Hemery, E., Servin, A.L. (1998). Antagonistic activity against *Helicobacter* infection *in vitro* and *in vivo* by the human *Lactobacillus acidophilus* strain LB. *Applied and Environmental Microbiology*, 64 (11): 4573-4580.

152.Cogan, T.M., Barbosa, M., Beuvier, E., Bianchi-salvadori, B.R.U.N.A., Cocconcelli, P.S., Fernandes, I., Gomez, J., Gomez, R., Kalantzopoulos, G., Ledda, A., Medina, M. (1997). Characterization of the lactic acid bacteria in artisanal dairy products. *Journal of Dairy Research*, *64*(3):409-421.

153.Colding, H., Hartzen, S.H., Roshanisefat, H., Andersen, L.P., Krogfelt, K.A. (1999). Molecular methods for typing of *Helicobacter pylori* and their applications. *FEMS Immunology and Medical Microbiology*, *24*(2):193-199.

154.Colina-Coca, C., de Ancos, B., Sánchez-Moreno, C. (2014). Nutritional composition of processed onion: S-Alk (en) yl-L-cysteine sulfoxides, organic acids, sugars, minerals, and vitamin C. *Food and Bioprocess Technology*, 7(1): 289-298.

155.Colin-Gonzalez, AL., Santana, RA., Silva-Islas, CA., Chanez-Cardenas, ME., Santamaria, A., Maldonado, PD. (2012).The antioxidant mechanisms underlying the aged garlic extract and S-allylcysteine-induced protection. Oxidative *Medicine*and*Cellular Longevity*, 2012:1-16.

156.Collins, M.D., Phillips, B.A., Zanoni, P. (1989). Deoxyribonucleic acid homology studies of *Lactobacillus* casei, *Lactobacillus* paracasei sp. Nov., subsp. *Paracasei* and subsp. *Tolerans*, and *Lactobacillus rhamnosus* sp. Nov., comb. Nov. *International Journal of Systematic Bacteriology*, 39: 105–108.

157.Colman, G., Tanna, A., Efstratiou, A., Gaworzewska, E. T. (1993). The serotypes of *Streptococcus* pyogenes present in Britain during 1980–1990 and their association with disease. *Journal of Medical Microbiology*, 39(3): 165-178.

158.Corr, S. C., Li, Y., Riedel, C. U., O'Toole, P. W., Hill, C., Gahan, C. G. (2007). Bacteriocin production as a mechanism for the anti-infective activity of *Lactobacillus salivarius* UCC118. *Proceedings of the National Academy of Sciences*, 104(18): 7617-7621.

159.Correa, P., Haenszel, W., Cuello, C., Tannenbaum, S., Archer, M. (1975). A model for gastric cancer epidemiology. *Lancet*, 306: 58-60

160.Corroler, D., Mangin, I., Desmasures, N., Gueguen, M. (1999). An ecological study of *Lactococci* isolated from raw milk in the camembert cheese registered designation of origin area. *Applied and Environmental Microbiology*, 65(2): 878-878.

161.Corsetti, A., Lavermicocca, P., Morea, M., Baruzzi, F., Tosti, N., Gobbetti, M. (2001). Phenotypic and molecular identification and clustering of lactic acid bacteria and yeasts from wheat (species *Triticum durum* and *Triticum aestivum*) sourdoughs of Southern Italy. *International Journal of Food Microbiology*, 64(1-2): 95-104.

162.Cotter, P.D., Hill, C., Ross, R.P. (2005). Bacteriocins: developing innate immunity for food. *Nature Reviews Microbiology*, 3(10): 777-788.

163. Coucheney, F., Desroche, N., Bou, M., Tourdot-Maréchal, R., Dulau, L.,Guzzo, J. (2005). A new approach for selection of *Oenococcus oeni* strains in order to produce malolactic starters. *International Journal of Food Microbiology*, 105(3): 463-470.
164. Covacci, A., Telford, J.L., Giudice, G.D., Parsonnet, J., Rappuoli, R. (1999). *Helicobacter pylori* virulence and genetic geography. *Science*, 284(5418): 1328-1333.
165. Crabtree, J.E., Figura, N., Taylor, J.D., Bugnoli, M., Armellini, D. Tompkins, D.S., (1992). Expression of 120 kilodalton protein and cytotoxicity in *Helicobacter pylori*. *Journal of Clinical Pathology*, 45(8): 733-734.
166. Cremonini, F., Di Caro, S., Covino, M., Armuzzi, A., Gabrielli,M., Santarelli, L., Nista, EC., Cammarota, G., Gasbarrini, G., Gasbarrini, A. (2002). Effect of different probiotic preparations on anti-*Helicobacter pylori* therapy-related side effects: a parallel group, triple blind, placebo controlled study. *Gastroenterology*, 97: 2744-2749.
167. Crociani, F., Alessandrini, A., Mucci, M. M., Biavati, B. (1994). Degradation of complex carbohydrates by *Bifidobacterium* spp. *International Journal of Food Microbiology*, 24(1-2): 199-210.
168. Crowe, S. E. (2019). *Helicobacter pylori* infection. *New England Journal of Medicine*, 380 (12): 1158-1165.
169. Cutler, R., Wilson, P. (2004). Antibacterial activity of a new, stable, aqueous extract of allicin against methicillin-resistant *Staphylococcus aureus*. *British Journal of Biomedical Science,* 61:71–74.
170. D'Elios, M. M., Manghetti, M., Almerigogna, F., Amedei, A., Costa, F., Burroni, D., Baldari, C. T., Romagnani, S., Telford, J. L., Del Prete, G. (1997). Different cytokine profile and antigen-specificity repertoire in *Helicobacter pylori*- specific T cell clones from the antrum of chronic gastritis patients with or without peptic ulcer. *European Journal* of *Immunology*, 27: 1751-1755
171. Dahlgren, R.M.T., Clifford, H.T., Yeo, P.F. (1985). The families of the monocotyledons. Ed. Springer-Verlag, Berlin. PP. 520.
172. Dallocca-Berno, N., Visioni Tezotto-Uliana, J., Tadeu dos Santos Dias, C.K., Ricardo, A. (2014). Storage temperature and type of cut affect the biochemical and physiological characteristics of fresh-cut purple onions. *Postharvest Biology and Technology*, 93:91-96.
173. Danesh, J. (1999). *Helicobacter pylori* infection and gastric cancer: systematic review of the epidemiological studies. *Alimentary Pharmacology and Therapeutics*, 13(7): 851-856.
174. Das, S.K., Das, G., Paramithiotis, S., Patra, J.K. (2022). Kimchi and sauerkraut lactic acid bacteria and human health. In: Lactic Acid Bacteria in Food Biotechnology, (Eds. Ramesh Ray), Elsevier, USA, Pp. 47-62.
175. Dash, B.K., Sultana, S., Sultana, N. (2011). Antibacterial activities of methanol and acetone extracts of fenugreek (*Trigonella foenum)* and coriander (*Coriandrum sativum*), *Life Sciences and Medicine Research,* 27: 65–72.
176. Davood, E., Ashraf, M.M., Hatef, S.A., Hosseini, Z. (2009). Optimization of *Helicobacter pylori* culture in order to prepare favorable antigens. *Journal of Bacteriology Research*, 1 (9): 101-104.
177. De Candolle, A. (1964). Origin of Cultivated Plants. Ed. Hafner, New York, PP. 272-272.
178. De Klerk H.C, Coetzee C, Jordan H. (1967). Bacteriocinogeny in *Lactobacillus fermenti*. *Nature*, 214 (5088): 609-609.
179. De Martel, C., Parsonnet, J. (2006). *Helicobacter pylori* infection and gender: A meta-analysis of population-based prevalence surveys. Digestive Diseases and Sciences, 51:2292-2301.
180. De Reuse, H., Bereswill, S. (2007). Ten years after the first *Helicobacter pylori* genome: comparative and functional genomics provide new insights in the variability and adaptability of a persistent pathogen. *FEMS Immunology and Medical Microbiology*, 50(2): 165-176.
181. De Vuyst, L. (2000). Technology aspects related to the application of functional starter cultures. *Food Technology and Biotechnology*, 38(2): 105-112.
182. De Waard, R., Garssen, J., Bokken, G.C.A.M., Vos, J.G. (2002). Antagonistic activity of *Lactobacillus casei* strain Shirota against gastrointestinal *Listeria monocytogenes* infection in rats. *International Journal of Food Microbiology,*73:93–100.
183. Debongnie, J.C., Pauwels, S., Raat, A., De Meeus, Y., Haot, J., Mainguet, P. (1991). Quantification of *Helicobacter pylori* infection in gastritis and ulcer disease using a simple and rapid carbon-14-urea breath test. *Journal of Nuclear Medicine*, 32(6): 1192-1198.
184. Deivasigamani, S., Swaminathan, C. (2018), Evaluation of seed test weight on major field crops, *International Journal of Research Studies in Agricultural Sciences*, 4(1): 8–11.
185. Delarra, C. (2007). Microbiologie pratique pour le laboratoire d'analyses ou de contrôle sanitaire. Ed. Tec et Doc - Lavoisier. Paris, France. PP 476.
186. Dellaglio, F., Dicks, L. M. T., Torriani, S.(1995). The genus *Leuconostoc*. The genera of lactic acid bacteria. Ed. Springer, Boston, MA, PP 235-278.
187. Delphine L, Bénédicte C, Annabelle F, Gilles L, Christel G, Martin P, Philippe G, Alexandra G. (2011). Using heme as an energy boost for lactic acid Bacteria. *Current Opinion in Biotechnology,* 22(2): 143-149.

188.Delport, W., Van der merwe, S.W. (2007). The transmission of *Helicobacter pylori*: The effects of analysis method and study population on inference. *Best Practice and Research Clinical Gastroenterology*, 21(2): 215–236.
189.Delves-Broughton, J., Blackburn, P., Evans, R.J., Hugenholtz, J. (1996). Applications of the bacteriocin, nisin. *Antonie Van Leeuwenhoek*, 69(2), 193-202.
190.Delvin, E.E., Brazier, J.L., Deslandres, C., Alvarez, F., Russo, P., Seidman, E. (1999). Accuracy of the [13C]-urea breath test in diagnosing *Helicobacter pylori* gastritis in pediatric patients. *Journal of Pediatric Gastroenterology and Nutrition*, 28(1): 59-62.
191.Demarne, F.E. (1985). Le Geranium rosat. *Parfums, Cosmétiques et Aromes*, 62:85–92.
192.Den Hoed, C.M., Van Eijck, B.C., Capelle, L.G., Van Dekken, H., Biermann, K., Siersema, P.D., Kuipers, E.J. (2011). The prevalence of premalignant gastric lesions in asymptomatic patients: predicting the future incidence of gastric cancer. *European Journal* of *Cancer,* 47: 1211-1218.
193.Derek, A.A., Joost, V.D.B., Inge, M.K.M., Jack, T.P., Antonius, J.A.V.M. (2009). Anaerobic homolactate Fermentation with *Saccharomyces cerevisiae* results In depletion of ATP and impaired metabolic activity. *FEMS Yeast Research*, 9(3): 349–357.
194.Dethier, B. (2010). Contribution à l'étude de la synthèse de l'alliine de L'ail. Master en Chimie et Bio industrie. Université de Liège. Belgique. PP238.
195.Dicks, L.M.T., Dellaglio, F., Collins, M.D. (1995). Proposal to reclassify *Leuconostocoenos* as *Oenococcus oeni* ,*International Journal of Systematic and Evolutionary Microbiology,* 45(2): 395-397.
196.Dicksved, J., Lindberg, M., Rosenquist, M., Enroth, H., Jansson, J.K., Engstrand, L. (2009). Molecular characterization of the stomach microbiota in patients with gastric cancer and in controls. *Journal* of *Medical Microbiology,* 58: 509-516.
197.Dixon, M.F., Genta, R., Yardley, J., Correa, P. (1994). The participants in the international workshop on the histopathology of gastritis, Houston. classification and grading of gastritis: The updated ydney system. *The American Journal of Surgical Pathology*, 20 (10): 1161-1181.
198.Djeridane, A., Yous, M., Nadjemi, B., Boutassouna, D., Stocker, P., Vidal, N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry,* 97:654-660.
199.Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., HuongHuynh, L., EdiSoetaredjo, F., Ismadji, S., Yi-Hsu, J. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, 22(3):296-302.
200.Doenges, J.L. (1939). Spirochaetes in the gastric glands of *Macacus rhesus* and of man without related disease. *Archives of Pathology and Laboratory Medicine,* 27: 469-477.
201.Dorant, E.V.D.B., van den Brandt, P.A., Goldbohm, R.A., Sturmans, F.E.R.D. (1996). Consumption of onions and a reduced risk of stomach carcinoma. *Gastroenterology*, 110(1): 12-20.
202.Dorsch, W., Wagner, H. (1991). New antiasthmatic drugs from traditional medicine, *International Archives of Allergy and Immunology,* 94:262-265.
203.Doukani, K, Mimoun, H . (2015).Physicochemical and nutritional characterization of *Arbutus unedo* L. from the Region of Tiaret (Algeria). *International Journal of Humanities, Arts, Medicine and Sciences,* 3(8):1-14.
204.Doukani, K., Tabak, S. (2015). Profil physicochimique du fruit "Lendj" (*Arbutus unedo* L.). *Revue Nature and Technology,* 12: 53-66.
205.Dower, W.J., Miller, J.F., Ragsdale, C.W. (1988). High efficiency transformation of *E. coli* by High voltage electroporation. *Nucleic Acids Research,* 16: 6127– 6145.
206.Drider, D., Bendali, F., Naghmouchi, K., Chikindas, M.L. (2016). Bacteriocins: Not Only Antibacterial Agents. *Probiotics and Antimicrobial Proteins,* 8: 177–182.
207.Dua, A., Gaurav, G., Balkar, S., Mahajan, R. (2013). Antimicrobial properties of methanolic extract of cumin (*Cuminum cyminum*) seeds. *International Journal of Research in Ayurveda and Pharmacy*, 4(1): 104– 107.
208.Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. (1956), Colorimetric method for determination of sugars and related substances, *Analytical Chemistry*, 28(3): 350–356.
209.Duke, A.J. (1986). Handbook of legumes of world economic importance. Ed. Plemus Press, New York and London. PP 345.
210.Duraffourd, C., D'Hervicourt, L., Lapraz, J.C. (1990). Cahiers de phytothérapie clinique. 1. Examens de laboratoires galéniques. Eléments thérapeutiques synergiques. Ed. Masson. Paris, France. PP 96.
211.Eaton, K.A. Krakowka, S. (1994). Effect of gastric pH on urease-dependent colonization of gnotobiotic piglets by *Helicobacter pylori*. *Infection and Immunity Journal,* 62: 3604-3607.
212.Eaton, K. A., Morgan, D. R., Krakowka, S. (1992). Motility as a factor in the colonisation of gnotobiotic piglets by *Helicobacter pylori*. *Journal of Medical Microbiology*, 37: 123-127.
213.Ebada, M.E. (2017). Cumin aldehyde: A potential drug candidate. *Journal of Pharmacology* and *Clinical Research,* 2(2): 1-4.

214.Ebrahimie, E., Habashi, A.A., Ghareyazie, B., Ghannadha, M., Mohammadie, M. (2003). A rapid and efficient method for regeneration of plantlets from embryo explants of cumin (*Cuminum cyminum*). *Plant Cell, Tissue and Organ Culture*, 75(1), 19-25.

215.Eddouks, M., Ouahidi, M.L., Farid, O., Moufid, A., Khalidi, A., Lemhadri, A. (2007). L'utilisation des plantes médicinales dans le traitement du diabète au Maroc. *Phytothérapie*, 5(4): 194-203.

216.Einafshar, S., Poorazrang, H., Farhoosh, R., Seiedi, S.M. (2012). Antioxidant activity of the essential oil and methanolic extract of cumin seed (*Cuminum cyminum*). *European Journal of Lipid Science and Technology,* 114(2): 168–174.

217.Ekwenye, U.N., Elegalam, N.N. (2005). Antibacterial activity of Ginger (*Zingiber officinale*) Roscoe and garlic (*Allium sativum* L.) extracts on *Escherichia coli* and *Salmonella typhi*. *International Journal of Molecular Medicine and Advance Sciences,* 1: 411-417.

218.El-Baradei, G., Delacroix-Buchet, A., Pery, P., Ogier, J.C. (2005). Occurrence of *Lactococcus graviae* in four types of Egyptian cheeses by specific Polymerase Chain Reaction assay. *Egyptian Journal of Dairy Science,* 33: 35-41.

219.El-Ghorab, A.H., Nauman, M., Anjum, F.M., Hussain, S., Nadeem, M. (2010). A comparative study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and cumin (*Cuminum cyminum*). *Journal of Agricultural and Food Chemistry,* 58(14): 8231-8237.

220.El-Haci, A., Atik-Bekkara, F., Didi, A., Gherib, M., Didi, M.A. (2012). Teneurs en polyphénols et pouvoir antioxydant d'une plante médicinale endémique du Sahara Algérien. *Pharmacognosie*,10 : 280-285.

221.El-Meleig, M., Ahmed, M., Arafa, R., Ebrahim, N., El-Kholany, E. (2010). Cytotoxicity of four essential oils on some human and bacterial cells. *Journal of Applied Sciences in Environmental Sanitation*, 5: 143-159.

222.El-Saber Batiha, G., Magdy Beshbishy, A.G. Wasef, L., Elewa, Y.H., A. Al-Sagan, A., Abd El-Hack, M.E., Taha, A.E., M. Abd-Elhakim, Y., Prasad Devkota, H. (2020). Chemical constituents and pharmacological activities of garlic (*Allium sativum* L.): A review. *Nutrients*, 12(3): 1-21.

223.El-Zimaity, H.M., Graham, D.Y. (1999). Evaluation of gastric mucosal biopsy site and number for identification of *Helicobacter pylori*or intestinal metaplasia: role of the Sydney system. *Human Pathology,* 30: 72-77.

224.Endo, A., Irisawa, T., Futagawa-Endo, Y., Takano, K., du Toit, M., Okada, S., Dicks, L.M. (2012). Characterization and emended description of *Lactobacillus* kunkeei as a fructophilic lactic acid bacterium. *International Journal of Systematic and Evolutionary Microbiology,* 62(3): 500-504.

225.Endo, A., Okada, S. (2006). *Oenococcus kitaharae* sp. nov., a non-acidophilic and non-malolactic-fermenting *oenococcus* isolated from a composting distilled shochu residue. *International Journal of Systematic and Evolutionary Microbiology*, 56(10): 2345-2348.

226.Ernst, P.B., Gold, B.D.(2000).The disease spectrum of *Helicobacter pylori*: the immuno-pathogenesis of gastroduodenal ulcer and gastric cancer. *Annual Review of Microbiology*, 54: 615-640.

227.Escherich, T. (1885) Die Darmbacterien des Neugeborenen und Sauglings. *Fortschitte der Medizin,* 3: 515–522.

228.Eslami, M., Yousefi, B., Kokhaei, P., Moghadas, A.J., Moghadam, B.S., Arabkari, V., Niazi, Z. (2019). Are probiotics useful for therapy of *Helicobacter pylori* diseases?. *Comparative Immunology, Microbiology and Infectious Diseases*, 64: 99-108.

229.Evans, D.J, Evans, D.G. (2000). *Helicobacter pylori* adhesins: review and perspectives. *Helicobacter*, 5: 183-195.

230.Evans, D.J., Evans, D.G., Graham, D.Y., Klein, P.D. (1989). A sensitive and specific serologic test for detection of *Campylobacter pylori* infection. *Gastroenterology*, 96: 1004-1008.

231.Fahad Mohammed, J., Al–Jasser, M.S. (2012), Chemical composition and fatty acid content of some spices and herbs under Saudi Arabia conditions, *The Scientific World Journal,*20(12): 1-5.

232.Faik M. (2000). Mise au point sur l'infestation gastrique par l'*Helicobacter pylori*. *Med Maghreb*,79:17–19.

233.Fang, S., Yang, W., Chu, X., Shang, X., She, C., Fu, X. (2011). Provenance and temporal variations in selected flavonoids in leaves of *Cyclocarya paliurus*. *Food Chemistry,*124:1382-1386.

234.FAO/STAT (2019). Statistics Division, Food and Agriculture Organization of the United Nations, Rome, Italy.

235.FAO/WHO. (2002). Guidelines for the evaluation of probiotics in Food. Food and Agriculture Organization of the United Nations/World Health Organization, London, Ontario.

236.Farhat, N., Hussain, S., Syed, S.K., Amjad, M., Javed, M., Iqbal, M., Hussain, M., Haroon, S.M., Raza, H., Butt, S.Z., Kiran, R. (2020). Dietary phenolic compounds in plants: Their antioxidant and pharmacological potential. *Postepy Biologii Komorki*, 47(3): 307-320.

237.Farnsworth, N.R., Bunapraphatsara, N. (1992). Thai medicinal plants recommended for primary health care system. Ed. Medicinal Plant Information Center, Thailand. PP 267-396.

238. Farthing, M.J.G. (2000). Diarrhoea, a significant worldwide problem. *International Journal of Antimicrobial Agents,* 14(1): 65–69.
239. Fayol-Messaoudi, D., Berger, C.N., Coconnier-Polter, M.H., Lievin-Le Moal, V., Servin, A.L. (2005). pH, Lactic acid, and non lactic acid dependent activities of probiotic *Lactobacilli* against *Salmonella enterica* Serovar *Typhimurium*. *Applied and Environmental Microbiology*, 71(10): 6008-6013.
240. Fazli, F.R.Y., Hardman, R. (1968). The spice fenugreek (*Trigonella Foenum*-graecum L.). Its commercial varieties of seed as a source of diosgenin. *Tropical Science*, 10:66-78.
241. Felhi, S., Daoud, A., Hajlaoui, H., Mnafgui, K., Gharsallah, N., Kadri, A. (2017). Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of *Ecballium elaterium* seeds and peels fruits. *Food Science and Technology,* 37(3):483-492.
242. Fenwick, G.R., Hanley, A.B. (1985). The genus *Allium*. *Critical Reviews in Food Science and Nutrition,* 22:199–377.
243. Ferguson, D.A., Jiang, C., Chi, D.S., Laffan, J. J., Li, C., Thomas, E. (1999). Evaluation of two string tests for obtaining gastric juice for culture, nested-PCR detection, and combined single-and double-stranded conformational polymorphism discrimination of *Helicobacter pylori*. *Digestive Diseases and Sciences*, 44 (10): 2056-2062.
244. Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D. Bray, F. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International Journal of Cancer*, 136(5): E359-E386.
245. Fetissov, S.O., Sinno, M.H., Coëffier, M., Bole-Feysot, C., Ducrotté, P., Hökfelt, T., Dechelotte, P. (2008). Autoantibodies against appetite-regulating peptide hormones and neuropeptides, putative modulation by gut microflora. *Nutrition*, 24(4): 348–359.
246. Filipe, M.I., Newell, D.G., Johnston, B.J., Caygill, C., Reed, P.I. (1995). *Helicobacter pylori* in patients with intestinal metaplasia and in controls: a serological and biopsy study in four UK centres. UK Sub-Group of the ECP- EURONUT-Intestinal Metaplasia Study Group. *European Journal of Cancer Prevention,*4: 175-180.
247. Fischer, W. (2011). Assembly and molecular mode of action of the *Helicobacter pylori* Cag type IV secretion apparatus. *The FEBS Journal*, 278(8): 1203-1212.
248. Flores, H.B., Salvana, A., Ang, E.L.R., Estanislao, N.I., Velasquez, M.E., Ong, J., Nolasco, E.R., Daez, M.L., Banez, V. (2010). M1138 Duration of proton-pump inhibitor-based triple therapy for *Helicobacter pylori* eradication: a meta-analysis. *Gastroenterology*, 138 (5): S-340.
249. Foligné, B., Dewulf, J., Breton, J., Claisse, O., Lonvaud-Funel, A., Pot, B. (2010). Probiotic properties of non-conventional lactic acid bacteria: immunomodulation by *Oenococcus oeni. International Journal of Food Microbiology*, 140(2-3): 136-145.
250. Ford, A.C., Forman, D., Hunt, R. H., Yuan, Y., Moayyedi, P. (2014). *Helicobacter pylori* eradication therapy to prevent gastric cancer in healthy asymptomatic infected individuals: systematic review and meta-analysis of randomised controlled trials. *British Medical Journal*, 348:1-13.
251. Foynes, S., Dorrell, N., Ward, S.J., Zhang, Z.W., McColm, A.A., Farthing, M.J., Wren, B.W. (1999). Functional analysis of the roles of FliQ and FlhB in flagellar expression in *Helicobacter pylori*. *FEMS Microbiology Letters*, 174(1): 33-39.
252. Franco, A.T., Israel, D.A., Washington, M.K., Krishna, U., Fox, J.G., Rogers, A.B., Neish, A.S., Collier-Hyams, L., Perez-Perez, G.I., Hatakeyama, M., Whitehead, R. (2005). Activation of β-catenin by carcinogenic *Helicobacter pylori*. *Proceedings of the National Academy of Sciences*, 102 (30): 10646-10651.
253. Franz, C.M., Holzapfel, W.H., Stiles, M.E. (1999). *Enterococci* at the crossroads of food safety?.*International Journal of Food Microbiology*, 47(1-2): 1-24.
254. Fratianni, F., Ombra, M.N., Cozzolino, A., Riccardi, R., Spigno, P., Tremonte, P., Coppola, R., Nazzaro, F. (2016). Phenolic constituents, antioxidant, antimicrobial and anti-proliferative activities of different endemic Italian varieties of garlic (*Allium sativum* L.). *Journal of Functional Foods*, 21: 240-248.
255. Fred, D. (1902). The electrical conductivity of plant juices; Botanical Gazette, *The University of Chicago Press journals,* 34(2):81-92.
256. Fredotović, Z., Šprung, M., Soldo, B., Ljubenkov, I., Budić-Leto, I., Bilušić, T., Puizina, J.(2017). Chemical composition and biological activity of *Allium cepa* L. and *Allium cornutum* methanolic extracts. *Molecules*, 22(3): 448.
257. Freedberg, A.S., Baron, L.E. (1940). The presence of spirochaetes in human gastric mucosa. *American Journal of Digestive Diseases*, **7**: 443-445.
258. Fuccio, L., Minardi, M.E., Zagari, R.M., Grilli, D., Magrini, N., Bazzoli, F. (2007). Meta-analysis: duration of first-line proton-pump inhibitor–based triple therapy for *Helicobacter pylori* eradication. *Annals of Internal Medicine*, 147(8): 553-562.

259.Fujii, A., Cook, E.S. (1973). Probiotics. Antistaphylococcal and antifibrinolytic activities of. omega.-guanidino acids and. omega.-guanidinoacyl-L-histidines. *Journal of Medicinal Chemistry*, 16(12): 1409-1411.

260.Fuller, R. (1992). History and development of probiotics. In: Probiotics, (Eds. Fuller, R.), Springer, Dordrecht. PP 1-8.

261.Gálvez, M., Martín-Cordero, C., Houghton, P.J., Ayuso, M.J. (2005). Antioxidant activity of methanol extracts obtained from Plantago species. *Journal of Agricultural and Food Chemistry,* 53(6):1927-1933.

262.Gangadharappa, H.V. (2017). *Cuminum cyminum*–A popular spice: An updated review. *Pharmacognosy Journal*, *9*(3): :292-301.

263.Gao, X., Björk, L., Trajkovski, V., Uggla, M. (2000). Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. *Journal of Agricultural and Food Chemistry,*80:2021-2027.

264.García, A., Salas-Jara, M.J., Herrera, C., González, C. (2014). Biofilm and *Helicobacter pylori*: from environment to human host. *World Journal of Gastroenterology*, 20(19): 5632.

265.Garcia-Castillo, V., Zelaya, H., Ilabaca, A., Espinoza-Monje, M, Komatsu, R., Albarracín, L., Kitazawa, H., Garcia-Cancino, A., Villena, J. (2018). *Lactobacillus fermentum* UCO-979C beneficially modulates the innate immune response triggered by *Helicobacter pylori* infection *in vitro*. *Beneficial Microbes*, 9:829–841.

