الجمهورية الجزائرية الديمقراطية الشعبية

People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
Ibn Khaldoun University –Tiaret–
Faculty of Nature and Life Sciences
Department of Biology

Dissertation

Submitted in partial fulfilment of

The requirements for the degree of

Master of Biological Sciences

Field: Nature and Life Sciences

Branch: Biological Sciences

Speciality: Cell and Molecular Biology

Title

Evaluation of the antioxidant, antimicrobial, hemolytic and anti-inflammatory activities of *Ocimum basilicum L.*

Presented by

- BENZERZOUR Youcef
- BERRABAH Billal Mohamed
- ABDELLAH Ahmed

Jury members:

President:	Mr. BENAISSA T.	MAA
Supervisor:	Mrs. AIT ABDERRAHIM L.	MCA
Examiner:	Mr. TAIBI K.	Prof

July2022/2023

Acknowledgement

First of all, thanks to ALLAH for having enlightened us and opened the doors of knowledge, and for having given us the will and the courage to elaborate this work.

All the expressions of esteem and gratitude in the world are insufficient to express our thanks to our parents who accompanied us throughout our study.

At the end of this work, we express our sincere thanks and our great respects to our supervisor Dr. AIT ABDERRAHIM L, for having accepted to supervise this work, and for her generosity, her kindness, her encouragement, her support and her confidence in us throughout this work.

Our thanks go to all the members of the jury Mr. BENAISSA Toufik and Mr. TAIBI Khaled who agreed to examine our work.

Special thanks go to Pr.TAIBI Khaled and Dr. BOUSSAID Mohamed, for accompanying us in the professional training of medicinal and aromatic plants.

Our thanks also go to all the teachers and all the persons in charge of the Molecular and Cellular Biology Department in the Faculty of Nature and Life Sciences, Ibn Khaldoun University of Tiaret

Thanks to all people who have helped our work in any way.

Dedication

I dedicate this modest work which is the fruit of my efforts to those I love most in the world, my father BENZERZOUR Mohamed and my mother Hirech.N.
For their sacrifices and encouragement all my life,

I can never thank them enough for giving me the best.

My dedications also go to:

To the BENZERZOUR and HIRECH families

All my dear teachers who taught me throughout my school life.

Mr.HAMDI Cheikh who helped me in my school life.

To all the people who, from near and far, have helped me.

Youcef

Dedication

I dedicate this modest work which is the fruit of my efforts To those I love most in the world, my father Hadj and my mother TAHERM. For their sacrifices and encouragement all my life,

I can never thank them enough for giving me the best.

My dedications also go to:

All my dear teachers who taught me throughout my school life.

To my friend who, from near and far, have helped me.

Billal

Dedication

I dedicate this modest work which is the fruit of my efforts To those I love most in the world, my father and my mother for their sacrifices and encouragement all my life,

I can never thank them enough for giving me the best.

My dedications also go to:

All my dear teachers who taught me throughout my school life.

To all the people who, from near and far, have helped me.

Ahmed

Abstract

Nowadays, a large number of aromatic and medicinal plants have been shown to possess very important biological properties that find numerous applications in various fields, namely medicine, pharmacy, cosmetology and agriculture. In this study, we evaluated some phytochemical content (total polyphenol compound content, total flavonoids content and condensed tannins) and biological activities (antioxidant, antibacterial, anti-inflammatory and hemolytic activities) of ethanolic and aqueous extracts of the aerial part (leaves and stems) of *Ocimum basilicum*.

Results show that generally the yield of leaves' ethanolic and aqueous extracts was higher than that of stems. However, ethanolic extract yield of leaves 19.42% was higher than all the other extracts. Moreover, the same result was observed regarding total phenolic and flavonoid content with high contents in the ethanolic leaf extract.. However, the condensed tannin content was almost the same in all four extracts. The ethanolic extracts of both leaves and stems as well as the aqueous extract of leaves showed the same inhibitory concentrations of 50 % (IC50) DPPH free radicals (0.22 mg/ml) and were lower than that of stems aqueous extract (0.38 mg/ml) demonstrating a better antioxidant effect.

Regarding the antibacterial activity, all the tested strains showed resistance to the four extracts even at a concentration of 100 mg/ml.

The hemolysis test indicates that the lowest hemolysis rates were recorded in treatments containing ethanolic extract of stems. However, the highest rates of hemolysis were recorded in the treatment containing aqueous leaf extract.

We noticed that the anti-inflammatory activity of leaf ethanolic extract was higher even at lower concentrations compared to the other extracts

Key words: *Ocimum basilicum* L., biological activities, polyphenol compound, flavonoids, condensed tannins, ethanolic extracts, aqueous extracts.

Résumé

De nos jours, un grand nombre de plantes aromatiques et médicinales ont été démontrées possédant des propriétés biologiques très importantes qui trouvent de nombreuses applications dans divers domaines, à savoir la médecine, la pharmacie, la cosmétologie et l'agriculture. Dans cette étude, nous avons évalué la quantité de certaines molécules phytochimiques (teneur en composes polyphenol totaux, teneur en flavonoïdes totaux et tanins condensés) ainsi que les activités biologiques (activités antioxydantes, antibactériennes, anti-inflammatoires et hémolytiques) d'extraits éthanoliques et aqueux de la partie aérienne (feuilles et tiges) du basilique *Ocimum basilicum*.

Les résultats montrent que généralement le rendement des extraits éthanoliques et aqueux des feuilles était supérieur à celui des tiges. Cependant, le rendement en extrait éthanolique des feuilles (19,42 %) était supérieur à celui de tous les autres extraits. De plus, le même résultat a été observé concernant la teneur totale en polyphénols et en flavonoïdes avec des teneurs élevés dans l'extrait éthanolique de feuilles. Cependant, la teneur en tanin condensé était presque la même dans les quatre extraits. Les extraits éthanoliques des feuilles et des tiges ainsi que l'extrait aqueux de feuilles présentent les mêmes concentrations inhibitrices de 50 % (IC50) des radicaux libres DPPH (0,22 mg/ml) et étaient inférieures à celles de l'extrait aqueux des tiges (0,38 mg/ml) démontrant une meilleure action antioxydante.

Concernant l'activité antibactérienne, toutes les souches testées ont montré une résistance aux quatre extraits même à une concentration de 100 mg/ml.

Le test d'hémolyse indique que les taux les plus faibles d'hémolyse ont été enregistrés dans les traitements contenant de l'extrait éthanolique des tiges. Cependant, les taux d'hémolyse les plus élevés ont été enregistrés dans le traitement contenant de l'extrait aqueux de feuilles.

Nous avons remarqué que l'activité anti-inflammatoire de l'extrait éthanolique de feuilles était plus élevée même à des concentrations plus faibles par rapport aux autres extraits.

Mots clés : *Ocimum basilicum* L., activités biologiques, polyphénols, flavonoïdes, tanins condensés, extraits aqueux, extraits éthanoliques.

الملخص

الريحان هو نبات طبي عطري ينتمي إلى عائلة لاميسيا , حاولنا من خلال هذه الدراسة بتقييم المحتوى الكيميائي النباتي (محتوى البوليفينول الكلي، إجمالي محتوى الفلافونويد والعفص المكثف) والأنشطة البيولوجية (النشاط المضاد للأكسدة، المضادة للبكتيريا، المضادة للالتهابات والانحلالي) للمستخلصات الإيثانولية والمائية للجزء العلوي (الأوراق والسيقان) من نبتة الريحان.

أظهرت النتائج أن إنتاجية المستخلصات المائية والإيثانولية للأوراق كانت أعلى بشكل عام من السيقان. ومع ذلك ، كان إنتاج المستخلص الإيثانولي للأوراق أعلى بنسبة 19.42٪ من جميع المستخلصات الأخرى. علاوة على ذلك ، لوحظت نفس النتيجة فيما يتعلق بمحتوى الفينول والفلافونويد الكلي بمستويات عالية في المستخلص الإيثانولي للأوراق. ومع ذلك، كان محتوى العفص المكثف متماثلًا تقريبًا في جميع المستخلصات الأربعة. أظهرت المستخلصات الإيثانولية لكل من وكذلك المستخلص المائي للأوراق نفس التركيزات المثبطة بنسبة50 % للجذور الحرة المؤكسدة(0.22 مجم / ملل لأوراق والسيقان وكانت أقل من المستخلص المائي للسيقان (0.38 مجم / مل)) مما يدل على تأثير مضاد للأكسدة أفضل.فيما يتعلق بالنشاط المضاد للبكتيريا، أظهرت جميع السلالات المختبرة مقاومة للمستخلصات الأربعة حتى بتركيز 100 مجم / مل.

