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Phytochemical constituents and antioxidant and antibacterial activities of medicinal plant hydro-ethanolic extract

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Dedication

First of all, I thank God for everything that I have achieved in my life, including the completion of this memorandum.

I dedicate this modest work to my parents :

My dear father who supported me from a young age, and the best mother for always supporting and stand with me, and all my family members, my brother and sister, to whom I dedicate my success, which they had a part in.

I thank my friends who are like brothers to me and I wish them the same success and more.

Imad

Dedication

First and foremost, I thank God Almighty for allowing us to conduct this scientific research with His success

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Résumé

Les plantes ont toujours joué un rôle très important pour l'humanité, car elles peuvent produire un grand nombre de molécules organiques complexes qui sont souvent impliquées dans des activités biologiques. L'objectif de cette étude est de detecter les composés phytochimiques et les propriétés antioxydantes et antibactériennes de l'extrait hydro-éthanolique de *Retama sphaerocarpa*.

Le rendement de l'extrait hydro-ethanolique de la partie aerienne de *Retama Sphaerocarpa* est de 23,18%. Les résultats de l'activité antioxydante montrent un pouvoir antioxydant important de l'extrait par rapport à la vitamine C, connue aussi sous le nom d'acide ascorbique, qui est un antioxydant privilégié et le test DPPH permet de conclure que le pourcentage d'inhibition des radicaux libres de DPPH est élevé dans l'extrait hydro-ethanolique de *Retama sphaerocarpa* avec un EC50 =29, 48µg/mL.

Les résultats obtenus par l'utilisation de la methode de microdilution montrent une activité antibactérienne intéressante.

Mots clés :

Retama Sphaerocarpa, Extrait hydro-ethanolique, Screening phytochimique, Activité antibactérienne, Activité antioxydante.

Abstract

Plants have always played a very important role for mankind, as they can produce a large number of complex organic molecules that are often involved in biological activities. The objective of this study was to detect the phytochemical compounds and the antioxidant and antibacterial properties of the hydro-ethanolic extract of *Retama sphaerocarpa*.

The yield of the hydro-ethanolic extract of the aerial part of *Retama Sphaerocarpa* was 23.18%. The results of the antioxidant activity show a significant antioxidant power of the extract compared to vitamin C, also known as ascorbic acid, which is a preferred antioxidant and the DPPH assay allows us to conclude that the percentage of DPPH free radical inhibition is high in the hydro-ethanolic extract of *Retama sphaerocarpa* with an EC50 =29, 48µg/mL.

The results obtained using the microdilution method show interesting antibacterial activity.

Key words :

Retama Sphaerocarpa, Hydro-ethanolic extract, Phytochemical screening, Antibacterial activity, Antioxidant activity.

ملخص

لطالما لعبت النباتات دورًا مهمًا جدًا للبشرية، حيث يمكنها إنتاج عدد كبير من الجزيئات العضوية المعقدة التي غالبًا ما تشارك في الأنشطة البيولوجية. كان الهدف من هذه الدراسة هو الكشف عن المركبات الكيميائية النباتية والخصائص المضادة للأكسدة والمضادة للبكتيريا في المستخلص المائي-الإيثانولي لنبات ريتاما سفيركاربا.

كان محصول المستخلص المائي-الإيثانولي للجزء الهوائي من ريتاما سفيروساربا 23.18%. تُظهر نتائج النشاط المضاد للأكسدة قوة كبيرة مضادة للأكسدة للمستخلص مقارنة بفيتامينC ، المعروف أيضاً بحمض الأسكوربيك، وهو مضاد أكسدة مفضل، ويسمح لنا اختبار DPPH باستنتاج أن نسبة تثبيط الجذور الحرة DPPH عالية في المستخلص المائي- الإيثانولي للجزء الهوائي من ريتاما سفيروساربا معEC50 = 29 ، 48 ميكروغرام/مل.

تُظهر النتائج التي تم الحصول عليها باستخدام طريقة التخفيف الجزئي نشاطاً مضاداً للبكتيريا مثيراً للاهتمام.

