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Title

Evaluation of the antioxidant, antimicrobial, hemolytic and anti inflammatory activities of *Capparis spinosa.*

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Dedications

I dedicate this memory

- *To the one who showered me with tenderness and hope, to the source of Unstoppable love, to the mother of fragile feelings who blessed me with these, my life, my mother fatima*
- *To my support in my life, who taught me, supported me and directed me Towards glory … my father* ***Abdelhadi*** *Most sincere thanks May God protect me.*
- *To my brother* ***Riadh*** *and his wife* ***Lou***
- *To my nephew* ***Nidal***
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- *To my friends* ***Sabrina , FATI , LYNA ,IMEN*** *and* ***AICHA***
- *To all my friends and colleagues* ***Amel*** *and* ***Malika*** *and All those who have helped me* ***Benaouda , Ilyes , Sabrina , and all my group***

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- *To the one who showered me with tenderness and hope, to the source of Unstoppable love, to the mother of fragile feelings who blessed me with these, my life, my mother Torkia____*
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As I stand on the precipice of a new chapter, I carry with me the love, guidance, and memories that have been bestowed upon me. This graduation is not just an individual accomplishment; it is a celebration of the collective support and love that has propelled me forward. I am eternally grateful for each and every one of you, and I dedicate this milestone to the incredible individuals who have made it possible

With heartfelt appreciation,

[Malika]

Abstract

Capparis spinosa, a shrub plant with a wide growing range and widespread natural distribution, has long been used in traditional medicine and food of the Mediterranean basin. The aim of this

Study is to evaluate the biological activities of natural populations of C. spinosa belonging to three regions from Algeria namely Mostaganem, Ammi moussa, and Tissemsilet.

The obtained a very closed results demonstrate that the stems and leaves have approximatly the same anti-inflammatory activity leaves. . Regarding DPPH radicals scavenging

activity, the leaves of plants collected from the different regions have higher DPPH ICs compared to the stems of plants, which means that they have weak antioxidant activity.

The phytochemical analysis showed that the ethanolic axtracts are rich mainly in polyphenols and flavonoids

However, the content of tanins is higher in stems.

These results demonstrates clearly that the different parts of C. spinosa L. have antioxidants and anti-inflammatory properties thanks to their phytochemical compounds. This supports the use of this plant in the preparation of medical remedies.

Resumé

Capparis spinosa, une plante arbustive avec une large plage de croissance et une distribution naturelle répandue, a longtemps été utilisée dans la médecine traditionnelle et l'alimentation du bassin méditerranéen. L'objectif de cette étude est d'évaluer les activités biologiques des populations naturelles de *C.spinosa* appartenant à trois régions d'Algérie, à savoir Mostaganem, Ammi Moussa et Tissemsilet.

Les résultats obtenus montrent très clairement que les tiges et les feuilles ont approximativement la même activité anti-inflammatoire. En ce qui concerne l'activité de piégeage des radicaux DPPH, les feuilles des plantes collectées dans les différentes régions présentent des valeurs IC plus élevées par rapport aux tiges des plantes, ce qui signifie qu'elles ont une faible activité antioxydante.

L'analyse phytochimique a montré que les extraits éthanoliques sont principalement riches en polyphénols et flavonoïdes. Cependant, la teneur en tanins est plus élevée dans les tiges. Ces résultats démontrent clairement que les différentes parties de *C spinosa L*. possèdent des propriétés antioxydantes et anti-inflammatoires grâce à leurs composés phytochimiques. Cela soutient l'utilisation de cette plante dans la préparation de remèdes médicaux.

ملخص

Capparis spinosa

هو نبات شجيرة متنامي ينتشر في نطاق واسع ويوزع طبيعيًا في منطقة البحر الأبيض المتوسط، وقد استُخدم منذ فترة طويلة في الطب التقليدي والغذاء في المنطقة. الهدف من هذه الدراسة هو تقييم الأنشطة البيولوجية للسلالات الطبيعية لهذه النبتة التي تنتمي إلى ثلاث مناطق في الجزائر، وهي مستغانم وعمي موسى وتيسمسيلت.

تظهر النتائج التي تم الحصول عليها أن السيقان والأوراق لها نشاط مضاد للالتهاب متقاربة جدا من بعضهما البعض. بالنسبة لنشاط امتصاص الجذور الحرةDPPH ، و أن الأوراق التي تم جمعها من المناطق المختلفة لها قيم IC لـ DPPH أعلى بالمقارنة مع السيقان، وهذا يعني أنها لديها نشاط مضاد للأكسدة ضعيف..

أظهر التحليل الفيتوكيميائي أن الاستخلاصات الإيثانولية غنية بشكل رئيسي في المركبات البوليفينولية والفلافونويدات. ومع ذلك، فإن محتوى التانين أعلى في السيقان. توضح هذه النتائج بوضوح أن أجزاء هذه النبتة مض .المختلفة لها خصائص مضادة للأكسدة ومضادة للالتهاب بفضل مركباتها الفيتوكيميائية. وهذا يدعم استخدام هذا النبات في إعداد العلاجات الطبية.

Summary

LIST OF ABREVATION

WHO: The World Health Organization

C. spinosa : Capparis spinosa

BSA : Bovine Serum Albumin

DPPH : 1,1diphenyl-2-picrylhydrazyl

PBS : Phosphate-buffered saline

IC50: Concentration of inhibition

DMSO : Dimethyl sulfoxide

MIC : Minimum inhibitory concentration

L38: Leaves Tissemsilt

S38: Stems Tissemsilt

L48: Leaves Ammi Moussa

S48 : Stems Ammi Moussa

L27: Leaves Mostaganem

S27: Stems Mostaganem

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Introduction

Introduction

Bacterial resistance to antibiotics is a major problem complicating the treatment of bacterial infections, especially through the spread of multi-resistant strains **(Ventola, 2015).** In recent years, research has therefore focused on the characterization of antimicrobial agents of natural origin, such as bacterial peptides, bacteriophages and bioactive plant molecules that can replace conventional antibiotics or act synergistically with them **(Kordali et al., 2008).**

Man has used a variety of plants found in the environment to treat and cure various illnesses. Today, plants seem to play an important role in healing throughout the world. Indeed, throughout our history, humans have always lived with plants, and are therefore used to consume them, which are not only pleasant in terms of taste and nutritional value, but also have undeniable medicinal value thanks to their compounds, making them a significant therapeutic asset compared with chemical treatments **(Karou et al., 2011; Benarba et al., 2015)**.

In general, medicinal plants naturally synthesize and accumulate secondary metabolites with potential pharmacological activity, such as alkaloids, sterols, flavonoids, glycosides, and tannins. **(Hussein et al., 2018; Singh et Sharma, 2020; Zhang et al., 2021)**.

Some 6377 plant species are used in Africa, of which over 400 are medicinal plants, contributing to 90 % of medical therapies. In 2004, it was estimated that about 75 % of the African population still uses plants for self-treatment **(Bennoune et al. 2012).**

Algeria has an important floral biodiversity, which represents an inexhaustible source of traditional and effective remedies due to the active principles found in these plants **(Benachour 2020).**

According to the **OMS (2013)**, in certain developing countries in Asia, Africa and Latin America, 80 % of people without access to modern medicine depend on traditional medicine, particularly in rural areas, because of the proximity, accessibility and low cost of care. In Algeria, the use of medicinal plants occupies an important place in traditional medicine and continues to be very active, particularly among local populations **(Bouzid et al., 2017; Lazli et al., 2019)**.

Capparis spinosa, or caper, is one of the rare shrubby species that has so many uses. In Algeria, the caper covers vast areas but in a sparse manner **(Benseghir-Boukhari and Seridi, 2007)**. The *C. spinosa* species has been the subject of several chemical investigations,

recording the presence of many types of secondary metabolites **a**nd so it has important medicinal qualities used in traditional medicine **(Benseghir-Boukhari and Seridi, 2007; Anwar et al., 2016).**

In fact, the roots, leaves and fruits of this species have been traditionally used to treat various ailments such as gastrointestinal disorders, skin diseases, earache, kidney and liver diseases, among others.

In addition, biological studies show significant activities such as antioxidant, antiinflammatory, immunomodulatory, antiviral, antiplaque antimicrobial, anticarcinogenic, antidiabetic and antihepatotoxic. Capers are also known for their diuretic, astringent and antirheumatic properties in traditional medicine **(Rajhi et al., 2019).**

In this context, this study is a part of the valorization of the Algerian natural biological resources, mainly medicinal plants, and to obtain maximum information about their properties.