يشير اختبار انحلال الدم إلى أنه تم تسجيل أقل معدلات انحلال الدم في العلاجات التي تحتوي على المستخلص الإيثانولي للسيقان. وسجلت أعلى معدلات انحلال الدم في المستخلص المائي للأوراق.

لاحظنا أن النشاط المضاد للالتهابات للمستخلص الإيثانولي للأوراق كان أعلى حتى عند التراكيز المنخفضة مقارنة بالمستخلصات الأخرى.

الكلمات المفتاحية : نشاط مضاد للأكسدة ، نشاط انحلالي ، نشاط مضاد للجراثيم ، نشاط مضاد للالتهابات، الريحان

List of figures

List of tables

List of abbreviations

WHO: World Health Organization

UV: Ultraviolet radiation

DPPH: 2; 2- diphenyl -1-picrylhydrazil

DPPH-H: 1, 1-diphenyl-2-picrylhydrazine

Rpm: Rotation per minute

I: Inhibition

Ac: Ascorbic Acid

GA: Gallic acid

Q: Quercetine

Eq: Equivalent

GAE: Gallic acid equivalent

QE: Quercetin equivalent

CE: Cyanidine

IC_{50}: 50% inhibitory concentration

ABS: Albumin Bovin Serum

$AlCl_3$: Aluminum Trichloride

Na_2CO_3: Sodium carbonate

PBS: Phosphate buffered saline

NaCl: Sodium chloride

TCA: Trichloroacetic acid

DMSO: dimethyl sulfoxide

KOH: Potassium hydroxide

CFU: Colony-forming unit

FCR: Folin-Ciocalteu

MeOH: Methanol

Aq: Aqueous

OB: *Ocimum basilicum*

SBA: Serum Bovine Albumin.

PBS: Phosphate buffer saline.

Table of content

2.2.2. Total phenolic content

2.2.3 Total flavonoid content

2.2.4. Condensed tannins content

2.2.5. DPPH radicals scavenging activity

2.2.6. Antibacterial activity

2.2.7. Hemolytic activity

2.2.8. Anti-inflammatory activity

Results and discussion

1. Plant extracts yields
2. Total phenolic content
3. Total flavonoid content
4. Condensed tannins
5. DPPH free radicals scavenging activity
6. Antibacterial activity
7. Hemolytic activity
8. Anti-inflammatory activity

Discussion

Conclusion

References

Introduction

Currently, the antioxidant properties of natural products are massively studied for the search of an additional supply of antioxidant compounds in order to prevent the damage of oxidative stress implicated in the pathogenesis of many human diseases such as certain cancers, cardiovascular diseases and degenerative diseases related to aging (Birben et al. 2012).

In fact, despite the advances in modern medicine, there is a very long history of healing with medicinal plants throughout the world, and there is currently a renewed interest in medicinal plants. According to the World Health Organization (WHO), more than 80% of the world's population uses traditional medicine for their health problems. The reason for this craze is that many diseases can be satisfactorily treated by plants at a relatively low cost. The healing power of these plants is due to the so-called active substances they contain (Bohui. 2018). At least 25% of modern medicines contain one or more active ingredients of plant origin (Benarba. 2015).

With an area of 2,381,741 square kilometers, Algeria is the largest country on the Mediterranean coast. It is known for its variety of medicinal and aromatic plants and their various popular uses in all regions of the country (Sahi. 2016).

The richness and diversity of the Algerian flora constitute a real phylogenetic reservoir, with about 4000 species and subspecies of vascular plants. However, the medicinal plants in Algeria still remain not well explored, as there are only a few thousand plant species, of which only 146 are medicinal (Hamel. 2018).

The Lamiaceae family is one of the most widespread in the plant kingdom. This plant family contains a large number of genera containing medicinal plants among which the genus Ocimum. This later has a number of species that are used to treat different ailments in traditional medicine especially the species *Ocimum basilicum* (known as sweet basil).

O. basilicum contains a variety of secondary metabolites, such as polyphenols, flavonoids and terpenes, with well-known potential biological effects that have been identified in this species (Güez et al. 2017). The secondary metabolites of the plants have important biological and pharmacological activities, such as antioxidants, anti allergic, antibacterial, hypoglycemic and anticancer (Stanković et al. 2011).

In this context, the present study aims to evaluate the antioxidant, antimicrobial, hemolytic and anti-inflammatory activities of aqueous and ethanolic extract of the aerial part (stems and leaves) of the plant *Ocimum basilicum*.

Literature review

Literature review

1. *Ocimum Basilicum* (Basil)

Ocimum basilicum (Basil) (Fig.1) is an aromatic herb belonging to the Lamiaceae family (Akbari et al. 2018). It is known as Lahbeq in Algeria and Rehan in Arabic (Chenni et al. 2016).

Basilicum is the Latin translation of the Greek basilikon, meaning king, and perhaps for the same reason the herb is known in French as “herbe royale” (Bariyah et al. 2012).

Figure1.*Ocimum Basilicum* (Aflatooni 2019; Aminian 2022).

2. Historical

In his De materia medica, Dioscorides, a Greek physician of the 1st century AD, mentions a widespread belief in Africa according to which the consumption of basil would remove the pain caused by a scorpion sting. Also, this plant was used by the Romans to fight flatulence, to promote the rise of milk and as an antidote and diuretic (Iserin P.2001)

3. Botanical description

Ocimum basilicum is an herbaceous plant of medium size, and with a soft or velvety touch (Bariyah et al.2012). Basil varieties vary in plant height and width, leaf size, leaf shape, leaf and flower color, inflorescence size, flowering time, seed size, color, and germination time. The height of mature basil plants at flowering can be divided into tall plants measuring between 45 and 75 cm, and small compact plants measuring between 15 and 38 cm. Plant width or lateral spread ranges from 31 to 55 cm (Novak et al. 2020)

The leaves of the grass are opposite, simple, entire, and ovate with a flat, spoon-shaped, or concave shape and smooth or wrinkled surfaces. They are usually toothed, 3-5 cm long in

cluster-shaped circles of 6 to 10 flowers. The color of the petals can be white, pink or purplish (Bariyah et al. 2012)

The seeds of basil varieties are described as grey, brown or black, 1.1 to 2.9 mm in length and 0.7 to 1.9 mm in width, with pitted surfaces. The time from sowing to germination ranges between 4 and 11 days, and the time from seed development until maturation varies between 6 and 28 days (Novak al. 2020)

4. Classification

For *O. basilicum*, five main botanical varieties are known, namely var. basilicum L., var. deformed Benth ., var. minimum L., var. purpurascens Benth., and var. thyrsi-florum L./Benth. Other varieties of *O. Basilicum* are var. Dianatnejaidii Salimi and var. Rashticus Salimi, originally from Iran (Pirmoradi et al. 2013).

The classification of *Ocimum basilicum* is presented in the following table:

Table 1. Botanical classification of *O. basilicum* (Bariyah 2012).

Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Ordre	Lamiales
Family	Lamiacaea
Genus	Ocimum
Species	*Ocimum basilicum*

5. Origin and geographical distribution of basil

Basil is one of the most important aromatic plants in tropical and subtropical regions of Asia and acclimatized in South and North America, Africa and Europe (Novak et al. 2020). There are more than 150 species distributed all over the world (Akbari et al. 2018)

Algeria, due to its biogeographical situation, and its different bioclimatic zones (humid, subhumid, semi-arid, arid or desert), offers a much-diversified flora (more than 3000 species and 1000 genera) of spontaneous aromatic and medicinal essences and cultivated plants. *Ocimum basilicum* is listed as one of the most widely used plants in Algeria with significant benefits due to its availability and ability to provide essential oils or extracts widely demanded by the food or pharmaceutical industries (Maidi et al. 2014).