266.García-González, M.A., Bujanda, L., Quintero, E., Santolaria, S., Benito, R., Strunk, M., Sopeña, F., Thomson, C., Pérez-Aisa, A., Nicolás-Pérez, D., Hijona, E. (2015). Association of PSCA rs2294008 gene variants with poor prognosis and increased susceptibility to gastric cancer and decreased risk of duodenal ulcer disease. *International Journal of Cancer*, 137(6):1362-1373.

267.Garnier, G., Bezanger Beauquesne, L., Debraux, G. (1961). Ressources médicinales de la flore Française 1st Ed. Maloine. Paris. PP 151.

268.Garrity, G. M., Brenner, D. J., Krieg, N. R., Staley, J. R., Manual, B. S. (2005). Systematic bacteriology. The Proteobacteria, Part C: The Alpha-, Beta-, Delta-, and *Epsilonproteo bacteria*, Bergey's Manual Trust, 2nd Ed. Springer, Boston, MA.PP 1-574.

269.Garvie E.I., (1986). Gram positive cocci - Genus *Leuconostoc*. In: Bergeys' Manual, Eds (Bergey, D. H., Sneathet P. H. A., John Holt G.) 9th Ed., the Williams and Wilkins Co., Baltimore. PP 1071-1075.

270.Garvie, E. I. (1960). The genus *Leuconostoc* and its nomenclature. *Journal of Dairy Research*, 27(2): 283-292.

271.Garza-Gonzalez, E., Perez-Perez, G.I., Maldonado- Garza, H.J., Bosques-Padilla, F.J. (2014). A review of *Helicobacter pylori* diagnosis, treatment, and methods to detect eradication. *World Journal of Gastroenterology*, 20:1438–49.

272.Gatta, G., Zigon, G., Capocaccia, R., Coebergh, J.W., Desandes, E., Kaatsch, P., Pastore, G., Peris-Bonet, R., Stiller, C.A., EUROCARE Working Group. (2009). Survival of European children and young adults with cancer diagnosed 1995–2002. *European Journal of Cancer*, *45*(6): 992-1005.

273.Gazuwa, S.Y., Makanjuola, E.R., Jaryum, K.H., Kutshik, J.R., Mafulul, S.G. (2013).The phytochemical composition of *Allium cepa* / *Allium sativum* and the effects of their aqueous extracts (cooked and raw forms) on the lipid profile and other hepatic biochemical parameters in female albino Wistar Rats. Asian Journal of Experimental Biological Sciences, 406-410.

274.Gebert, B., Fischer, W., Weiss, E., Hoffmann, R., Haas, R. (2003). *Helicobacter pylori* vacuolating cytotoxin inhibits T lymphocyte activation. *Science*, 301(5636): 1099-1102.

275.Gene, E., Calvet, X., Azagra, R., Gisbert, JP., Triple, VS. (2003). Quadruple therapy for treating *Helicobacter pylori* infection: an updated meta-analysis. *Alimentary Pharmacology and Therapeutics,* 18: 543–544.

276.Gezici, S., Sekeroglu, N. (2019 b). Neuroprotective potential and phytochemical composition of acorn fruits. *Industrial Crops and Products*, 128: 13-17.

277.Gezici, S., Sekeroglu, N. (2019a), Current perspectives in the application of medicinal plants against cancer: novel therapeutic agents, *Anti-Cancer Agents in Medicinal Chemistry,* 19 (1): 101–111.

278.Ghasemi, G., Fattahi, M., Alirezalu, A. Ghosta, Y. (2019). Antioxidant and antifungal activities of a new chemovar of cumin (*Cuminum cyminum* L.). *Food Science and Biotechnology*, 28(3): 669–677.

279.Gimenez, M.A., Solanes, R.E., Gimenez, D.F. (1988).Growth of *Clostridium botulinum* in media with garlic, *Revista Argentina de Microbiología,* 20:17–*24.*

280.Gisbert, J.P., Calvet, X. (2011). non-bismuth quadruple (concomitant) therapy for eradication of *Helicobater pylori*. *Alimentary Pharmacology and Therapeutics*, 34(6), 604-617.

281.Gisbert, J.P., Morena, F. (2006). Systematic review and meta-analysis: levofloxacin-based rescue regimens after *Helicobacter pylori* treatment failure. *Alimentary Pharmacology and Therapeutics*, (23):35–44.

282.Gohari, A.R., Saeidnia, S. (2011). A review on phytochemistry of *Cuminum cyminum* seeds and its standards from field to market. *Pharmacognosy Journal*, 3(25): 1–5.

283. Gomes, A.M., Malcata, F.X. (1999). *Bifidobacterium* spp. and *Lactobacillus acidophilus*: biological, biochemical, technological and therapeutical properties relevant for use as probiotics. *Trends in Food Science and Technology*, 10(4-5): 139-157.
284. Goodwin, C.S., Armstrong, J. A., Chilvers, T., Peters, M., Collins, M.D., Sly, L., McConnell, W., Harper, W.E. (1989). Transfer of *Campylobacter pylori* and *Campylobacter mustelae* to *Helicobacter* gen. nov. as *Helicobacter pylori* comb. nov. and *Helicobacter mustelae* comb. nov., respectively. *International Journal of Systematic and Evolutionary Microbiology*, 39(4): 397-405.
285. Goodwin, C.S., McCulloch, R.K., Armstrong, J.A., Wee, S.H. (1985). Unusual cellular fatty acids and distinctive ultrastructure in a new spiral bacterium (*Campylobacter pyloridis*) from the human gastric mucosa. *Journal* of *Medical Microbiology,*19: 257-267.
286. Gorinstein, S., Leontowicz, H., Leontowicz, M., Namiesnik, J., Najman, K., Drzewiecki, J., Cvikrová, M., Martincová, O., Katrich, K., Trakhtenberg, S. (2008). Comparison of the main bioactive compounds and antioxidant activities in garlic and white and red onions after treatment protocols. *Journal of Agricutural and Food Chemistry,* 56 :4418–4426.
287. Gorinstein, S., Park, Y., Heo, B., Namieśnik, J., Kruszewska, H., Leontowicz, M., Ham, K., Cho, J., Kang, S. (2009). A comparative study of phenolic compounds and antioxidant and anti proliferative activities in frequently consumed raw vegetables. *European Food Research and Technology,* 228:903-911.
288. Gosciniak, G, Przondo-Mordarska, A, Iwanczak, B, Blitek, A. (2003). *Helicobacter pylori* Antigens in stool specimens of gastritis children before and after treatment. *Journal* of *Pediatric Gastroenterology and Nutrition*, 36: 376-380.
289. Graham, D.Y. (2015). *Helicobacter pylori* update: gastric cancer, reliable therapy, and possible benefits. *Gastroenterology*, 148 (4): 719-731.
290. Graham, D.Y. (1998). Antibiotic resistance in *Helicobacter pylori*: implications for therapy. *Gastroenterology*, 115: 1272-1277.
291. Graham, D.Y., Fischbach, L. (2010). *Helicobacter pylori* treatment in the era of increasing antibiotic resistance. *Gut,* 59:1143–1153.
292. Green, F.N., Baur, R., Thomson, M., McCarthy, L**.** (1997). An example of chartreuse skin colour in onion (*Allium cepa* L.) cultivar Greenella. Genetic *Resources* and *Crop Evolution,*44: 491-493.
293. Gressmann, H., Linz, B., Ghai, R., Pleissner, K.P., Schlapbach, R., Yamaoka, Y., Kraft, C., Suerbaum, S., Meyer, T.F., Achtman, M. (2005). Gain and loss of multiple genes during the evolution of *Helicobacter pylori*. *PLOS Genetics*, 1(4): 419-428.
294. Grigoroff, S. (1905). Etude sur le lait fermenté comestible: le" Kissélo-mléko" de Bulgarie. *La Revue Médicale Suisse*, 25: 714-720.
295. Groenen, M.J., Kuipers, E.J., Hansen, B.E., Ouwendijk, R.J.T. (2009). Incidence of duodenal ulcers and gastric ulcers in a Western population: back to where it started. *Canadian Journal of Gastroenterology*, 23(9): 604-608.
296. Grove, DI., McLeay, RA., Byron, KE., Koutsouridis, G. (2001). Isolation of *Helicobacter pylori* after transport from a regional laboratory of gastric biopsy specimens in saline, Portagerm *pylori* or cultured on chocolate agar. *Pathology*, 33: 362-364.
297. Grubben, J.H., Denton, D.A. (2004). Plant resources of tropical Africa. Ed. PROTA Foundation, Netherlands. PP 298-298.
298. Gruhlke, M. C., Nwachwukwu, I., Arbach, M., Anwar, A., Noll, U., Slusarenko, A. J. (2011). Allicin from garlic, effective in controlling several plant diseases, is a reactive sulfur species (RSS) that pushes cells into apoptosis. *Modern Fungicides and Antifungal Compounds VI. 16th International Reinhardsbrunn Symposium,* PP 325-330.
299. Guiraud, J.P. (1998). Microbiologie alimentaire. Ed. Dunod, Paris, PP88.
300. Gulsen, G., Erol, A. (2010). Antimicrobial effect of garlic (*Allium sativum*). *Journal of Animal and Veterinary Advances,* 9: 1-4.
301. Gupta, D. (2013). Comparative analysis of spices for their phenolic content, flavonoid content and antioxidant capacity. *American International Journal of Research in Formal, Applied and Natural Sciences,* 4:38-42.
302. Haamadi, A.A., Risan, M.H., AboAlmaali, H.M., Sayah, H.A., Abbas, A.H. (2021). Used of probiotic production of *Saccharomyces boulardii* to eradication triple therapy of *Helicobacter pylori* infection. *Scientific Journal of Medical Research,* 5 (18): 40-45.
303. Hachem, C.Y., Clarridge, J.E., Evans, D.G., Graham, D.Y. (1995). Comparison of agar based media for primary isolation of *Helicobacter pylori*. *Journal of Clinical Pathology*, 48: 714-716.
304. Halliwel, B., Guteridge, J.M.C. (1992). Free radicals antioxidants and human diseases. *Journal of Laboratory and Clinical Medicine,* 119(6): 598-620.
305. Hamilton-Miller, J.M.T. (2003). The role of probiotics in the treatment and prevention of *Helicobacter pylori* infection. *International Journal of Antimicrobial Agents*, 22 (4): 360-366.

306.Hamilton-Miller, J.M.T., Gibson, G.R., Bruck, W. (2003). Some insights into the derivation and early uses of the word probiotic. *British Journal of Nutrition*, 90(4): 845-845.
307.Hammes, W.P., Vogel, R.F. (1995). The genus *Lactobacillus*. In: The genera of lactic acid bacteria. (Eds. Wood, B.J.B., Holzapfel, W.H.), Springer, Boston, MA. PP 19-54.
308.Han, S.W., Flamm, R., Hachem, C.Y., Kim, H.Y., Clarridge, J.E., Evans, D.G., Beyer, J., Drnec, J., Graham, D.Y. (1995). Transport and storage of *Helicobacter pylori* from gastric mucosal biopsies and clinical isolates. *European Journal of Clinical Microbiology and Infectious Diseases*, 14 (4): 349-352.
309.Haouala, R., Hawala, S., El-Ayeb, A., Khanfir, R., Boughanmi, N. (2008). Aqueous and organic extracts of *Trigonella Foenum*-graecum L. inhibit the mycelia growth of fungi. *Journal of Environmental Sciences*, 20 (12): 1453-1457.
310.Haram, M.A. (1991), The protein quality of Sudanese fenugreek seeds. Master Thesis, University of Khartoum, Sudan.
311.Harish, K., Varghese, T. (2006). Probiotics in humans–evidence based review. *Calicut Medical Journal*, 4(4): 1-11.
312.Hegazy, A.I., Ibrahim, M.I. (2009). Evaluation of the nutritional protein quality of wheat biscuit supplemented by fenugreek seed flour. *World Journal of Dairy and Food Sciences*, 4:129-135.
313.Hegnauer, R. (1963). Chemotaxonomy of plants. A survey of the distribution and systematic significance of plant substances. Volume II. Monocotyledoneae. Ed. *Verlag Birkhäuser Basel Stuttgart.Germany.*PP 540.
314.Helambe, S.S, Dande, R.P. (2011). Fenugreek (*Trigonella Foenum Graceum*): An overview. *International Journal of Current Pharmaceutical Review and Research*, 2(4): 169-187.
315.Herdian, H., Istiqomah, L., Damayanti, E., Suryani, A.E., Anggraeni, A.S., Rosyada, N., Susilowati, A. (2018). Isolation of cellulolytic lactic-acid bacteria from Mentok (*Anas moschata*) gastro-intestinal tract. *Tropical Animal Science Journal*, 41(3): 200-206.
316.Herter, C.A., Kendall, A.I. (1910). The influences of dietary alterations on the types of intestinal flora. *Journal of Biological Chemistry,* 7: 203-217.
317.Hertog, M.G.L., Hollman, P.C.H., Katan, M.B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. *Journal of Agricultural* and *Food Chemistry,*40: 2379-2383.
318.Heyman, M. (2000). Effect of lactic acid bacteria on diarrheal diseases. *Journal of the American College of Nutrition*, 19 (2): 137S-146S.
319.Hildebrand, P., Bardhan, P., Rossi, L., Parvin, S., Hasan, M., Ahmad, M.M., Glatz–Krieger, K., Terracciano, L., Bauerfeind, P. (2001). Recrudescence and reinfection with *Helicobacter pylori*after eradication therapy in Bangladeshi adults.*Gastroenterology,*121: 792-798.
320.Himanshu, S., Saroj, S., Sarada, S., Rakesh, S., Mohan, K. (2014). Anti-diarrhoeal investigation from aqueous extract of *Cuminum cyminum* Linn. seed in albino rats. *Pharmacognosy Research*, 6:204-209.
321.Hinneburg, I., Dorman, D., Hiltunen, R. (2006). Antioxidant activities of extracts from selected herbs and spices. *Food Chemistry,* 97:122 129.
322.Ho, B., Marshall, B.J. (2000). Accurate diagnosis of *Helicobacter pylor*i: serologic testing. *Gastroenterology Clinics of North America*, 29(4): 853-862.
323.Hocker, M., Hohenberger, P. (2003). *Helicobacter pylori* virulence factors-one part of a big picture. *Lancet*, 362: 1231-1233.
324.Holzapfel, W.H., Haberer, P., Snel, J., Schillinger, U., In't Veld, J.H.H. (1998). Overview of gut flora and probiotics. *International Journal of Food Microbiology*, 41(2): 85-101.
325.Hong, W., Chen, H., Chen, Y. , Chen, M. (2009). Effects of kefir supernatant and lactic acid bacteria isolated from kefir grain on cytokine production by macrophage. *International Dairy Journal,*19(4): 244–251.
326.Hoves, H., Norgaard, H., Mortensen, B.P. (1999). Lactic acid bacteria and human gastrointestinal tract. *European Journal of Clinical Nutrition*, 53: 339–350.
327.Hu, F.Z., Ehrlich, G.D. (2008). Population-level virulence factors amongst pathogenic bacteria: Relation to infection outcome. *Future Microbiology*, 3:31-42.
328.Huang, F.C., Chang, M.H., Hsu, H.Y., Lee, P.I., Shun, C.T. (1999). Long-term follow-up of duodenal ulcer in children before and after eradication of *Helicobacter pylori*. *Journal of Pediatric Gastroenterology and Nutrition*, 28 (1):76-80.
329.Huili, P., Guangyong, Q., Zhongfang, T., Zongwei, L., Yanping, W., Yimin, C. (2011). Natural populations of lactic acid bacteria associated with silage fermentation as determined by phenotype, 16S ribosomal RNA and recA gene analysis. *Systematic and Applied Microbiology,* 34(3): 235-241.
330.Hurduc, V., Plesca, D., Dragomir, D., Sajin, M., Vandenplas, Y. (2009). Arandomized, open trial evaluating the effect of *Saccharomyces boulardii* on the eradication rate of *Helicobacter pylori* infection in children. *Acta Paediatrica,* 98: 127-131

331. Hussein, R.A., El-Anssary, A.A. (2019). Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. In: Herbal medicine, (Eds. Hussein, R.A., El-Anssary, A.A.), Books on Demand, UK. PP 314.
332. Hutchinson, J. (1959). The families of flowering plants. 3 rd Ed. Clarr Press. Oxford. PP 508.
333. Ichikawa, H., Sugimoto, M., Uotani, T., Sahara, S., Yamade, M., Iwaizumi, M., Yamada, T., Osawa, S., Sugimoto, K., Miyajima, H., Yamaoka, Y. (2015). Influence of prostate stem cell antigen gene polymorphisms on susceptibility to *Helicobacter pylori*-associated diseases: a case-control study. *Helicobacter*, 20(2): 106-113.
334. Ilver, D., Arnqvist, A., Ogren, J., Frick, I.M., Kersulyte, D., Incecik, E.T., Berg, D.E., Covacci, A., Engstrand, L., Borén, T. (1998). *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. *Science*, 279(5349): 373-377.
335. Imrie, C., Rowland, M., Bourke, B., Drumm, B. (2001). Is *Helicobacter pylori* infection in childhood a risk factor for gastric cancer?. *Pediatrics*, 107(2): 373-380.
336. Ioku, K., Aoyama, Y., Tokuno, A., Terao, J., Nakatani, N., Takei, Y. (2001).Various cooking methods and the flavonoid content in onion. *Journal of Nutritional Science and Vitaminology,* 47: 78-83.
337. Jack, R.W., Tagg, J.R., Ray, B. (1995). Bacteriocins of Gram-positive bacteria. *Microbiological Reviews*, 59 (2): 171-200.
338. Jain, A.K., Vargas, R., Gotzkowsky, S., McMahon, F.G. (1993). Can garlic reduce levels of serum lipids? A controlled clinical study. *American Journal of Medicine,* 94 (6): 632–635.
339. Jakszyn, P., Bingham, S., Pera, G., Agudo, A., Luben, R., Welch, A., Boeing, H., Del Giudice, G., Palli, D., Saieva, C., Krogh, V. (2006). Endogenous versus exogenous exposure to N-nitroso compounds and gastric cancer risk in the european prospective investigation into cancer and nutrition (EPIC-EURGAST) study. *Carcinogenesis*, 27(7): 1497-1501.
340. Jamal, Z., Miot-Sertier, C., Thibau, F., Dutilh, L., Lonvaud-Funel, A., Ballestra, P., Le Marrec, C. Dols-Lafargue, M. (2013). Distribution and functions of phosphotransferase system genes in the genome of the lactic acid bacterium *Oenococcus oeni*. *Applied and Environmental Microbiology*, 79(11): 3371-3379.
341. James, D.G., Price, T.S.(2002). Fecundity in two-spotted spider mite (*Acari tetranychidae*) is increased by direct and systemic exposure to imidacloprid. *Journal* of *Economic Entomology,* 95: 729-732.
342. Jang, H.J., Lee, H.J., Yoon, D.K., Ji, D.S., Kim, J.H., Lee, C.H. (2017). Antioxidant and antimicrobial activities of fresh garlic and aged garlic by-products extracted with different solvents. *Food Science* and *Biotechnology,* 27: 219–225.
343. Jani, R., Udipi, S.A., Ghugre, P.S. (2009). Mineral content of complementary foods. *The Indian Journal of Pediatrics*, 76(1):37-44.
344. Jastrzebski, Z., Kruszewska, H., Leontowicz, M., Namieśnik, J., Zachwieja, Z., Barton, H., Pawelzik, E., Arancibia, P., Toledo, F., Gorinstein, S. (2007).The bioactivity of processed garlic (*Allium sativum* L.) as shown *in vitro* and *in vivo* studies on rats. *Food and Chemical Toxicology,*45:1626-1633.
345. Jeantet, R., Croguennec, T., Mahaut, M., Schuck, P., Brule, G. (2008). Les produits laitiers. Ed. Technique et Documentation, Lavoisier, Paris. PP 26– 27.
346. Jilani, M.S. Ahmed, P. Waseem, K., Kiran, M. (2010). Effect of plant spacing on growth and yield of two varieties of onion (*Allium cepa* L.), *Pakistan Journal of Science*, 62(1):37- 41.
347. Jiménez-Soto, L.F., Kutter, S., Sewald, X., Ertl, C., Weiss, E., Kapp, U., Rohde, M., Pirch, T., Jung, K., Retta, S.F., Terradot, L. (2009). *Helicobacter pylori* type IV secretion apparatus exploits β1 integrin in a novel RGD-independent manner. *PLOS Pathogens*, 5(12): 1-14.
348. Jin, Y.L., Ai, H.L., Cheng, J., Wu, M.Y. (2009). First description of a novel *Weissella* species as an opportunistic pathogen for rainbow trout *Oncorhynchus mykiss* (Walbaum) in China. *Veterinary Microbiology*, 136(3-4): 314-320.
349. Joglekar, M., Mandal, M., Somaiah, M. P., Murthy, S. (2012). Comparative analysis of antioxidant and antibacterial properties of *Aegle marmelos*, *Coriandrum sativum* and *Trigonella Foenum* graecum. *Acta Biologica Indica,* 1(1):105-108.
350. Johnson, R., Vitha, M.F. (2011). Chromatography selectivity triangle, *Journal of Chromatography,*4:559-560.
351. Johnson-Henry, K.C., Mitchell, D.J., Avitzur, Y., Galindo-Mata, E., Jones, N.L., Sherman, P.M. (2004). Probiotics reduce bacterial colonization and gastric inflammation in *H. pylori*-infected mice. *Digestive Diseases and Sciences*, 49 (7): 1095-1102.
352. Joly-Guillou, M. L. (2006). Intérêt du E-test dans le suivi de l'antibiothérapie. *Réanimation*, 15(3): 237-240.
353. Jones, D. (1978). Composition and differentiation of the genus *Streptococcus*. In: *Streptococci*, Society for Applied Bacteriology Symposium Series No. 7, Ed. Academic Press, London, UK, PP 1–49.
354. Jonkers, D., van den Broek, E., van Dooren, I., Thijs, C., Dorant, E., Hageman, G., Stobberingh, E. (1999). Antibacterial effect of garlic and omeprazole on *Helicobacter pylori*. *The Journal of Antimicrobial Chemotherapy*, 43(6): 837–839.

355. Josenhans, C., Labigne, A., Suerbaum, S. (1995). Comparative ultrastructural and functional studies of *Helicobacter pylori* and *Helicobacter mustelae flagellin* mutants: both flagellin subunits, FlaA and FlaB, are necessary for full motility in *Helicobacter* species. *Journal of Bacteriology*, 177: 3010-3020.
356. Jung, K.O., Kil, J.H., Kim, K.H., Park, K.Y. (2003). Effect of kimchi and its ingredients on the growth of *Helicobacter pylori*. *Preventive Nutrition and Food Science*, 8(2): 149-153.
357. Junqueira, A.C.M., Ratan, A., Acerbi, E., Drautz-Moses, D.I., Premkrishnan, B.N., Costea, P.I., Linz, B., Purbojati, R.W., Paulo, D.F., Gaultier, N.E. Subramanian, P. (2017). The microbiomes of blowflies and houseflies as bacterial transmission reservoirs. *Scientific Reports*, 7(1):1-15.
358. Juszczak, L., Fortuna, T. (2003). Viscosity of concentrated strawberry juice. effect of temperature and soluble solids content; *Electronic Journal of Polish Agricultural Universities,* 6(2): 1-7.
359. Juszczak, L., Fortuna, T., Kosla, A. (2003), Sensory and rheological properties of Polish commercial mayonnaise, *Nahrung /Food,* 47 (4): 232–235.
360. Kabir, S. (2001). Detection of *Helicobacter pylori* in faeces by culture, PCR and enzyme immunoassay. *Journal of Medical Microbiology,*50: 1021-1029.
361. Kabrah, M.A.M., Faidah, H.S., Ashshi, A.M., Turkistani, M.S.A. (2016). Antibacterial effect of onion. *Scholars Journal of Applied Medical Sciences,* 4: 4128-4133.
362. Kailasapathy, K., Chin, J. (2000). Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. *Immunology and Cell Biology*, 78(1): 80-88.
363. Kallel, F., Driss, D., Chaari, F., Belghith, L., Bouaziz, F., Ghorbel, R., Chaabouni, E.S. (2014).Garlic (*Allium sativum* L.) husk waste as a potential source of phenolic compounds: Influence of extracting solvents on its antimicrobial and antioxidant properties. *Industrial Crops* and *Products,* 62: 34-41.
364. Kanamarlapudi, S.K. Muddada, S. (2017). Characterization of exopolysaccharide produced by *Streptococcus thermophilus* CC30. *BioMed Research International*, 2017:1-11.
365. Kanani, B., Khosrowshahi, A., Khaledabad, M.A., Pourahmad, R. (2018). Probiotic yogurt formulated with nettle (*Urtica dioica*) extract, a compound with dual functionalities: *Bifidobacterium* growth promoter and *Helicobacter pylori* growth inhibitor. *Biomedical Journal*, 1- 7.
366. Kandler, O., Kunath, P. (1983). *Lactobacillus* kefir sp. nov., a component of the microflora of kefir. *Systematic and Applied Microbiology*, 4(2): 286-294.
367. Kandler, O., Weiss, N. (1986). *Lactobacillus*. In: Bergey's manual of systematic bacteriology, (Eds. Sneath PHA., Mair NS., Sharpe ME., Holt JG.). Williams and Wilkins, London, PP 1209–1034.
368. Kandoliya, U.K., Bodar, N.P., Bajaniya, V.K., Bhadja, N.V., Golakiya, B.A. (2015). Determination of nutritional value and antioxidant from bulbs of different onion (*Allium cepa*) variety: A comparative study. *International Journal of Current Microbiology and Applied Sciences,*4(1): 635-641.
369. Kang, J.M., Kim, N., Lee, D.H., Park, Y.S., Kim, Y.R., Kim, J.S., Jung, H.C., Song, I.S. (2007). Second-line treatment for *Helicobacter pylori* infection: 10-day moxifloxacin-based triple therapy versus 2-week quadruple therapy. *Helicobacter*, 12(6):623-628.
370. Kang, N., Yuan, R., Huang, L., Liu, Z., Huang, D., Huang, L., Gao, H., Liu, Y., Xu, Q.M. Yang, S. (2019). Atypical nitrogen-containing flavonoid in the fruits of cumin (*Cuminum cyminum* L.) with anti-inflammatory activity. *Journal of Agricultural and Food Chemistry*, 67 (30): 8339-8347.
371. Kang, S., Guo, Y., Rao, J., Jin, H., You, H.J., Eog Ji, G. (2021). *In vitro* and *in vivo* inhibition of *Helicobacter pylori* by *Lactobacillus plantarum* pH3A, monolaurin, and grapefruit seed extract. *Food and Function*, 12(21): 11024-11032.
372. Kaoru, A., Tadashi, N., Tomoaki, N., Masashi, K., Akemi, Y. (2006). Effects of fertilization, crop year, variety, and provenance factors on mineral concentrations in onions. *Journal of Agricultural and Food Chemistry,*54 (9):3341-3350.
373. Kassaian, N., Azadbakht, L., Forghani, B., Amini, M. (2009). Effect of fenugreek seeds on blood glucose and lipid profiles in type 2 diabetic patients. *International Journal for Vitamin* and *Nutrition* Research, 79(1):34-39.
374. Kaviarasan, S., Vijayalakshmi, K., Anuradha, C. (2004). Polyphenol-rich extract of fenugreek seeds protect erythrocytes from oxidative damage. *Plant Foods for Human Nutrition,*4:143-147.
375. Kawai, M., Furuta, Y., Yahara, K., Tsuru, T., Oshima, K., Handa, N., Takahashi, N., Yoshida, M., Azuma, T., Hattori, M., Uchiyama, I. (2011). Evolution in an oncogenic bacterial species with extreme genome plasticity: *Helicobacter pylori* East Asian genomes. *BMC Microbiology*, 11(1):1-28.
376. Kawasaki, M., Kawasaki, T., Ogaki, T., Itoh, K., Kobayashi, S., Yoshimizu, Y., Aoyagi, K., Iwakawa, A., Takahashi, S., Sharma, S. Acharya, G.P. (1998). Seroprevalence of *Helicobacter pylori* infection in Nepal: low prevalence in an isolated rural village. *European Journal of Gastroenterology and Hepatology*, 10(1):47-50.
377. Kenji, O., Qiao, Z., Satoru, S., Shogo, Y., Tsutomu, T., Hideki, F., Akihiko, K. (2009). Efficient production of optically pure D-Lactic Acid from raw corn starch by using a genetically modified L-Lactate

dehydrogenase gene-deficient and Amylase secreting *Lactobacillus plantarum* strain. *Applied and Environmental Microbiology,* 75(2): 462-467.