The specific aim of this study is to determine the phytochemical composition and evaluate some biological activities of the aqueous and ethanolic extracts of stems and leaves of *C. spinosa* belonging to three different regions from Algeria, namely Mostaganem, Ammi Moussa (Relizane) and Tissemsilet.

Literature Review

Literature review

1. Generalities

The Mediterranean flora is home to representatives of subtropical or tropical plant families, including the Capparidacee family **(Paccalet, 1981).** The small family Capparidaceae is represented in Algeria by the thorny caper (*Capparis spinosa.)* **(Lapie et Maige, 1914),** which is a spontaneous shrubby perennial, xerophytic and heliophilous **(Benseghir-Boukhari and Seridi, 2007)**.

The caper, *Capparis spinosa* L. (Fig. 1), is a native Mediterranean shrub that thrives in North Africa, Italy, Greece, Central Asia, Iran and other regions of the world **(Zarei et al. 2021)**. The plant has been part of the Mediterranean food for almost 5,000 years, dating back to the middle Bronze Age **(Güleryüz et al. 2009)**.

Figure 1. *Capparis spinosa* (Giuseppe MAZZA).

2. Botanical classification

Capparis spinosa is commonly known by several names; *câprier* in French, caper in English, and *Kabbar* in Arabic, and Taylalouth in Tamazight (**Meddour, 2011; Chedraoui et al. 2017)**. According to APG (Angiosperm phylogeny Group) (2016). The classification of is as follows:

- **Kingdom:** Plantae
- **Sub-kingdom**: Tracheobionta
- **Class:** Magnoliopsida
- **Sub-class:** Dilleniidae
- **Order:** Capparales
- **Family:** Capparacea
- **Genus:** Capparis
- **Species :** *Capparis spinosa*

3. Botanical description

Capparis spinosa is an upright shrub with flexuous, thorny stems bearing smooth, green and often a little reddish, with spines at the base. The flowers are axillary, solitary, composed of four large green sepals and four white petals with pink veins, numerous very long stamens and a very long pistil which and a very long pistil that emerges from the flower. The fruit is ovoid and oblong, up to 3 cm long. The seeds are black, smooth and kidney-shaped, 2 to 3 mm long. The roots are fleshy, well-developed and deep **(Inocenio et al., 2006; Sher and Alyemeni, 2010; AlSoqeer, 2011; Fici, 2014).**

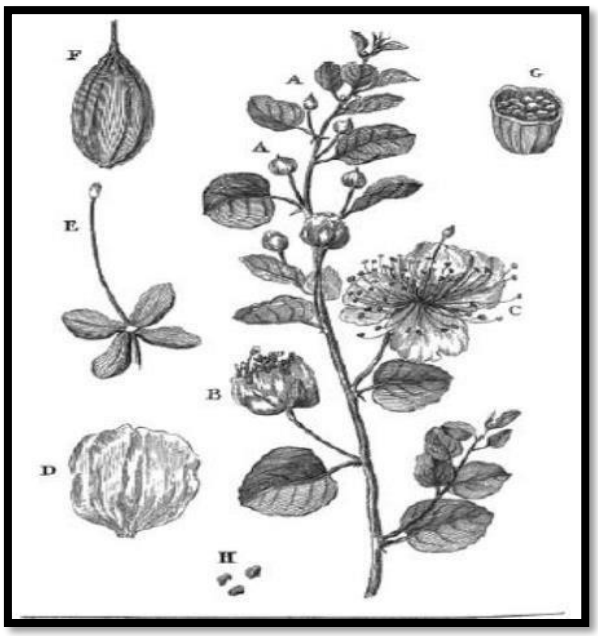


Figure 2. The different parts of *Capparis spinosa* L. (Rozier, 1782).

A: flowering bud**, B:** bud ready to open**, C and D:** the flower composed of four petals arranged in a pink, white, notched, large and open; the stamens, of indeterminate number from sixty to one hundred, coloured red**, E:** the pistil is green throughout its length, larger than the stamens, and reddish at its apex**. F: fruit**, a fleshy berry with a single compartment, shown cut horizontally at **G,** containing white, kidney-shaped **H:** seeds.

4. Distribution and ecology

The caper is a shrubby perennial plant that is widespread in the countries of the Mediterranean basin, and in dry environments along the coast of Europe, from North Africa across the Mediterranean basin to southern Asia and Australia **(Benzidane, 2014).**

The caper has morphological and physiological characteristics that give it a high tolerance to drought. It therefore has the particularity of growing in the most ungrateful soils and on steep

slopes, hence its ecological value in combating erosion in arid and semi-arid zones. It has been reported in the most xerophilous sites. **(Maire, 1965; Ozenda, 1983; Kadik, 1986).**

5. Geographical repartition

5.1. In the world

The capers grow naturally in the steppes of North Africa and in the Mediterranean countries, growing wild on walls or along rocky shores, although they have recently been cultivated and traded **(Rhimi et al. 2012; Moufid et al. 2015).**

C. spinosa is distributed from Morocco to the Black Sea, along the Atlantic coast of the Canary Islands, east of the Caspian Sea, Europe, North Africa, western Asia, Australia and Afghanistan **(Chedraoui et al. 2017)**. It is not known whether the plant is native to these areas (Fig. 3). The caper species may have originated in the tropics and then spread to the Mediterranean and Central Asia **(Faran 2014).**

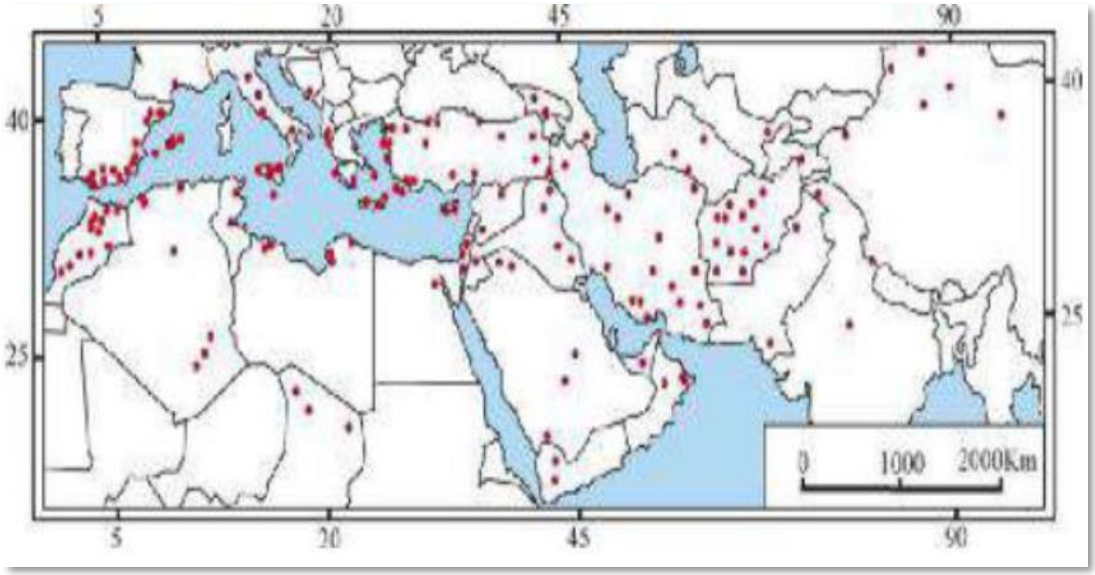


Figure 3. Distribution of *Capparis spinosa L*. (•) in the Mediterranean basin (Jiang et al., 2007).

5.2. In Algeria

The caper harvest is best in June in Algeria and is mainly used for traditional medicines. It covers large areas in Algeria, albeit scattered. It was recently rediscovered by foresters who began studying its evolution. This could indeed be planted in agriculturally unsuitable areas to green areas where it would not be possible to crowd out sensitive species. During the field season, it was discovered that two phenotypically distinct caper plants can coexist in the same season without ecological variation. Between the cultivar inermis there are less prickly cultivars, some of which appear to bear fruit only rarely **(Benseghir-Boukhari and Seridi 2007).**

6. Genetic diversity

Capparis spinosa displays significant variation in its morphology, which can be attributed to factors such as phenotypic plasticity, eco-geographical differentiation, topographical changes, and hybridization processes. These factors contribute to the occurrence of intermediate forms, resulting in a complex and diverse structure within wild *C. spinosa* populations (**Chedraoui et al., 2017)**. Recognizing the limitations of relying solely on physical characteristics for distinguishing between Capparis species, alternative approaches have emerged, such as the utilization of molecular markers. These molecular-based methods have gained popularity as they offer a more effective means to overcome the challenges encountered in *Capparis* taxonomy. By analysing genetic diversity and relationships among Capparis species, valuable insights can be gained for genetic resource conservation and the establishment of a Capparis breeding program. Such studies can guide the selection of appropriate parental lines, enabling the creation of optimal cross combinations to enhance desirable traits with broad applications in agriculture, food industry, and medicine **(Aichi-Yousfi et al., 2016).**

7. Traditional use

In ancient times, *Capparis spinosa* was used to treat paralysis in the Romans and diabetes in the Moroccans. The Egyptians also used the roots to treat liver and kidney diseases **(Tlili et al., 2011).** Capers are considered a unique ingredient in Italian cuisine, particularly in Sicilian and southern Italian cuisine. They are commonly used on pizzas and in salads, pasta sauces and meat dishes **(Panico et al., 2005)**.