6. Importance and use of *Ocimum basilicum*

The basil is an aromatic medicinal plant with multiple uses: medicine, food industry, cosmetics industry …etc. Basil mainly acts on the nervous and the digestive systems. It is used to treat flatulence and indigestions (Novak et al. 2020). The properties of this plant can reduce cardiovascular disease, prevent cancer and maintain healthy skin (Shahrajabian et al. 2020).

As a popular remedy, basil leaves have long been used to treat various health problems such as anxiety, fever, infections, arthropod bites, stomach pain, coughs, headaches, as well as a diuretic and antipyretic; it can also control and lower blood sugar, with antispasmodic and anti-diabetic effects (Nadeem et al. 2022)

People use its seeds as a source of dietary fiber (Shahrajabian et al. 2020). Basil essential oil is used as a flavoring in confectionery, salads, ice cream and perfumes, and in the formulation of soft drinks and toothpaste (Aflatooni et al.2019).

7. Properties of *Ocimum basilicum*

Basil leaves contain a variety of secondary metabolites, such as polyphenols, tannins and flavonoids that give the plant its biological properties. The components of basil essential oil have antioxidant, anti-inflammatory and antibacterial activities (Shahrajabian et al. 2020).

Methodology

Methodology

1. Objective of the study

The present study aims to evaluate in vitro some biological activities (antioxidant, antimicrobial, anti-inflammatory and anti-hemolytic) of aqueous and ethanolic extracts of the aerial part (stems and leaves) of *Ocimum basilicum*. In addition, a phytochemical analysis is performed to determine its content of polyphenols, flavonoids and tannins.

2. Methodology

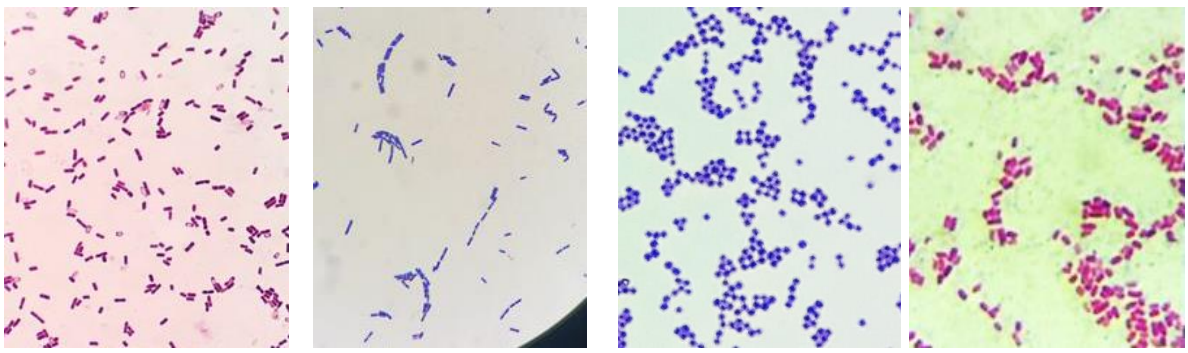
2.1. Materials

2.1.1. Plant sample

The plant *Ocimum basilicum* was collected in the Wilaya of El Oued (east Algeria).

2.1.2. Bacterial strains

The antibacterial activity of basil extracts was evaluated against the Gram-positive strains *Staphylococcus aureus* and *Bacillus cereus* and the Gram-negative strains *Escherichia coli*, and *Pseudomonas aeruginosa*. These strains were maintained by subculturing on nutrient agar and the purity was checked by microscopic observations (Fig. 2).



P. aeruginos *B. cereus* *S. aureus* *E. coli*

Figure 2. Microscopic observation of the tested bacterial strains.

2.1.3. Human blood samples

Human blood was collected from healthy, non-smoking volunteers not taking anti-inflammatory drugs, in order to assess the hemolytic effects of the plant extracts. Blood (O positive) samples were collected in heparin tubes by venipuncture from the volunteers and used directly for analysis.

2.2. Methods

2.2.1. Preparation of the aqueous and ethanolic extracts of *O. basilicum*

a. Aqueous extraction

The aqueous extract (Fig. 3) was prepared according to the modified method described by (Ghedadba et al. 2014). The dehydrated leaves and stems were ground using a kitchen grinder. The powdered leaves and stems were then macerated for 24 h in water by suspending 50 g of each powder in 500 ml of distilled water in an Erlenmeyer flask, which was then covered with aluminum paper, in order to preserve the darkness, preserving the metabolites as much as possible against the effects of oxidation by photons.

The homogenate was filtered on Whatman filter paper and then the collected filtrate was condensed using a rotary evaporation set at 500 rpm and at 40° C. The extract obtained was maintained in sterile vials and stored in a refrigerator at 4°C.

b. Ethanolic extraction

The ethanolic extract was prepared according to Ghedadba 2014.Extraction was carried out by maceration for 24 hours in ethanol 70 %. 50 g of dry ground leaves and stems are mixed, separately, with 500 ml ethanol in an Erlenmeyer flask which is subsequently wrapped with aluminum foil, in order to preserve the darkness. Mechanical agitation was performed for 15 min to accelerate the extraction process; the heterogeneous mixture was then filtered on Whatman filter paper (Fig. 3). The organic extracts were preserved in sterile vials and stored in a refrigerator at -20°C. The organic extract was evaporated using rotavapor at 40 °C.

c. Determination of extraction yield

Extraction yield is the ratio of the amount of analytic extracted to the amount of plant sample. (Bohui et al. 2018).