378. Khalid, K. (2011). An overview of lactic acid bacteria. *International Journal of Bioscience,* 1(3): 1-13.
379. Khalifa, M.M., Sharaf, R.R., Aziz, R.K. (2010). *Helicobacter pylori*: A poor man's gut pathogen?.*Gut Pathogens*, 2:2-12
380. Khalil, M.O., Morton, L.M., Devesa, S.S., Check, D.P., Curtis, R.E., Weisenburger, D.D., Dores, G.M. (2014). Incidence of marginal zone lymphoma in the United States, 2001-2009 with a focus on primary anatomic site. *British Journal* of *Haematology,* 165: 67-77.
381. Khare, C.P. (2002). Indian Medicinal Plants. In: An illustrated dictionary. Ed. Spinger. USA. PP 818.
382. Khulusi, S., Mendall, M.A., Patel, P., Levy, J., Badve, S., Northfield, T.C. (1995). *Helicobacter pylori* infection density and gastric inflammation in duodenal ulcer and non-ulcer subjects. *Gut*, 37(3): 319-324.
383. Kikuchi, S., Kurosawa, M., Sakiyama, T. (1999). *Helicobacter pylori* risk associated with sibship size and family history of gastric diseases in Japanese adults. *Japanese Journal of Cancer Research*, 89:1109-1112.
384. Kim, J.S., Kang, O.J., Gweon, O.C. (2013). Comparison of phenolic acids and flavonoids in black garlic at different thermal processing steps. *Journal of Functional Foods*, 5(1): 80-86.
385. Kim, J.U., Kim, Y.H., Han, K.S., Oh, S.J., Whang, K.Y., Kim, J.N., Kim, S.H. (2006). Function of cell-bound and released exo-polysaccharides produced by *Lactobacillus rhamnosus* ATCC 9595. *Journal of Microbiology and Biotechnology*, 16(6): 939-945.
386. Kim, J.U., Kim, Y.H., Han, K.S., Oh, S.J., Whang, K.Y., Kim, J.N. Kim, S.H. (2006). Function of cell-bound and released exopolysaccharides produced by *Lactobacillus rhamnosus* ATCC 9595. *Journal of Microbiology and Biotechnology*, 16(6): 939-945.
387. Kim, K.H., Lee, K.W., Kim, D.Y., Park, H.H., Kwon, I.B., Lee, H.J. (2004).Extraction and fractionation of glucosyl-transferase inhibitors from cacao-bean husk. *Process Biochemistry,* 39: 2043-2046.
388. Kim, M., Lee, S.J., Seul, K.J., Park, Y.M., Ghim, S.Y.( 2009). Characterization of antimicrobial substance produced by *Lactobacillus paraplantarum* KNUC25 isolated form Kimchi. *Korean Journal of Microbiology and Biotechnology*, 37:24–32.
389. Kindermann, A., Konstantopoulos, N., Lehn, N., Demmelmair, H., Koletzko, S. (2001). Evaluation of two commercial enzyme immunoassays, testing immunoglobulin G (IgG) and IgA responses, for diagnosis of *Helicobacter pylori* infection in children. *Journal of Clinical Microbiology*, 39:3591-3596.
390. Klaenhammer, T. R. (1993). Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiology Reviews*, 12(1-3): 39-85.
391. Koenen, EV. (2001). Medicinal poisonous and edible plant in Namibia. 9th Ed. Klaus Hess Verlag, Berlin, Germany. PP 336.
392. Kok, J., Buist, G., Zomer, A.L., Van Hijum, S.A., Kuipers, O.P. (2005). Comparative and functional genomics of *Lactococci*. *FEMS Microbiology Reviews*, 29(3): 411-433.
393. Kolb, H. (1955). Die Behandlung akuter infekte unter dem Gesichtswinkel der Prophylaxe chronischer Leiden. Über die Behandlung mit physiologischen Bakterien. *Microecology and Therapy*, 1: 15-19.
394. Koletzko, S., Konstantopoulos, N., Bosman, D., Feydt-Schmidt, A., Van der Ende, A., Kalach, N., Raymond, J., Rüssmann, H. (2003). Evaluation of a novel monoclonal enzyme immunoassay for detection of *Helicobacter pylori* antigen in stool from children. *Gut*, 52 (6): 804-806.
395. Kolho, K.L., Korhonen, J., Verkasalo, M., Lindahl, H., Savilahti, E., Rautelin, H. (2002). *Helicobacter pylori* serology at diagnosis and follow-up of biopsy-verified infection in children. *Scandinavian Journal of Infectious Diseases*, 34 (3): 177-182.
396. Kollath, W. (1953). Ernahrung und Zahnsystem. *Deutsche Zahnarztliche Zeitschrift*, 8: 7-16.
397. König, H., Fröhlich, J. (2017). Lactic acid bacteria. In: Biology of microorganisms on grapes, in must and in wine, (Eds. Helmut König, Gottfried Unden, Jürgen Fröhlich). Springer, Cham. PP 395.
398. Krienitz, W. (1906). Ueber das Auftreten von Spirochäten verschiedener Form im Mageninhalt bei Carcinoma ventriculi. *DMW-Deutsche Medizinische Wochenschrift*, 32(22): 872-872.
399. Krishnamurthy, K. H. (2013). Medicinal Plants: Jeeraka or cumin and caraway (*Cuminum cyminum* and *Carum carvi*). *Journal of New Approaches to Medicine and Health,* 21(2): 1-11.
400. Krishnapura, S. (2017). Cumin (*Cuminum cyminum*) and black cumin (*Nigella sativa*) seeds: Traditional uses, chemical constituents, and nutraceutical effects. *Food Quality and Safety*, 2(1): 1-16.
401. Krishnasamy, V., Seshu, D.V. (1989). Seed germination rate and associated characters in rice, *Crop Science*, 29: 904–908.
402. Krzewinski, F., Brassart, C., Gavini, F., Bouquelet, S. (1996). Characterization of the lactose transport system in the strain *Bifidobacterium bifidum* DSM 20082. *Current Microbiology*, 32(6): 301-307.
403. Krzyżek, P., Junka, A., Słupski, W., Dołowacka-Jóźwiak, A., Płachno, B.J., Sobiecka, A., Matkowski, A., Chodaczek, G., Płusa T., Gościniak, G., Zielinska, S. (2021). Antibiofilm and antimicrobial enhancing activity of *Chelidonium majus* and *Corydalis cheilanthifolia* extracts against multidrug-resistant *Helicobacter pylori*. *Pathogens*, 10 (8): 1008-1033.

404. Kuda, T., Iwai, A., Yano, T. (2004). Effect of red pepper *Capsicum annuum* var. conoides and garlic *Allium sativum* on plasma lipid levels and cecal microflora in mice fed beef tallow. *Food and Chemical Toxicology,* 42: 1695–1700.
405. Kumar Amit, N., Dilipkumar, P., Kousik, S. (2015). Screening of polysaccharides from tamarind, fenugreek and jack fruit seeds as pharmaceutical excipients*, International Journal of Biological Macromolecules,* 79: 756–760.
406. Kumar, A., Kassavetis, G.A., Geiduschek, E.P., Hambalko, M., Brent, C.J. (1997). Functional dissection of the B component of RNA polymerase III transcription factor IIIB: a scaffolding protein with multiple roles in assembly and initiation of transcription. *Molecular and Cellular Biology,* 17 (4):1868-1880.
407. Kumari, N., Kumar, M., Lorenzo, J.M., Sharma, D., Puri, S., Pundir, A., Dhumal, S., Bhuyan, D.J., Jayanthy, G., Selim, S., Abdel-Wahab, B.A. (2022). Onion and garlic polysaccharides: A review on extraction, characterization, bioactivity, and modifications. *International Journal of Biological Macromolecules*, 219: 1047–1061.
408. Kumari, R., Singh, A., Yadav, A.N., Mishra, S., Sachan, A., Sachan, S.G. (2020). Probiotics, prebiotics, and synbiotics: current status and future uses for human health. In: New and future developments in microbial biotechnology and bioengineering. (Eds. Vijai Gupta) Elsevier, USA. PP 314.
409. Kumura, H., Tanoue, Y., Tsukahara, M., Tanaka, T., Shimazaki, K. (2004). Screening of dairy yeast strains for probiotic applications. *Journal of Dairy Science*, 87(12): 4050-4056.
410. Kunyanga, C., Imungi, J., Okoth, M., Biesalski, H., Vadivel, V. (2011).Antioxidant and antidiabetic properties of condensed tannins in acetonic extract of raw and processed food ingredients from Kenya. *Journal of Food Science*, 76: 560-567.
411. Kurihara, H., Asami, S., Shibata, H., Fukami, H., Tanaka, T. (2003). Hypolipemic effect of *Cyclocarya paliurus* (Batal) Iljinskaja in lipid-loaded mice. *Biological and Pharmaceutical Bulletin,* 26:383-385.
412. Kwok, T., Zabler, D., Urman, S., Rohde, M., Hartig, R., Wessler, S., Misselwitz, R., Berger, J., Sewald, N., König, W., Backert, S. (2007). *Helicobacter* exploits integrin for type IV secretion and kinase activation. *Nature*, 449(7164): 862-866.
413. Lachman, J., Hosnedl, V., Pivec, V. (1997). Changes in the content of polyphenols in barley grains and pea seed after controlled accelerated ageing treatment. Scientia Agriculturae Bohemica, 28: 17–30.
414. Lachman, J., Pronek, D., Hejtmánková, A., Dudjak, J., Pivec, V., Faitova, K. (2002). Total polyphenol and main flavonoid antioxidants in different onion (*Allium cepa* L.) varieties. *Horticultural Science*, 30 (4): 142–147.
415. Lafon-Lafourcade, S., Carre, E., Ribereau-Gayon, P. (1983). Occurrence of lactic acid bacteria during the different stages of vinification and conservation of wines. *Applied and Environmental Microbiology*, 46(4): 874-880.
416. Lage, A. P., Godfroid, E., Fauconnier, A., Burette, A., Butzler, J.P., Bollen, A., Glupczynski, Y. (1995). Diagnosis of *Helicobacter pylori* infection by PCR: comparison with other invasive techniques and detection of cagA gene in gastric biopsy specimens. *Journal of Clinical Microbiology*, 33(10): 2752–2756.
417. Laheij, R.J.F., Straatman, H.M.P.M., Jansen, J.B.M.J., Verbeek, A.L.M. (1998). Evaluation of commercially available *Helicobacter pylori* serology kits: a review. *Journal of Clinical Microbiology*, 36(10): 2803-2809.
418. Laheij, R.J., de Boer, W.A., Jansen, J.B., van Lier, H.J., Sneeberger, P.M., Verbeek, A.L. (2000). Diagnostic performance of biopsy-based methods for determination of *Helicobacter pylori* infection without a reference standard. *Journal of Clinical Epidemiology*, 53(7): 742-746.
419. Laine, L., Hunt, R., El-Zimaity, H., Nguyen, B., Osato, M. Spénard, J. (2003). Bismuth-based quadruple therapy using a single capsule of bismuth biskalcitrate, metronidazole, and tetracycline given with omeprazole versus omeprazole, amoxicillin, and clarithromycin for eradication of *Helicobacter pylori* in duodenal ulcer patients: a prospective, randomized, multicenter, North American trial. *The American Journal of Gastroenterology*, 98 (3): 562-567.
420. Lakra, A.K., Domdi, L., Hanjon, G., Tilwani, Y.M., Arul, V. (2020). Some probiotic potential of *Weissella confusa* MD1 and *Weissella cibaria* MD2 isolated from fermented batter. *LWT - Food Science and Technology,* 125:1-26.
421. Lam, E.K., Tai, E.K., Koo, M.W., Wong, H.P., Wu, W.K., Yu, L., So, W.H., Woo, P.C., Cho, C.H. (2007). Enhancement of gastric mucosal integrity by *Lactobacillus rhamnosus* GG. *Life Sciences*, *80*(23): 2128-2136.
422. Lambinon, J., Delvosalle, L., Duvigneaud, J. (2004) . Nouvelle flore de la Belgique, du Grand-Duché de Luxembourg, du Nord de la France et des Régions voisines (Ptéridophytes et Spermatophytes). 4th Ed. Meise, Patrimoine du Jardin Botanique National de Belgique. PP 152-153.
423. Lancaster, JE., Shaw, ML. (1989). G-Glutamyl peptides in the biosynthesis of S-alk(en)yl-L-cysteine sulfoxides (flavor precursors) in *Allium*. *Phytochemistry*, 28:455–460.
424. Lanzotti, V. (2006). The analysis of onion and garlic. *Journal of Chromatography A,* 1112: 3–22.

425.Laroubi A, Touhami M, Farouk L, Zrara I, Aboufatima R, Benharref A. (2007) Prophylaxis effect of *Trigonella Foenum graecum* L. seeds on renal stone formation in rats. *Phytotherapy Research*, 21(10): 921-925.

426.Larpent, J.P. (1996). Les bactéries lactiques In: Microbiologie alimentaire : Aliments fermentés et fermentation alimentaires. (Eds. Bourgeois C., Larpent J.).Tec and Doc, Lavoisier. PP 1072.

427.Larpent, J.P., Larpent-gourgaud, M. (1985). Elément de microbiologie. Ed. Hermann, Paris, PP 250.

428.Lauwers, G.Y., Riddell, R.H. (1999). Gastric epithelial dysplasia. *Gut*, 45(5): 784-784.

429.Lawande, K.E. (2012). Onion. In: Handbook of herbs and spices. (Eds. Peter KV.).Wood head Publishing. UK. PP. 319.

430.Lawrence, B.M. (2000). Progress in essential oils-tansy oil, cajuput oil, and St John's bread or carob extract. *Perfumer and Flavorist*, 25(1): 33-48.

431.Lawson, L.D. (1998). Garlic: a review of its medicinal effects and indicated active compounds. *Blood*, 179, 62.

432.Lazouni, H.A., Benmansour, A., Taleb-Bendiab, S.A.D Chabane, S.A.R.I. (2007). Composition des constituants des huiles essentielles et valeurs nutritives du *Foeniculum vulgare* Mill. *Sciences and Technologie C,* 2007: 7-12.

433.Ledda, A., Floris, R., Mannu, L., Scintu, M.F. (1996). Studies on the microbial population and ecosystem of Pecorino Sardo cheese made from raw ewe's milk. Flora 3rd Plenary Meeting, Thessaloniki, PP 89–95.

434.Lee, A., O'Rourke, J., De Ungria, M.C., Robertson, B., Daskalopoulos, G., Dixon, M.F.(1997). A standardized mouse model of *Helicobacter pylori* infection: introducing the Sydney strain. *Gastroenterology,* 112:1386-1397.

435.Lee, E.J., Yoo, K.S., Jifon, J., Patil, B.S., (2009). Characterization of short-day onion cultivars of 3 pungency levels with flavor precursor, free amino acid, sulphur and sugar contents. *Journal of Food Science,*74, 475–480.

436.Lee, H.A., Kim, J.Y., Kim, J., Nam, B., Kim, O. (2020). Anti-*Helicobacter pylori* activity of a complex mixture of *Lactobacillus paracasei* HP7 including the extract of *Perilla frutescens* var. acuta and *Glycyrrhiza glabra*. *Laboratory Animal Research*, 36(1):1-8.

437.Lefief, A. (2012). L'ail Malin. Ed. LEDUC. France. PP147.

438.Leighton, T., Glinther, C., Fluss, L., Harte, W.K., Cansado, J., Notario, V. (1992). Molecular characterization of quercetin and quercetin glycosides in *Allium* vegetables: their effects on cell transformation. ACS Symposium series. *Journal* of the American *Chemical Society,* 507: 220-238.

439.Lenkova, M., Bystrická, J., Tóth, T., Hrstkova, M.(2016). Evaluation and comparison of the content of total polyphenols and antioxidant activity of selected species of the genus *Allium.* Journal of Central European *Agriculture*, 17(4):1119-1133.

440.Lesbros-Pantoflickova, D., Corthesy-Theulaz, I., Blum, A.L. (2007). *Helicobacter pylori* and probiotics. *The Journal of Nutrition*, 137(3): 812S-818S.

441.Li, R., Jiang, Z.T. (2004). Chemical composition of the essential oil of *Cuminum cyminum* L. from China. *Flavour and Fragrance Journal*, 19(4): 311–313.

442.Li, Z., Zou, D., Ma, X., Chen, J., Shi, X., Gong, Y., Man, X., Gao, L., Zhao, Y., Wang, R., Yan, X. (2010). Epidemiology of peptic ulcer disease: endoscopic results of the systematic investigation of gastrointestinal disease in China. *Official Journal of the American College of Gastroenterology*, 105(12): 2570-2577.

443.Liguori, L., Califano, R., Albanese, D., Raimo, F., Crescitelli, A., Di Matteo, M. (2017). Chemical composition and antioxidant properties of five white onion (*Allium cepa* L.) landraces. *Journal of Food Quality*, 2017:1-9.

444.Lilly, D., Stillwell, R. (1965). Probiotics: growth-promoting factors produced by microorganisms. *Science,* 147: 747-748.

445.Lim, T.K. (2015). Edible medicinal and non medicinal plants. Ed. Springer Science & Business Media, London. PP 203.

446.Lim, Y.Y., Lim, T.T., Tee, J.J. (2007). Antioxidant properties of several tropical fruits: a comparative study. *Food Chemistry*, 103:1003–1008.

447.Lin, C.K., Hsu, P.I., Lai, K.H., Lo, G.H., Tseng, H.H., Lo, C.C., Peng, N.J., Chen, H.C., Jou, H.S., Huang, W.K., Chen, J.L. (2002). One-week quadruple therapy is an effective salvage regimen for *Helicobacter pylori* infection in patients after failure of standard triple therapy. *Journal of Clinical Gastroenterology*, 34(5): 547-551.

448.Lin, J., Huang, W.W. (2009). A systematic review of treating *Helicobacter pylori* infection with Traditional Chinese Medicine.*World Journal of Gastroenterology*, 15: 4715-4719.

449.Ling, N.B. (2005). *Helicobacter pylori* infection in paediatric patients with dyspeptic symptoms. PhD. Thesis, Microbiology, University of Singapore, PP192.

450.Liou, J.M., Lin, J.T., Chang, C.Y., Chen, M.J., Cheng, T.Y., Lee, Y.C., Chen, C.C., Sheng, W.H., Wang, H.P., Wu, M.S. (2010). Levofloxacin-based and clarithromycin-based triple therapies as first-line and

second-line treatments for *Helicobacter pylori* infection: a randomised comparative trial with crossover design. *Gut*, 59(5): 572-578.
451.Liu, J., Guo, W., Yang, M.L., Liu, L.X., Huang, S.X., Tao, L., Zhang, F., Liu, Y.S. (2018). Investigation of the dynamic changes in the chemical constituents of Chinese “laba” garlic during traditional processing. *RSC Advances,* 8: 41872–41883.
452.Liu, S.G., Ren, P.Y., Wang, G.Y., Yao, S.X., He, X.J. (2015). Allicin protects spinal cord neurons from Glutamate-induced oxidative stress through regulating the heat shock protein 70 /inducible nitric oxide synthase pathway. *Food and Function*, 6:321–330.
453.Liu, W. H., Hsu, C.C., Yin, M.C. (2008). *In vitro* anti-*Helicobacter pylori* activity of diallyl sulphides and protocatechuic acid. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 22(1): 53-57.
454.Liu, Y., Qian, C., Ding, S., Shang, X., Yang, W., Fang, S. (2016). Effect of light regime and provenance on leaf characteristics, growth and flavonoid accumulation in *Cyclocarya paliurus* (Batal) Iljinskaja coppices. *Botanical Studies,*57 (28):1-13.
455.Liu, Z., Shen, J., Zhang, L., Shen, L., Li, Q., Zhang, B., Zhou, J., Gu, L., Feng, G., Ma, J., You, W.C. (2008). Prevalence of A2143G mutation of *H. pylori*-23S rRNA in Chinese subjects with and without clarithromycin use history. *BMC Microbiology*, 8(1): 1-8.
456.Lofgren, J.L., Whary, M.T., Ge, Z., Muthupalani, S., Taylor, N.S., Mobley, M., Potter, A., Varro, A., Eibach, D., Suerbaum, S., Wang, T.C., (2011). Lack of commensal flora in *Helicobacter pylori*-infected INS-GAS mice reduces gastritis and delays intraepithelial neoplasia. *Gastroenterology,* 140: 210-220.
457.Loh, J.T., Torres, V.J., Scott Algood, H.M., McClain, M.S., Cover, T.L. (2008). *Helicobacter pylori* HopQ outer membrane protein attenuates bacterial adherence to gastric epithelial cells. *FEMS Microbiology Letters*, 289 (1): 53-58.
458.Lorca, G., Wadström, T., Font de Valdez, G., Ljungh, Å. (2001). *Lactobacillus acidophilus* autolysins inhibit *Helicobacter pylori in Vitro*. *Current Microbiology*, 42: 39-44.
459.Lu, H., Hsu, P. I., Graham, D. Y., Yamaoka, Y. (2005). Duodenal ulcer promoting gene of *Helicobacter pylori.Gastroenterology*, 128(4): 833-848.
460.Lu, X., Wang, J., Al-Qadiri, H., Ross, C., Powers, J., Tang, J., Rasco, B. (2011).Determination of total phenolic content and antioxidant capacity of onion (*Allium cepa*) and shallot (*Allium oschaninii*) using infrared spectroscopy. *Food Chemistry*, 129 (2): 637-644.
461.Luther, J., Higgins, P.D., Schoenfeld, P.S., Moayyedi, P., Vakil, N., Chey, W.D. (2010). Empiric quadruple vs. triple therapy for primary treatment of *Helicobacter pylori* infection: Systematic review and meta-analysis of efficacy and tolerability. *Official Journal of the American College of Gastroenterology*, 105(1): 65-73.
462.Lutomsky, J, (1983). Inhalts und Wirkstoffe des *Alliums* arten. *Allium Konferenz. Freizing*, 19 (23): 164-187.
463.Luzza, F., Suraci, E., Larussa, T., Leone, I., Imeneo, M. (2014). High exposure, spontaneous clearance, and low incidence of active *Helicobacter pylori* infection: the Sorbo San Basile study. *Helicobacter,* 19: 296-305.
464.Macedo, A.C., Malcata, F.X. (1996). Changes in the major free fatty acids in Serra cheese throughout ripening. *International Dairy Journal*, 6(11-12): 1087-1097.
465.Madec, J., Decousser, J., Fortine, N., Haenni, M., Jouy, E., Kempf, I., Laurentie, M., Lupo, A., Morvan, H., Pinsard, J., Sanders, P., Woronoff-Rehn, N. (2018). Comité de l’antibiogramme de la société française de la microbiologie. Recommandation vétérinaire. 16 p.
466.Magboul, B.I. (1986). Chemical and amino acid composition of fenugreek Seeds grown in Sudan, *Food Chemistry*, 22:1–5.
467.Mahachai, V., Sirimontaporn, N., Tumwasorn, S., Thong-Ngam, D., Vilaichone, R.K. (2011). Sequential therapy in clarithromycin-sensitive and-resistant *Helicobacter pylori* based on Polymerase Chain Reaction molecular test. *Journal of Gastroenterology and Hepatology*, 26(5): 825-828.
468.Mahady, G.B., Pendland, S.L., Stoia, A., Chadwick, L.R. (2003). *In vitro* susceptibility of *Helicobacter pylori* to isoquinoline alkaloids from *Sanguinaria canadensis* and *Hydrastis canadensis*. *Phytotherapy Research*, 17: 217-221.
469.Mahady, G.B., Matsuura, H., Pendland, S.L. (2001). Allixin, a phytoalexin from garlic, inhibits the growth of *Helicobacter pylori in vitro*. *The American Journal of Gastroenterology*, 96(12): 3454.
470.Mahdavi, J., Sondén, B., Hurtig, M., Olfat, F.O., Forsberg, L., Roche, N., Ångström, J., Larsson, T., Teneberg, S., Karlsson, K.A., Altraja, S. (2002). *Helicobacter pylori* SabA adhesin in persistent infection and chronic inflammation. *Science*, 297(5581): 573-578.
471.Maisuthisakul, P., Suttajit, M., Pongsawatmanit, R. (2007). Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. *Food Chemistry*, 100:1409-1418.

472.Malaty, H.M., El-Kasabany, A., Graham, D.Y., Miller, C.C., Reddy, S.G., Srinivasan, S.R., Yamaoka, Y., Berenson, G.S. (2002). Age at acquisition of *Helicobacter pylori* infection: a follow-up study from infancy to adulthood. *The Lancet*, 359(9310): 931-935.
473.Malaty, H.M., Graham, D.Y. (1994). Importance of childhood socioeconomic status on the current prevalence of *Helicobacter pylori* infection. *Gut.*, 35:742-745.
474.Malfertheiner, P., Bazzoli, F., Delchier, J.C., Celiñski, K., Giguère, M., Rivière, M., Mégraud, F. Pylera Study Group (2011). *Helicobacterpylori* eradication with a capsule containing bismuth subcitrate potassium, metronidazole, and tetracycline given with omeprazole versus clarithromycin-based triple therapy: a randomised, open-label, non-inferiority, phase 3 trial. *Lancet*, 377 (9769): 905-913.
475.Malfertheiner, P., Dent, J., Zeijlon, L., Sipponen, P., Veldhuyzen Van Zanten, S.J.O., Burman, C.F., Lind, T., Wrangstadh, M., Bayerdörffer, E., Lonovics, J. (2002). Impact of *Helicobacter pylori* eradication on heart-burn in patients with gastric or duodenal ulcer disease results from a randomized trial programme. *Alimentary Pharmacology and Therapeutics*, 16 (8):1431-1442.
476.Malfertheiner, P., Link, A., Selgrad M. (2014). *Helicobacter pylori*: Perspectives and time trends.Nature Reviews. *Gastroenterology and Hepatology,*11:628-638
477.Malfertheiner, P., Megraud, F., O'Morain, C., Bazzoli, F., El-Omar, E., Graham, D., Hunt, R., Rokkas, T., Vakil, N., Kuipers, E.J. (2007). Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus Report. *Gut*, *56*(6): 772-781.
478.Malfertheiner, P., Megraud, F., O'morain, C., Hungin, A.P.S., Jones, R., Axon, A., Graham, D.Y., Tytgat, G. European *Helicobacter Pylori* Study Group (EHPSG). (2002). Current concepts in the management of *Helicobacter pylori* infection. The Maastricht 2-2000 Consensus Report. *Alimentary Pharmacology and Therapeutics*, 16(2): 167-180.
479.Mallor, C. Balcells, M. Sales, E. (2011). Genetic variation for bulb size, soluble solids content and pungency in the Spanish sweet onion variety Fuentes de Ebro. Response to selection for low pungency. *Plant Breeding*, 130:55-59.
480.Man, J.C., Rogosa, M., Sharp M.E. (1960). A medium for the cultivation of *Lactobacilli*. *Journal of Applied Microbiology,* 23: 130-135.
481.Manjegowda, S. Dharmesh, S. (2012). Anti-*Helicobacter pylori*, proton pump inhibitory and antioxidant properties of selected dietary/medicinal plants. *International Journal of Phytomedicine*, 4: 573.
482.Marchal N., Bourdon J.L., Richard C. (1991). Les milieux de culture : pour l'isolement et l'identification biochimiques des bactéries. Ed. Doin. Paris. PP 3-511.
483.Marelli, B., Perez, AR., Banchio, C., de Mendoza, D., Magni, C. (2011). Oral immunization with live *Lactococcus lactis* expressing rotavirus VP8 subunit induces specific immune response in mice. *The Journal of Virological Methods*,175(1):28–37.
484.Marina, S., Butt, M. S., Masood, B., Shehzad, A., Tanweer, S. (2014). Chemical and mineral analysis of garlic: a golden herb. *Pakistan Journal of Food Sciences*, 24(1):108-110.
485.Marotti, M., Piccaglia, R. (2002). Characterization of flavonoids in different cultivars of onion (*Allium cepa* L.). *Journal of Food Science*, 67(3): 1229-1232.
486.Marshall, B. (2002). *Helicobacter pylori*: 20 years on. *Clinical Medicine*, 2 (2): 147-152.
487.Marshall, B.J., Armstrong, J.A., Mc Gechie, D.B., Glancy, R.J. (1985a). Attempt to fulfil Koch's postulates for *pylori*c *Campylobacter*. *Medical Journal of Australia,*142: 436-439.
488.Marshall, B.J., Goodwin, C.S. (1987). Revised nomenclature of *Campylobacter pyloridis*. *International Journal of Systematic Bacteriology,* 37: 68.
489.Marshall, B.J., Mc Gechie, D.B., Rogers, P.A., Glancy, R.J. (1985b). *Pylori*c *Campylobacter* infection and gastroduodenal disease. *Medical Journal of Australia,*142: 439-444.
490.Marshall, B.J., Warren, J.R. (1984). Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *The Lancet*, 1:1311-1315.
491.Mashkor, I.M. (2014). Phenolic content and antioxidant activity of fenugreek seeds extract. *International Journal* of *Pharmacognosy* and *Phytochemical Research,* 6:841-844.
492.Masood, M.I., Qadir, M.I., Shirazi, J.H., Khan, I.U. (2011). Beneficial effects of lactic acid bacteria on human beings. *Critical Reviews in Microbiology*, 37(1): 91-98.
493.M'Buyamba-Kabangu, J.R., Tambwe, M. (1990). The efficacy of beta-adrenoceptor and calcium-entry blockers in hypertensive blacks. *Cardiovascular Drugs and Therapy*, 4: 389-394.
494.McColl, K.E.L. (1991). Fullarton, G.M., Chittajalu, R., El Nujumi, A.M., MacDonald, A.M.I., Dahill, S.W., Hilditch, T.E.. Plasma gastrin, daytime intragastric pH, and nocturnal acid output before and at 1 and 7 months after eradication of *Helicobacter pylori* in duodenal ulcer subjects. *Scandinavian Journal of Gastroenterology,* 26: 339-346.
495.McColl, K. E., el-Omar, E. M. Gillen, D. (1997). Alterations in gastric physiology in *Helicobacter pylori* infection: causes of different diseases or all epiphenomena? *Italian Journal of Gastroenterology and Hepatology,* 29: 459-464.