Dried caper leaves are also used as a substitute for the enzyme rennet, which is mainly used in the production of high-quality cheeses **(Panico et al., 2005; Musallam et al., 2011)**.

The various parts of *C. spinosa*, including the fruit and roots, are widely used in traditional medicine for the treatment of a number of diseases, and to date **(Mansour et al., 2016)** have been used mainly as a remedy for: fever, headache and toothache, rheumatism, convulsions, menstrual pain, skin and kidney diseases, liver diseases, diabetes, haemorrhoids, ulcers, gout .... **(Zhang and Feei Ma, 2018)**. In Algeria, capers are used for nutritional and medicinal purposes by rural people in the northern regions of Sétif, Bordj Bou Arreridj and Mila. It is known that caper root bark is used in the Sétif region to treat rheumatism **(Benseghir-Boukhari and Seridi, 2007)**. The following table summarises some of the traditional uses of *Capparis spinosa*.

Table 1. Some traditional uses of *Capparis spinosa*

Indication	Used part	Method of use	Reference
Rheumatism, cold, migraine	Leaves and fruits Decoction	Infusion, ointment	Ould El Hadj et al. (2003)
Cough and diabetes	Fruits	Decoction	Kusmenoglu et al. (1997)
Hypertension and diabetic complications	Dried fruits Powder	Orally with a glass of water	Sher and Alyemeni (2010)
Flu and related infections	Flower buds	Infusion	Sher and Alyemeni (2010)
Eye infections	flower buds of capers and root bark	External use	Aniyathi et al. (2009); Sher and Alyemeni (2010)
Hydropsy, anemia, and rheumatism	Root bark	Decoction	Chopra et al. (1986); Brown (1995); Aghel et al. (2007); Aniyathi et al. (2009)
Rheumatism and gastrointestinal complications Flower	buds with root	Infusion	Sher and Alyemeni (2010)
Diuresis and appetite stimulation	Stem bark	Decoction before meals	Sher and Alyemeni (2010)
Hepatoprotective	Root bark	Infusion	Sher and Alyemeni (2010)
Capillary weakness and skin diseases	Flower buds and root bark	External use	Aghel et al. (2007); Sher and Alyemeni (2010)

8. Biochemical composition of *Capparis spinosa* L.

Capparis spinosa has been studied for its biochemical content, which is influenced by several factors such as geographical and environmental conditions, harvest date and size, preservation procedures, genotype and extraction methods. All these studies have indicated the richness of capers in phenolic compounds and flavonoids (Sozzi and Vicente, 2006; Tlili et al., 2010 a).

Several bioactive chemical components have been identified from different parts of *C. spinosa* :

- **Flavonoids**: various flavonoids have been identified, including rutin, quercetin and kaempferol and their derivatives (Sharrif moghaddasi et al., 2012)
- **Alkaloids**: such as spermidines, alkaloids isolated from roots (Fu et al., 2008).
- **Terpenoids:** tocopherols (α-tocopherol, γ-tocopherol and δ-tocopherol), vitamin C (Tesoriere et al., 2007) and carotenoids (lutein and β-carotene), (Matthous and Ozcan, 2005; Tlili et al., 2009a; Tlili et al., 2009b) and fatty acids, mainly oleic, linoleic and linolenic acids (Matthous and Ozcan, 2005; Tlili et al., 2010b).
- **Carbohydrates** (glucose, arabinose, mannose and galactose, etc.) (Demir et al., 2008).
- **Proteins** (Demir et al., 2008).
- **Glucosinolates**: the caper is one of the species rich in glucosinolates and isothiocyanates (Calis et al., 1999; Calis et al., 2002).
- **Mineral elements:** mainly K, Mg, Ca, Na, Zn, Cu, Fe and P (Rodrigo et al., 2006; Giuffrida et al., 2002).

The therapeutic efficacy of the plant's organs, based on ethnobotanical references seems to be producing results for natural anti-cancer and anti-inflammatory treatments **(Benseghir-Boukhari and Seridi, 2007)**. The antioxidant activity of *C. spinosa* extracts has been demonstrated in several studies **(Aichour, 2017; Wojdyło et al., 2019).** According to **Meddour (2013)** aqueous and methanolic extracts have a polyphenolic content and strong antiradical activity. It also has anti-diabetic, hypolipidaemic and anti-obesity effects **(Eddouks et al., 2004; Lemhadri et al., 2007)**. *Capparis spinosa* is an important source of various bioactive secondary metabolites of interest to humanity **(Fadili et al., 2017; Zhang et Ma, 2018).**

Table 2. Some important *Capparis spinosa* components.

Plant parts	Their composition	Reference
Roots	- Spermidine, alkaloids, Degradation products of glucosinolates, Glucosinolates	(Khatib et al., 2016), (Fu et al., 2008), (Afsharypuor et al., 1998), (Satyanarayana et al., 2008).
Aerial part	- Terpene, Glycosinolates, Vitamin E, Alkaloids, Polyphenols	(Ascrizzi et al., 2016; Matthaus et al., 2002; Matthaus et al., 2005; Yang et al., 2010; Wojdyło et al., 2019.

These secondary metabolites are commonly associated with heat tolerance and play a role in abiotic stress responses **(Wahid 2007; Benzidane et al. 2020).** These phytochemicals have also been shown to have antioxidant and antimicrobial properties **(Krimat et al. 2014).** The seeds are high in protein, oil and fibre and could be used as food.

They synthesise mixtures of two proteins: a lectin with haemagglutinating activity and a nonlectin protein without haemagglutinating activity. Both proteins have antifungal properties (**TziBun et al. 2011).** Due to their strong antioxidant activity and high phenolic content, caper seeds have been identified as an important source of antioxidant molecules for the food and pharmaceutical industries **(Tlili et al. 2015)**. The oil content of the seeds ranges from 27.3 to 37.6 g/100 g, dominated by linoleic acid (24.6-50.5%), oleic acid and its isomer, vaccenic acid, tocopherols, sterols (sitosterol, campesterol, stigmasterol and 3-avenasterol) and glucosinolates, as well as the minerals Al, P, Na, Mg and Fe **(Akbar 2020)**. *C. spinosa* berries contain 5% carbohydrates, 3% fibre, 29% protein and 2% fat. They contain a moderate amount of vitamin C (4mg/100g fw) **(Allaith 2016).**

- **Polyphenols**

Phenolic compounds are one of the most important groups in plants. They are widespread products of the secondary metabolism of plants and contain phenolic groups, with or without other functions, and have at least 9,000 different known structures **(Bahorun, 1997)**. Polyphenols are a group of molecules with a variety of structures that are widely used in phytotherapy. They are generally derived from cinnamic acid formed by the shikimate and acetate-malonate pathways. acetate-malonate pathway and can be divided into different classes based on their molecular structure **(Gorham, 1977; Mohmmadi, 2013)**. They are very effective in plant tolerance to various stresses, so these compounds play an essential role in theessential role in the balance and adaptation of the plant in its natural environment **(Macheix et al., 2005)**.

- **Flavonoids**

Flavonoids are molecules of plant origin. They are pigments that give colour to flowers, fruits and in some cases leaves (Milane., 2004). They are the most representative group of phenolic compounds, these molecules have a variety of chemical structures and structures and properties **(Benhammou, 2011)**. The chemical nature of flavonoids depends on their structural class, degree of hydroxylation and methoxylation. hydroxylation and methoxylation, the degree of polymerisation, substitutions and conjugations on the conjugations on the ring, i.e. the presence of a C2-C3 double bond, the 3-O group and the4-oxo function **(YAO et al., 2004)**.These are benzo-y-pyran derivatives. Their basic structure is that of a diphenylpropane with 15 carbon atoms (C6- C3 - C6), consisting of two aromatic rings labeled A and B, linked by an oxygenated heterocycle, which is designated by the letter C **(Dacosta, 2003)**.