Yield% = Weight of the dry extract x 100/ initial weight of the dry plant

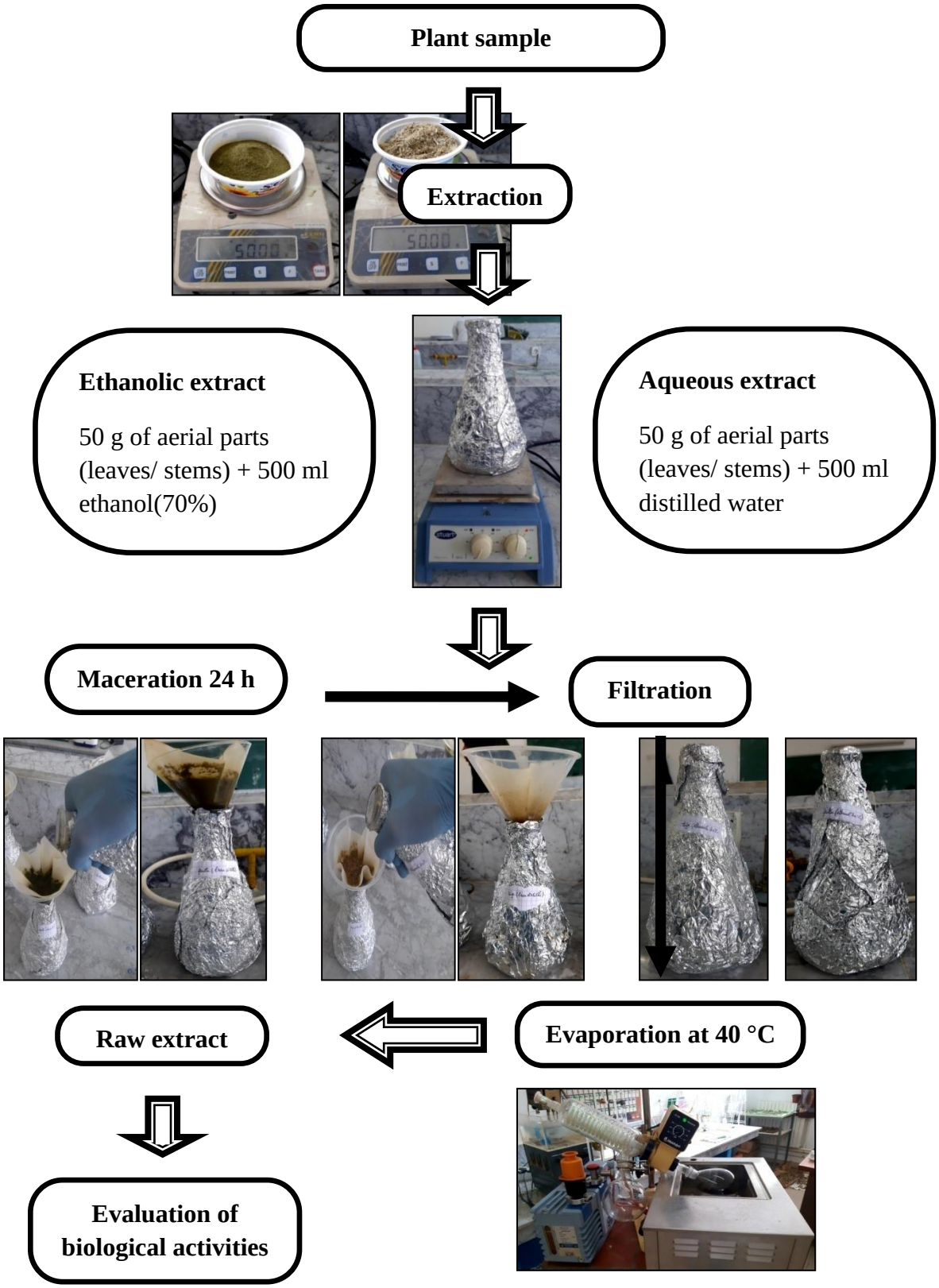


Figure 3. Steps of preparation of ethanolic and aqueous extracts of *Ocimum basilicum.*

2.2.2. Total phenolic content

Folin Ciocalteu Reagent (FCR) is a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMO_{12}O_{40}$), which is reduced to a mixture of tungsten oxide (W_8O_{23}) and molybdenum oxide (MO_8O_{23}) during the oxidation of phenol producing a blue color proportional to the total phenol content, with maximum absorbance around 750-765 nm

Total phenolic assay is performed following the modified method described byGhedadba2014. A 200 µl of each plant extract (dissolved in ethanol 20%) is added to 1ml diluted Folin-Ciocalteu reagent (10 folds). After 5 min, 800µl of sodium carbonate (7.5 %)is added, the whole is incubated in the shade for 30 min, and then the reading was taken at 765 nm by a spectrophotometer. The total polyphenol content is calculated from the calibration curve and the results are expressed as mg gallic acid equivalent (GAEq)/g extract. All the experiments were replicated at least three times

2.2.3. Total flavonoid content

The concentration of total flavonoids was determined spectrophotometrically using the aluminum trichloride ($AlCl_3$) colorimetric method, based on the formation of a highly stable complex between aluminum chloride and the oxygen atoms present on carbons 4 and 5 of flavonoids (Ghedadba2014).

Briefly, 1mL of an ethanolic solution of $AlCl_3$ (2%) is added to 1ml of plant extract (dissolved in ethanol 20%) and incubated for 15 minutes at room temperature. The absorbance is read at 430 nm. Total flavonoid content is calculated from the calibration curve and the results are expressed as mg quercetin equivalent(QEq)/g extract. All the experiments were replicated at least three times.

2.2.4. Condensed tannins content

Condensed tannins are quantified using a colorimetric method described by Haddouchi et al. 2016.Using the vanillin method with HCl. This method depends on the reaction of vanillin with tannins and the formation of red complexes anthocyanidols.

50 µl of each extract is added to 1.5 ml of 4% vanillin and 750 µl of HCl. After homogenization, the mixture is incubated at room temperature for 20 minutes. Absorbance is measured against a blank at 550 nm. The concentration of condensed tannins is deduced from calibration curves using catechin as the standard, and the result is expressed as mg catechin equivalent per g dry plant.

2.2.5. DPPH radicals scavenging activity

The radical scavenging activity of extracts was measured using 1,1-diphenyl-2-picrylhydrazine (DPPH). The antioxidant reacts with the stable free radical DPPH (dark purple color) and converts it to 1,1-diphenyl-2-picrylhydrazine with a corresponding color change (yellow) (Elmsellem et al. 2019).

0.2 ml of plant extracts added to 1 ml of DPPH solution (2 mg in 100 ml of methanol), the mixture is then incubated in the dark for 30 min. After then, absorbance is measured at 517 nm. The IC50 value of the standard, which is the concentration of the standard required to inhibit 50% of the DPPH free radical was calculated using log dose inhibition curve. Lower absorbance of their action mixture indicated higher free radical activity. The percentage of the radical scavenging activity (RSA) was calculated based on the following equation(Bentabet 2014):

DPPH Scavenged (%) = [(Acontrol – Asample)/ Acontrol]× 100

2.2.6. Antibacterial activity

Antimicrobial activity is a method designed to determine the antagonistic and inhibitory effect of bimolecular (or other compounds) known as antagonists on the growth of microorganisms known as targets. Inspired by antibiotic susceptibility testing, the method used in our study consists in impregnating Whatman paper disks placed on the surface of a suitable agar (Mueller Hinton agar), already inoculated with standardized microbial suspension, with a solution of the antagonist to be tested (in our case, extracts); the method is called the "disk diffusion method". The disks can be replaced by wells dug into the agar and filled with the solution to be tested (well diffusion method) (Fig. 4). Antimicrobial activity is then assessed by measuring the diameter of the zones of inhibition around the disks or wells (Tyagi et al. 2011).

Figure 4. Inoculation of agar wells with plant extract.

2.2.7. Hemolytic activity

The test of the hemolytic effect of extracts (aqueous and ethanolic) of the aerial parts (stems and leaves) of *Ocimum basilicum* was carried out, in vitro, on an erythrocyte suspension of human blood incubated in phosphate-buffered saline (PBS), pH 7.4 (Haddouchi2016).

3 ml freshly collected blood from a single healthy donor in a heparinized tube is centrifuged at 1500 rpm for 3 minutes. After removal of the plasma, the pellet is washed 3 times with a solution of PBS, each wash is followed by centrifugation (1500 rpm for 5 minutes), the pellet thus obtained is resolubilized again by the same volume of plasma removed, the erythrocyte suspension thus obtained is diluted 20 times with PBS.

Place 1ml of the prepared erythrocyte suspension in each hemolysis tube, together with 1ml of the extract at different concentrations (5, 2.5 and 1.25 mg/ml). Incubate tubes at 37°C for 30 min., then centrifuge (1500 rpm for 10 min) (Fig. 5).

The absorbance (Abs) of each tube is read at 540nm using a UV-Visible spectrophotometer against the PBS blank.

Under the same conditions and using the same experimental procedures, we prepared a negative control. It consists of 1ml erythrocyte suspension and 1ml phosphate buffer (PBS), in the absence of extract. The positive control contains 1ml erythrocyte suspension and 1ml distilled water, in the absence of extract.

The percent hemolysis for each extract was calculated using the following formula: **%hemolysis = (Abs540nm of sample - Abs540nm of negative control) / (Abs 540nm of positive control - Abs 540nm of negative control) x 100**

N.B. In all activities and for each concentration, the experiment is repeated 3 times.