الكلمات المفتاحية :

Retama Shaerocarpa، المستخلص المائي الإيثانولي المائي، الفحص الكيميائي النباتي، النشاط المضاد للبكتيريا، النشاط المضاد للأكسدة.

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List of abbreviations

Symbols	Title
MIC	Minimum Inhibitory Concentration
BMC	Minimum Bactericidal Concentration
CFU	Colony Forming Units
EC 50	Half Maximal Effective Concentration
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
EUCAST	European Committee on Antimicrobial Susceptibility Testing
MHB	Mueller Hinton Broth

Summary

1. Introduction

Different plants and plant-derived products are being used as medicine since the dawn of human civilization. Around 75% - 80% of the world's population residing in the developing countries rely on herbal remedies for primary healthcare because of their cultural acceptability and fewer side effects (Khanal ***et al***., 2020). Research using medicinal plants has significantly contributed to the development of new therapeutic strategies based on bioactive compounds (Bezerra ***et al***., 2022). Classes of compounds comprise alkaloids, which have antitumoral and antiviral properties, flavonoids, which exhibit several anti-inflammatory, antioxidant, antiallergic and anticarcinogenic characteristics, tannins, that contribute to treatments for arterial hypertension, fungi, bacteria and burns, saponins, which exhibit antiviral activity and act on cell membranes, steroids/triterpenes, which are natural anti-inflammatories (Amanda ***et al***., 2020)**.**

Moreover, many medicinal plants have an antioxidant activity that is attracting more and more the attention of several research teams for its role in the fight against several diseases such as cancer, atherosclerosis, cerebro-vascular condition, diabetes, hypertension, and Alzheimer's disease. Numerous physiological and biochemical processes produced oxygen-centered free radicals and other reactive oxygen species. Antioxidants are capable of scavenging free radicals, which can oxidize many biological macromolecules (DNA, proteins, and lipids) in cells and tissues. (Kougnimon ***et al.***, 2018).

Make improper use and abuse of antibiotics have accelerated the emergence of multi-resistant bacteria, which is now a real problem of antibiotics and public health . The use of natural resources in general and medicinal plants in particular becomes one of the most important and interesting avenues for the search for new and more effective antibacterial products. Medicinal plants are known to contain substances which could be used for treatment purposes or used to produce drugs. Plants have long been an inspiration for the search for new medicines, contributing to the well-being and health of humans. Today, the permanent resistance of certain bacteria to classical antibiotics leads to the search for new active ingredients (medicines) based on plants as sources of compounds that can overcome the cases of resistance in order to put under control new infections (Mounerou ***et al.***, 2018).

Retama sphaerocarpa, commonly known as "white broom," is a medicinal plant native to the Mediterranean region and North Africa. This plant has gained attention due to its potential therapeutic properties, including antioxidant and antibacterial activities. Studies have revealed that extracts from *Retama sphaerocarpa* contain a rich array of phytochemicals

such as flavonoids, phenolic acids, and alkaloids, which contribute to its antioxidant capacity (González-Burgos ***et al***., 2011). These compounds scavenge free radicals and inhibit oxidative stress, thereby protecting cells from damage and reducing the risk of chronic diseases. Additionally, *Retama sphaerocarpa* extracts have exhibited significant antibacterial activity against various pathogenic bacteria, including both Gram-positive and Gram-negative strains (Iglesias ***et al***., 2017). The antibacterial efficacy of *Retama sphaerocarpa* extracts can be attributed to the presence of bioactive compounds that disrupt bacterial cell membranes or interfere with essential bacterial metabolic processes. Further research into the phytochemical composition and biological activities of *Retama sphaerocarpa* could unveil its potential as a natural source of antioxidants and antibacterial agents for pharmaceutical and nutraceutical applications (Sánchez-García ***et al***., 2018).

For this purpose, our experiment aims to identify the chemical compounds of *Retama Sphaerocarpa* and to determine their antioxidant and antibacterial effects.