- **Tannins**

Tannins are the complex form of phenolic compounds with water-soluble molecules. watersoluble molecules, with the presence of various hydroxyl groups. which facilitates their combination with proteins (**Mangan, 1988; Makkar, 2003 ; Kamra et al, 2006 ; Khenaka, 2011 ; Mcsweeney et al., 2001)**. They are widespread in the plant kingdom, but are particularly abundant in certain families **(Ghesterm et al., 2001)**. They can be found in different organs: bark leaves, fruits, roots and grains (**Khanbabae and Ree, 2001)**. Tannins have the potential to be the most important class of secondary metabolites in the defence of plants against herbivory, and because of their mode of action, they are generally considered to be a generalised defensive substance whose adverse effects are largely are largely independent of molecular structure **(Hemingway and Lak, 1992).**

9. Biological properties of *Capparis spinosa* L Anti-oxidant activity

Protection against the deleterious effects of reactive oxygen species (ROS) is achieved by several mechanisms, including non-enzymatic proteins, enzymes such as superoxide dismutases (SODs) and glutathione peroxidases (GPx), and dietary antioxidants such as carotenoids, tocopherols (vitamin E), ascorbic acid (vitamin C), and polyphenols **(Ferreira et al. 2019; Goyal et al. 2020)**. Non-enzymatic proteins such as transferrin, ceruloplasmin and ferritin act as ROS scavengers. Enzymes such as SODs, GPx and catalase (CAT) play a crucial role in mitigating oxidative stress in living organisms **(Valko et al. 2005)**. Antioxidants such as carotenoids (e.g. beta-carotene, lycopene and lutein), tocopherols (vitamin E), ascorbic acid (vitamin C) and polyphenols (e.g. resveratrol, quercetin and catechins) are essential antioxidants for humans as they play a crucial role in scavenging ROS and reducing oxidative stressrelated damage **(Kapoor et al. 2018; Rothwell et al. 2019)**. Therefore, by consuming a balanced diet enriched with adequate amounts of these essential antioxidants, we can combat the adverse effects of oxidative stress on our bodies.

The use of antioxidants is widespread in primary and secondary prevention. Well-known antioxidants such as beta-carotene, ascorbic acid, anthocyanins, polyphenols and flavonoids are widely used in this context **(Bjelakoic et al. 2007)**. Flavonoids can scavenge free radicals produced by our bodies in response to environmental aggressions that promote cellular ageing **(Karbin et al. 2015)**.

Methodology

III. Methodology

III.1. Aim of the study

This study aimed to quantify some phytochemicals components of *C. spinosa* from different regions of Algeria. As well, their biological activities (antibacterial, antioxidant, antiinflammatory, and hemolytic) were evaluated.

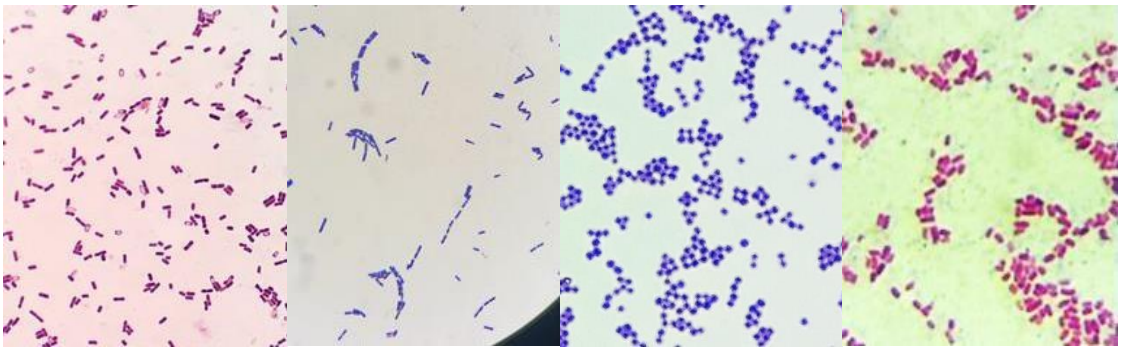
III.2. Materials

1. Plant material

The plant *C. spinosa* was harvested from different regions in Algeria: Ammi Moussa (Relizane), Tissemsilt and Mostaganem.

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. 4).



P. aeruginosa *B. cereus* *S. aureus* *E. coli* **Figure 4.** Microscopic observation of the tested bacterial strains.

III.3. Methods

3.1. Powder Preparation:

After the drying of plant parts (Fig. 5) (Stems and leaves) in the open air, leaves and stems are ground using an electric grinder to obtain a powder. The resulting powders are put in closed bottles away from the light.

stems

Leaves

Figure 5. parts of the *Capparis spinosa* plant

3.2. Preparation of plant extracts

Extracts were prepared by the modified method of **Diallo et al. (2004).**

3.3. Maceration

Plant extracts were prepared using 50 g of powder mixed with 500 ml of distilled water for the aqueous extract and with 500 ml ethanol 70 % for the ethanolic extract. The whole is stirred for 15 minutes in the darkness at ambient temperature then left to macerate for 24 h. The obtained mixture was filtered using Whatman paper

3.4. Evaporation

3.4.1. Rotavapor processes

The filtrate was evaporated using a rotavapor (Fig. 6) which allows the solvent to be removed under vacuum by means of the following steps:

- Start heating the water bath. Wait until the desired temperature is reached.
- Pour the solution into the evaporating flask.
- Continue to evaporate until the solvent has completely disappeared.
- Remove the flask from the Rotavapor and allow it to cool.
- Scrape the flask with a spatula and transfer the contents to a Petri dish.
- Place the Petri dishes in the oven (40°C) until the extracts are solid.

Rotavapor or evaporator

The final dry extract

Figure 6. The evaporation stage.

3.4.2 The rotator constitution

It consists in particular of (Fig 4):

- A flask (1) containing the mixture of solvent to be evaporated
- A water bath with temperature control
- A serpentine cooler for the liquefaction of solvent vapors
- A flask (2) for the recovery of the liquid solvent (after liquefaction of the vapors)
- A motor for the rotation of the flask with the mixture to be evaporated to obtain more uniform evaporation.

It is connected to a system for reducing the pressure inside the apparatus (example: a pump). The flask (1) contains the mixture whose solvent is to be evaporated. The solvent evaporates and the vapors produced are condensed by the refrigerant in a different container from the flask (1): the recovery flask (2).

3.4.3 Evaluation of the antimicrobial activity

The antimicrobial activity of the extracts was determined by the agar diffusion method cited by **(Treki et al., 2009).** To prepare this medium, start by weighing 38 g of powder and combining it with 1 L of water. Thoroughly mix the ingredients to ensure homogeneity, and then heat the mixture while stirring. Bring it to a boiling point and let it boil for about one minute. Once the boiling is complete, the agar needs to be sterilized to ensure a sterile

environment. This can be done by placing the agar in an autoclave and subjecting it to a sterilization process at a temperature of 121.1°C for 15 minutes.

3.4.3.1 Disc method

The procedure began by streaking the different bacterial strains using the streaking method. These streaked plates were then placed in an incubator at 37°C for a period of 18 to 24 hours. This incubation period allowed the growth of a young bacterial culture with isolated colonies. These isolated colonies were then used to prepare the inoculum. They were immersed in tubes containing a sterile solution of distilled water, with the aim of achieving a specific initial cell density or turbidity similar to 0.5 McFarland units. We used four strains: *Escherichia coli, Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus* .

After adjusting the turbidity of the inoculum suspension, a swab was immersed in the suspension and used to streak the entire surface of the Mueller Hinton agar three times. After each swab, the Petri dish was rotated approximately 60° to ensure even distribution of the inoculum. Swabbing was also performed around the periphery of the agar surface.

To investigate the antimicrobial activity, sterile Whatman paper discs were saturated with increasing concentrations (10mg/ml,15mg/ml,20mg/ml) of dried extracts dissolved in dimethyl sulfoxide 70% (DMSO). These discs were then carefully placed on the surface of the Mueller Hinton agar using forceps. Following the application of the discs, the Petri dishes were incubated for a period of 24 hours at a temperature of 37°C.

3.4.3.2 Wells method

In this procedure, the bacteria are introduced into agar medium that contains five wells.

Among these wells, four are filled with 20 µl of extract, while the fifth well is filled with

DMSO.

4. Biological activity

4.1. Evaluation of the antioxidant activity

1. DPPH test

1.1. Protocol

The DPPH radical reduction activity was carried out using the method of **Brand Williams et al. (1995)**

with some modifications**.** A volume of 1 ml of DPPH solution was introduced into 200 ul of a solution

Figure 7. DPPH solution with sample before incubation

containing plant extracts at varying concentrations (0,05 mg/ml, 0,01 mg/ml, 0,005 mg/ml, 0,001g/ml,).