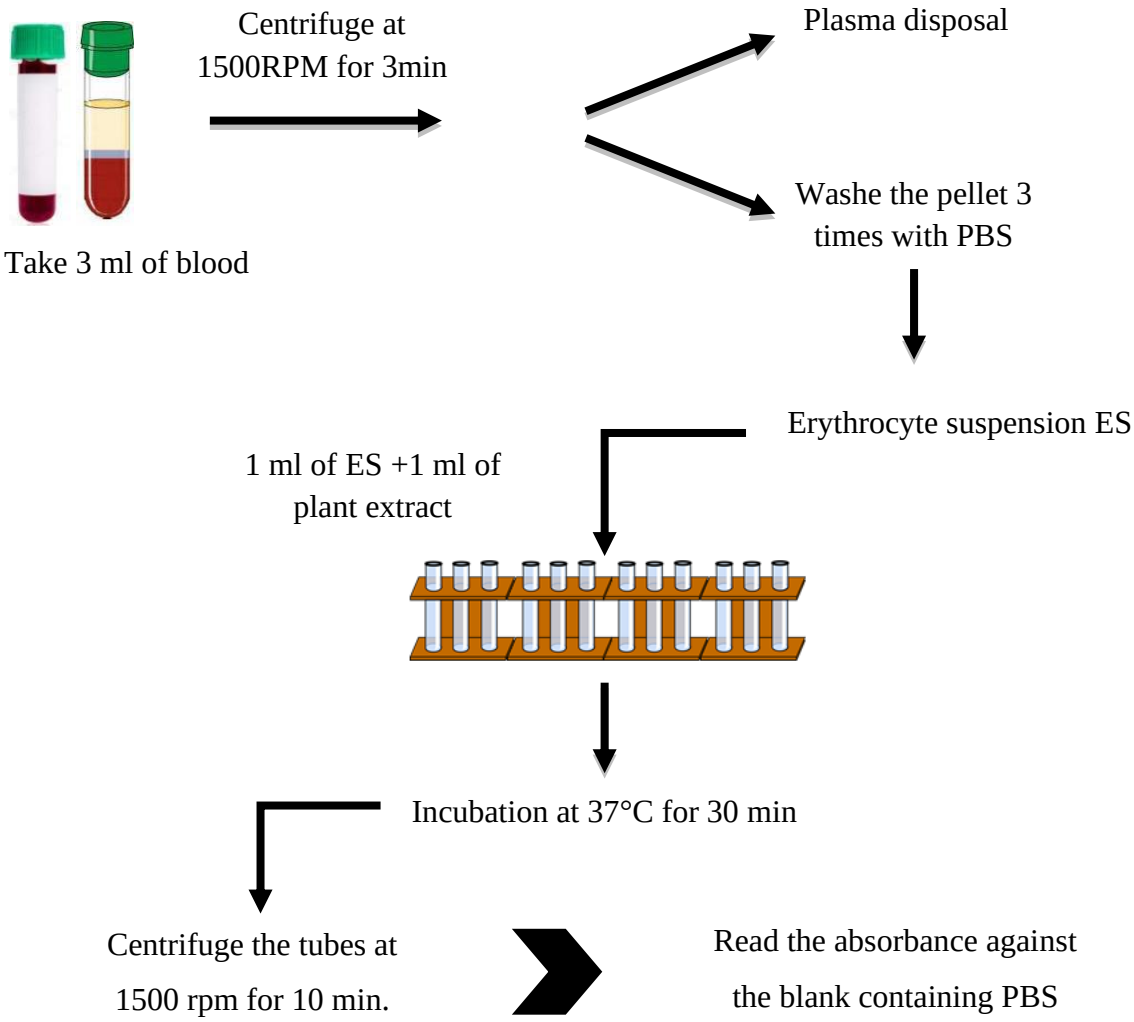


Figure 5. Hemolytic activity protocol

2.2.8. Anti-inflammatory activity

The principle of this technique is based on the ability of the extracts to reduce the thermal denaturation of BSA (Bouhlali et al. 2016). Weigh 75mg of extract and dissolve in 5ml of 50% DMSO to obtain a concentration of 15mg /ml, and then apply a series of dilutions. Foreach concentration, the experiment is repeated 3 times.

0.5 ml of extracts or standard, are mixed with 0.5 ml of 0.2% BSA prepared in Tris HCL (pH 6.6). The tubes are incubated at 37°C for 15 min, and then placed in a water bath at 72°C for 5

min. After the tubes have cooled, turbidity is measured at 660 nm in a spectrophotometer. The standard used is sodium diclofénac under the same conditions as the extracts. The positive control contains 0.5 ml BSA and 0.5 ml Tris HCL buffer.

The percentage inhibition of albumin denaturation was calculated using the following formula:

% inhibition = [(OD of test control solution-OD of test solution/DO of test control solution)] x 100.

OD: optical density.

Control represents 100% of denatured proteins; and results are compared with diclofénac sodium.

Results and discussion

Results

1. Plant extracts yields

Determining the yield of the extract studied is an important step in determining the percentage of extract obtained by the extraction method used. Ethanolic and aqueous extracts yields of the aerial part (leaves and stems) of *Ocimum basilicum* are shown in figure 6:

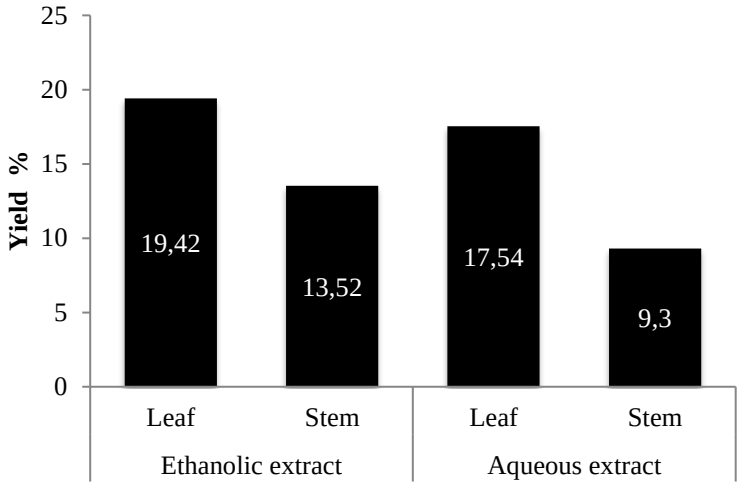


Figure 6. Ethanolic and aqueous extracts yields from*Ocimum basilicum*.

Our results demonstrate that the yield of both ethanolic and aqueous extracts of basil leaves obtained is higher than that of stems. However, ethanolic extracts yield are higher than that of aqueous extracts regarding leaves and stems

2. Total phenolic content

Results show that the total phenolic content of the ethanolic extract of leaves (77,40 ± 1,53 mg GAE/ g extract) is higher compared to the other extracts (Fig. 7).

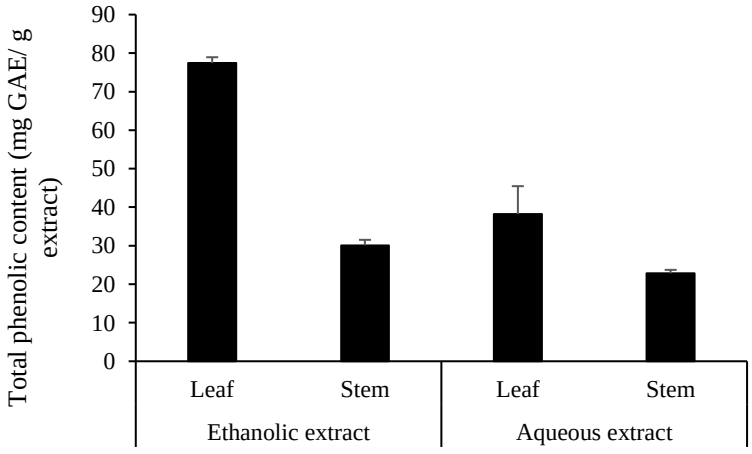


Figure 7. Total phenolic content of *O. basilicum* ethanolic and aqueous extracts.

3. Total flavonoid content

The total flavonoid content of leaves' ethanolic extract (9.65 ± 0.15 mg QE/g) is higher compared to the other extracts followed by the aqueous extract of leaves (6.99 ± 0.09 mg QE/g) (Fig. 8).

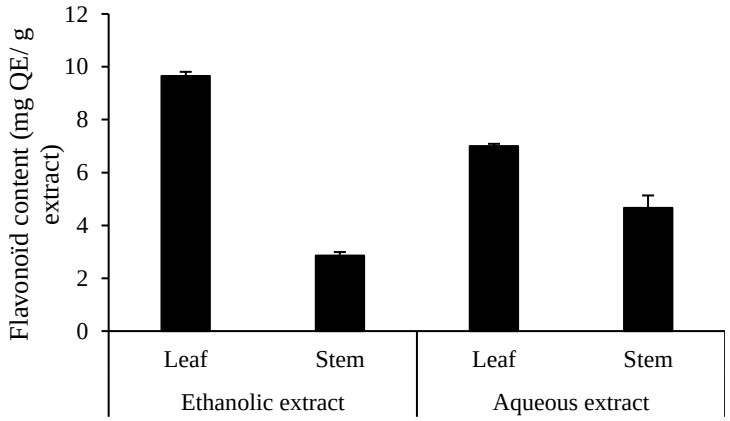


Figure 8. Total flavonoid content of *O. basilicum* ethanolic and aqueous extracts.