496.Medjoudj, H. (2007). Etude du comportement au décharge de six légumes:Carotte, courgette cardon, pomme de terre, ail et oignon. Master Thesis, Nutrition and Food Sciences, University of Constantine. Algeria. PP 34.

497.Medouakh, L., Tabak, S., Benkada, A., Mahi, M., Rouissat, L., Yagoubi, A., Bensoltane, A. (2006). Isolation and characterization of *Helicobacter pylori* from patients suffering from gastroduodenal ulcer disease. *The Egyptian Journal of Applied Sciences*, 21(3): 406-417.

498.Megha, P., Swati, J., Bharat, M. (2019). Phytochemical screening and comparative study of antioxidant activity of *Cuminum cyminum* L. and *Nigella sativa* L. *Journal of Scientific Research and Reports,* 8(2):1356-1364.

499.Meghwal, M., Goswami, T.K. (2012). A review on the functional properties, nutritional content, medicinal utilization and potential application of fenugreek. *Journal of Food Processing and Technology,* 3(9):181.

500.Megraud, F. (1993). Epidemiology of *Helicobacter pylori* infection. *Gastroenterology Clinics of North America*, *22*(1): 73-88.

501.Megraud, F. (1994). *H. pylori* resistance to antibiotics. In: *Helicobacter pylori:* basic mechanisms to clinical cure, (Eds. Richard H. Hunt, Guido N. J. Tytgat). Springer. USA. PP 612.

502.Megraud, F., Lamouliatte, H. (2003). the treatment of refractory *Helicobacter pylori* infection. *Alimentary Pharmacology and Therapeutics*, 17(11): 1333-1343.

503.Megraud, F., Lehours, P. (2007). *Helicobacter pylori* detection and antimicrobial susceptibility testing. *Clinical Microbiology Reviews*, 20(2): 280-322.

504.Megraud, F., Trimoulet pascale, Lamouliatte, H., Boyanova, L. (1991). Bactericidal effect of amoxicillin on *Helicobacter pylori* in an *in vitro* model using epithelial cells. *Antimicrobial Agents and Chemotherapy*, 35(5): 869-872.

505.Mehrafarin, A., Rezazadeh, S. H., Naghdi Badi, H., Noormohammadi, G.H., Zand, E., Qaderi, A. (2011). A review on biology, cultivation and biotechnology of fenugreek (*Trigonella foenum-graecum* L.) as a valuable medicinal plant and multipurpose. 10(37): 6-24

506.Menard, A., Buissonnière, A., Prouzet-Mauléon, V., Sifré, E., Megraud, F. (2016). The GyrA encoded gene: a pertinent marker for the phylogenetic revision of *Helicobacter* genus. *Systematic and Applied Microbiology*, 39(2): 77-87.

507.Mengmei, M., Taihua, M., Hongnan, S., Miao, Z., Jingwang, C., Zhibin, Y. (2015), Optimization of extraction efficiency by shear emulsifying assisted enzymatic hydrolysis and functional properties of dietary fiber from deoiled cumin (*Cuminum cyminum* L.), *Food Chemistry*, 179: 270–277.

508.Mercan, N., Guvensen, A., Celik, A., Katircioglu, H. (2007). Antimicrobial activity and pollen composition of honey samples collected from different provinces in Turkey. *Natural Product Research*, 21(3): 187-195.

509.Meredith T.J. (2008): The complete book of garlic: a guide for gardeners, growers, and serious cooks. Ed. Timber Press. Portland, Columbia. PP 332.

510.Merga, Y.J., O'hara, A., Burkitt, M.D., Duckworth, C.A., Probert, C.S., Campbell, B.J., Pritchard, D.M. (2016). Importance of the alternative NF-κB activation pathway in inflammation-associated gastrointestinal carcinogenesis. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 310 (11): G1081-G1090.

511.Meriga, B., Mopuri, R., MuraliKrishna, T. (2012). Insecticidal, antimicrobial and antioxidant activities of bulb extracts of *Allium sativum*. *Asian Pacific Journal* of *Tropical Medicine,* 5: 391–395.

512.Metchnikoff, I.I. (2004). The prolongation of life: Optimistic studies. Springer, New York, USA, PP 44-53.

513.Michaela, S., Reinhard, W., Gerhard, K., Christine, M.E. (2009). Cultivation of anaerobic and facultatively anaerobic bacteria from space craft associated clean rooms. *Applied and Environmental Microbiology,* 11(75): 3484-3491.

514.Michetti, P., Dorta, G., Wiesel, P.H., Brassart, D., Verdu, E., Herranz, M., Felley, C., Porta, N., Rouvet, M., Blum, A.L., Corthesy-Theulaz, I. (1999). Effect of whey-based culture supernatant of *Lactobacillus acidophilus* (johnsonii) La1 on *Helicobacter pylori* infection in humans. *Digestion*, 60 (3): 203-209.

515.Midolo, P.D., Lambert, J.R., Hull, R., Luo, F., Grayson, M.L. (1995). *In vitro* inhibition of *Helicobacter pylori* NCTC 11637 by organic acids and lactic acid bacteria. *Journal of Applied Bacteriology*, 79 (4): 475-479.

516.Miean, K.H., Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolinand apigenin) content of edible tropical plants. *Journal of Agricultural and Food Chemistry,*49:3106-3112.

517.Miehlke, S., Schneider-Brachert, W., Kirsch, C., Morgner, A., Madisch, A., Kuhlisch, E., Haferland, C., Bästlein, E., Jebens, C., Zekorn, C., Knoth, H. (2008). One-week once-daily triple therapy with esomeprazole, moxifloxacin, and rifabutin for eradication of persistent *Helicobacter pylori* resistant to both metronidazole and clarithromycin. *Helicobacter*, 13(1): 69-74.

518.Mijac, V.D., Duki, S.V., Opavski, N.Z., Duki, M.K., Ranin, L.T. (2006). Hydrogen peroxide producing *Lactobacilli* in women with vaginal infections. *European Journal of Obstetrics and Gynecology and Reproductive Biology*, 129(1): 69–76.
519.Mikaili, P., Maadirad, S., Moloudizargari, M., Aghajanshakeri, S., Sarahroodi, S. (2013). Therapeutic uses and pharmacological properties of garlic, shallot, and their biologically active compounds. *Iranian Journal* of *Basic Medical Sciences,*16: 1031–1048.
520.Miller, G.L. (1972), Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Analytical Chemistry,* 31: 426–428.
521.Miller, H.E., Rigelhof, F., Marquart, L., Prakash, A., Kanter, M. (2000).Antioxidant content of whole grain breakfast cereals, fruits and vegetables. *Journal of the American College of Nutrition,*19: 1-8.
522.Mimoune, N., Hamiroune, M., Boukhechem, S., Mecherouk, C., Harhoura, K., Khelef, D., Kaidi, R. (2022). Pathological findings in cattle slaughtered in northeastern Algeria and associated risk factors. *Veterinary Sciences*, 9(7): 330.
523.Mintah, S.O. , Asafo-Agyei, T., Archer, M., Junior, P.A. , Boamah, D., Kumadoh, D., Appiah, A., Ocloo, A., Boakye, Y.D., Agyare, C. (2019). Medicinal plants for treatment of prevalent diseases. In: Pharmacognosy- medicinal plants. (Eds. Perveen, S. and Al-Taweel, A.). Books on Demand, Germany. PP 320.
524.Miron, T., Rabinkov, A., Mirelman, D., Wilchek, M., Weiner, L. (2000). The mode of action of allicin:Its ready permeability through phospholipid membranes may contribute to its biological activity. *Biochimica et Biophysica Acta,*1463:20–30.
525.Mirzaei, F., Venkatesh, H.K. (2012). Efficacy of phyto medicines as supplement in feeding practices on ruminant's performance: a review. *Global Journal of Research on Medicinal Plants and Indigenous Medicine*, 1(9): 391-403.
526.Mishra, S., Singh, V., Rao, G., Jain, A.K., Dixit, V.K., Gulati, A.K., Nath, G. (2008). Detection of *Helicobacter pylori* in stool specimens: comparative evaluation of nested PCR and antigen detection. *The Journal of Infection in Developing Countries*, 2(03): 206-210.
527.Mnif, S., Aifa, S. (2015). Cumin *(Cuminum cyminum* L.) from traditional uses to potential biomedical applications. *Chemistry and Biodiversity*, 12(5):733-742.
528.Moghaddam, M.N. (2010). *In vitro* anti-bacterial activity of cumin (*Cuminum cyminum* L.) and tarragon (*Artemisia dracunculus* L.) extracts against clinical isolates of *Helicobacter pylori*. *Planta Medica*, 76(12): 476-484.
529.Mohd, K., Akowah, A., Ismail, Z. (2006). Antioxidant activity and phenolic content of *Orthosiphon stamineus Benth* from different geographical origin. *Journal* of *Sustainability Science* and *Management,* 1:14-22.
530.Mohsen, S.M., Bazaraa, W.A., Doukani, K. ( 2009). Purification and characterization of *Aspergillus niger* U-86 polygalacturonase and its use in clarification of pomegranate and grape juices. *The 4th Conference on Recent Technology in Agriculture,* 84: 805-816.
531.Mole, S., Waterman, P.G. (1987). A critical analysis of techniques for measuring tannins in ecological studies. *Oecologia*,72:137-147.
532.Momtaz, H., Dabiri, H., Souod, N., Gholami, M. (2014). Study of *Helicobacter pylori* genotype status in cows, sheep, goats and human beings. *BMC Gastroenterology*, 14(1): 1-7.
533.Monojit, D., Samyak, C., Amalesh, N., Moulisha, B., Pallab, K.H. (2017). Pharmacognostic and preliminary physicochemical study of vidangadi louham an ayurvedic antidiabetic herbo mineral preparation, *Journal of Ayurvedic and Herbal Medicine*, 3(1):15–26.
534.Moradi, N., Moradi, K. (2013). Physiological and pharmaceutical effects of fenugreek (*Trigonella Foenum-graecum* L.) as a multipurpose and valuable medicinal plant. *Global Journal of Medicinal Plant Research*, 1(2):199-206.
535.Moravec, J., Kvasnicka, S., Velicka, U. (1974). Correlation between bulb weightand other characters in cultivars of bolting garlic. *Bulletin Vyzkumry VstavZelinarsky Olamoue*. 18:15-23.
536.Moro, E. (1900). *Bacillus acidophilus*: A contribution to the knowledge of the normal intestinal bacteria of infants. *Monatsschrift Kinderheilkunde,* 52: 38-55.
537.Morris, A.J., Ali, M.R., Nicholson, G.I., Perez-Perez, G.I., Blaser, M.J. (1991). Long-term follow-up of voluntary ingestion of *Helicobacter pylori*. *Annals of Internal Medicine*, 114 (8): 662-663.
538.Morris, A., Nicholson, G. (1987). Ingestion of *Campylobacter pyloridis* causes gastritis and raised fasting gastric pH. *American Journal of Gastroenterology*, 82(3):192-199.
539.Moumen, F. (2016).Valorization of cultivated and spontaneous culinary plants in western Algeria: case of the *Allium* genus. PhD Thesis in Environmental Science, University of Sidi Bel Abbes, Algeria, PP171.
540.Mower, C. (2013). The difference between yellow, white and red onions. *The Cooking Dish.* 2013: 03-24.

541.Muhammad, T.S., Masood, S.B., Faqir, M.A., Amer J. (2009), Influence of black cumin fixed and essential oil supplementation on markers of myocardial necrosis in normal and diabetic rats, *Pakistan Journal of Nutrition,* 8: 1450–1455.

542.Mullaicharam, A.R., Geetali, D., Uma, M. (2013). Weight serum cholesterol levels and the nitrogen medicinal values of fenugreek, *International Journal of Biological and Chemical Sciences,* 4:130– 134.

543.Muller-Harvey, I. (2006). Unraveling the conundrum of tannins in animal nutrition and health. *Journal of Agriculture*, *Food*, *Environment and Animal Sciences,* 86:2010-2037.

544.Multon, J.L. (1991). Techniques d'analyses et de contrôle dans les industries agroalimentaires. Ed. Tech et Doc –Lavoisier, France. PP 121–137.

545.Musumba, C., Jorgensen, A., Sutton, L., Van Eker, D., Moorcroft, J., Hopkins, M., Pritchard, D.M., Pirmohamed, M. (2012). The relative contribution of NSAIDs and *Helicobacter pylori* to the aetiology of endoscopically-diagnosed peptic ulcer disease: observations from a tertiary referral hospital in the UK between 2005 and 2010. *Alimentary Pharmacology and Therapeutics*, 36(1): 48-56.

546.Myllyluoma, E., Kajander, K., Mikkola, H., Kyrönpalo, S., Rasmussen, M., Kankuri, E., Sipponen, P., Vapaatalo, H., Korpela, R. (2007). Probiotic intervention decreases serum gastrin-17 in *Helicobacter pylori* infection. *Digestive and Liver Disease*, 39(6): 516–523.

547.Nadeem, M., Riaz, A. (2012). Cumin (*Cuminum cyminum*) as a potential source of antioxidants. *Pakistan Journal of Food Sciences*, 22(2): 101–107.

548.Naidu, M.M., Shyamala, B.N., Naik, J.P., Sulochanamma, G., Srinivas, P. (2011). Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds. LWT-*Food Science and Technology*, 44(2):451-456.

549.Naima, R., Oumam, M.M., Hannache, H., Sesbou, A., Charrier, B., Pizzi, A.P., Charrier El Bouhtoury, F. (2015). Comparison of the impact of different extraction methods on polyphenols yields and tannins extracted from Moroccan Acacia mollisima barks. *Industrial Crops and Products,*70:245-252.

550.Nakhaei, M., Ramezani, M., Karamoddin, M.K., Malekzadeh, F. (2006). *In vitro* anti-*Helicobacter pylori* activity of cumin (*Cuminumcyminum* L.) and tarragon (*Artemisia dracunculus* L.) extracts. *Iranian Journal of Basic Medical Sciences*, 9(3): 193-200.

551.Nam, H., Ha, M., Bae, O., Lee, Y. (2002). Effect of *Weissella confusa* strain PL9001 on the adherence and growth of *Helicobacter pylori*. *Applied and Environmental Microbiology*, 68(9): 4642-4645.

552.Namavar, F., Sparrius, M., Veerman, E.C., Appelmelk, B.J., Vandenbroucke-Grauls, C.M. (1998). Neutrophil-activating protein mediates adhesion of *Helicobacter pylori* to sulfated carbohydrates on high-molecular-weight salivary mucin. *Infection and Immunity*, 66(2): 444-447.

553.Naseri, V., Hozhabri, F., Kafilzadeh, F. (2012). Assessment of *in vitro* digestibility and fermentation parameters of alfalfa hay-based diet following direct incorporation of fenugreek seed *(Trigonella foenum*) and asparagus root *(Asparagus officinalis*). *Journal of Animal Physiology and Animal Nutrition,* 97(4):773-784.

554.Nasr, N.M., Khider, M., Metry, W., Atallah, K. (2017). Antibacterial activity of lactic acid bacteria against *Helicobacter pylori* evidence by *in vivo* and *in vitro* studies. *International Journal of Current Microbiology and Applied Sciences,*6(12): 4235-4247.

555.Nath, K.V.S., Rao, K.N.V., Banji, D., Sandhya, S., Sudhakar, K., Saikumar, P., Sudha, P., Chaitanya, R.K. (2010). A comprehensive review on *Allium cepa*. *Journal of Advanced Pharmaceutical Research,* 1(2): 94-100.

556.Nathiya, S., Durga, M., Devasena, T. (2014). Therapeutic role of *Trigonella Foenum*-graecum (fenugreek)– a review. *International Journal of Pharmaceutical Sciences Review and Research*, *27*(2): 74-80.

557.National Committee for Clinical Laboratory Standards. (1998). Methods for Antimicrobial Susceptibility Testing for Bacteria that Grow Aerobically; Approved Standard, 4$^{th}$ Ed. NCCLS document M7-A4. Wayne, PA.

558.National Research Council. (1989). Dietary fiber, in committee on diet and health, Food and Nutrition Board, Commission Life Sciences. Ed. Diet and health implications for reducing chronic disease risk. National Academy Press, Washington. PP 291–309.

559.Nazima, S., Adeel, A. (2013). A study on viscosity, surface tension and volume flow rate of some edible and medicinal oils, *International Journal of Science, Environment and Technology,* 2(6): 1318–1326.

560.NF EN 12092. (2002). Adhesives. Determination of viscosity.

561.Ngan, L. Tan, M.T. , Hoang, N., Thanh, D.T., Linh, N.T.T., Hoa, T.T.H., Nguyen, M., Trung Hieu, T.(2021). Antibacterial activity of *Hibiscus rosasinensis* L. red flower against antibiotic-resistant strains of *Helicobacter pylori* and identification of the flower constituents. *Brazilian Journal of Medical and Biological Research*, 54(7):1-10.

562.Ni, Y.H., Lin, J.T., Huang, S.F., Yang, J.C., Chang, M.H. (2000). Accurate diagnosis *Helicobacter pylori* infection by stool antigen test and 6 other currently available tests in children. *The Journal of Pediatrics*, 136(6): 823-827.

563.Nilsson, H.O., Blom, J., Al-Soud, W.A., Ljungh, A., Andersen, L.P., Wadström, T. (2002). Effect of cold starvation, acid stress, and nutrients on metabolic activity of *Helicobacter pylori*. *Applied and Environmental Microbiology*, 68(1): 11-19.

564.Nirmala, M.J., Durai, L., Rao, K.A., Nagarajan, R. (2020). Ultrasonic nanoemulsification of *Cuminum cyminum* essential oil and its applications in medicine. *International Journal of Nanomedicine*, 15: 795-807.

565.Nista, E.C., Candelli, M., Zocco, M.A., Cazzato, I.A., Cremonini, F., Ojetti, V., Santoro, M., Finizio, R., Pignataro, G., Cammarota, G. Gasbarrini, G. (2005). Moxifloxacin-based strategies for first-line treatment of *Helicobacter pylori* infection. *Alimentary Pharmacology and Therapeutics*, 21(10):1241-1247.

566.Nostro, A., Cannatelli, M.A., Crisafi, G., Musolino, A.D., Procopio, F., Alonzo, V.(2004). Modifications of hydrophobicity, *in vitro* adherence and cellular aggregation of *Streptococcus mutans* by *Helichrysum italicum* extract, *Letters in Applied Microbiology*, 38(5): 423-427.

567.Nostro, A., Cellini, L., Di Bartolomeo, S., Di Campli, E., Grande, R., Cannatelli, M.A. (2005). Antibacterial effect of plant extracts against *Helicobacter pylori*. *Phytotherapy Research,* 19: 198–202.

568.Nuutila, A.M., Puupponen-Pimiä, R., Aarni, M., Oksman-Caldentey, K.M. (2003).Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chemistry*, 81(4): 485–493.

569.Nwinuka, N.M., Ibeh, G.O., Ekeke, G.I. (2005). Proximate composition and levels of some toxicants in four commonly consumed spices. *Journal of Applied* Sciences and *Environmental Management,* 9(1): 150-155.

570.O'Mahony, R., Al-Khtheeri, H., Weerasekera, D., Fernando, N., Vaira, D., Holton, J., Basset, C. (2005). Bactericidal and anti-adhesive properties of culinary and medicinal plants against *Helicobacter pylori. World Journal of Gastroenterology*, 11(47):7499-7507.

571.Oberman, H., Libudzisz, Z. (1998). Fermented milks. In: Microbiology of fermented foods. (Eds. Wood, B.J.B.). Springer, Boston, MA. PP 308-350.

572.Odamaki, T., Yonezawa, S., Kitahara, M., Sugahara, Y., Xiao, J.Z., Yaeshima, T., Iwatsuki, K., Ohkuma, M. (2011). Novel multiplex polymerase chain reaction primer set for identification of *Lactococcus* species. *Letters in Applied Microbiology*, 52(5): 491-496.

573.O'Gara, E.A., Hill, D.J., Maslin, D.J. (2000). Activities of garlic oil, garlic powder, and their diallyl constituents against *Helicobacter pylori*. *Applied and Environmental Microbiology*, 66(5): 2269-2273.

574.Oh, J.D., Kling-Bäckhed, H., Giannakis, M., Xu, J., Fulton, R.S., Fulton, L.A., Cordum, H.S., Wang, C., Elliott, G., Edwards, J., Mardis, E.R. (2006). The complete genome sequence of a chronic atrophic gastritis *Helicobacter pylori* strain: evolution during disease progression. *Proceedings of the National Academy of Sciences*, 103 (26): 9999-1004.

575.Ohnishi, N., Yuasa, H., Tanaka, S., Sawa, H., Miura, M., Matsui, A., Higashi, H., Musashi, M., Iwabuchi, K., Suzuki, M., Yamada, G. (2008). Transgenic expression of *Helicobacter pylori* CagA induces gastrointestinal and hematopoietic neoplasms in mouse. *Proceedings of the National Academy of Sciences*, 105(3): 1003-1008.

576.Okada, M., Nishimura, H., Kawashima, M., Okabe, N., Maeda, K., Seo, M., Ohkuma, K., Takata, T. (1999). A new quadruple therapy for *Helicobacter pylori*: influence of resistant strains on treatment outcome. *Alimentary Pharmacology and Therapeutics*, 13(6): 769-774.

577.Okey, E.N., Akwaji, P.I., Umana, E.J., Markson, A.A.A. (2015). *In vitro* and *in vivo* biological evaluation of crude extracts of *Dioscorea dumetorum* and *Psidium guajava* leaves in the control of storage rot of onion (*Allium cepa* L.) bulbs. *International Journal of Pharmacology, Phytochemistry and Ethnomedicine*, 1: 81-92.

578.Okolo, F.A, Ocheja, J.O, Lalabe, B.C, Ejiga, P.A (2012). Digestibility, performance and bio-economics of growing west African dwarf goats fed diets containing graded level of cashew nut shell. *International Journalof Agriculture andRural Development* ,15(2): 1000-1007.

579.Okwu, DE. (2005). Phytochemicals, vitamins and mineral contents of two Nigerian medicinal Plants. *International Journal of Molecular Medicine* and *Advance,* 1: 375-381.

580.Oloyede, O.I (2005). Chemical profile of unripe pulp of *Carica Papaya. Pakistan Journal of Nutrition,* 4(6): 379-38.

581.Omolo, M.A., Wong, Z.Z., Mergen, K., Hastings, J.C., Le, N.C., Reil, H.A., Case, K.A., Baumler, D.J. (2014). Antimicrobial properties of chili peppers. *Journal of Infectious Diseases and Therapy*, 2 (4): 1-8.

582.O'Toole, P.W., Kostrzynska, M., Trust, T.J. (1994). Non-motile mutants of *Helicobacter pylori* and *Helicobacter* mustelae defective in flagellar hook production. *Molecular Microbiology,* 14:691-703.

583.Otunola, G.A., Oloyede O.B., Adenike T., Oladiji T., Afolayan A.J. (2010). Comparative analysis of the chemical composition of three spices *Allium sativum, Zingiber officinale* Rosc. and *Capsicum frutescens* L. commonly consumed in Nigeria. *African Journal of Biotechnology,*9(41):6927-6931.

584.Ourouadi, S., Moumene, H., Zaki, N., Boulli, A.A., Ouatmane, A., Hasib, A. (2016). Garlic (*Allium sativum*): A source of multiple nutraceutical and functional components. *Journal of Chemical, Biological and Physical Sciences*, 7(1): 9-21.