Materials and methods

2. Materials and Methods

2.1. Botanical description of *Retama sphaerocarpa*

Retama sphaerocarpa is a shrub that grows from 1 to 3 m high and can exceptionally up to 4 m. Its bark is smooth and free of thorns, and grey or greenish-grey in color. The young branches are quadrangular and ridged, with 8 to 10 V-shaped ribs (four of which are more developed) that are jonciform and very flexible, with the stem acting as an assimilator. At first they are sericoloured, then crested, with simple clothing made of short hairs and postulate. The youngest terminal branches act as modular growth units, able to produce new branches from lateral axillary buds (Salvador ***et al***., 2013).The leaves are generally somewhat deformed and arching downwards, the calyx ob-conical or sub-globose and appearing slightly swollen glabrous, bilabiate with two large acute upper teeth and three smaller lower teeth with a well-developed receptacle at the base 2 to 3.2 mm long to the tip of the upper teeth (Cullen ***et al***., 2013). Its root system is dimorphic, with both extensive lateral roots and a main taproot the lateral roots are finely branched and can reach 15m in length, while the taproot is generally devoid of fine branches and can reach depths of over 30m (Prieto ***et al***., 2010). *Retama sphaerocarpa* has yellow hermaphrodite flowers, yellowish, arranged in lateral clusters on short racemes, (EL fennoun, 2012). It flowers from April to June, depending on locality and altitude. Pollination is entomophilous, mainly by bees and ants. The fruit is an ovoid legume about 5-8 mm, straw-yellow in colour, almost always containing a single seed which is dark green (Salvador ***et al***., 2013).

Figure 01: Picture of *R. sphaerocarpa* (L)Boisse

2.2. Geographic Distribution of *Retama Sphaerocarpa*

Retama sphaerocarpa is a plant that grows in mountainous rocky and clay pastures, open forests, and prairie stream banks (Maire, 1987). This species is found in Spain, Portugal and North Africa and is very rare in the Sahara Desert. In Algeria, *R. sphaerocarpa* is found in Ain Sefra, Oued M'zab, Constantine, Maillot and Bouira. (Quezel et Santa, 1962).

2.3. Classification of *Retama sphaerocarpa*

Retama Sphaerocarpa belongs to the legume family, the most important in the plant kingdom. of the plant kingdom. There are 750 genera and between 16,000 and 19,000 species. Only 10% of known species are examined for root nodulation (Duhoux et Nicole. 2004).

According to Quezel and Santa (1962), retams are classified in the following taxon.

Kingdom : Plant

Phylum : Spermaphytes

Subphylum : Angiosperms

Class : Dicotyledons

Order : Fabales

Superfamily : Legumes

Family : Fabaceae

Subfamily : Papilionaceae

Genus : Retama

Species : R. Sphaerocarpa

2.4.Chemical Composition Of *Retama Sphaerocarpa*

2.4.1. Coumarins

Coumarins are benzopyrone compounds belonging to the flavonoid group of secondary metabolites. Presently there are more than 1300 coumarins have been identified in plants, bacteria, and fungi. Coumarins have their specific fingerprints as antiviral, antimicrobial, antioxidant, antiinflammatory, antiadipogenic, cytotoxic, apoptosis, antiprolilferative, antitubercular, and cytotoxicity agents. Due to their wide range of pharmacological values, coumarins and their derivatives have gained more importance in synthesis and production. Organisms such as cultivated microbes, including mushrooms and lower fungi, have provided much of the chemical diversity inspiring syntheses and filling the pharmaceutical pipeline.

Natural coumarins possess anticancer, antiHIV, antidiabetic, and other effects. Coumarins have been found to exhibit antioxidative properties and radical scavenging activities in various studies (Tsivileva, 2013).

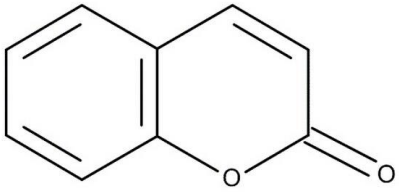


Figure 2: base structure of couarins (Gagui & Guelil. 2021)

2.4.2. Tannins

Are substances of the polyphenol family, possessing a molecular weight between 500 and 3000 Da (Delcambre, 2010). They are found in the bark, leaves and fruit of many plants, and are divided into two types, hydrolysable tannins and condensed tannins. plants, and are divided into two types, hydrolysable tannins and condensed tannins (Hemingway, 1992). Tannins have a number of therapeutic properties, and are used for tanning animal skinrepairing tissue damage caused by eczema and as an anti-constipant (Iserin. 2001).