4. Condensed tannins

Results showed that condensed tannins content was almost the same in both types of extracts and in both leaves and stems (Fig. 9).

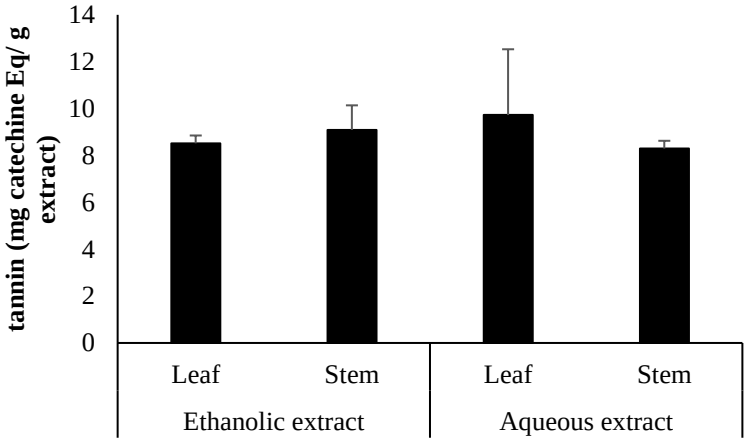


Figure 9. Condensed tannin content of *O. basilicum* ethanolic and aqueous extracts.

5. DPPH free radicals scavenging activity

The DPPH scavenging activity of *O. basilicum* ethanolic and aqueous extracts was expressed as the inhibitory concentration of 50 % radicals. The IC50 is inversely proportional to a compound's antioxidant capacity, because it expresses the amount of antioxidant required to reduce the free radical concentration by 50%. The smaller the IC50 value, the greater the antioxidant activity of a compound. Results (Table 2) showed that ethanolic extract of leaves

and stems as well as the aqueous extract of leaves of basil have the same IC50 with no significant difference. The aqueous extract of the stems showed relatively higher IC50 compared to the other extracts. However, all extracts showed high IC50 values compared to ascorbic acid.

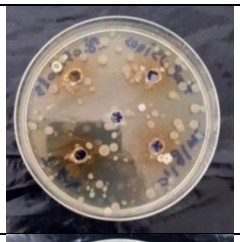
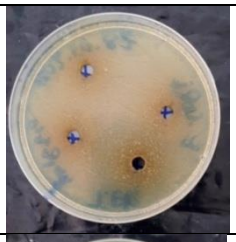
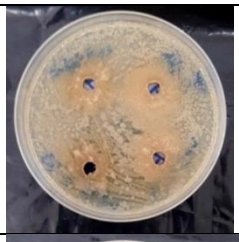
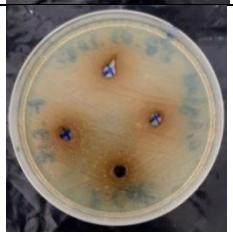
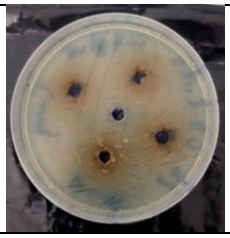
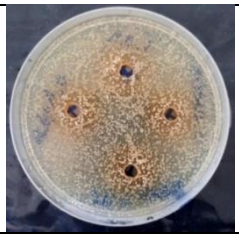
Table 2. IC50 of ethanolic and aqueous extracts of *O. basilicum* compared to ascorbic acid.

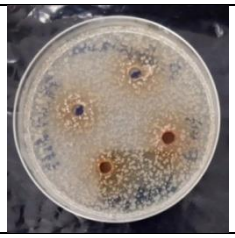
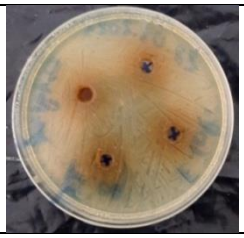
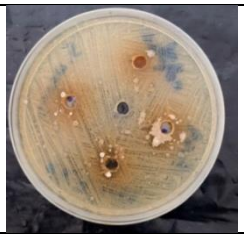
	IC50 (mg/ ml)			
	Ethanolic extract		Aqueous extract	
Acide ascorbique	leaves	stems	leaves	stems
0.0025 mg/ml	0.22	0.22	0.25	0.38

6. Antibacterial activity

All the tested bacterial strains (Gram positive and Gram negative) showed resistance to all extracts of *O. basilicum* at a concentration of 100 mg/ml (Table 3).

Table 3. Results of the antibacterial assay by well diffusion method.

	Tested bacterial strains			
Extract	***P. aeruginosa***	***E. coli***	***B. cereus***	***S. aureus***
Stem EtOH				
Leaf EtOH				
StemAq				

Leaf Aq				

7. Hemolytic activity

In general, the rate of hemolysis increased with the concentration of *O.basilicum* plant extract in the reaction medium. The lowest hemolysis rates were recorded in treatments containing ethanolic stem extract. However, the highest rates of hemolysis were recorded in the treatment containing aqueous leaf extract (Fig 10).

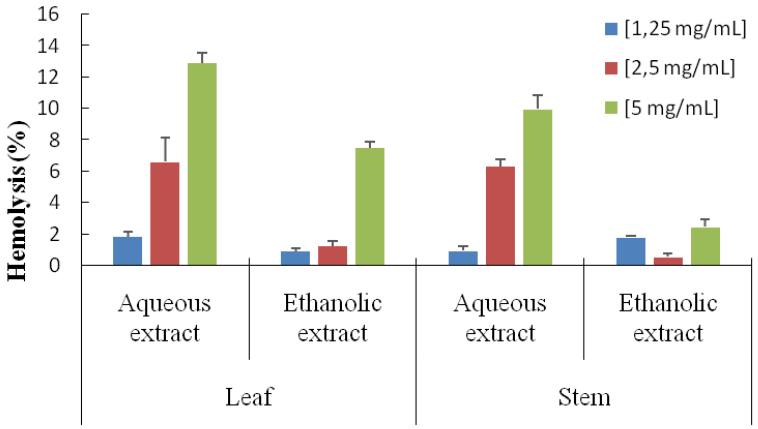


Figure 10. Effects of aqueous and ethanolic extracts of *O. basilicum* on the evolution of the hemolysis rate.

The hemolysis rate of the aqueous stem extract rose from 0.9% below the concentration of 1.25 mg/ml, to 10% when the extract concentration increased to 5 mg/ml. Similarly, the hemolysis rate of the aqueous leaf extract rose from 1.8% at the low concentration of 1.25 mg/ml to 12.9% at the high concentration of 5 mg/ml. In addition, the hemolysis rate of the ethanolic stem extract rose from 1.8% at a concentration of 1.25 mg/ml to only 2.5% when the extract concentration was increased to 5 mg/ml. Similarly, the hemolysis rate of ethanolic leaf extract rose from 0.9% at a low concentration of 1.25 mg/ml to 7.5% at a high concentration of 5 mg/ml.

8. Anti-inflammatory activity

Anti-inflammatory capacity increases in vitro when the extract concentration is increased.

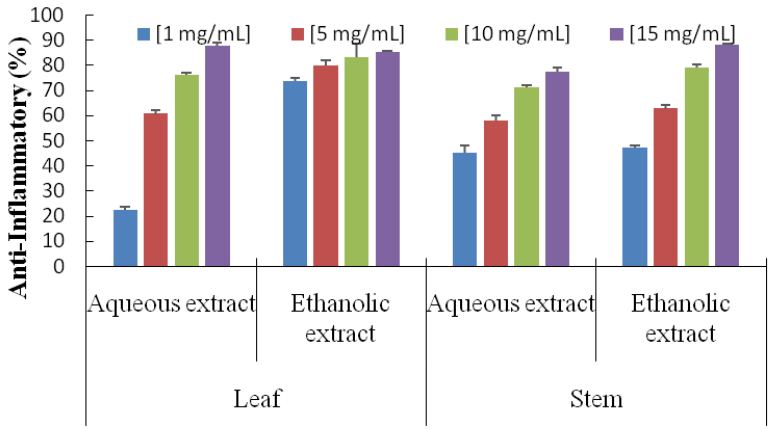


Figure 11. Anti-inflammatory activity of *O.basilicum* aqueous and ethanolic extracts.