585.Ouwehand, A.C., Kirjavainen, P.V., Shortt, C., Salminen, S. (1999). Probiotics: mechanisms and established effects. *International Dairy Journal*, 9(1): 43-52.
586.Owen, R.J. (1998). *Helicobacter*-species classification and identification. *British Medical Bulletin,54*(1): 17-30.
587.Özkutlu, F., Kara, S.M., Şekeroğlu, N. (2007). Determination of mineral and trace elements in some spices cultivated in Turkey, *Acta Horticulturae,* 756: 321–328.
588.Palframan, S.L., Kwok, T., Gabriel, K. (2012). Vacuolating cytotoxin A (VacA), a key toxin for *Helicobacterpylori* pathogenesis. *Frontiers in Cellular and Infection Microbiology*, 2: 92:1-9.
589.Palmer, E.D. (1954). The state of the gastric mucosa of elderly persons without upper gastrointestinal symptoms. *Journal of the American Geriatrics Society*, 2 (3): 171-173.
590.Pandey, B.P. (1980). Economic Botany for degree honours and postgraduate students. Ed. S-Chand and Company. Ram Nagar, India. PP 416.
591.Pandian, R.S., Anuradha, C.V., Viswanathan, P. (2002). Gastroprotective effect of fenugreek seeds (*Trigonella foenum graecum*) on experimental gastric ulcer in rats. *Journal of Ethnopharmacology*, 81(3): 393-397.
592.Paoluzi, O.A., Visconti, E., Andrei, F., Tosti, C., Lionetti, R., Grasso, E., Ranaldi, R., Stroppa, I., Pallone, F. (2010). Ten and eight-day sequential therapy in comparison to standard triple therapy for eradicating *Helicobacter pylori* infection: a randomized controlled study on efficacy and tolerability. *Journal of Clinical Gastroenterology*, 44 (4): 261-266.
593.Parida, A.K., Das, A.B., Sanada, Y., Mohanty, P. (2004). Effects of salinity on biochemical components of the mangrove, *Aeceras corniculatum.Aquat Bot*., 80:77-87.
594.Park, J., Park, Y.K., Park, E. (2009). Antioxidative and antigenotoxic effects of garlic (*Allium sativum* L.) prepared by different processing methods. *Plant Foods for Human Nutrition,* 64: 244-249.
595.Park, K., Lee, C. (1996). Identification of isorhamnetin 4'-glucoside in onions. *Journal of Agricultural* and *Food Chemistry,* 44: 34-36.
596.Park, S. Y., Chin, K. B. (2010). Evaluation of antioxidant activities of ethanol extracted garlic and onion as affected by pre-heating for the application of meat products. *Korean Journal for Food Science of Animal Resources*, 30: 641-648.
597.Parker, R.B. (1974). Probiotics, the other half of the antibiotic story. *Animal Nutrition and Health,* 29: 4-8.
598.Parsonnet, J., Friedman, G.D., Vandersteen, D.P., Chang, Y., Vogelman, J.H., Orentreich, N., Sibley, R.K. (1991). *Helicobacter pylori* infection and the risk of gastric carcinoma. *New England Journal of Medicine*, 325(16): 1127-1131.
599.Pasha, I., Shabbir, M., Asim, H.M.A., Afzal, B., Chughtai, MF., Ahmad, S., Muhammad, M.S. (2017). Biochemical evaluation of *Trigonella foenum graecum* (fenugreek) with special reference to phenolic acids. *Biological Sciences*, 60:154-16.
600.Pathak Nimish, L., Kasture Sanjay, B., Bhatt Nayna, M., Rathod Jaimik, D. (2011). Phytopharmacological properties of *Coriander sativum* as a potential medicinal tree: an overview. *Journal* of *Applied Pharmaceutical Science,*1(4): 20-25.
601.Patil, A.K., Baghel, R.P.S., Nayak, S., Malapure, C.D., Govil, K., Kumar, D. (2017). Cumin (*Cuminum cyminum*): As a Feed Additive for Livestock. *Journal of Entomology and Zoology Studies*, 5(3): 365–369.
602.Paucar-Carrión, C., Espinoza-Monje, M., Gutiérrez-Zamorano, C., Sánchez-Alonzo, K., Carvajal, RI., Rogel-Castillo, C., Sáez-Carrillo, K., García-Cancino, A. (2022). Incorporation of *Limosi Lactobacillus fermentum* UCO-979C with anti *Helicobacter pylori* and immunomodulatory activities in various ice cream bases. *Foods*,11(3):333.
603.Peach, H.G., Pearce, D.C., Faris, S.J. (1997). *Helicobacter pylori* infection in an Australian regionalcity: Prevalence and risk factors. *The Medical Journal of Australia*, 167:310-313.
604.Pearson, A.D., Ireland, A., Bamforth, J., Walker, C., Booth, L., Hawtin, P., Holdstock, G., Millward-Sadler, H. (1984). Polyacrylamide gel electrophoresis of spiral bacteria from the gastric antrum. *Lancet*, 323(8390):1349-1350.
605.Pearson, R., Steigbigel, R., Davis, H., Chapman, S.(1980). Method of reliable determination of minimal lethal antibiotic concentrations. *Antimicrobial Agentsand Chemotherapy*, 18 (5): 699-708.
606.Pennisi, E. (1999). First food-borne pathogen sequenced. *Science,* 283: 1243.
607.Percival, S.I., Suleman, L. (2014). Biofilms and *Helicobacter pylori*: Dissemination and persistence within the environment and host. *World Journal of Gastrointestinal Pathophysiology*, 5:122-132
608.Peres, C.M., Hernandez-Mendonza, A., Bronze, M.R., Peres, C., Xavier Malcata, F. (2015). Synergy of olive bioactive phytochemicals and probiotic strain in control of *Escherichia coli*. *LWT -Food Science and Technology*, 64(2) :938-945.
609.Pérez-Martín, F., Seseña, S., Izquierdo, P.M., Palop, M.L. (2013). Esterase activity of lactic acid bacteria isolated from malolactic fermentation of red wines. *International Journal of Food Microbiology*, 163(2-3): 153-158.

610.Persson, C., Canedo, P., Machado, J.C., El-Omar, E.M., Forman, D. (2011). Polymorphisms in inflammatory response genes and their association with gastric cancer: a HuGE systematic review and meta-analyses. *American Journal of Epidemiology,* 173: 259-270.

611.Peter, K.V. (2003). Handbook of herbs and spices . Ed. Woodhead Publishing, Cambridge, UK. PP 164-167.

612.Peters, C., Schablon, A., Harling, M., Wohlert, C., Costa, J.T., Nienhaus, A. (2011). The occupational risk of *Helicobacter pylori* infection among gastroenterologists and their assistants. *BMC Infectious Diseases*, 11(1): 1-10.

613.Petri, E., Rodríguez, M., García, S. (2015). Evaluation of combined disinfection methods for reducing *Escherichia coli* O157: H7 population on fresh-cut vegetables. *International Journal of Environmental Research and Public Health*, 12 (8): 8678-8690.

614.Petropoulos, G.A. (1973). Agronomic, genetic and chemical studies of *Trigonella Foenum-graecum* L. PhD Thesis, Bath University, England. PP 145.

615.Petropoulos, G.A. (2002). Fenugreek : the genus *Trigonella*. Ed. reprint, London. PP 226.

616.Petropoulos, S.A., Fernandes, Â., Barros, L., Ferreira, I.C., Ntatsi, G. (2015).Morphological, nutritional and chemical description of Vatikiotiko, an onion local landrace from Greece, *Food Chemistry*, 182: 156-163.

617.Philippe, H., Anne-Claire, C.M., Dennis, A.R., Patrick, B., Christophe, F., Rodolphe, B. (2009). Comparative analysis of CRISPR loci in lactic acid bacteria genomes. *International Journal of Food Microbiology*, 131 (1): 62-70.

618.Piccolomini, R.P., Bonaventura, G.D., Festi, D., Catamo, G., Laterza, F., Neri, M. (1997). Optimal combination of media for primary isolation of *Helicobacter pylori* from gastric biopsy specimens. *Journal of Clinical Microbiology,*35: 1541-1544.

619.Pilar, C.M., Samuel, A., Karola, B., Trinidad, M., Ananias, P., Jorge, B.V. (2008). Current applications and future trends of lactic acid bacteria and their bacteriocins for the biopreservation of aquatic food products. *Food and Bioprocess Technology,* 1(1): 43- 63.

620.Pinchuk, I.V., Bressollier, P., Verneuil, B., Fenet, B., Sorokulova, I.B., Megraud, F. , Urdaci, M.C. (2001). *In vitro* anti-*Helicobacter pylori* activity of the probiotic strain *Bacillus subtilis* 3 is due to secretion of antibiotics. *Antimicrobial Agents and Chemotherapy*, 45(11): 3156-3161.

621.Pizzale, L., Bortolomeazzi, R., Vichi, S., Überegger, E., Conte, L. (2002). Antioxidant activity of sage *(Salvia officinalis* and *Salvia fruticosa*) and oregano *(Origanum onites* and *Origanum indercedens*) extracts related to their phenolic compound content. *Journal of the Science of Food and Agriculture,*82:1645-1651.

622.Plat-Sinnige, M.J.T., Verkaik, N.J., van Wamel, W.J., de Groot, N., Acton, D.S., van Belkum, A. (2009). Induction of *Staphylococcus aureus*-specific IgA and agglutination potency in milk of cows by mucosal immunization. *Vaccine*, 27(30): 4001-4009.

623.Plummer, S., Weaver, M.A., Harris, J.C., Dee, P., Hunter, J. (2004). *Clostridium* difficile pilot study: effects of probiotic supplementation on the incidence of C. difficile diarrhoea. *International Microbiology*, 7(1): 59-62.

624.Polishchuk, R.S., Polishchuk, E.V., Mironov, A. A. (1999). Coalescence of Golgi fragments in microtubule-deprived living cells. *European Journal of Cell Biology*, 78(3): 170-185.

625.Prajapati, V., Karen, H.D., Prajapati, P.H., Sen, D.J., Patel, C.N. (2018). Chemistry and histochemistry of Gram staining of dyes on bacterial peptidoglycan. *World Journal of Pharmaceutical Research*, 7(16): 490-535.

626.Prescott, L.M., Harley, J.P., Kelin, D.A. (2002). Microbiology, Bacteria: The Low G+ C Gram positives. 5th Ed. Boston: Mc Graw Hill, 529–530.

627.Price, M.L., Van Scoyoc, S. (1978).A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural* and *Food Chemistry,*26:1214-1218.

628.Prinz, C., Schöniger, M., Rad, R., Becker, I., Keiditsch, E., Wagenpfeil, S., Classen, M., Rösch, T., Schepp, W., Gerhard, M. (2001). Key importance of the *Helicobacter pylori* adherence factor blood group antigen binding adhesin during chronic gastric inflammation. *Cancer Research*, 61(5): 1903-1909.

629.Pronovost, A.D., Rose, S.L., Pawlak, J.W., Robin, H., Schneider, R. (1994). Evaluation of a new immunodiagnostic assay for *Helicobacter pylori* antibody detection: correlation with histopathological and microbiological results. *Journal of Clinical Microbiology*, 32 (1): 46-50.

630.Purseglove J.W. (1985). Tropical Crops Monocotyledons. Ed. Longman Singapore Publishers, PTC, PP 271-279.

631.Rababah, T., Hettiarachchy, N., Horax, R. (2004). Total phenolics and antioxidant activities of fenugreek, green Tea, black Tea, grape Seed, ginger, rosemary, gotu kola, and ginkgo extracts, Vitamin E, and tert-Butylhydroquinone. *Journal of Agricultural* and*Food Chemistry*, 52:5183-5186.

632.Rabinowitch, H.O. , Currah, L. (2002). *Allium* Crop Science: Recent Advances. Ed. CABI Publishing, UK. PP 585.

633.Rad, R., Brenner, L., Krug, A., Voland, P., Mages, J., Lang, R., Schwendy, S., Reindl, W., Dossumbekova, A., Ballhorn, W., Wagner, H. (2007). Toll-like receptor–dependent activation of antigen-presenting cells affects adaptive immunity to *Helicobacter pylori*. *Gastroenterology*, 133(1):150-163.

634.Radler, F. (1975). metabolism of organic acids by lactic acid bacteria. In: Lactic acid bacteria in beverages and food. Ed. Academic Press, London. PP 17.

635.Rafik El–Mahdy, A., El–Sebaiy, L.A (1982). Effect of germination on the nitrogenous constituents, protein fractions, in vitro digestibility and antinutritional factors of fenugreek seeds (*Trigonella foenum graecum* L.), *Food Chemistry*, 8(4): 253–262.

636.Rahmani, M., Hamel, L., Toumi-Benali, F., Dif, M.M., Moumen, F., Rahmani, H. (2018). Determination of antioxidant activity, phenolic quantification of four varieties of fenugreek *Trigonella foenum graecum* L. seed extract cultured in West Algeria. *Journal of Materials and Environmental Science,* 9(6):1656-1661.

637.Rahmani, M., Toumi-Benali, F., Hamel, L., Dif, M.M. (2015). Aperçu ethnobotanique et phyto-pharmacologique sur *Trigonella foenum graecum* L. *Phytothérapie*, 1-3.

638.Rajni, M., Joshi, R., Beenu, T. (2016). Effect of domestic processing on physico chemical and nutritional quality of fenugreek cultivars, *Asian Journal of Dairy and Food Research,* 35 (4): 338–344.

639.Ramos, F.A., Takaishi, Y., Shirotori, M., Kawaguchi, Y., Tsuchiya, K., Shibata, H., Higuti, T., Tadokoro, T., Takeuchi, M. (2006). Antibacterial and antioxidant activities of quercetin oxidation products from yellow onion (*Allium cepa*) skin. *Journal of Agricultural and Food Chemistry*, 54(10): 3551-3557.

640.Randhir, R., Lin, Y.T., Shetty, K., Lin, Y.T. (2004). Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. *Asian Pacific Journal of Clinical Nutrition*, 13 (3):295-307.

641.Randhir, R., Shetty, K. (2007). Improved α-amylase and *Helicobacterpylori* inhibition by fenugreek extracts derived via solid-state bioconversion using *Rhizopus oligosporus*. *Asian Pacific Journal of Clinical Nutrition*, 16(3): 382-392.

642.Ranilla, L.G., Apostolidis, E., Shetty, K. (2012). Antimicrobial activity of an Amazon medicinal plant (*Chancapiedra)(Phyllanthus niruri* L.) against *Helicobacter pylori* and lactic acid bacteria. *Phytotherapy Research*, 26(6): 791-799.

643.Rasul Suleria, H.A., Butt, M. S., Khalid, N., Sultan, S., Raza, A., Aleem, M., Abbas,M. (2015). Garlic (*Allium sativum*): diet based therapy of 21st century. *Asian Pacific Journal of Tropical Disease*, 5(4):271-278.

644.Raymond, J., Lamarque, D., Kalach, N., Chaussade, S., Burucoa, C. (2010). High level of antimicrobial resistance in French *Helicobacter pylori* isolates. *Helicobacter*, 15 (1): 21-27.

645.Raymond, J., Sauvestre, C., Kalach, N., Bergeret, M., Dupont, C. (2000). Immunoblotting and serology for diagnosis of *Helicobacter pylori* infection in children. *The Pediatric Infectious Disease Journal*, 19(2): 118-121.

646.Raymond, J., Sauvestre, C., Kalach, N., Bergeret, M., Dupont, C. (2000). Immunoblotting and serology for diagnosis of *Helicobacter pylori* infection in children. *The Pediatric Infectious Disease Journal*, 19(2): 118-121.

647.Rebey, B.I., Bourgou, S., Aidi Wannes, W., Hamrouni, I., Limam, F., Marzouk, B. (2010). Essential oils, phenolics, and antioxidant activities of different parts of cumin (*Cuminum cyminum* L.). *Journal of Agricultural and Food Chemistry* ,58(19):10410-10418.

648.Rebey, B.I., Bourgou, S., Benslimene Debez, I., Jabri Karoui, I., Sellami Hamrouni, I., Msaada, K., Limam, F., Marzouk, B. (2012). Effects of extraction solvents and provenances on phenolic contents and antioxidant activities of cumin (*Cuminum cyminum* L.) seeds. *Food and Bioprocess Technology,*5:2827-2836.

649.Rebey, B.I., Bourgou, S., Sriti, J., Msaada, K., Limam, F., Marzouk, B. (2011). Essential oils and fatty acids composition of Tunisian and Indian cumin (*Cuminum cyminum* L.) seeds: A comparative study. *Journal of the Science of Food and Agriculture,*91(11):2100-2107.

650.Rehman, K. (2003). Garlic and aging: new insight into an old remedy. *Ageing Research Reviews,*2:39-56.

651.Rettger, L. F., Cheplin, H. A. (1920a). The transformation of the intestinal flora, with special reference to the implantation of *Bacillus acidophilus*. I. Feeding experiments with albino rats. *Proceedings of the National Academy of Sciences*, 6: 423–426.

652.Rettger, L.F., Horton, G.D. (1914). A comparative study of the intestinal flora of white rats kept on experimental and ordinary mixed diets. *Zentralblatt fur Bakteriologie und Parasitenkunde*, 73: 362–372.

653.Rice-Evans, C.A., Miller, N.J., Paganga, G. (1996).Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology* and Medicine, 20(7): 933-956.

654.Robin, J.M. Rouchy, A. (2001). Les probiotiques. *Nutrithérapie Info*, 6: 1-4.

655.Rochard, B.P., Schmid, F. (2000). A review of methods to measure and calculate train resistances. *Proceedings of the Institution of Mechanical Engineers, Part F: Journal of Rail and Rapid Transit*, *214*(4): 185-199.

656.Rodas, A.M., Chenoll, E., Macian, M.C., Ferrer, S., Pardo, I., Aznar, R. (2006). *Lactobacillus* vini sp. nov., a wine lactic acid bacterium homofermentative for pentoses. *International Journal of Systematic and Evolutionary Microbiology*, 56(3): 513-517.

657.Rodolfo, J, Koroch, A, Simon, J, Hitiamana, N. (2006). Quality of geranium oils: case studies in southern and eastern Africa. *Journal of Essential Oil Research*, 18:116-121.

658.Rodriguez Galdon, B., Tascon Rodriguez, C., Rodriguez Rodriguez, E., Diaz Romero, C. (2008). Organic acid contents in onion cultivars (*Allium cepa* L.). *Journal of Agricultural and Food Chemistry,* 56: 6512–6519.

659.Rodriguez, J., Perez, C., Ramirez, H., Zambrano, J., (1998). Caracterizacìon de algunosparàmetros de calidad en la cebolla bajo diferentes èpocas de cosecha. *Agronomia Trop*ical, 48: 33–40.

660.Roesler, B.M. Zeitune, J.M.R. (2014). Molecular epidemiology of *Helicobacter pylori* in Brazilian patients with early gastric cancer and a review to understand the prognosis of the disease. In: Trends in *Helicobacter pylori* infection. (Eds. Bruna Roesler). Intech Open, London, UK, PP 392.

661.Roesler, B.M. (2019). Introductory chapter: *Helicobacter pylori*-An overview of an old human microorganism. In: *Helicobacter pylori new approaches of an old human microorganism*. (Eds. Bruna Maria Roesler). Intech Open, London, UK. PP 134.

662.Rohde, M., Püls, J., Buhrdorf, R., Fischer, W., Haas, R. (2003). A novel sheathed surface organelle of the *Helicobacter pylori* cag type IV secretion system. *Molecular Microbiology*, 49(1): 219-234.

663.Roland, F., Bourbon, D., Szturm, S. (1947). Rapid differentiation of entero-bacteria without action on lactose. *Annales de l'Institut Pasteur*. 73 (9): 914-916.

664.Rolfe, R.D. (2000). The role of probiotic cultures in the control of gastrointestinal health. *The Journal of Nutrition*, 130(2): 396S-402S.

665.Romaniuk, P.J., Zoltowska, B., Trust, T.J., Lane, D.J., Olsen, G.J., Pace, N.R., Stahl, D. (1987). *Campylobacter pylori*, the spiral bacterium associated with human gastritis, is not a true *Campylobacter* sp. *Journal of Bacteriology*, 169(5): 2137-2141.

666.Romano, M., Cuomo, A., Gravina, A.G., Miranda, A., Iovene, M.R., Tiso, A., Sica, M., Rocco, A., Salerno, R., Marmo, R., Federico, A. (2010). Empirical levofloxacin-containing versus clarithromycin-containing sequential therapy for *Helicobacter pylori* eradication: a randomised trial. *Gut*, 59(11):1465-1470.

667.Rönnqvist, D., Forsgren-Brusk, U., Husmark, U., Grahn-Håkansson, E. (2007). *Lactobacillus fermentum* Ess-1 with unique growth inhibition of vulvo-vaginal candidiasis pathogens. *Journal of Medical Microbiology*, 56(11): 1500-1504.

668.Rose, P., Whiteman, M., Moore, P. , Zhu, Y.( 2005). Bioactive S-alk(en)yl cysteine sulfoxide metabolites in the genus *Allium*: the chemistry of potential therapeutic agents, *Natural Product Reports*, 22:351-368.

669.Rosebeck, S., Madden, L., Jin, X., Gu, S., Apel, I.J., Appert, A., Hamoudi, R.A., Noels, H., Sagaert, X., Van Loo, P., Baens, M. (2011). Cleavage of NIK by the API2- MALT1 fusion oncoprotein leads to noncanonical NF-kappaB activation. *Science*, 331: 468-472.

670.Ross, I.A. (2001). Morinda citrifolia. In: Medicinal Plants of the World. Ed. Humana Press, Totowa, NJ. PP 309-317.

671.Ross, Z.M., O'Gara, E.A., Hill, D.J., Sleightholme, H.V., Maslin, D.J. (2001). Antimicrobial properties of garlic oil against human enteric bacteria: evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. *Applied and Environmental Microbiology,* 67 (1):475–480.

672.Rossland, E., Langsrud, T., Granum, P.E. , Sorhaug, T. (2005). Production of antimicrobial metabolites by strains of *Lactobacillus* or *Lactococcus* co-cultured with *Bacillus cereus* in milk. *International Journal of Food Microbiology*, 98(2): 193-200.

673.Rüssmann, H., Kempf, V.A., Koletzko, S., Heesemann, J., Autenrieth, I.B. (2001). Comparison of fluorescent in situ hybridization and conventional culturing for detection of *Helicobacter pylori* in gastric biopsy specimens. *Journal of Clinical Microbiology*, 39(1): 304-308.

674.Rusynyk, R.A., Still, C.D. (2001). Lactose intolerance. *Journal of Osteopathic Medicine*, 101(s41): 10-12.

675.Ruzsovics, A., Molnar, B., Unger, Z., Tulassay, Z., Pronai, L. (2001). Determination of *Helicobacter pylori* cagA, vacA genotypes with real-time PCR melting curve analysis. *Journal of Physiology*, 95(1-6): 369-377.

676.Ryan, S.J., Walsh, P.D. (2011). Consequences of non-intervention for infectious disease in African great apes. *PloS One*, 6(12): 1-9.

677.Sacco, F., Spezzaferro, M., Amitrano, M., Grossi, L., Manzoli, L., Marzio, L. (2010). Efficacy of four different moxifloxacin-based triple therapies for first-line *H. pylori* treatment. *Digestive and Liver Disease*, 42(2):110-114.

678.Sachdeva, A., Nagpal, J. (2009). Effect of fermented milk-based probiotic preparations on *Helicobacter pylori* eradication: a systematic review and meta-analysis of randomized-controlled trials. *European Journal of Gastroenterology and Hepatology*, 21(1): 45-53.

679.Sadeghi, AR., Pourahmad, R., Mokhtare, M. (2017). Enrichment of probiotic yogurt with broccoli sprout extract and its effect on *Helicobacter pylori*. *Applied Food Biotechnology*, 4(1):53-57.

680.Sakhria, M., Hichem, A., Hafsia, B., Abdelfettah, E., Najla, H. (2016). Phytochemical study and protective effect of *Trigonella foenum graecum* (Fenugreek seeds) against carbon tetrachloride-induced toxicity in liver and kidney of male rat. *Biomedicine and Pharmacotherapy*, 88:19-26.
681.Salaün, L., Ayraud, S., Saunders, N.J. (2005). Phase variation mediated niche adaptation during prolonged experimental murine infection with *Helicobacter pylori*. *Microbiology*, 151(3): 917-923.
682.Salehi Surmaghi, M.H. (2008). Medicinal plants and herbal therapy. *Tehran University Medical Journal*, 1: 253-260.
683.Salmerón-Manzano, E., Garrido-Cardenas, J.A., Manzano-Agugliaro, F. (2020). Worldwide research trends on medicinal plants. *International Journal of Environmental Research and Public Health*, 17 (10): 3376.
684.Salminen, S., Ouwehand, A., Benno, Y., Lee, Y.K. (1999). Probiotics: how should they be defined?. *Trends in Food Science and Technology*, 10(3): 107-110.
685.Sampath, K., Debjit, P., Chiranjib, Pankaj, T., Rakesh., K. (2010). *Allium sativum* and its health benefits. *Journal of Chemical and Pharmaceutical Research,* 2:135-146.
686.Samuels, A.L., Windsor, H.M., Ho, G.Y., Goodwin, L.D., Marshall, B.J. (2000). Culture of *Helicobacter pylori* from a gastric string may be an alternative to endoscopic biopsy. *Journal of Clinical Microbiology*, 38(6): 2438-2439.
687.Sangoyomi, T.E., Owoseni, A.A., Okerokun, O. (2010). Prevalence of enteropathogenic and lactic acid bacteria species in wara: A local cheese from Nigeria. *African Journal of Microbiology Research*, 4(15): 1624-1630.
688.Santas, J., Almajano, M.P. ,Carbó, R. (2010). Antimicrobial and antioxidant activity of crude onion (*Allium cepa*, L.) extracts. *International Journal of Food Science and Technology*, 45(2): 403-409.
689.Santhosha, S.G., Jamuna, P., Prabhavathin, S.N. (2013). Bioactive components of garlic and their physiological role in health maintenance. *Food Bioscience,* 3: 59-74.
690.Sarafa, A., Joseph, J., Kayode, R., Kolawole, F. (2016). Comparative phytochemical analysis and use of some Nigerian spices. *Croatian Journal of Food* Scienceand *Technology,*11 (3-4):145-151.
691.Sascha, S., Magdalena, K. (2010). Mass spectrometry tools for the classification and identification of bacteria. *Nature Reviews Microbiology,* 8: 74-82.
692.Sazawal, S., Hiremath, G., Dhingra, U., Malik, P., Deb, S., Black, R.E. (2006). Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials. *Lancet Infectious Diseases*, 6(6): 374-382.
693.Schleifer, K.H., Ludwig, W. (1995). Phylogenetic relationships of lactic acid bacteria. In: The genera of lactic acid bacteria, Ed: (Brian, J.B., Wood, W.H.N, Holzapfel).Springer, Boston, MA. PP 398.
694.Scholte, G.H., van Doorn, L.J., Cats, A., Bloemena, E., Lindeman, J., Quint, W.G., Meuwissen, S.G., Kuipers, E.J. (2002). Genotyping of *Helicobacter pylori* in paraffin-embedded gastric biopsy specimens: relation to histological parameters and effects on therapy. *The American Journal of Gastroenterology*, 97(7):1687-1695.
695.Schütz, H., Radler, F. (1984). Anaerobic reduction of glycerol to propanediol-1.3 by *Lactobacillus brevis* and *Lactobacillus buchneri. Systematic and Applied Microbiology*, 5(2): 169-178.
696.Sekeroglu, N., Meraler, S.A., Ozkutlu, F., Kulak, M. (2012). Variation of mineral composition in different parts of mahaleb. *Asian Journal of Chemistry,* 24(12): 5824-5828.
697.Selgrad, M., Malfertheiner, P. (2011). Treatment of *Helicobacter pylori*. *Current Opinion in Gastroenterology*, 27(6): 565-570.
698.Sellappan, S., Akoh, C.C. (2002).Flavonoids and antioxidant capacity of Georgia-grown Vidalia onions. *Journal of Agricultural* and *Food Chemistry,* 50 (19): 5338-5342.
699.Sendl, A. (1995). *Allium sativum* and *Allium ursinum*: Part 1 Chemistry, analysis, history, botany. *Phytomedicine*, 1(4): 323-339.
700.Senkovich, O.A., Yin, J., Ekshyyan, V., Conant, C., Traylor, J., Adegboyega, P., McGee, D.J., Rhoads, R.E., Slepenkov, S.,Testerman, T.L. (2011). *Helicobacter pylori* AlpA and AlpB bind host laminin and influence gastric inflammation in gerbils. *Infection and Immunity*, 79(8): 3106-3116.
701.Senouci, M.B., Abdelouahid, D. (2010). Antibacterial activity of essential oils of some Algerian aromatic plants against multidrug resistant bacteria. *Journal of Essential Oil Bearing Plants*, *13*(3), 362-370.
702.Sepehri, Z., Bagheri, G., Mohasseli, T., Javadian, F., Anbari, M., Nasiri, A.A., Baigi, G.S. (2014). Antibacterial Activity of *Cuminum cyminum* and *Piper nigrum* against antibiotic-resistant *Klebsiella pneumonia. Bulletin* of *Environment*, *Pharmacology* and *Life Sciences*, 3(3): 17–19.
703.Sethi, N., Kaura, S., Dilbaghi, N., Parle, M., Pal, M. (2014). Garlic: a pungent wonder from nature. *International Research Journal* of *Pharmacy,* 5(7): 523-529.
704.Sewald, X., Gebert-Vogl, B., Prassl, S., Barwig, I., Weiss, E., Fabbri, M., Osicka, R., Schiemann, M., Busch, D.H., Semmrich, M., Holzmann, B. (2008). Integrin subunit CD18 Is the T-lymphocyte receptor for the *Helicobacter pylori* vacuolating cytotoxin. *Cell Host and Microbe*, 3(1): 20-29.