Following a 30-minute incubation at room temperature, the absorbance was determined at 517 nm. All concentrations were repeated in triplicate (Fig. 7).

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 the reaction mixture indicated higher free radical activity. The percentage of the radical scavenging activity (RSA) was calculated based on the following equation:

DPPH Scavenged (%) =[(A control – A sample)/ A control]× 100.

4.2. Anti-inflammatory activity

4.2.1. Protocol

A volume of 0,5 mL of each extract with a different concentration (1 mg/ml, 5 mg/ml, 10 mg/ml, 15 mg/ml) was mixed with 0,5 mL of Bovine Serum Albumin solution (0.2 %) prepared in Tris-HCI buffer (50 mM, pH 6.6). The mixture was allowed to stand for 15 min at 37°C and then heated in a bath water at 72 °C for 5 min. The absorbance was recorded at 660 nm using a UV-visible spectrophotometer after cooling to room temperature. The experiment was performed in triplicate.

Diclofenac sodium was used as a standard. The protective effect of samples against the denaturation of BSA was presented as inhibition percentages calculated using the formula:

Inhibition (%) = [(Abs control - Abs sample) / Abs control x 100

4.3. Hemolytic activity

4.3.1. Preparation of Phosphate-buffered saline (PBS):

To prepare 1L of Pbs dilute 8g of sodium chloride (NaCl), 0.2g of potassium chloride (KCl), 1.44g of monobasic sodium phosphate (NaH2PO4) and 0.24g of dibasic potassium phosphate (K2HPO4) in 1L of distilled water, after stirring until the products are completely dissolved then adjust solution pH to 7.4 with hydrochloric acid (HCl) or sodium hydroxide (NaOH).

4.3.2. Preparation of the red blood cell suspension

Healthy volunteers' blood cells (O positive) were collected and subjected to centrifugation at 1500 rpm. The resulting pellet was then washed using a phosphate buffer saline solution (PBS) with a pH of 7.2 ± 0.2. The centrifugation was carried out at 1500 rpm for 5 minutes, and the cells were subsequently suspended in a 4% PBS solution **(Kalita et al., 2011).**

4.3.3. Protocol

For the determination of hemolytic activity, the method described by **Kumar et al. (2011)** was followed. Briefly, 1 mL of red blood cell suspension was mixed with 1 mL of extracts at different concentrations prepared in PBS (5 mg/ml, 10 mg/ml,/15 mg/mL). The mixture was then incubated at 37°C for 30 minutes, followed by centrifugation at 1500 rpm for 10 minutes. The resulting supernatant (Fig. 8) was measured at a wavelength of 540 nm. For the positive and negative controls, distilled water and PBS, respectively, were used under the same conditions. Each experiment was replicated three times. The percentage of hemolysis was calculated according to the following equation:

$$\text{Hemolysis (\%)} = (\text{At} - \text{An} / \text{Ac} - \text{An}) \times 100$$

Where: At: absorbance of the tested extract.

An: absorbance of the negative control (PBS).

Ac: absorbance of the positive control (distilled water)

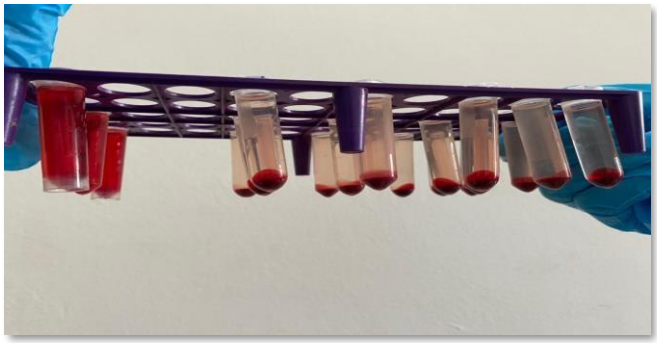


Figure 8. Hemolytic test.

5. Phytochemical characterization

5.1. Total polyphenols content

Total polyphenol content was estimated by the Folin-Ciocalteu method. Two hundred microliters of the sample of a concentration (5mg/ml) were added to 1 ml of Folin-Ciocalteu reagent diluted. After 5 minutes, 800 µl of saturated sodium carbonate (7,5%) was added. After 2 h incubation at room temperature, absorbance was measured at 765 nm. All tests were repeated in triplicate.

5.2. Total flavonoids content

The aluminum trichloride method is used to quantify flavonoids in *C.spinosa* extracts. 1 ml of sample with a concentration of 0,5 mg/ml (prepared in ethanol 10%) is added to 1 ml of AlCl3

solution (2% in methanol). After 10 minutes of incubation the absorbance was read at 430 nm (all tests were repeated in triplicate). (**Bahorun et al., 2003).**

5.3. Condensed tannins content

Add 50 µl of sample with a concentration of 5 mg/ml extract to 1500 µl of vanillin/methanol solution (4 %). Mix by vortexing and add 750 µl of concentrated hydrochloric acid (HCl). The resulting mixture was allowed to react for 20 min at room temperature. The absorbance was measured at 550 nm against a blank using a spectrophotometer.

Results

Results

The biological properties of leaves and stems of *Capparis spinosa* collected from different regions of Algeria were investigated. In addition to the comparison made between the different parts, the regional effect has been highlighted to demonstrate the effect of environmental variation on the biological activity of this species.

1. Antioxidant activity

• **DPPH assay**

The DPPH assay is based on the reduction of 2,2-diphenyl-1-picrylhydrazyl, a stable free radical. In fact, the DPPH assay has been used extensively to evaluate the antioxidant ability of samples to reduce DPPH by donating hydrogen to form neutral DPPH. The results were expressed as IC50, defined as the concentration of the substrate at 50 % inhibition.

In general, it seems that the leaves of plants collected from the different regions have higher DPPH ICs compared to the stems of plants, which means that they have weak antioxidant activity (Fig. 9).

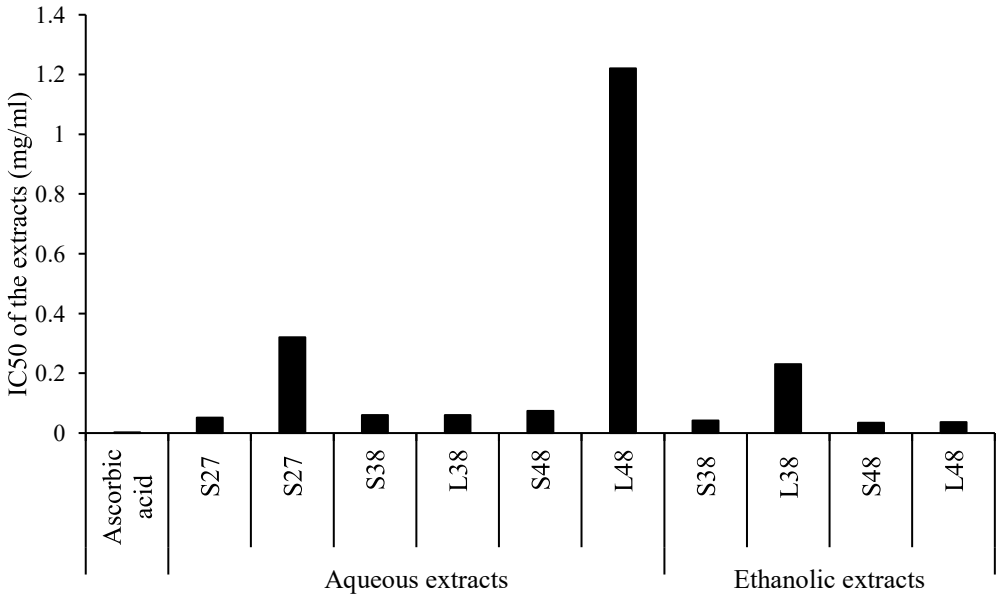


Figure 9. Antioxidants activity (IC50) of aqueous and ethanolic extracts of leaves and stems of *Capparis spinosa* L.

The aqueous extract of *C. spinosa* leaves from the region of Relizane L48 showed the highest IC50 value (1.22 mg/ml) compared to the other extracts. Whereas, the ethanolic extract of stems of plant from the same region (Relizane) showed the lowest IC50 value (0.034 mg/ml).

2. Phytochemicals

2.1. Polyphenols

The results obtained indicate the equivalent amounts of gallic acid present in the aqueous and ethanol extracts of *Capparis spinosa*.