The lowest anti-inflammatory levels were recorded in treatments containing aqueous leaf extract22.5%, at a concentration of 1 mg/ml. whereas, the highest value was recorded in stem ethanolic extract 87.9 % at a concentration of 5mg/ml. We noticed that the anti-inflammatory activity of leaf ethanolic extract was higher even at lower concentrations compared to the other extracts (Fig. 11).

Discussion

In recent years, modern medicine has undergone significant and unprecedented development. However, it remains powerless in the face of many illnesses, which is why people are turning to alternative medicine especially as it is more available, less expensive and contains no chemicals (Bohui2018).

Plants are an important source of bioactive molecules for drug discovery. Isolated bioactive molecules serve as starting materials for laboratory synthesis of drugs as well as a model for the production of biologically active compounds (Dhanani et al. 2017).

Among the plants known for their biological effects, basil, a Lamiaceae plant widely used for its culinary and therapeutic properties.

The present work is part of the evaluation of some biological activities (antioxidant, antimicrobial, hemolytic and anti-inflammatory) of the aqueous and ethanolic extracts of the aerial part (stems and leaves) of the plant *Ocimum basilicum*.

All the extracts were prepared by maceration procedure and the extraction yield was higher in ethanolic leaves extract. The choice of extraction technique is important as it allows the extraction and discovery of bioactive constituents from the plant. As well, it allows the selection of those desirable soluble constituents and leaving those not required. Various extraction techniques most commonly used include maceration, percolation, infusion, decoction, etc., other recent techniques like ultrasounds are also used

The extraction yield is comparable to conventional extraction and in some cases, it is even higher. However, extract yield as well as the bioactivities of the extract prepared using different extraction methods have been reported to vary in several studies (Dhanani et al. 2017)

The highest percentage of hemolysis was recorded in the treatment containing aqueous leaf extract.

Our result is considered very high compared to that found by (Prasath et al. 2019) in the same species (*Ocimum basilicum*) the yield of plant extractions depends essentially on their genotypic properties the manner and duration of storage.

Our results show that the *Ocimum basilicum* L. studied is less rich in phenolic and flavonoids compounds compared to the contents obtained in previous works of Fathiazad et al. (2012); Prasath et al. (2019) and Nadeem et al. (2022). In fact, the total polyphenol and flavonoids contents of a plant extract such *Ocimum basilicum* L. can vary considerably in the same species from one region to another and in the same plant parts. These variations depend

essentially on: their origin, variety, harvesting season, soil, geographical location, the various diseases that can affect the plant, the maturity of the plant and the harsh climatic conditions of the places where they grow (high temperature, high sun exposure, drought and salinity which stimulate the biosynthesis of secondary metabolites.

Besides, the variation of phytochemicals content in the same plant parts can also be linked to the distribution of secondary metabolites, which can change during plant development.The decrease in secondary metabolites may also be due to evaporation losses and/or biosynthesis activity that continues long after the plant material has been harvested the time of harvest and the extraction method used(ـertout et al.2016).

Condensedtannins are water-soluble phenolic metabolites commonly found in almost all plants parts. They play a key role inthe health promoting properties of the fruit. Tannin-rich plant shave shown high radical scavenging activity conferring protection against lipid peroxidation (Berrabah et al. 2019).

In this study, the different extracts showed ability to scavenge DPPH free radicals at different concentrations. Several studies have demonstrated the direct correlation between the antioxidant activity and phenolic content. This activity is believed to be mainly due to the redox properties of phenolic compounds, which plays an important role in adsorbing and neutralizing free radicals. Besides, other bioactive compounds such as dietary fibers and microelements contribute to the antioxidant activity (Ait Abderrahim et al. 2019).

The results obtained show that basil extracts are inactive on all the tested bacterial strains which does not correlate with the other studies. According to (Al-Ghamdi et al. 2020), the antimicrobial activities of plants have been attributed to bioactive components in plants such as polyphenols, tannins, flavonoids, etc.

Hemolytic activity was assessed to determine the toxicity of the aqueous and ethanolic extracts of the plant. Results show that aqueous leaf extract has a high hemolytic potential of around 12.9%.

This study revealed that all the tested aqueous and ethanolic extract have anti-inflammatory activity which correlate with the work of (Osei Akoto .2020). Several studies demonstrated the correlation between the antioxidant activity and anti-inflammatory activity.

Conclusion

Conclusion

O. basilicum although known for its virtues from a long time constitutes a potential source of phytochemicals with therapeutic properties.

Throughout this study we demonstrated that the aqueous and ethanolic extracts of the aerial parts of basil have beneficial biological activities. Furthermore, the ethanolic extracts showed increased phytochemicals content with higher antioxidant and anti-inflammatory activities compared to the aqueous extract. In addition, the hemolytic test showed that the tested extracts are not toxic to cell membranes.

However, no antibacterial action was observed even at high concentrations with all tested extracts.

As a follow-up to this work, it would be interesting to:

- Test other extraction techniques to find the optimum method that gives the best yield and biological activity.
- Isolate the natural molecules responsible for the biological activities of leaves and other plant parts.
- Carry out tests on a wide range of microorganisms.

References

References

1. Ait AbderrahimL.,Taibi K., Ait Abderrahim C. 2019. Assessment of the antimicrobial and antioxidant activities of *Ziziphuslotus* and *Peganum harmala*. Iranian Journal of Science and Technology. Transaction Science. 43:409–414. https://doi.org/10.1007/s40995-017-0411-x.
2. Berrabah H., Taïbi K., Ait Abderrahim L., Boussaid M. 2019. Phytochemical composition and antioxidant properties of prickly pear (*Opuntia ficus-indica* L.) flowers from the Algerian germplasm. Journal of Food Measurement and Characterization. https://doi.org/10.1007/s11694-019-00032-8.
3. Dhanani T., Shah S., Gajbhiye NA., Kumar S. 2017. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of with *ania somnifera*. Arabian Journal of Chemistry. 10(1): S1193-S1199.
4. Sahi L.2016. La dynamique des plantes aromatiques et médicinales en Algérie [Troisième partie]. In : Ilbert H. (ed.), Hoxha V. (ed.), Sahi L. (ed.), Courivaud A. (ed.), Chailan C. (ed.). Le marché des plantes aromatiques et médicinales : analyse des tendances du marché mondial et des stratégies économiques en Albanie et en Algérie. Montpellier : Ciheam / FranceAgriMer.104 (Options Méditerranéennes : Série B. Etudes et Recherches; n. 73).
5. Hamel T., Sadou S., Seridi R., Boukhdir S., Boulemtafes A. 2018. Pratique traditionnelle d'utilisation des plantes médicinales dans la population de la péninsule de l'edough (nord-est algérien). Ethnopharmacologia, (59).75.
6. Akbari G., Soltani E., Binesh S., Amini F. 2018. Cold tolerance, productivity and phytochemical diversity in sweet basil (*Ocimum basilicum* L.) accessions. Industrial Crops and Products, 124, 677–684. doi:10.1016/j.indcrop.2018.08.048
7. Bohui G., Adima, A., Niamké F., N'Guessan J. 2018. Etude comparative de trois méthodes d'extraction des flavonoïdes totaux à partir des feuilles de plantes médicinales : *Azadirachta indica* et *Psidium guajava*. Journal de la Société Ouest-Africaine de Chimie 46. 50-58.
8. Benarba B., Belabid L., Righi K., Bekkar A., Elouissi M., Khaldi A., Hamimed A. 2015. Ethnobotanical Study of Medicinal Plants used by Traditional Healers in Mascara (North West of Algeria). Journal of Ethnopharmacology. (175). 626-637.
9. Güez C., Souza R ., Fischer P., Leão M., Duarte J., Boligon A ., Athayde M ., Zuravski L., Oliveira , L ., Machado. 2017. Evaluation of basil extract (*Ocimumbasilicum*L.) on

oxidative, anti-genotoxic and anti-inflammatory effects in human leukocytes cell cultures exposed to challenging agents. Brazilian Journal of Pharmaceutical Sciences. 53(1): 1-12.