705.Sgorbati, B., Biavati, B., Palenzona, D. (1995). The genus *Bifidobacterium*. In: The genera of lactic acid bacteria, (Eds. Wood B. J. B. , Holzapfel W. H).Springer, Boston, MA. PP 279-306.

706.Sgouras, D.N., Panayotopoulou, E.G., Martinez-Gonzalez, B., Petraki, K., Michopoulos, S., Mentis, A. (2005). *Lactobacillus* johnsonii La1 attenuates *Helicobacter pylori*-associated gastritis and reduces levels of proinflammatory chemokines in C57BL/6 mice. *Clinical and Vaccine Immunology*, 12(12): 1378-1386.

707.Sgouras, D., Maragkoudakis, P., Petraki, K., Martinez-Gonzalez, B., Eriotou, E., Michopoulos, S., Kalantzopoulos, G., Tsakalidou, E., Mentis, A. (2004). *In vitro* and *in vivo* inhibition of *Helicobacter pylori* by *Lactobacillus casei* strain Shirota. *Applied And Environmental Microbiology*. 70(1): 518-526.

708.Shabir, I., Pandey, V.K., Dar, A.H., Pandiselvam, R., Manzoor, S., Mir, S.A., Shams, R., Dash, K.K., Fayaz, U., Khan, S.A., Jeevarathinam, G. (2022). Nutritional profile, phytochemical compounds, biological activities, and utilisation of onion peel for food applications. *Sustainability*, 14(19): 1-15.

709.Shan, B., Cai, Y.Z., Sun, M., Corke, H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of Agricultural and Food Chemistry*, 53:7749-7759.

710.Shang, A., Cao, S.Y., Xu, X.Y., Gan, R.Y., Tang, G.Y., Corke, H., Mavumengwana, V., Li, H.B. (2019). Bioactive compounds and biological functions of garlic (*Allium sativum* L.). *Foods,* 8: 1-31.

711.Sharma, R.D. (1986). An evaluation of hypocholesterolemic factor of fenugreek seeds (T. *Foenum graecum*) in rats. *Nutrition Reports International*, 33(4): 669-677.

712.Shenoy, C., Patil, M.B., Kumar, R., Patil, S. (2009). Preliminary phytochemical investigation and wound healing activity of *Allium cepa* Li. (*Liliaceae*). *International Journal of Pharmacy and Pharmaceutical Sciences*, 2(2): 167-175.

713.Shimada, K., Fujikawa, K., Yahara, K., Nakamura, T. (1992). Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion. *Journal of Agricultural and Food Chemistry,* 40: 945-948.

714.Shimon, L.J., Rabinkov, A., Shin, I., Miron, T., Mirelman, D., Wilchek, M., Frolow, F. (2007). Two structures of alliinase from *Allium sativum* L.: Apo form and ternary complex with aminoacrylate reaction intermediate covalently bound to the PLP cofactor. *Journal of Molecular Biology,* 366: 611–625.

715.Shokrzadeh, M., Ebadi, A.G. (2006). Antibacterial effect of garlic (*Allium sativum* L.) on *Staphylococcus aureus*. *Pakistan Journal* of *Biological Sciences,* 9: 1577–1579.

716.Shovon B.S., Abida S.A., Muhammad H.S., Muhammed A.I., Ahtashom M.M. Asaduzzaman A.A. (2013). Analysis of the proximate composition and energy values of two varieties of Onion: A comparative Study. *International Journal of Nutrition and Food Sciences*, 2 (5):246-253.

717.Sikandar, K.S., Haider, S.S., Kazmi, S.U. (2013). *Helicobacter pylori*: Gastric ulcer and cancer causing burg. *International Journal of Advanced Research*, 1: 399-405.

718.Silva, C.P.D., Sousa, M.S.B., Siguemoto, É.S., Soares, R.A.M., Arêas, J.A.G. (2014). Chemical composition and antioxidant activity of jatobá-do-cerrado (*Hymenaea stigono carpa* Mart.) flour. *Food Science and Technology*, 34(3):597-603.

719.Silva, M., Jacobus, N. V., Deneke, C., Gorbach, S.L. (1987). Antimicrobial substance from a human *Lactobacillus* strain. *Antimicrobial Agents and Chemotherapy*, 31(8): 1231-1233.

720.Simon, P.W., Jenderek, M.M. (2010). Flowering, seed production, and the genesis of garlic breeding. *Plant breeding reviews*, 23: 211-244.

721.Simpson, W.J., Taguchi, H. (1995). The genus *Pediococcus*, with notes on the genera *Tetratogenococcus* and *Aerococcus*. In: The genera of lactic acid bacteria. (Eds. Wood, B.J.B. , Holzapfel, W.H.) . Springer, Boston, MA. PP 125-172.

722.Singh, B.N., Singh, B.R., Singh, R.L., Prakash, D., Singh, D.P., Sarma, B.K., Singh, H.B. (2009). Polyphenolics from various extracts/fractions of red onion (*Allium cepa*) peel with potent antioxidant and antimutagenic activities. *Food and Chemical Toxicology,* 47(6):1161-1167.

723.Singh, N., Yadav, S.S., Kumar, S., Narashiman, B. (2021). A review on traditional uses, phytochemistry, pharmacology, and clinical research of dietary spice *Cuminum cyminum* L. *Phytotherapy Research*, *35*(9): 5007-5030.

724.Singh, S. (2018). Agrometeorological requirements for sustainable vegetable crops production. *Journal* of *Food Protection,* 2: 1-22.

725.Singleton, V.L., Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *The American Journal of Enology and Viticulture,*16(3):144-158.

726.Siow, H.L., Gan, C.Y. (2016). Extraction, identification, and structure–activity relationship of antioxidative and α-amylase inhibitory peptides from cumin seeds (*Cuminum cyminum*). *Journal of Functional Foods*, *22*: 1-12.

727.Sirimontaporn, N., Thong-Ngam, D., Tumwasorn, S., Mahachai, V. (2010). Ten-day sequential therapy of *Helicobacter pylori* infection in Thailand. *American Journal of Gastroenterology,*105: 1071–1075.

728.Sivam, G.P. (2001). Protection against *Helicobacter pylori* and other bacterial infections by garlic. *The Journal of Nutrition*, 131(3): 1106S-1108S.

729.Sivam, G.P., Lampe, J.W., Ulness, B., Swanzy, S.R., Potter, J.D. (1997). *Helicobacter pylori in vitro* susceptibility to garlic (*Allium sativum*) extract. *Nutrition and Cancer*, 27: 118–21.

730.Sivan, P., Lampe, J., Lunes, B., Swanzy, S., Potter, J. (1997). *Heliobacter pylori in vitro* susceptibility to garlic (*Allium sativum*) extract. *Nutrition and Cancer Research,* 27 (2), 118-121.

731.Škerget, M., Majhenič, L., Bezjak, M., Knez, Z. (2009). Antioxidant, radical scavenging and antimicrobial activities of red onion (*Allium cepa* L) skin and edible part extracts. *Chemical* and *Biochemical Engineering*, 23(4): 435–444.

732.Smit, G., Smit, B.A., Engels, W.J. (2005). Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. *FEMS Microbiology Reviews*, 29(3): 591-610.

733.Sobala, G.M., Crabtree, J.E., Dixon, M.F., Schorah, C.J., Taylor, J.D., Rathbone, B.J., Heatley, R.V., Axon, A.T. (1991). Acute *Helicobacter pylori* infection: clinical features, local and systemic immune response, gastric mucosal histology, and gastric juice ascorbic acid concentrations. *Gut*, 32(11): 1415-1418.

734.Sobhani, I., Flourié, B., Lavergne, A., Colimon, R., Mignon, M., Modigliani, R., Rambaud, J.C. (1991). *Helicobacter pylori* and gastroduodenal pathology. Second part: Clinical aspects and therapy. *Gastroenterologie Clinique et Biologique*, 15(5): 412-420.

735.Sofowora, A. (1993). Recent trends in research into African medicinal plants. *Journal of Ethnopharmacology,* 38(2-3): 197-208.

736.Solnick, J.V., Hansen, L.M., Salama, N.R., Boonjakuakul, J.K., Syvanen, M. (2004). Modification of *Helicobacter pylori* outer membrane protein expression during experimental infection of rhesus macaques. *Proceedings of the National Academy of Sciences*, 101(7): 2106-2111.

737.Soto-vargas, V.C., Gonzalez, R.E., Sance, M.M., Galmarini, C.R. (2016).Organosulfur and phenolic content of garlic (*Allium sativum* L.) and onion (*Allium cepa* L.) and its relationship with antioxidant activity**.** *Acta Horticulturae*, 1143: 277-290.

738.Souza, G.A., Ebaid, G.X., Seiva, F.R., Rocha, K.H., Galhardi, C.M., Mani, F., Novelli, E.L. N. (2011). Acetyl cysteine an *Allium* plant compound improves high-sucrose diet-induced obesity and related effects. *Evidence Based Complementary and Alternative Medicine,* 2011: 1-7.

739.Srinivasan, K. (2018). Cumin (*Cuminum cyminum*) and black cumin (*Nigella sativa*) seeds: Traditional uses, chemical constituents, and nutraceutical effects. *Food Quality and Safety*, 2(1): 1-16.

740.Srinivasan, K., Kaul, C.L., Ramarao, P. (2005). Partial protective effect of rutin on multiple low dose streptozotocin-induced diabetes in mice. *The Indian Journal of Pharmacology,*37:327-328.

741.Stalikas, D. (2007). Extraction, separation, and detection methods for phenolic acids and flavonoids. *Journal of Separation Science,* 30 (18):3268-3295.

742.Steer, H.W., Colin-Jones, D.G. (1975). Mucosal changes in gastric ulceration and their response to carbenoxolone sodium. *Gut*, 16(8): 590-597.

743.Steinmetz, K.A, Potter, J.D. (1991) Vegetables, fruit, and cancer. Epidemiology. *Cancer Causes Control,* 2: 325–357.

744.Stiles, M.E., Holzapfel, W.H. (1997). Lactic acid bacteria of foods and their current taxonomy. *International Journal of Food Microbiology*, 36(1): 1-29.

745.Strasburger, E., Sitte, P., Weiler, E.W., Kadereit, J.W., Bresinsky, A., Körner, C. (1978). Lehrbuch der Botanik für Hochschulen. Ed. Gustav Fischer Verlag Stuttgart, New York. PP 1124.

746.Strzałkowska, N., Jasińska, K., Jóźwik, A. (2018). Physico-chemical properties of lactose, reasons for and effects of its intolerance in humans–a review. *Animal Science Papers and Reports*, 36(1): 21-31.

747.Sulieman, A. Elhadi. (1995), Fenugreek supplemented backed products, quality aspects. Master Thesis, University of Gezira, Wad Medani, Sudan. PP 48.

748.Sultana, B., Anwar, F. (2008). Flavonols (kaempeferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. *Food Chemistry*, 108 (3): 879-884.

749.Sun, Q., Liang, X., Zheng, Q., Liu, W., Xiao, S., Gu, W., Lu, H. (2010). High efficacy of 14-day triple therapy-based, bismuth-containing quadruple therapy for initial *Helicobacter pylori* eradication. *Helicobacter*, 15 (3): 233-238.

750.Sung, J.J.Y., Kuipers, E.J., El-Serag, H. B. (2009). Systematic review: the global incidence and prevalence of peptic ulcer disease. *Alimentary Pharmacology and Therapeutics*, 29(9): 938-946.

751.Suvarna, V., Sarkar, M., Chaubey, P., Khan, T., Sherje, A., Patel, K., Dravyakar, B. (2018). Bone health and natural products-an insight. *Frontiers in Pharmacology*, 9: 981.

752.Suzuki, M., Mimuro, H., Kiga, K., Fukumatsu, M., Ishijima, N., Morikawa, H., Nagai, S., Koyasu, S., Gilman, R.H., Kersulyte, D., Berg, D.E. (2009). *Helicobacter pylori* CagA phosphorylation-independent function in epithelial proliferation and inflammation. *Cell Host and Microbe*, 5(1): 23-34.

753.Suzuki, N., Wakasugi, M., Nakaya, S., Okada, K., Mochida, R., Sato, M., Kajiyama, H., Takahashi, R., Hirata, H., Ezure, Y., Koga, Y. (2002). Production and application of new monoclonal antibodies specific for a fecal *Helicobacter pylori* antigen. *Clinical and Vaccine Immunology*, 9(1): 75-78.

754.Swati, D., Pradeep, S., Jyoti, R., Renu, T., Arti, B. (2014). Phytochemical analysis of seeds of certain medicinal plants. *International Research Journal of Pharmacy,* 5:102-105.

755.Tabaestani, H., Sedaghat, N., Saeedi Pooya, E., Alipour, A. (2013). Shelf life improvement and postharvest quality of cherry tomato (*Solanum lycopersicum* L.) fruit using basil mucilage edible coating and cumin essential oil, *International Journal of Agronomy and Plant Production*, 4(9): 2346–2353.

756.Tabak, S., Bensoltane, A. (2012). L'activité antagoniste des bactéries lactiques (*Streptococcus thermophilus, Bifidobacterium bifidum* et *Lactobacillus bulgaricus*) vis-à-vis de la souche *Helicobacter pylori* responsable des maladies gastroduodénales. *Nature and Technology*, (6): 71- 79.

757.Tabak, S., Hamdi, B., Miara, M. D., Vitali, L. A. (2022). *In Vitro* anti-*Helicobacter pylori* and antioxidant activities of plants used in Algerian traditional medicine for gastrointestinal disorders. *Journal of Bioresource Management*, 9(4): 1-7.

758.Tabak, S., Maghnia, D., Bensoltane, A. (2012). The antagonistic activity of the lactic acid bacteria (*Streptococcus thermophilus, Bifidobacterium bifidum and Lactobacillus bulgaricus*) against *Helicobacter pylori* responsible for the gastroduodenals diseases. *Journal of Agricultural Science and Technology*, *2* (5A): 709.

759.Tabarsa, M., You, S., Yelithao, K., Palanisamy, S., Prabhu, N.M., Nan, M. (2020). Isolation, structural elucidation and immuno-stimulatory properties of polysaccharides from *Cuminum cyminum*. *Carbohydrate Polymers*, 230: 1-39.

760.Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y. and Ouellette, M. (2018). Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infectious Diseases*, 18(3): 318-327.

761.Talarico, S., Whitefield, S.E., Fero, J., Haas, R., Salama, N.R. (2012). Regulation of *Helicobacter pylori* adherence by gene conversion. *Molecular Microbiology*, 84(6): 1050-1061.

762.Talley, N.J., Cameron, A.J., Shorter, R.G., Zinsmeister, A.R., Phillips, S.F. (1988). *Campylobacter pylori* and Barrett's esophagus. *Mayo Clinic Proceedings*, 63:1176 –1180.

763.Tan, P.V., Boda, M., Etoa, F.X. (2010). *In vitro* and *in vivo* anti-*Helicobacter*/*Campylobacter* activity of the aqueous extract of *Enantia chlorantha*. *Pharmaceutical Biology*, 48 (3): 349-356.

764.Tanaka, A., Watanabe, K., Tokunaga, K., Hoshiya, S., Imase, K., Sugano, H., Shingaki, M., Kai, A., Itoh, T., Ishida, H., Takahashi, S.I. (2003). Evaluation of *Helicobacter pylori* stool antigen test before and after eradication therapy. *Journal of Gastroenterology and Hepatology*, 18 (6): 732-738.

765.Tang, Y., Zhu, J., Chen, L., Zhang, S., Lin, J. (2008). Associations of matrix metalloproteinase-9 protein polymorphisms with lymph node metastasis but not invasion of gastric cancer. *Clinical Cancer Research*, 14: 2870-2877.

766.Tannock, G.W. (2005). New perceptions of the gut microbiota: implications for future research. *Gastroenterology Clinics*, *34*(3): 361-382.

767.Tassou, C., Koutsoumanis, K., Nychas, G.J.E., (2000). Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil. *Food Research International,* 33:273–280.

768.Taylor, D.E., Eaton, M., Chang, N., Salama, S.M. (1992). Construction of a *Helicobacter pylori* genome map and demonstration of diversity at the genome level. *Journal of Bacteriology*, 174(21): 6800-6806.

769.Tedesco, I., Carbone, V., Spagnuolo, C., Minasi, P., Russo, G.L. (2015). Identification and quantification of flavonoids from two southern Italian cultivars of *Allium cepa* L., Tropea (Red Onion) and Montoro (Copper Onion), and their capacity to protect human erythrocytes from oxidative stress. *Journal of Agricultural and Food Chemistry*, 63(21): 5229-5238.

770.Tegtmeyer, N., Wessler, S. , Backert, S. (2011). Role of the cag-pathogenicity island encoded type IV secretion system in *Helicobacterpylori* pathogenesis. *The FEBS Journal*, 278(8):1190–1202.

771.Terzaghi, B.E., Sandine, W.E. (1975). Improved media for lactic streptococci and their bacteriophages. *Applied and Environmental Microbiology,* 29 : 807 – 813.

772.Thamaraikannan, M., Sengottuvel, C. (2012). Cumin: Can India maintain a monopoly. *Facts For You*, 32:11-14.

773.Thippeswamy, N.B., Naidu, A. (2005). Antioxidant potency of cumin varieties cumin, black cumin and bitter cumin – on antioxidant systems. *European Food Research* and *Technology,* 220:472-476.

774.Thomas, D.J., Parkin, K.L. (1994). Quantification of alk (en) yl-L-cysteine sulfoxides and related amino acids in Alliums by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, 42(8): 1632-1638.

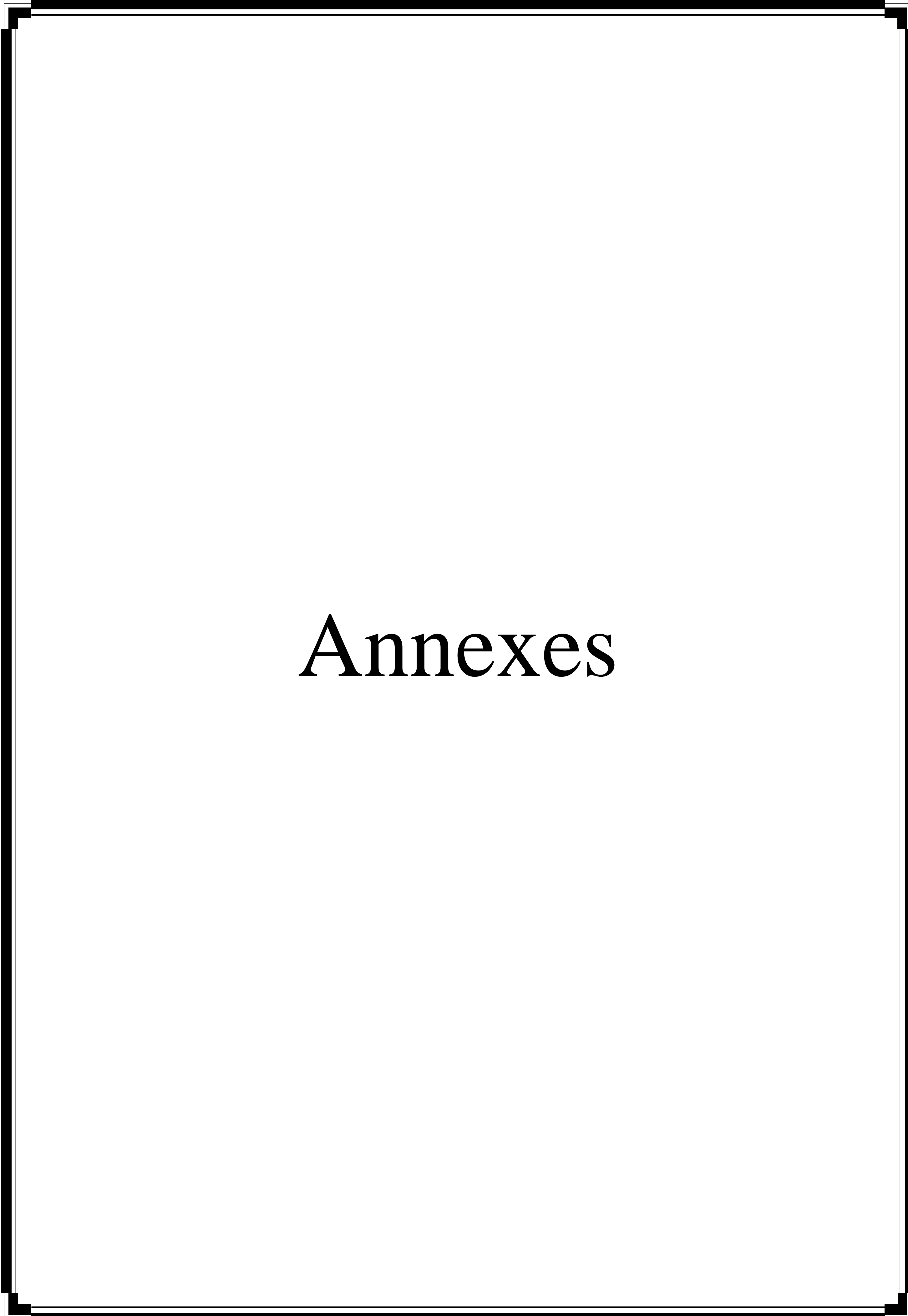
775.Tissier, H. (1908). Recherches sur la flore intestinale normale des enfants agés d'un an à cinq ans. *Annales de l'Institut Pasteur*, 22: 189-208.

776.Todd, R.K. (1993). Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiology Reviews,* 12(1-3): 39-85.

777. Tokunaga, Y., Shirahase, H., Hoppou, T., Kitaoka, A., Tokuka, A., Ohsumi, K. (2000). Density of *Helicobacter pylori* infection evaluated semi quantitatively in gastric cancer. *Journal of Clinical Gastroenterology*, 31(3): 217-221.
778. Torres, L.E., Melián, K., Moreno, A., Alonso, J., Sabatier, C.A., Hernández, M., Bermúdez, L., Rodríguez, B.L. (2009). Prevalence of vacA, cagA and babA2 genes in Cuban *Helicobacter pylori* isolates. *World Journal of Gastroenterology*, 15(2): 204-210.
779. Trease, G.E., Evans, W.C. (1989). Pharmacognsy. 11th Ed. Elsevier, UK. PP 616.
780. Udayan, P.S., Venkatesh, S. (2005). Some common medicinal plant used by the Nayaka community, savandurga forest of Magadi taluk, Bangalore District, Karnataka. *Indian Journal of Natural Remedies*, 5(1): 35-40.
781. Udayasekhara Rao, P., Sesikeran, M.D.P., Srinivasa, R., .Nadamuni Naidu, V., Vikas R. (1996), Short term nutritional and safety evaluation of fenugreek, *Nutrition Research*, 16(9):1495–1505.
782. Ujowundu, C.O.F.N. Kalu, E.C. Nwosunjoku, R.N. Nwaoguikpe, R.I. Okechukwu, K.O. Igwe. (2011). Iodine and inorganic mineral contents of some vegetables, spices and grains consumed in South eastern Nigeria. *African Journal of Biochemistry Research,* 5(2):57-64.
783. Uma Pradeep, K., Geervani, P., Eggum, B.O. (1993). Common Indian spices: Nutrient composition, consumption and contribution to dietary value. *Plant Foods* for *Human Nutrition,* 44:137-148.
784. Uma, D.B., Ho, C.W., Wan, A.W.M. (2010). Optimization of extraction parameters of total phenolic compounds for Henna (*Lawsonia inermis*) leaves. *Sains Malaysiana*, 39:119-128.
785. Vaira, D., Holton, J. (1998). Vector potential of houseflies (*Musca domestica*) for *Helicobacter pylori*. *Helicobacter*, 3:65-66
786. Vaira, D., Zullo, A., Hassan, C., Fiorini, G., Vakil, N. (2009). Sequential therapy for *Helicobacter pylori* eradication: the time is now. *Therapeutic Advances in Gastroenterology*, 2(6): 317-322.
787. Vakil, N., Affi, A., Robinson, J., Sundaram, M., Phadnis, S. (2000). Prospective blinded trial of a fecal antigen test for the detection of *Helicobacter pylori* infection. *The American Journal of Gastroenterology*, 95(7): 1699-1701.
788. Vakil, N., Lanza, F., Schwartz, H., Barth, J. (2004). Seven-day therapy for *Helicobacterpylori* in the United States. *Alimentary Pharmacology and Therapeutics,*20:99–107.
789. Vale, F.F., Vitor, J.M. (2010). Transmission pathway of *Helicobacterpylori*: Does food play a role inrural and urban areas? .*International Journal of Food Microbiology*,138:1-22.
790. Valkonen, K.H., Wadstrom, T., Moran, A.P. (1994). Interaction of lipopolysaccharides of*Helicobacterpylori*with basement membrane protein laminin. *Infection and Immunity,*62: 3640-3648.
791. Van Der Hulst, R.W., Keller, J.J., Rauws, E.A., Tytgat, G.N. (1996). Treatment of *Helicobacter pylori* infection: a review of the world literature. *Helicobacter*, 1(1): 6-19.
792. Van Doorn, L.J., Figueiredo, C., Megraud, F., Pena, S., Midolo, P., Queiroz, D.M.D.M., Carneiro, F., Vanderborght, B., Maria Da Glória, F.P., Sanna, R., De Boer, W. (1999). Geographic distribution of vacA allelic types of *Helicobacter pylori*. *Gastroenterology*, 116(4): 823-830.
793. Van Doorn, O.J., Bosman, D.K., Van't Hoff, B.W., Taminiau, J.A., Fibo, J., Van der Ende, A. (2001). *Helicobacter pylori* stool antigen test: a reliable non-invasive test for the diagnosis of *Helicobacter pylori* infection in children. *European Journal of Gastroenterology and Hepatology*, 13(9): 1061-1065.
794. Van Zwet, A.A., Vandenbrouke-Grauls, C.M.J.E., Thijs, J.C., Van Der Wouden, E.J., Gerrits, M.M., Kusters, J.G. (1998). Stable amoxicillin resistance in *Helicobacter pylori*. *Lancet*, 352(9140): 1595-1595.
795. Vandenbergh, P.A. (1993). Lactic acid bacteria, their metabolic products and interference with microbial growth. *FEMS Microbiology Reviews*, 12(1-3): 221-237.
796. Varcoe, J.J., Krejcarek, G., Busta, F., Brady, L. (2003). Prophylactic feeding of *Lactobacillus acidophilus* NCFM to mice attenuates overt colonic hyperplasia. *Journal of Food Protection*, 66(3): 457-465.
797. Varon, C., Megraud, F. (2013). Infection à *Helicobacter pylori* et cancer gastrique. *Revue Francophone des Laboratoires*, 2013 (456): 67-76.
798. Vasiljevic, T., Shah, N.P. (2008). Probiotics from Metchnikoff to bioactives. *International Dairy Journal*, 18: 714-728.
799. Vavilov, N.I. (1951) The Origin, Variation, Immunity and Breeding of Cultivated Plants. *Chronica Botonica*, 13: 1-366.
800. Vavilov, N.I. (1926). Studies in the origin of cultivated plants. *Leningrad.* 147-148.
801. Vazquez-Armenta, F.J., Ayala-Zavala, J.F., Olivas, G.I., Molina-Corral, F.J., Silva-Espinoza, B.A. (2014). Antibrowning and antimicrobial effects of onion essential oil to preserve the quality of cut potatoes. *Acta Alimentaria*, 43(4): 640-649.
802. Velioglu, Y.S.G., Mazza, L., Gao, Oomah, B.D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural* and *Food Chemistry,*46: 4113- 4117.
803. Vergin, F. (1954) Anti- und Probiotika (Anti- and probiotics). *Hippokrates,* 25(4): 116– 119.