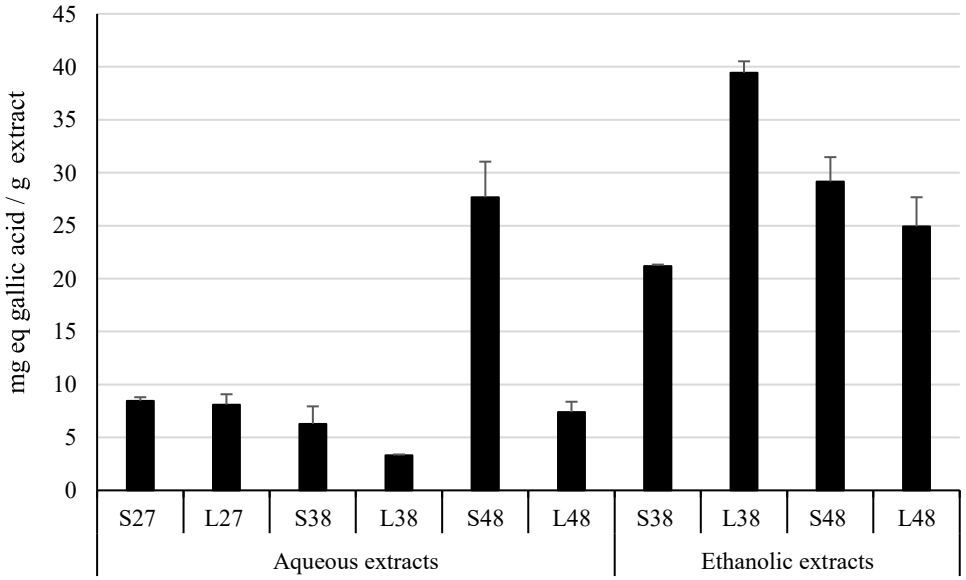


Figure 10. Polyphenols content in aqueous and ethanolic extracts of leaves and stems of *Capparis spinosa* L.

We notice that the ethanolic extracts of both plant parts (stems and leaves) demonstrated higher levels of gallic acid equivalents compared to the aqueous extracts with the leaves extract of the region of Tissemsilt showing the higher value (39.44 ± 1 mg GAE/ g extract). However, the aqueous extract of stems from the regions of Relizane showed higher phenolic content compared to the other aqueous extracts.

2.2. Flavonoids

The samples were analysed to measure flavonoids content as quercetin equivalent concentrations. All extracts showed relatively the same content in flavonoids except for the aqueous extracts of stems and leaves of the plants harvested from the region of Tissemsilt where flavonoids content were very low compared to the other extracts (Fig. 11).

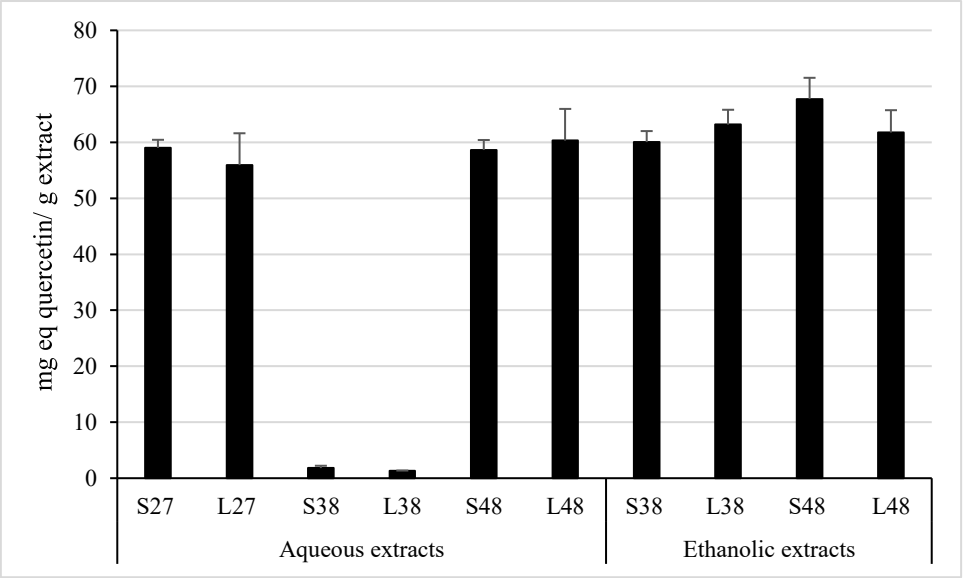


Figure 11. Favonoids content in aqueous and ethanolic extracts of leaves and stems of *Capparis spinosa* L.

2.3. Condensed tannins

The results of this study highlight the equivalent concentrations of catechin, a potentially beneficial antioxidant compound, in different samples of aqueous and ethanolic extracts.

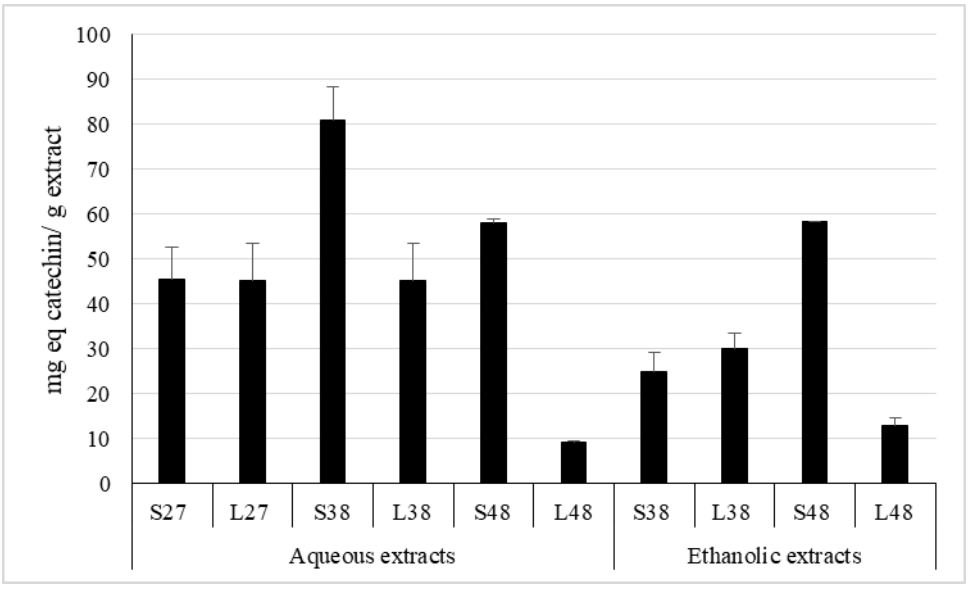


Figure 10. Condensed tannins content in aqueous and ethanolic extracts of leaves and stems of *Capparis spinosa* L.

Generally, we noticed that the aqueous extracts showed higher condensed tannins contents compared to ethanolic extracts. However, it should be noted that the aqueous and ethanolic

extracts of leaves of the region of Relizane showed very low contents compared to the other extracts (Fig. 10).

3. Anti-inflammatory activity

The anti-inflammatory activity of the aqueous and ethanolic extracts can be assessed by examining the percentages of inhibition of inflammation for each concentration of extract tested. We noticed that at different concentrations of plant extract the anti-inflammatory activity changes. At low concentrations 1mg/ml, both types of extracts and both plant parts demonstrated higher anti-inflammatory activity compared to the other concentrations. Indeed, we observed that with the increase in the concentrations some plant extracts, the antiinflammatory activity decreases such as in the ethanolic extract of stems of the region of Tissemsilt (Fig. 11).