10. Stanković M. 2011. Total phenolic content, Flavonoid concentration and antioxidant activity of *Marrubium peregrinum*. extracts. Kragujevac Journal of Science. 33. 63-72
11. Pirmoradi M., Moghaddam M., Farhadi N. 2013. Chemotaxonomic Analysis of the Aroma Compounds in Essential Oils of Two Different *Ocimum basilicum* L. Varieties from Iran. Chemistry & Biodiversity, 10(7), 1361–1371. doi:10.1002/cbdv.201200413
12. Chenni M., El Abed D., Rakotomanomana N., Fernandez X., Chemat F. 2016. Comparative Study of Essential Oils Extracted from Egyptian Basil Leaves (Ocimumbasilicum L.) Using Hydro-Distillation and Solvent-Free Microwave Extraction. Molecules, 21(1), 113. doi:10.3390/molecules21010113
13. Aflatooni H., Ebrahimi M., Ramshini H., Akbari G.2019. Evaluation of Some Morphological Characteristics of Different Basil Masses (Ocimumbasilicum L.).Gene Technol, 8(1), 148.
14. Aminian A., Mohebbati R., BoskabadyM . (2022). The Effect of *Ocimumbasilicum* L. and Its Main Ingredients on Respiratory Disorders: An Experimental, Preclinical, and Clinical Review. *Frontiers in pharmacology*, *12*, 805391. https://doi.org/10.3389/fphar.2021.805391
15. Iserin P.2001.larousse-des-plantes-medicinales.Larous
16. Bariyah S., Ahmed D., Ikram M.2012.Ocimumbasilicum: A Review on Phytochemical and Pharmacological Studies. Pakistan Journal of Chemistry, 2, 78-85.https://doi.org/10.15228/2012.v02.i02.p05
17. Novak J., Blüthner W. 2020. Medicinal, Aromatic and Stimulant Plants. Handbook of Plant Breeding. doi:10.1007/978-3-030-38792-1
18. Maidi L.2014. Mise en évidence des activités antimicrobienne et antioxydante des huiles essentielles et des extraits de *Ocimum basilicum* L. (Lamiaceae) de la région d'El Assafia (W. de Laghouat) Algérie. Journal of Advanced Research in Science and Technology, 8(2), 45-59.
19. Shahrajabian M., Sun W., Cheng Q.2020. Chemical components and pharmacological benefits of Basil (OcimumBasilicum): a review. International Journal of Food Properties, 23(1), 1961–1970. doi:10.1080/10942912.2020.1828456
20. Nadeem H., Akhtar S., Sestili P., Ismail T., Neugart S., Qamar M., Esatbeyoglu T. 2022. Toxicity, Antioxidant Activity, and Phytochemicals of Basil (*Ocimum basilicum* L.)

Leaves Cultivated in Southern Punjab, Pakistan. *Foods*, *11*(9), 1239. http://dx.doi.org/10.3390/foods11091239

21. Adetunji C., Palai S., Ekwuabu C., Egbuna C., AdetunjiJ ., Ehis-Eriakha, C . Mtewa, A. 2021. General principle of primary and secondary plant metabolites: Biogenesis, metabolism, and extraction. Preparation of Phytopharmaceuticals for the Management of Disorders, 3–23. doi:10.1016/b978-0-12-820284-5.00018-6
22. Anjali., Kumar S., Korra T., Thakur R., Arutselvan R., Kashyap A., Nehela Y., ChaplyginV ., Minkina T., Keswani C. 2023. Role of Plant Secondary Metabolites in Defence and Transcriptional Regulation in Response to Biotic Stress. *Plant Stress*. 8.100154 https://doi.org/10.1016/j.stress.2023.100154
23. Mehinagic E., Bourles E., Jourjon F. 2011. Composés des fruits d'intérêt nutritionnel: impact des procédés de transformation sur les polyphénols. Revue suisse Viticulture, Arboriculture, Horticulture. 43 (6). 364–368
24. MassauxC.2012. Polyphénols: des alliés pour la santé. Abeilles Cie, 149, 2..
25. Karthika, R., Meenatchi, P., Sundaram, R., Purushothaman, A. (2017). Phytochemical Analysis, Antioxidant and Antibacterial Activities of Two Traditionally Used Indian Medicinal Plants. Asian Journal of Biology, 4(3), 1 11.https://doi.org/10.9734/AJOB/2017/37961
26. Ghedadba N., Bousselsela H., Hambaba L., Benbia S., Mouloud Y. 2014. Évaluation de l'activité antioxydante et antimicrobienne des feuilles et des sommités fleuries de Marrubiumvulgare L. Phytothérapie, 12(1), 15–24. doi:10.1007/s10298-014-0832-z
27. Bibi Sadeer N., Montesano D., Albrizio S., Zengin G., Mahomoodally M. 2020. The Versatility of Antioxidant Assays in Food Science and Safety-Chemistry, Applications, Strengths, and Limitations. *Antioxidants (Basel, Switzerland)*, *9*(8), 709. https://doi.org/10.3390/antiox9080709
28. Haddouchi F., Chaouche T., Halla N. 2016. Screening phytochimique, activités antioxydantes et pouvoir hémolytique de quatre plantes sahariennes d'Algérie. Phytothérapie. doi:10.1007/s10298-016-1086-8
29. Elmsellem H., Ouadi E., Mokhtari M., Bendaif H., Steli H., Aouniti A., Almehdi A ., Abdel-Rahman I., Kusuma H., Hammouti B. 2019. A natural antioxidant and an environmentally friendly inhibitor of mild steel corrosion: a commercial oil of basil (*Ocimum basilicum* L.).

30. Bentabet N., Boucherit-Otmani Z., Boucherit K.2014. Composition chimique et activité antioxydante d'extraits organiques des racines de *Fredolia aretioides* de la région de Béchar en Algérie. Phytothérapie, 12(6), 364–371. doi:10.1007/s10298-014-0834-x
31. Vlase L., Benedec D., Hanganu D., Damian 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. doi:10.3390/molecules19055490
32. Tyagi A., Malik A. 2011. Antimicrobial potential and chemical composition of Mentha piperita oil in liquid and vapour phase against food spoiling microorganisms. Food Control, 22(11), 1707–1714. doi:10.1016/j.foodcont.2011.04.002
33. Bouhlali D., Sellam K., Bammou M., Alem C., Filali-ZehzoutiY. 2016. In vitro Antioxidant and anti-inflammatory properties of selected Moroccan medicinal plants - .Journal of Applied Pharmaceutical Science, *6*, 156-162.
34. Fertout N., Latreche A., Mehdadi Z., Bengherraz Z.2016. Activité antibactérienne de quatre extraits de Teucriumpolium L. du mont de Tessala (Algérie occidentale). Bulletin de la Société Royale des Sciences de Liège.
35. Karthik K., Rathna B., Kumar P., Venupriya R., SunilKumar K., Singh R. 2013. Evaluation of anti-inflammatory activity of Canthium parviflorum by in-vitro method. 1 , 729-730.
36. Fathiazad F., Matlobi A., Khorrami A., Hamedeyazdan S., Soraya H., Hammami M., Garjani A. 2012. Phytochemical screening and evaluation of cardioprotective activity of ethanolic extract of *Ocimum basilicum* L. (basil) against isoproterenol induced myocardial infarction in rats. DARU Journal of Pharmaceutical Sciences, 20(1), 87. doi:10.1186/2008-2231-20-87
37. Benedecad D., Vlaseb L., Hanganua D., Oniga I.2012. Antioxidant potential and polyphenolic content of Romanian *Ocimumbasilicum*.
38. Prasath S., Bharathi S., Subramanian S. 2019. Antioxidant Properties of *Ocimum basilicum* Leaves Extract: An in vitro study. Der Pharmacia Lettre. 11(1): 33-41.
39. Al-Ghamdi A., Abdalla M., Fadlelmula A. 2020. Phytochemical, total phenolic contents, and antioxidant and antimicrobial activities of *Ocimumbasilicum* L. leaf extract in AL-BAHA area, SAUDI ARABIA. International Journal of Advanced Research.