804. Verma, S. (2016). A review study on valuable ayurvedic plant *cuminum cyminum* linn: Apiaceae. *World Journal of Pharmaceutical and Life Sciences*, 2(6): 108–113.
805. Vigentini, I., Praz, A., Domeneghetti, D., Zenato, S., Picozzi, C., Barmaz, A., Foschino, R. (2016). Characterization of malolactic bacteria isolated from Aosta Valley wines and evidence of psychrotrophy in some strains. *Journal of Applied Microbiology*, 120(4): 934-945.
806. Villani, F., Aponte, M., Blaiotta, G., Mauriello, G., Pepe, O., Moschetti, G. (2001). Detection and characterization of a bacteriocin, garviecin L1-5, produced by *Lactococcus garvieae* isolated from raw cow's milk. *Journal of Applied Microbiology*, 90(3): 430-439.
807. Vítor, J.M., Vale, F.F. (2011). Alternative therapies for *Helicobacterpylori*: probiotics and phytomedicine. *FEMS Immunology and Medical Microbiology,* 63: 153-16.
808. Vlase, L., Benedec, D., Hanganu, D., Damia, G., Csillag, I., Sevastre, B., Tilea, I. (2014). Evaluation of antioxidant and antimicrobial activities and phenolic profile for *Hyssopus officinalis*, *Ocimum basilicum* and *Teucrium chamaedrys*. *Molecules*, 19(5): 5490-5507.
809. Wagner, R.D., Pierson, C., Warner, T., Dohnalek, M., Hilty, M., Balish, E. (2000). Probiotic effects of feeding heat-killed *Lactobacillus* acidophilus and *Lactobacillus casei* to *Candida albicans*-colonized immunodeficient mice. *Journal of Food Protection*, 63(5): 638-644.
810. Wallace, T.D., Bradley, S., Buckley, N.D., Green-Johnson, J.M. (2003). Interactions of lactic acid bacteria with human intestinal epithelial cells: effects on cytokine production. *Journal of Food Protection*, 66(3): 466-472.
811. Wallock-Richards, D., Doherty, C.J., Doherty, L., Clarke, D. J., Place, M., Govan, J.R., Campopiano, D.J. (2014). Garlic revisited: antimicrobial activity of allicin-containing garlic extracts against *Burkholderia cepacia* complex. *PLoS One*, 9(12): 1-13.
812. Wang, S.W., Yu, F.J., Lo, Y.C., Yang, Y.C., Wu, M.T., Wu, I.C., Lee, Y.C., Jan, C.M., Wang, W.M., Wu, D.C. (2003). The clinical utility of string-PCR test in diagnosing *Helicobacter pylori* infection. *Hepato-Gastroenterology*, 50(53):1208-1213.
813. Wang, X.Q., Terry, P.D. ,Yan, H. (2009). Review of salt consumption and stomach cancer risk: epidemiological and biological evidence. *World Journal of Gastroenterology,* 15: 2204-2213.
814. Wang, Y.C. (2014). Medicinal plant activity on *Helicobacter pylori* related diseases. *World Journal of Gastroenterology*, 20(30): 10368-10382.
815. Wang, Y.C., Wu, D.C., Liao, J.J., Wu, C.H., Li, W.Y., Weng, B.C. (2009). *In vitro* activity of *Impatiens balsamina* L. against multiple antibiotic-resistant *Helicobacter pylori*. *The American Journal of Chinese Medicine*, 37: 713-722.
816. Wang, Y.C., Huang, T.L. (2005). Anti-*Helicobacter pylori* activity of *Plumbago zeylanica* L. *FEMS Immunology And Medical Microbiology*, 43(3): 407-412.
817. Wani, S.A., Kumar, P. (2018). Fenugreek: A review on its nutraceutical properties and utilization in various food products. *Journal of the Saudi Society of Agricultural Sciences*, 17(2): 97-106.
818. Weyermann, M., Rothenbacher, D., Brenner, H. (2009). Acquisition of *Helicobacter pylori* infection in early childhood: Independent contributions of infected mother, father and siblings. *The American Journal of Gastroenterology*,104:182-189.
819. Williams, A.M., Fryer, J.L., Collins, M.D. (1990). *Lactococcus piscium* sp. nov. a new *Lactococcus* species from salmonid fish. *FEMS Microbiology Letters*, 68(1-2): 109-113.
820. Wolfgang, H. (2008). Welche heilpflanzeistdas. Ed. Delachaux et Niestle SA,Paris. PP 169.
821. Woo, J.S., EI-Zimaity, H.M., Genta, R.M., Yousfi, M.M., Graham, D.Y. (1996). The best gastric site for obtaining a positive rapid urease test. *Helicobacter*, 1(4): 256-259.
822. Working Party of the European *Helicobacter pylori* Study Group. (1997). Technical annex: tests used to assess *Helicobacter pylori* infection. *Gut*, *41* (2): S10-S18.
823. World Health Organization. (1999). Monographs on selected medicinal plants. 2nd Ed. Ravello-Salerno, Italy. PP 362.
824. World Health Organization. (2000). Global water supply and sanitation assessment 2000 report. Geneva, PP 204–205.
825. World Health Organization. (2002). The world health report 2002: reducing risks, promoting healthy life. PP 248.
826. Wu, D.C., Hsu, P.I., Wu, J.Y., Opekun, A.R., Kuo, C.H., Wu, I.C., Wang, S.S., Chen, A., Hung, W.C., Graham, D.Y. (2010). Sequential and concomitant therapy with four drugs is equally effective for eradication of *H. pylori* infection. *Clinical Gastroenterology and Hepatology*, 8(1): 36-41.
827. Xi, W., Zhang, Y., Sun, Y., Shen, Y., Ye, X., Zhou, Z. (2014). Phenolic composition of Chinese wild mandarin (*Citrus reticulata* Balnco.) pulps and their antioxidant properties. *Industrial Crops and Products,* 52:466-474.
828. Yadav, S.L., Ghasolia, R.P., Yadav, R., Yadav, P. (2019). Studies on survey and epidemiology of *Rhizoctonia solani* causing root rot of fenugreek. *International Journal of Seed Spices*, 9: 91-95.

829. Yakult Central Institute for Microbiological Research. (1998). *Lactobacillus casei* strain Shirota. Tokyo, Japan.
830. Yamaguchi, H., Osaki, T., Kurihara, N., Taguchi, H., Hanawa, T., Yamamoto, T., Kamiya, S. (1997). Heat-shock protein 60 homologue of *Helicobacter pylori* is associated with adhesion of *H. pylori* to human gastric epithelial cells. *Journal of Medical Microbiology*, 46(10): 825-831.
831. Yamamoto, S., Uemura, N., Okamoto, S., Yamaguchi, S., Mashiba, H., Tachikawa, T. (2000). A new rapid test for detecting anti–*Helicobacter pylori* antibody excreted into urine. *Helicobacter*, 5(3): 160-164.
832. Yamaoka, Y. (2010). Mechanisms of disease: *Helicobacter pylori* virulence factors. *Nature Reviews Gastroenterology and Hepatology,* 7: 629-641.
833. Yang, J.C., Yang, H.C., Shun, C.T., Wang, T.H., Chien, C.T., Kao, J.Y. (2013). Catechins and sialic acid attenuate *Helicobacter pylori*-triggered epithelial caspase-1 activity and eradicate *Helicobacter pylori* infection. *Evidence-Based Complementary and Alternative Medicine*, 2013:1-13.
834. Yang, J.C., Shun, C.T., Chien, C.T., Wang, T.H. (2008). Effective prevention and treatment of *Helicobacter pylori* infection using a combination of catechins and sialic acid in AGS cells andBALB/c mice. *The Journal of Nutrition,*138: 2084-2090.
835. Yaser Al Muneer, A., Abdelhafid, B., Daoudi, C., Lazoni, A. (2013). Chemical and phytochemical analysis of some antidiabetic plants in Yemen. *International Research Journal* of *Pharmacy,* 2:72-76.
836. Yin, M., Cheng, W. (1998). Antioxidant activity of several *Allium* Members. *Journal of Agricultural and Food Chemistry*, 46 (10): 4097-4101.
837. Yin, M., Cheng,W. (2003). Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. *Meat Science*, 63:23-28.
838. Yoo, K.S., Pike, L., Crosby, K., Jones, R., Leskovar, D. (2006). Differences in onion pungency due to cultivars, growth environment, and bulb sizes. *Scientia Horticulturae,* 110: 144–149.
839. Yoon, H., Kim, N., Lee, B.H., Hwang, T.J., Lee, D.H., Park, Y.S., Nam, R.H., Jung, H.C., Song, I.S. (2009). Moxifloxacin-containing triple therapy as second-line treatment for *Helicobacter pylori* infection: effect of treatment duration and antibiotic resistance on the eradication rate. *Helicobacter*, 14(5): 429-437.
840. Yoon, J.Y., Cha, J.M., Hong, S.S., Kim, H.K., Kwak, M.S., Jeon, J.W., Shin, H.P. (2019). Fermented milk containing *Lactobacillus paracasei* and *Glycyrrhiza glabra* has a beneficial effect in patients with *Helicobacter pylori* infection: A randomized, double-blind, placebo-controlled study. *Medicine*, 98(35):1-8.
841. Yordanov, D., Boyanova, L., Markovska, R., Ilieva, J., Andreev, N., Gergova, G., Mitov, I. (2017). Influence of dietary factors on *Helicobacter pylori* and CagA seroprevalence in Bulgaria. *Gastroenterology Research and Practice*, 2017: 1-7.
842. You, W.C., Zhang, L., Gail, M.H., Ma, J.L., Chang, Y.S., Blot, W.J., Li, J.Y., Zhao, C.L., Liu, W.D., Li, H.Q., Hu, Y.R. (1998). *Helicobacter pylori* infection, garlic intake and precancerous lesions in a Chinese population at low risk of gastric cancer. *International Journal of Epidemiology*, *27*(6): 941-944.
843. Zacharof, MP. Lovitt, RW. (2012). Bacteriocins produced by lactic acid bacteria a review article. *Apcbee Procedia*. 2 (2012): 50-56.
844. Zaidi, S.F., Yamada, K., Kadowaki, M., Usmanghani, K., Sugiyama, T. (2009). Bactericidal activity of medicinal plants, employed for the treatment of gastrointestinal ailments, against *Helicobacter pylori*. *Journal of Ethnopharmacology*, 121: 286-291.
845. Zambrano J., Ramirez H., Manzaro J.(1994). Efectos de cortos periodos a bajatemperatura sobre algunos parametros de calidad de cebollas *Allium cepa* L. *Agronomia Tropical,* 44,731-742.
846. Zeng, Y., Li, Y., Yang, J., Pu, X., Du, J., Yang, X., Yang, T., Yang, S. (2017). Therapeutic role of functional components in Alliums for preventive chronic disease in human being. *Evidence Based Complementary and Alternative Medicine,*2017: 1-13.
847. Zhang, L., Feng, S., Xu, J. (2014). Profile of phytochemicals and antioxidant activities of different solvent extracts of cumin seeds. *Applied Mechanics and Materials,* 16(12): 675-677.
848. Zheng, P.Y., Hua, J., Ng, H.C., Ho, B. (1999). Unchanged characteristics of *Helicobacter pylori* during its morphological conversion. *Microbios*, 98(389): 51-64.
849. Zia, T., Hasnain, S.N., Hasan, S.K. (2001). Evaluation of the oral hypoglycaemic effect of *Trigonella Foenum-graecum* L.(methi) in normal mice. *Journal of Ethnopharmacology*, 75(2-3): 191-195.
850. Zill-E-Huma Vian, M.A., Fabiano-Tixier, A.S., El-maataoui, M., Dangles, O., Chemat, F. (2011).A remarkable influence of microwave extraction: Enhancement of antioxidant activity of extracted onion varieties. *Food Chemistry*, 127: 1472–1480.
851. Zou, Y., Lu, Y., Wei, D. (2004). Antioxidant activity of a flavonoid rich extract of *Hypericum perforatum* L. *in vitro. The Journal of Agricultural and Food Chemistry,* 52: 5032-5039.
852. Żuk-Gołaszewska, K., Wierzbowska, J. (2017). Fenugreek: productivity, nutritional value and uses. *Journal of Elementology,* 22(3): 1067-80.



# Annexes

**Annexe 1:** Photos of plants used in the present study

A: Garlic plant

B: Onion plant

C: Fenugreek seeds

D: Cumin seeds

**Annexe 2:** Photos of commercialized used probiotics

| *Bifidobacterium* | *Lactobacillus rhamnosus* GG |
|---|---|
| *Lactobacillus helviticus* | *Lactobacillus rhamnosus* LA80 |

**Annexe 3:** Steps of preperation of plants powder

1. Steps of preperation of garlic powder

A: Peeling and cleaning

B: Sliced into chips

C: Drying

D: Grinding and screening

2. Steps of preperation of onion powder

A: Peeling and cleaning

B: Sliced into chips

C: Drying

D: Grinding and screening

3. Steps of preperation of fenugreek powder

A: Sorting

B: Grinding and screening

4. Steps of preperation of cumin powder

A: Sorting

B: Grinding and screening

5. Storage of plant powders

Stored plant powders in glass containers

**Annexe 4:** Reading table of API Campy

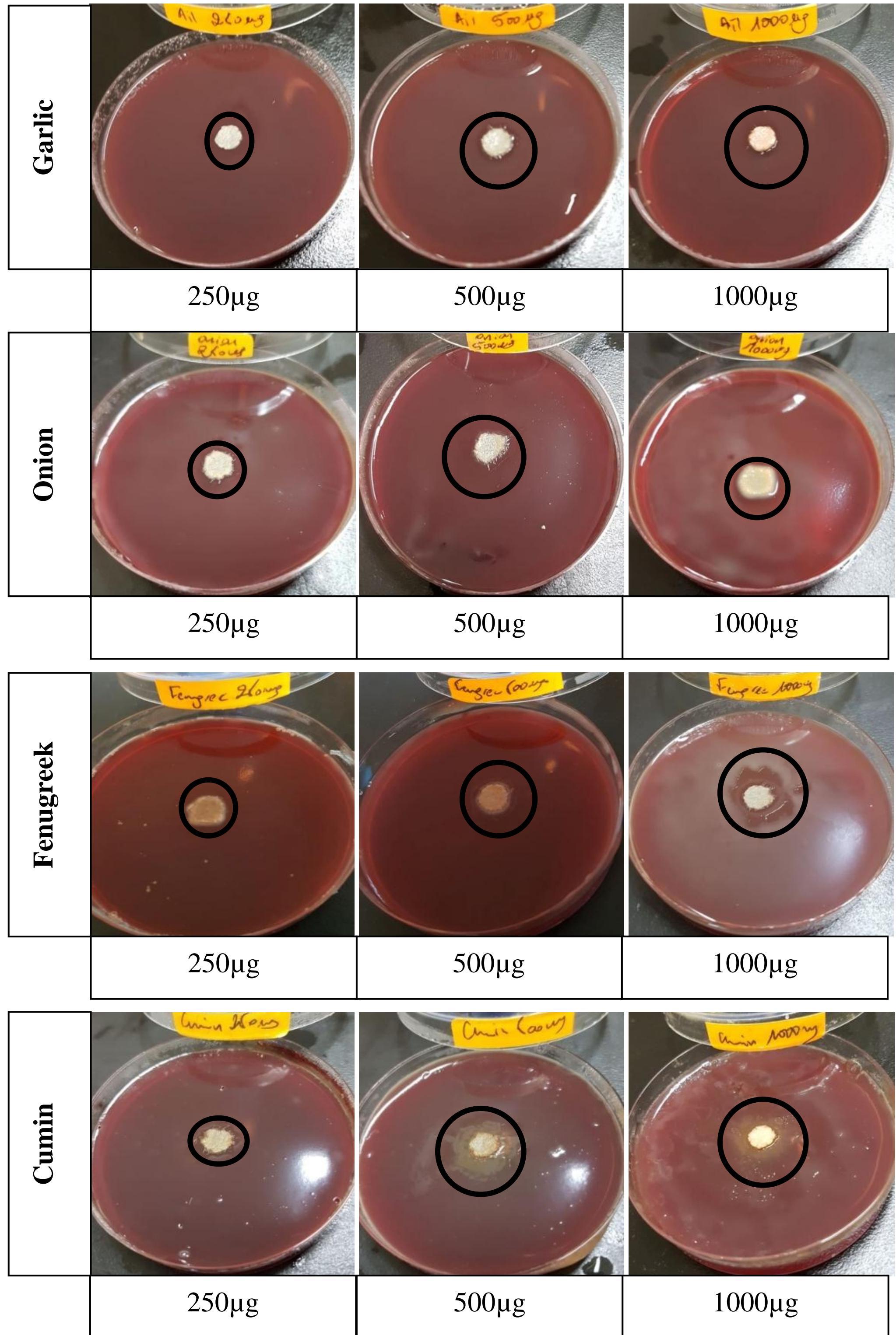
READING TABLE

| TESTS | ACTIVE INGREDIENTS | QTY (mg/cup.) | REACTIONS | RESULTS | |
|---|---|---|---|---|---|
| | | | | NEGATIVE | POSITIVE |
| URE | urea | 0.216 | UREase | yellow | orange / red |
| NIT | potassium nitrate | 0.1 | Reduction of NITrates | NIT 1 + NIT 2 / 5 min<br>colorless | pink / red |
| EST | 5-bromo-4-chloro-3-indoxyl-acetate | 0.029 | ESTerase | colorless<br>pale blue | turquoise |
| HIP | sodium hippurate | 0.2 | HIPpurate | NIN / 5 min<br>colorless<br>bluish-grey | violet |
| GGT | γL-glutamic acid-ß-naphthylamide | 0.0272 | Gamma Glutamyl Transferase | FB / 5 min<br>colorless | dark orange |
| TTC | triphenyltetrazolium chloride | 0.02 | Reduction of Triphenyl Tetrazolium Chloride | colorless<br>pale pink | pink / red<br>or deposit in base of cupule |
| PyrA | pyroglutamic acid-ß-naphthylamide | 0.038 | Pyrrolidonyl Arylamidase | FB / 5 min (PyrA → PAL)<br>colorless | orange |
| ArgA | L-arginine-4-methoxy-ß-naphthylamide | 0.056 | L-Arginine Arylamidase | colorless | orange |
| AspA | aspartic acid-ß-naphthylamide | 0.039 | L-Aspartate Arylamidase | colorless | orange |
| PAL | 2-naphthyl phosphate | 0.024 | ALkaline Phosphatase | colorless | purple |
| $H_2S$ | sodium thiosulfate | 0.076 | production of $H_2S$ | colorless | black |
| \|GLU\| | D-glucose | 1.56 | assimilation (GLUcose) | transparent<br>(no growth or sensitivity) | opaque (even if weak)<br>(growth or resistance) |
| \|SUT\| | sodium succinate | 1.36 | assimilation (sodium SUccinaTe) | | |
| \|NAL\| | nalidixic acid | 0.084 | growth inhibition (NALidixic acid) | | |
| \|CFZ\| | sodium cefazoline | 0.224 | growth inhibition (sodium CeFaZoline) | | |
| \|ACE\| | sodium acetate | 1.1 | assimilation (sodium ACEtate) | | |
| \|PROP\| | propionic acid | 1.16 | assimilation (PROPionate) | | |
| \|MLT\| | malic acid | 1.56 | assimilation (MaLaTe) | | |
| \|CIT\| | trisodium citrate | 2.28 | assimilation (trisodium CITrate) | | |
| \|ERO\| | erythromycin | 0.014 | susceptibility - therapeutic prediction (ErythrOmycin) | | |

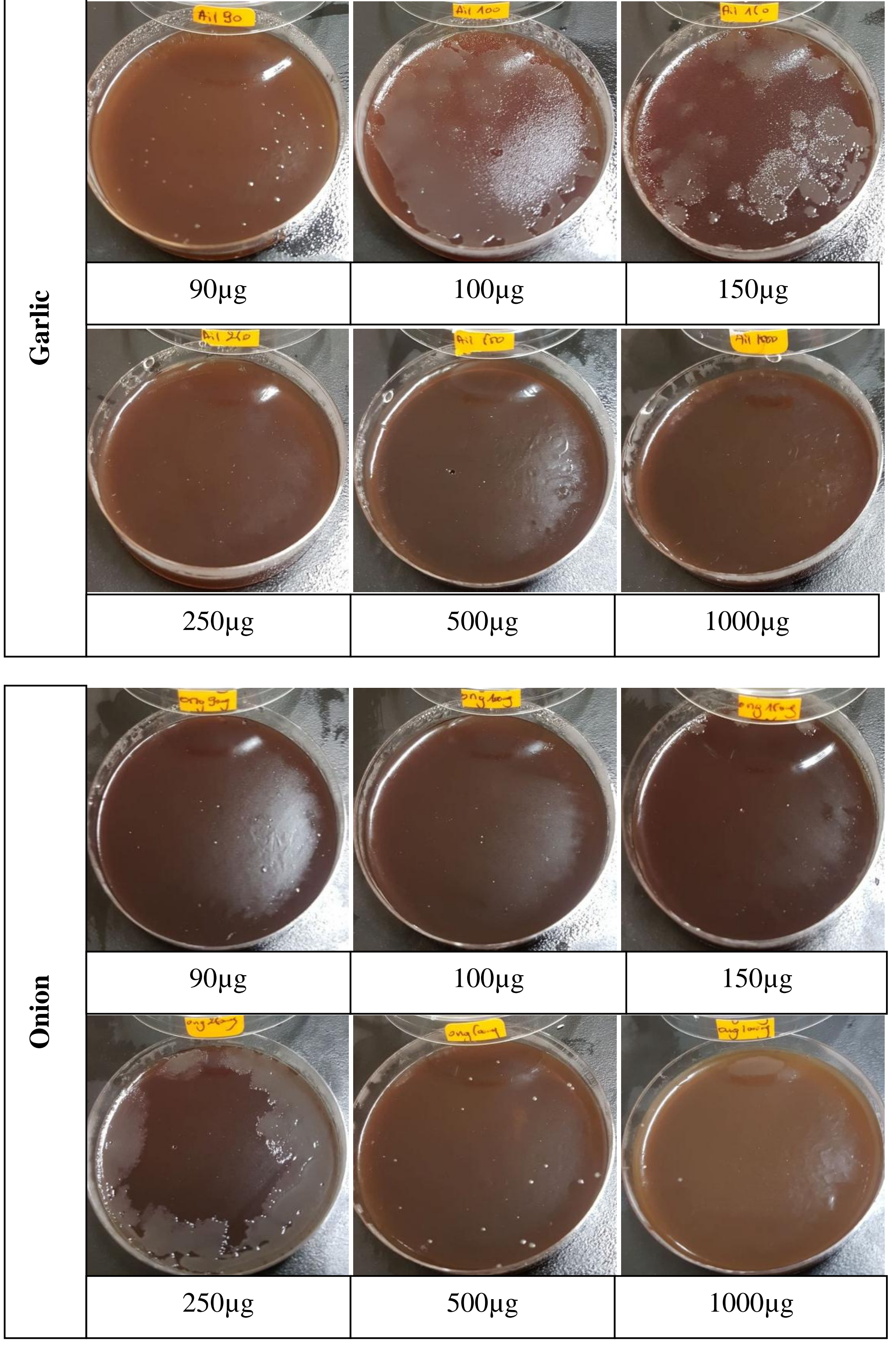
**Annexe 5:** Results of phytochemical screening of plant extracts

| Phytochemical compounds | Garlic | Onion | Fenugreek | Cumin |
|---|---|---|---|---|
| **Flavonoids** | | | | |
| **Tannins** | | | | |
| **Reducing compounds** | | | | |
| **Terpenoids** | | | | |
| **Alkaloids** | | | | |
| **Cardiac glycosides** | | | | |
| **Saponosides** | | | | |
| **Sterols and triterpenes** | | | | |
| **Anthocyanins** | | | | |

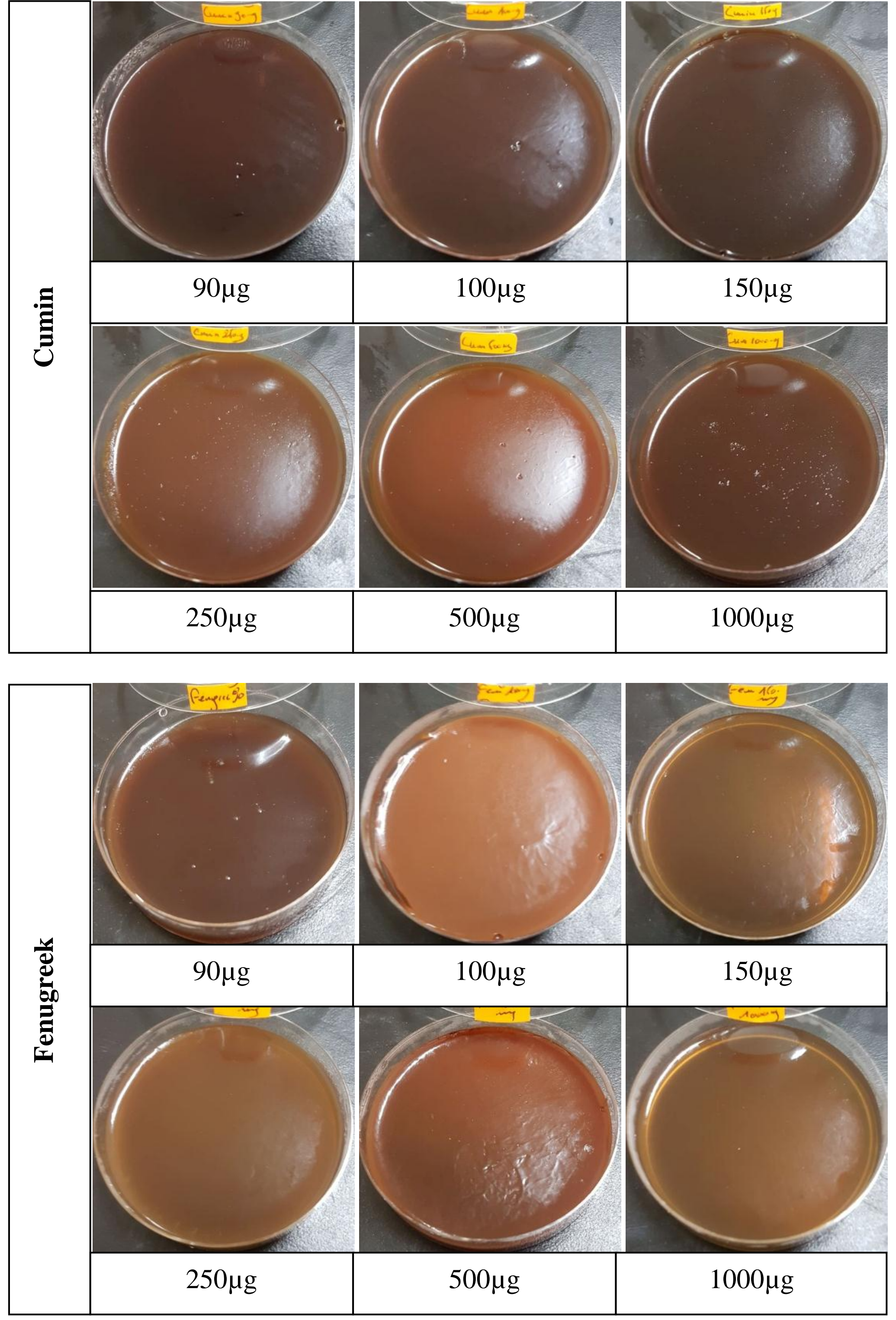
**Annexe 6:** Results of determination of DZI of plant extracts against *H. pylori*