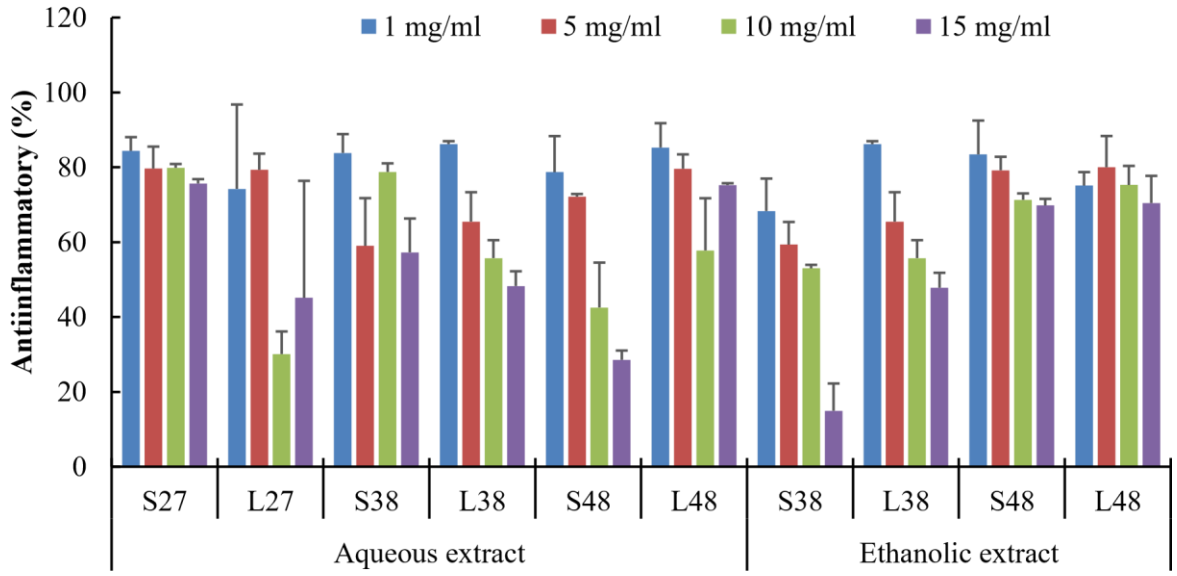


Figure 11. In vitro anti-inflammatory activity of aqueous and ethanolic extracts of leaves and stems of *Capparis spinosa* L.

4. Hemolytic activity

The hemolytic activity of aqueous and ethanolic extracts can be assessed by determining the percentage of erythrocyte lysis for each concentration. Overall, these results suggest that both aqueous and ethanolic extracts have hemolytic activity, although this may vary depending on the concentration and type of extract. It is important to note that high percentages of lysis may indicate toxicity to red blood cells, whereas lower percentages of lysis may indicate less toxicity.

We notice that higher hemolysis occurred at 10 and 15 mg/ml concentration of stems ethanolic extract of Tissemsilt, followed by the same concentrations for the aqueous extract of stems of the region of Mostaganem and the ethanolic extract of stems of the region of Relizane. However, the lowest hemolytic activity was observed in the both aqueous extracts of leaves of Tissemsilt and Relizane (Fig. 12).

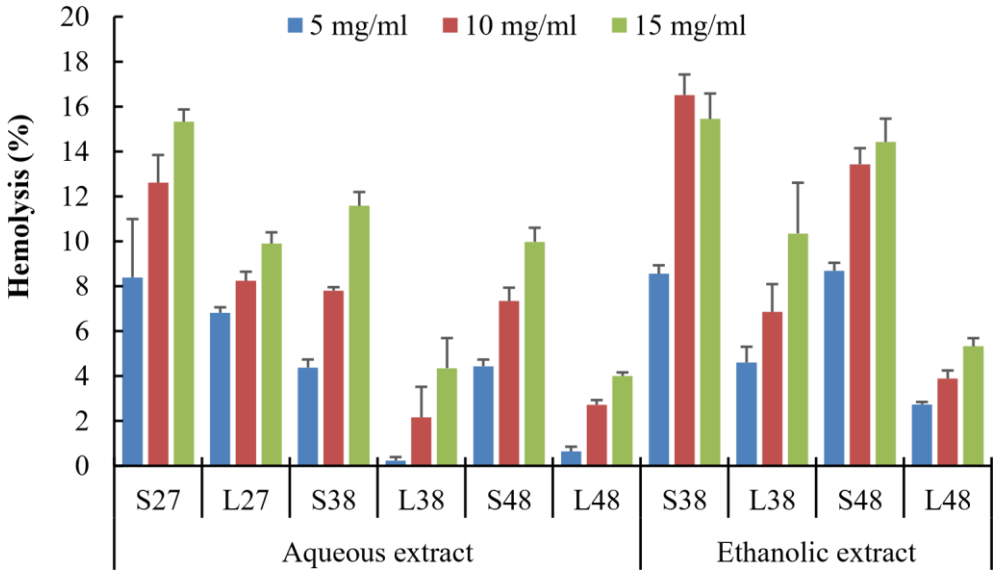


Figure 12. Hemolytic activity of aqueous and ethanolic extracts of leaves and stems of *Capparis spinosa* L.

5. Antimicrobial activity

No antibacterial action was observed with all extracts against the tested strains even at a hiogh concentration of 100 mg/ml (Fig. 13).

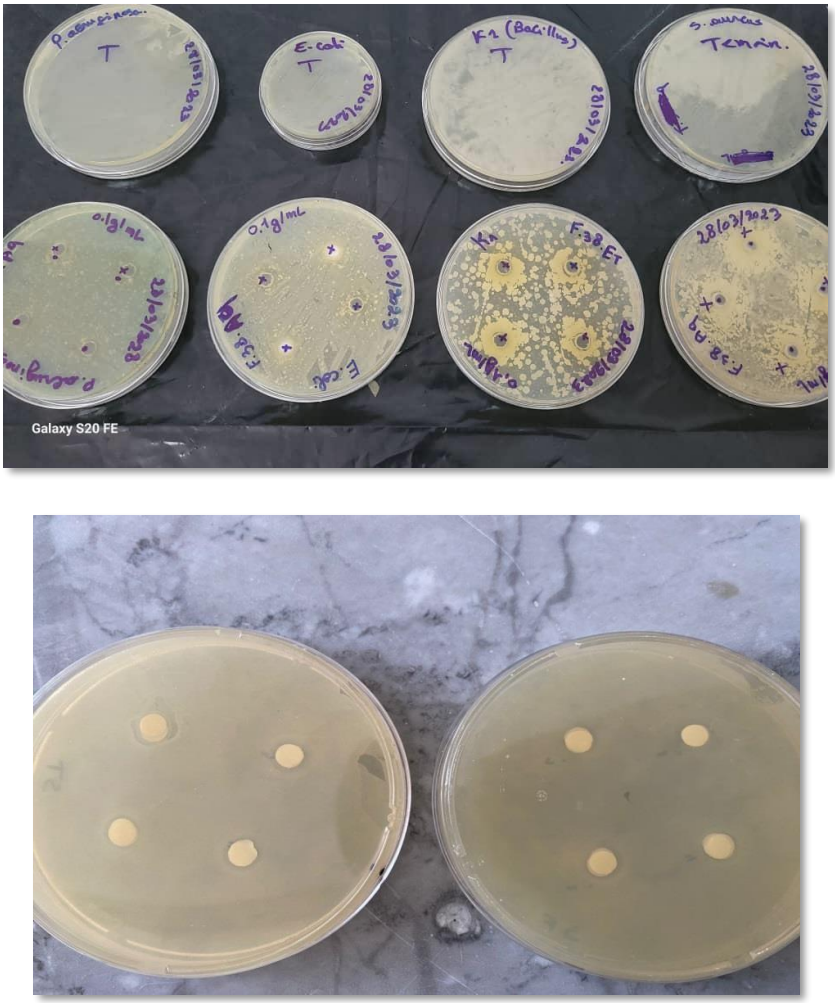


Figure 13. Results obtained from the antibacterial assay.

Discussion

Discussion

C. spinosa L., a plant commonly found in Mediterranean countries like Algeria, holds a prominent position in traditional medicine due to its extensive therapeutic properties (Benzidane et al. 2013; Mahboubi and Mahboubi 2014). Researchers have successfully isolated and identified numerous valuable chemical compounds from the various parts of this plant (Gull 2015).

Numerous studies have been conducted to explore the diverse biological activities exhibited by extracts of *C. spinosa* through in vitro and in vivo tests. This species has been identified to possess several pharmacological properties, while ongoing investigations continue to unveil additional potentials (Moufid et al. 2015). The objective of this study was to assess the anti-inflammatory and antioxidant activities, as well as the levels of phenolic compounds, flavonoids, and tannins in the leaves and stems of three populations of *C. spinosa* naturally growing in different regions of western Algeria : Ammi Moussa, Mostaganem, and Tissemsilt. The aim was to enhance the understanding of this underexplored species in Algeria. The findings revealed that *C. spinosa* serves as a valuable source of phytochemical compounds with diverse therapeutic activities. Furthermore, the phytochemical profiles exhibited variations between organs (leaves and stems) and regions highlighting the impat=ct of the environment.

In this study, *C. spinosa* aqueous extract was evaluated for antioxidant activity using DPPH assay. DPPH is a free radical compound that is widely used to determine the ability of various samples to scavenge free radicals, as evidenced by a colour change from deep purple to light yellow caused by the donation of hydrogen from an antioxidant to DPPH. The results showed that leaves extract from Ammi moussa region possess higher ICso values compared to all regions extracts. A study by Saeed et al. (2015) reported that aqueous extracts of *Capparis spinosa* had an IC50 value of 0.066 mg/mL, which is within the same range as the IC50 values obtained in this study. Similarly, another study by Bouzabata et al. (2009) found that ethanolic extracts of *Capparis spinosa* had an IC50 value of 0.082 mg/mL, which is also consistent with the results obtained in this study.