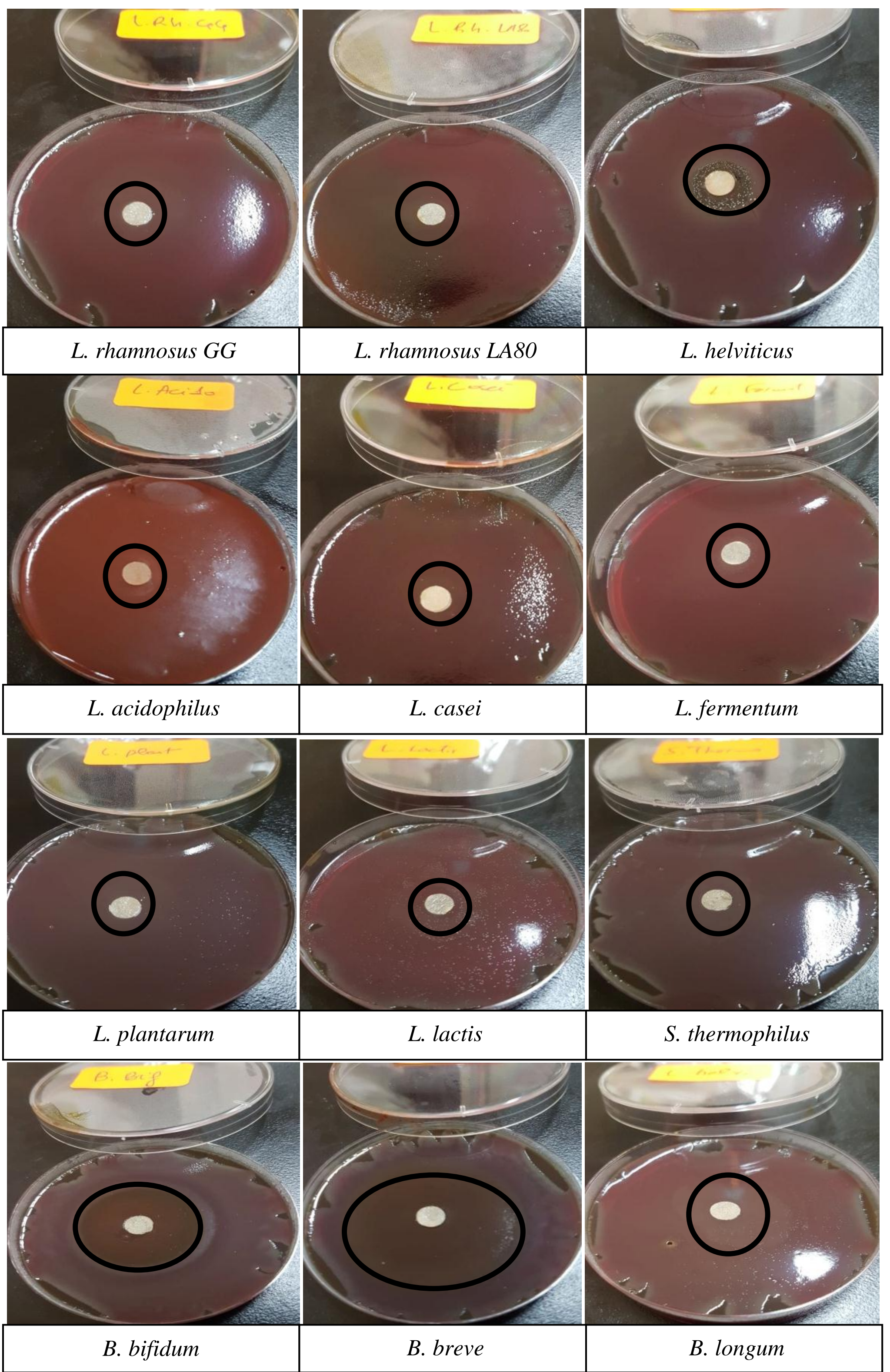
**Annexe 7:** Results of determination of MIC and MBC of plant extracts against *H. pylori*



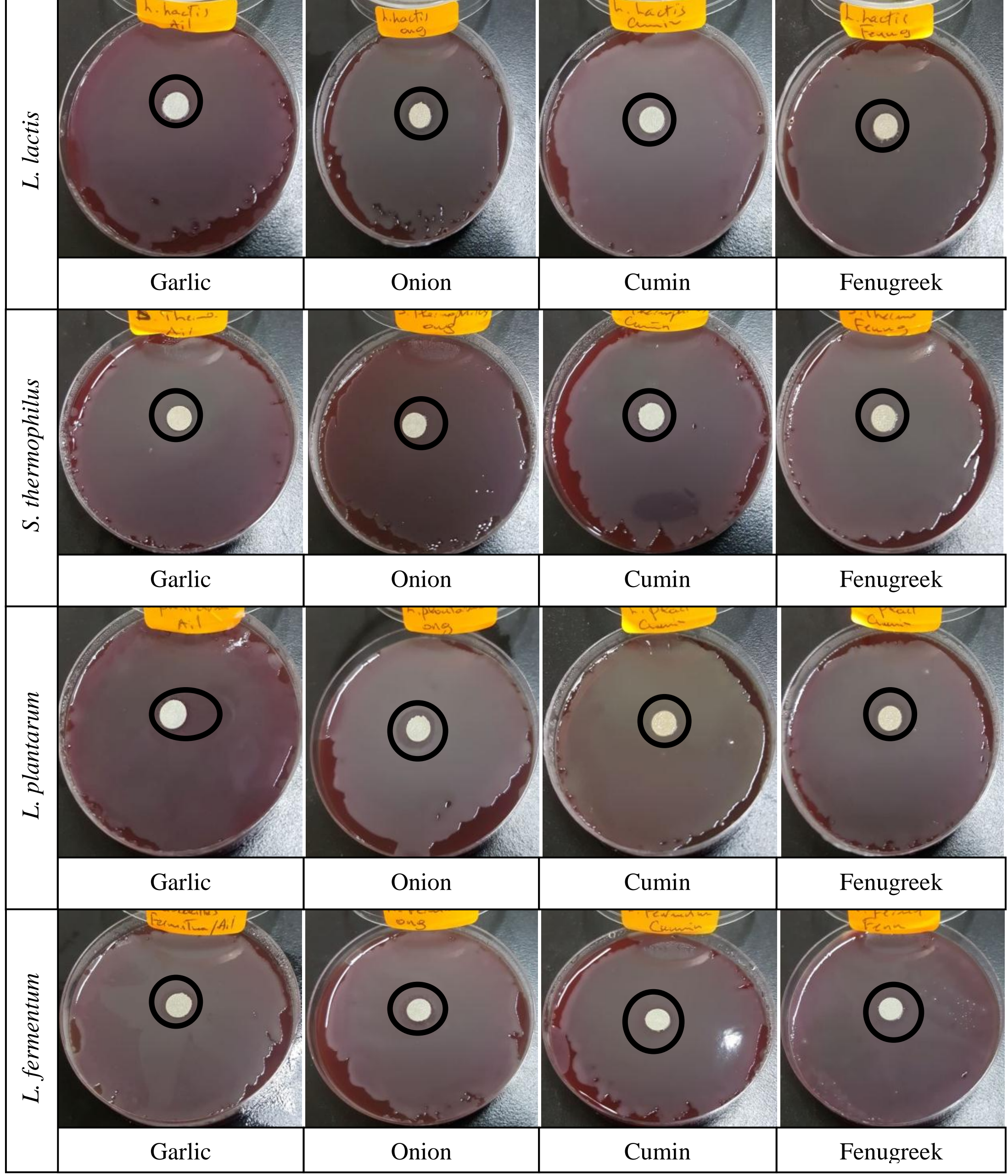
**Annexe 8**: Results of determination of MIC and MBC of plant extracts against *H. pylori*

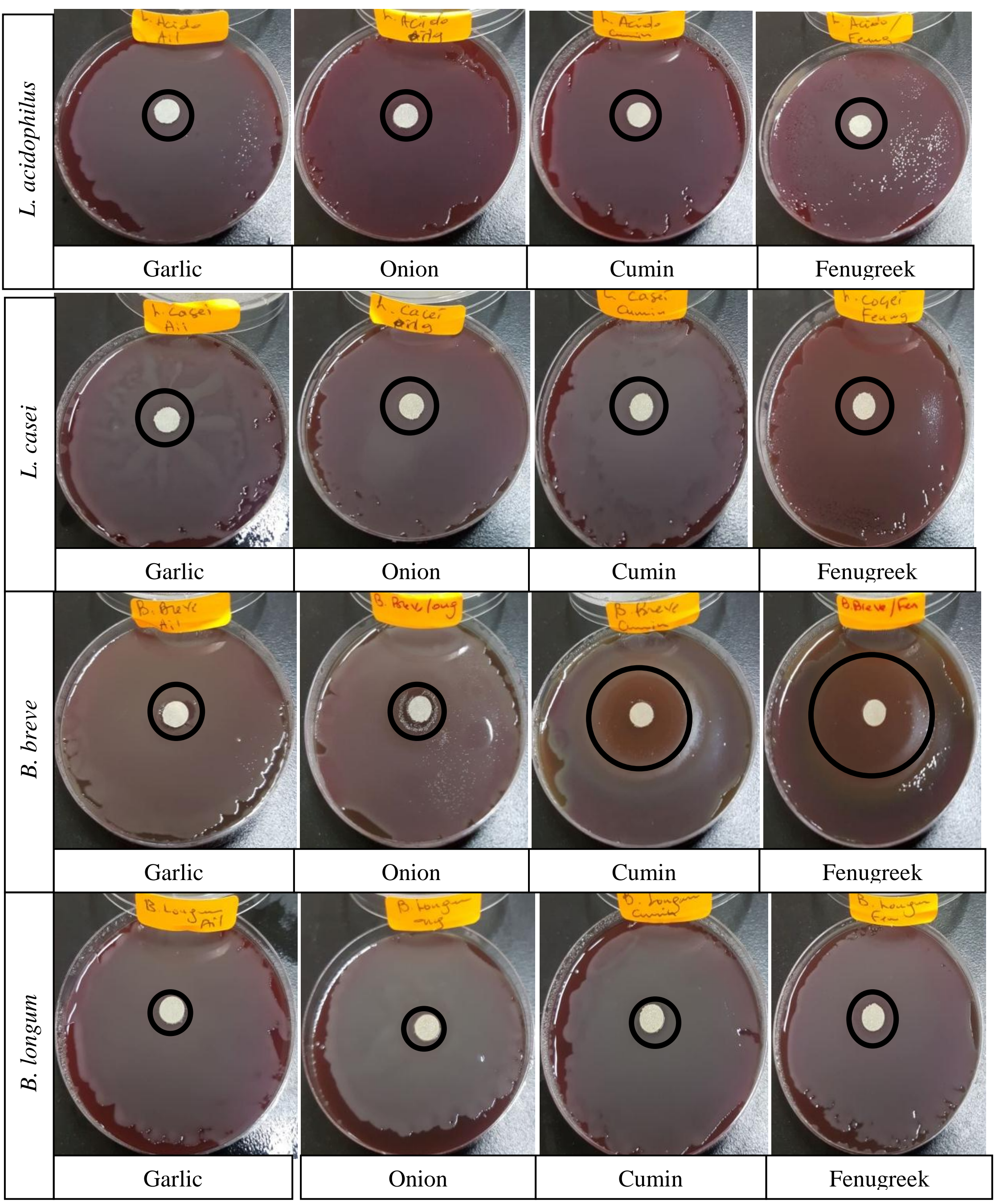


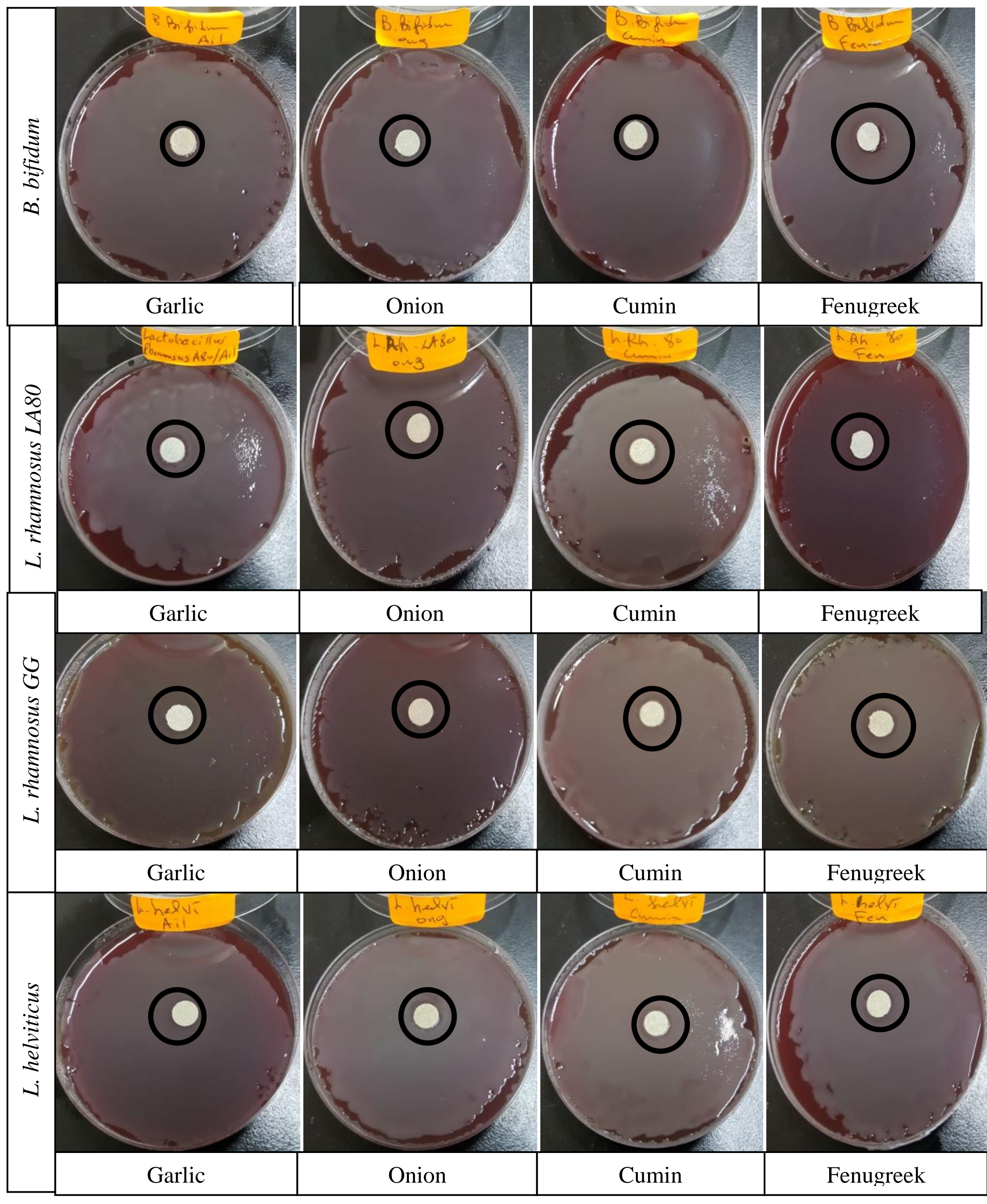
**Annexe 9:** Results of determination of DZI of probiotics against *H. pylori*



**Annexe 10:** Results of DZI of medicinal plants with probiotics against *H. pylori*




L. acidophilus
Garlic
Onion
Cumin
Fenugreek
L. casei
Garlic
Onion
Cumin
Fenugreek
B. breve
Garlic
Onion
Cumin
Fenugreek
B. longum
Garlic
Onion
Cumin
Fenugreek


B. bifidum
Garlic
Onion
Cumin
Fenugreek
L. rhamnosus LA80
Garlic
Onion
Cumin
Fenugreek
L. rhamnosus GG
Garlic
Onion
Cumin
Fenugreek
L. helviticus
Garlic
Onion
Cumin
Fenugreek

**Annexe 11:** Photos of *invivo* study of combined mixtures against *H.pylori*

| Grp | Hematoxylin-Eosin (H&E) stain | May-Grünwald Giemsa stain | Grp | Hematoxylin-Eosin (H&E) stain | May-Grünwald Giemsa stain |
|---|---|---|---|---|---|
| **NC** | **Inflamation: 3/4** | **Colonization: 3/4** | **TFE1 + TBB** | **Inflamation: 0/4** | **Colonization: 0/4** |
| **PC** | **Inflamation:2/4** | **Colonization:3/4** | **HP+TFE** | **Inflamation: 0/4** | **Colonization: 1/4** |
| **TFE** | **Inflamation: 2/4** | **Colonization: 2/4** | **HP+TBB** | **Inflamation: 0/4** | **Colonization: 1/4** |
| **TFE2** | **Inflamation: 1/4** | **Colonization: 1/4** | **HP+TFE+BB** | **Inflamation: 0/4** | **Colonization: 0/4** |
| **TBB** | **Inflamation: 0/4** | **Colonization: 1/4** | | NC : Negative control<br>PC : Positive control<br>TFE1 :Treated with fenugreek extract 150 µg/kg<br>TFE2 : Treated with fenugreek extract 300 | |

| | | | | µg/kg<br>TBB : Treated with *B. breve* |
|---|---|---|---|---|

**Annexe 12:** Composition of culture media

**Composition of Muller Hinton Agar (Senouci et Abdelouahid, 2010)**

| Ingredients | g/l |
|---|---|
| Beef extract | 2.00 |
| Acid hydrolysate of casein | 17.50 |
| Starch | 1.50 |
| Agar | 17.00 |

**Composition of Columbia Agar (Marchal et *al.*, 1991)**

| Ingredients | g/l |
|---|---|
| Pancreatic digest of casein | 12.0 |
| Peptic digest of animal tissue | 5.0 |
| Yeast extract | 3.0 |
| Beef extract | 3.0 |
| Corn starch | 1.0 |
| Sodium chloride | 5.0 |
| Agar | 13.5 |
| Sheep blood, defibrinated | 5 % |

| Ingredients | g/l |
|---|---|
| Tryptone | 5.0 |
| Soya peptone | 5.0 |
| Meat digest | 5.0 |
| Yeast extract | 2.5 |
| Ascorbic acid | 0.5 |
| Magnesium sulphate | 0.25 |
| Di-sodium-glycerophosphate | 19.0 |
| Agar | 11.0 |

**Composition of M17 Agar (Trezaghi et Sandine, 1975)**

**Composition of MRS Agar (Manet *al.,* 1960)**

| Ingredients | g/l |
|---|---|
| Peptone | 10 |
| Yeast extract | 5 |
| Meat extract | 10 |
| Glucose | 20 |
| Sodium acetate | 5 |
| Magnesium sulfate | 0.1 |
| Manganese sulfate | 0.05 |

| Disodium phosphate | 2 |
|---|---|
| Agar | 15 |

**Composition of urea indole broth (Roland et *al.,* 1947)**

| Ingredients | g/l |
|---|---|
| Dipotassium phosphate | 1 |
| Monopotassium phosphate | 1 |
| Phenol red | 0.025 |
| Sodium chloride | 5 |
| Urea | 20 |
| L-Tryptophan | 3 |

**Composition of Brain Heart Infusion (BHI) (Marchal et *al*., 1991)**

| Ingredients | g/l |
|---|---|
| Calf brain, infusion from | 200 |
| Beef heart, infusion from | 250 |
| Proteose peptone | 10 |
| Dextrose | 2 |
| Sodium chloride | 5 |
| Disodium phosphate | 2.5 |

**Abstract**

Combined use of probiotics and herbal medicine seems to result a decrease of the side effects of drugs used against *H. pylori* infection . It may also be a proper replacement for the treatment of this infection. The objectives of the present study were to study the physicochemical and the phytochemical compounds of garlic (*Allium sativum*), red onion (*Allium cepa*), fenugreek (*Trigonella foenum-graecum L.*) and cumin (*Cuminum cyminum L.*), to characterize their phenolic compounds using HPLC method, to evaluate the antioxidant properties of these plants and to investigate anti-*Helicobacter pylori* activities of the mentioned plant extracts, some probiotics such as *Lactobacillus, Bifidobacterium, Lactococcus and Streptococcus*, and their combinations (*invitro* and *invivo*).

The results of the physicochemical analysis found that these plants are good source of proteins, fats, fibers and energy. Also, the phytochemical analysis showed that plant extracts are rich in secondary metabolites namely total polyphenols, flavonoids, condensed and hydrolysable tannins. Chromatographic analysis of the samples identified five phytochemical molecules for red onion extract (gallic acid, quercitin, rutin, hyperoside and karempferol); one molecule for garlic extract (gallic acid); eight molecules for cumin extract (caffeic acid, isoquercetin, vanillic acid, myricetin 3-0, rutinoside, syringaresinol, citrusine, rosmarinic acid, p-coumaric acid) and seven molecules for fenugreek extract (gallic acid, sinapic acid, caffeic acid, asterogenic acid, pyrogallol, hyperoside and ferulic acid). The results of antioxidant activity measured by the DPPH method showed that all studied plants possessed a good antioxidant activities particularly fenugreek with an IC50 of 343.75 ±4.73 µg/ml. On the other hand, the results of anti-*H. pylori* activities revealed the presence of important inhibitory zones especially for fenugreek extract and *Bifidobacterium breve* combination (29 mm). Preliminary studies on the mode of action of probiotics against *H. pylori* revealed that the inhibition was due to lactic acid and bacteriocins. Also when *H. pylori*-infected rats were administered *B. breve*, the infection rate of *H. pylori* was significantly reduced, while the combination of *B. breve* and fenugreek extract effectively inhibited *H. pylori*. In addition, the *B. breve* and fenugreek extract complex mixture significantly reduced the stomach inflammation in *H. pylori* infected rats.



**Key Words:** *Helicobacter pylori*, Probiotics, Garlic, Onion, Fenugreek, Cumin, Gastro-duodenal diseases, Combined effect.

**Résumé**

L'effet combiné des probiotiques et des plantes médicinales entraînent une diminution des effets secondaires des médicaments utilisés contre l'infection à *H. pylori*. Il peut également être un substitut approprié pour le traitement de cette infection. Les objectifs de la présente étude étaient d'étudier les profils physicochimiques et phytochimiques de l'ail (*Allium sativum*), de l'oignon rouge (*Allium cepa*), du fenugrec (*Trigonella foenum-graecum* L.) et du cumin (*Cuminum cyminum* L.), de caractériser leur composés phénoliques en utilisant la méthode d'HPLC, d'évaluer les propriétés antioxydantes de ces plantes et d'étudier les activités anti-*Helicobacter pylori* des extraits de plantes mentionnées, et de certains probiotiques tels que *Lactobacillus, Bifidobacterium, Lactococcus* et *Streptococcus*, et leurs combinaisons (*invitro* et *invivo*). Les résultats de l'analyse physico-chimique ont révélé que ces plantes sont une bonne source de protéines, de graisses, de fibres et d'énergie. Aussi, l'analyse phytochimique a montré que les extraits végétaux sont riches en métabolites secondaires à savoir les polyphénols totaux, les flavonoïdes, les tanins condensés et hydrolysables. L'analyse chromatographique des échantillons a identifié cinq molécules phytochimiques pour l'extrait d'oignon rouge (acide gallique, quercitine, rutine, hyperoside et karempférol) ; une molécule pour l'extrait d'ail (acide gallique) ; huit molécules pour l'extrait de cumin (acide caféique, isoquercétine, acide vanillique, myricétine 3-0, rutinoside, syringarésinol, citronine, acide rosmarinique, acide p-coumarique) et sept molécules pour l'extrait de fenugrec (acide gallique, acide sinapique, acide caféique, acide astérogène acide, pyrogallol, hyperoside et acide férulique). Les résultats d'activité antioxydante mesurée par la méthode DPPH ont montré que toutes les plantes étudiées possédaient une bonne activité antioxydante particulièrement le fenugrec avec une IC50 de 343.75 ±4.73 µg/ml. En revanche, les résultats de l'anti-*H. pylori* ont révélé la présence des zones inhibitrices importantes notamment pour l'association (extrait de fenugrec et *Bifidobacterium breve*)(29 mm). Des études préliminaires sur le mode d'action des probiotiques contre *H. pylori* ont montré que l'inhibition était due à l'acide lactique et aux bactériocines. De plus, lorsque des rats infectés par *H. pylori* ont reçu *B. breve*, le taux d'infection de *H. pylori* a été considérablement réduit, tandis que la combinaison de *B. breve* et d'extrait de fenugrec a inhibé efficacement *H. pylori*. Enfin, le mélange complexe d'extraits de fenugrec et *B. breve* a considérablement réduit l'inflammation de l'estomac chez les rats infectés par *H. pylori*.

Mots clés : *Helicobacter pylori*, Probiotiques, Ail, Oignon, Fenugrec, Cumin, Maladies gastro-duodénales, Effet combiné.

**الملخص**

يؤدي التأثير المشترك للبكتيريا حامض اللاكتيك والنباتات الطبية إلى انخفاض الآثار الجانبية للأدوية المستخدمة ضد عدوى بكتيريا المعدة, وقد يكون أيضًا بديلًا مناسبًا لعلاج هذه العدوى. هدف البحث الحالي إلى دراسة الخصائص الفيزيوكيميائية للثوم (*Allium sativum*) ، البصل الأحمر(*Allium cepa*) ،الحلبة (*Trigonella foenum-graecum* L.) والكمون (*Cuminum cyminum* L.) ، والتعرف على مركباتها الفينولية. باستخدام طريقة الكروماتوغرافيا العالية الأداء ، وتقييم الخصائص المضادة للأكسدة لهذه النباتات ودراسة الفعاليات المضادة لبكتيريا *Helicobacter pylori* للمستخلصات النباتية المذكورة ، وبعض أنواع بكتيريا حامض اللاكتيك مثل *Lactobacillus*، *Streptococcus*، *Bifidobacterium* و *Lactococcus* خارج و داخل الجسم ,و. أظهرت نتائج التحليل الفيزيوكيميائي أن هذه النباتات هي مصدر جيد للبروتينات والدهون والألياف والطاقة .كما أظهر التحليل الكيميائي النباتي أن المستخلصات النباتية غنية بنواتج الايض الثانوية وهي البوليفينول الكلي والفلافونويد والعفص المكثف والمتحلل بالماء . أعطى التحليل الكروماتوغرافي للعينات خمسة مركبات كيميائية نباتية لمستخلص البصل الأحمر (حمض الغاليك ، كيرسيتين ، روتين ، هايبروسيد وكاريمبفيرول) ؛ مركب واحد لمستخلص الثوم (حمض الجاليك) ؛ ثمانية مركبات لمستخلص الكمون (حمض الكافيك ، أيزوكيرسيتين ، حمض الفانيليك ، ميريستين 3-0 ، الروتينوسيد ، سيرينجارسينول ، الليمونين ، حمض روزمارينيك ، حمض الكوماريك) وسبعة مركبات لمستخلص الحلبة (حمض الغاليك ، سينابيك ، حمض الكافيك ، حمض الأستيروجينيك حمض ، بيروجالول ، hyperoside وحمض الفيروليك) .أظهرت نتائج النشاط المضاد للأكسدة التي تم قياسها بواسطة طريقة تثبيط الجذر الحر أن جميع النباتات المدروسة لديها نشاط مضاد للأكسدة جيد ، خاصة الحلبة مع تركيزمثبط بنسبة 50% من 343.75 ± 4.73 ميكروغرام / مل و في المقابل فإن نتائج مضادات بكتيريا المعدة عن وجود مناطق مثبطة مهمة ، لا سيما بالنسبة لاتحاد مستخلص الحلبة و *Bifidobacterium breve* (29 ملم ) و أظهرت الدراسات الأولية حول طريقة عمل بكتيريا حامض اللاكتيك ضد بكتيريا المعدة أن التثبيط كان بسبب حمض اللاكتيك والبكتريوسينات .علاوة على ذلك ، عندما أعطيت الفئران المصابة بالبكتيريا المعدة *B. breve* ، انخفض معدل الإصابة ببكتيريا المعدة بشكل كبير ، في حين أدى الجمع بين بكتيريا *B. breve* ومستخلص الحلبة إلى تثبيط فعالية *H. pylori*بالإضافة إلى ذلك ، قلل المزيج المعقد من مستخلص الحلبة و*B. breve* التهاب المعدة بشكل ملحوظ في الفئران المصابة بالبكتيريا الحلزونية .

**الكلمات الدالة**: *Helicobacter pylori* ، بكتيريا حامض اللاكتيك ، الثوم ، البصل ، الحلبة ، الكمون ، أمراض معدية معوية، التأثير المشترك.