However, there are also differences in the results obtained in different studies, which could be due to variations in plant materials, extraction methods, and assay conditions. For instance, another study by Zouari et al. (2015) found that different parts of *Capparis spinosa* (leaves, flowers, and fruits) had different antioxidant activities, with the leaves showing the highest activity. Furthermore, the same study reported that the ethanolic extracts had higher

antioxidant activity than the aqueous extracts, which is contrary to the results obtained in this study.

Further studies are needed to better understand the antioxidant potential of *Capparis spinosa* and to optimize the extraction and assay methods.

Interestingly, it should be noted that the antioxidant activity of *C. spinosa* extracts is due to their richness in phenolic and flavonoid compounds, which have the ability to scavenge DPPH radicals and neutralise free radicals (Benzidane 2014).

The reducing power of *C. spinosa* extracts is probably due to the presence of phenolic compounds, whose hydroxyl groups can be attributed to electron donors, as it can also be related to the content of thiols and sulphur compounds. It can therefore be considered an important indicator of potential antioxidant activity (Rahi et al. 2021). Therefore, antioxidant activity is mainly due to natural antioxidants such as polyphenols, flavonoids and related compounds, which play an essential role in preventing oxidative damage caused by free and nitrogen radicals present in biological systems and foods. They are therefore very important in the prevention and management of various human chronic diseases, such as cardiovascular diseases, cancer, diabetes and degenerative diseases (Krimat et al. 2014).

The results obtained showed that the aqueous extracts of *C. spinosa* have a remarkable content of bioactive molecules, including polyphenols, flavonoids and tannins. Polyphenols are one of the most abundant and widespread families of compounds in the plant kingdom. It appears that the highest levels of polyphenols are found in ethanolic extract than aqueous extracts especially the stems. Chedraoui et al. (2017) reported 33.55 mg GAE/g DW of total phenolics in Tunisian aqueous leaf extract of *C. spinosus*. In contrast, Arrar et al. (2013) found that the amount of polyphenols in leaves was 56.98±14.24 mg GAEq/g.

Antioxidant activities are thought to help promote optimal health by protecting cellular components from damage caused by free radicals (Akkari et al. 2016). Nevertheless, differences in phytochemical composition were observed among the populations studied. These differences may be due to several factors, and the concentration of polyphenols and flavonoids may vary depending on different extraction methods, genetic factors and climatic conditions in different regions (Mar et al. 2019).

Flavonoids are a group of polyphenols that are widely distributed throughout the plant kingdom, where they serve as antioxidants, antimicrobials, photoreceptors, visual attractants and food repellents (Benzidarie 2014). The results show that the content of flavonoids was higher in ethanolic extract than aqueous extracts. According to Amar et al. (2013) and Benzidarie (2014), the content of flavonoids in leaves was estimated to be 6.7-40.2 mg

QE/mg and 6.69-0.24 mg QE/mg extract, respectively. These results are consistent with the study conducted by Ameni (2018), who reported that all plant organs contain polyphenols produced by plant secondary metabolism. Furthermore, Gull et al. (2015) showed that Capparis species are rich sources of flavonoids, including flavonoids, flavonols and flavons, in addition to their seed oil, which is rich in quercetin, 3-O-gentiobioside, and their fruits, which contain apigenin and kaempferol

Several phytochemical studies on capers show that they contain alkaloids, polyphenols, flavonoids (such as kaempferol, rutin and quercitin derivatives), phenolic acids, especially hydroxycinnamic acids (caffeic acid, ferulic acid, pcumaric acid and cinnamic acid), glucosinolates (glucocapparin, glucoiberin, sinigrin and glucobrassicin), vitamins C and E (Bennoune et al. 2012). Inocencio et al. (2000) stated that the wide variation in flavonoid content of capers from different regions may be due to environmental and physiological factors.

Tannins are secondary metabolites produced by plants. They have a wide range of structural variations (Belghoul 2020). The results obtained showed that the highest levels of tannins were found in the stems compared to the leaves. Rajhi et al. (2019) showed that the tannin content in the methanolic extract of the leaves was estimated to be 11.4±0.9 mg CE/g DW. The presence of phenolic compounds such as flavonoids and tannins in the crude aqueous extracts of *C. spinosa* showed potential as an in vitro dose-dependent antioxidant. dependent antioxidant activity (Akkari et al. 2016). The difference in tannin content between plant parts in three regions is due to the fact that the total phenolic content varies depending on different plant species and parts, growth conditions and stages, extraction technique and other factors (Mohebali et al. 2016).

According to Meddour (2019), the distribution of secondary metabolites may differ between organs and during plant growth, which may be related to climatic conditions (high temperature, sun exposure, drought and salinity) that favour the biosynthesis of secondary metabolites such as polyphenols. Akkari et al. (2016) showed that the total polyphenol content in the leaves of *C. spinosa* was higher in the flowering stage than in the vegetative stage. Studies have shown that the secondary metabolites of plants, including capers, are extremely sensitive to stress, biodiversity, perturbations and environmental changes. This influences genetic changes either by mutation or by methylation and acetylation as they enter into obtaining epigenetic changes, where several parameters are highlighted in this case, such as light, temperature, humidity, pressure and water, which also play an important role, and its limited presence in *C. spinosa* contributes to a better photosynthetic performance. In addition,

the variation in mineral content, which is generally related to the harvest date, bud size and nitrogen-fixing microorganisms in the rhizosphere of *C. spinosa* (Ephraim et al. 2014). In terms of antibacterial activity, we noticed none on the tested strains. Proestos et al. (2006) studied the leaves of *C. spinosa* and found that this extract was inactive against *E. coli* but showed suspicious activity against *S. aureus*. Similarly, ethyl acetate and n-butanol extracts of the leaves showed moderate to high activity ranging from 10 to 30 mm against *E. coli* and *S. aureus* (Fadlelmula et al., 2019). Mahboubi and Mahboubi (2014) indicated that root extracts (ethanol, methanol and ethyl acetate) showed higher antimicrobial activity compared to the fruit extracts. However, there are also differences in the results obtained in different studies, which could be due to differences in plant materials, extraction methods and test conditions. Another study done by Benachour et al. (2020) showed that *Bacillus cereus* was the most sensitive to *C. spinosa* essential oil, while E. coli was the most resistant to oils.

Many medicinal plants have anti-inflammatory properties. This can help the body respond to infection and injury.

We worked on two extractions, one aqueous and the other ethanolic, of the leaves and El Azhary et al. (2017) demonstrated that the anti-inflammatory activity of human blood mononuclear cells induced mainly by *C. spinosa* leaf extracts was due to the regulation of cytokine gene expression, which plays a primordial role in cell signalling and inflammatory response. These extracts have the ability to suppress the expression of interleukin-17 (IL-17), a pro-inflammatory cytokine, and to promote the expression of IL-4, an anti-inflammatory cytokine, in peripheral blood mononuclear cells (PBMC) (Kulisic-Bilusic et al. 2010).

Conclusion

Conclusion

Medicinal plants have been used since ancient times as therapeutic agents, as they represent a valuable source of medicinal compounds with proven potential for treating diseases. Drug resistance in bacteria is a very serious problem, primarily caused by the indiscriminate use of antibiotics. Consequently, conventional drugs fail to control pathogenic infections effectively. However, plants are considered an inexhaustible source of natural bioactive substances and compounds with fewer side effects.

This study aimed to valorize the natural substances found in the Algerian flora. The objective focused on the phytochemical study of Stems and Leaves of Capparis spinosa and the evaluation of its antibacterial activity. According to the results of qualitative phytochemical analysis, the ethanolic axtracts are rich mainly in polyphenols and flavonoids

However, the content of tanins is higher in stems.

The results we obtained show that the stems and leaves have similar anti-inflammatory effects. When it comes to removing DPPH radicals, the leaves of plants collected from different regions have higher levels compared to the stems. This suggests that the leaves have weaker antioxidant properties.

The antibacterial activity of different extracts of C. spinosa was evaluated against various bacterial strains in numerous studies using different parts of the plant and diverse methods. The results of these studies clearly demonstrate that our plant has not a anti microbial effect .

In conclusion, we hope to make our modest contribution to the study of medicinal plants, acknowledging their economic importance and potential in the pharmaceutical industry.

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