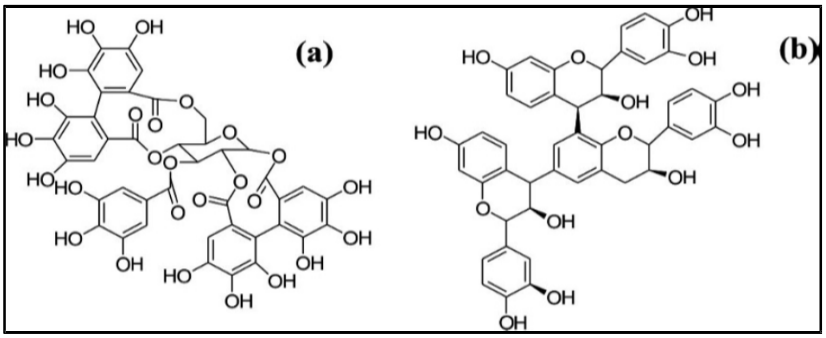


Figure 3 : Structure of (a) hydrolysable (b) condensed tannins**.** (Naczk, 2006)

2.4.3. Saponsides

The term saponsides and derived from the word soap, are heterosides, can be found in the following forms triterpene and steroid (Robinet, 1951). They have surface-active properties (William, 2003).

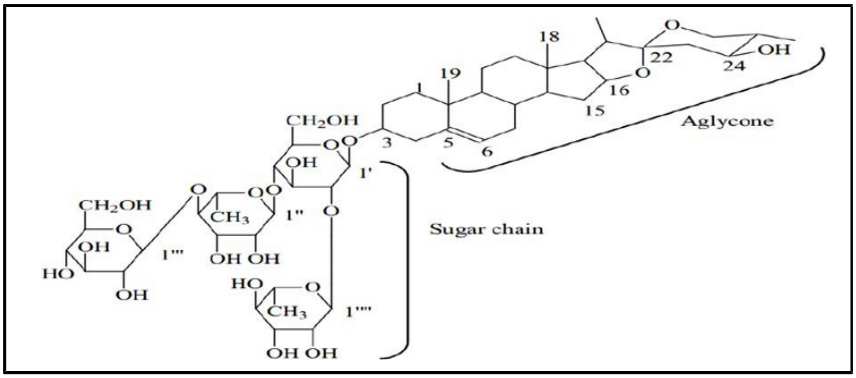


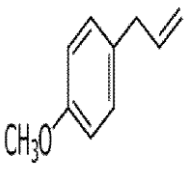
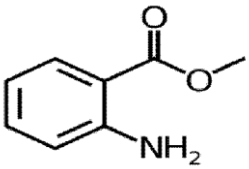
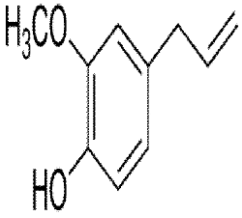
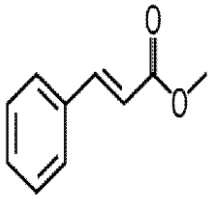
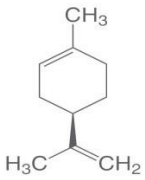
Figure 4: Structure of saponpsides (Moghimipour, 2015)

2.4.4. Essential oil

They are oily, volatile and odorous products, most often localised in secretory secreting organs. Essential hulias are used as anti-inflammatories; they also expels intestinal gas. The chemical composition of an E.H. is fairly complex, and there are generally many constituents in it. These mainly belong to two main chemical types:

Terpenic compounds such as monoterpenes, sesquiterpenes, diterpenes and terpenes. and aromatic aromatic compounds derived from phenylpropane, which are very often alkyls and prophenylphenols, sometimes aldehydes (Iserin, 2001).

Table 1: Some examples of compounds in the essential oil of retama species**(Gagui Asma &Guelil Sassia 2021)**

				
Estragole	Methylanthranilate	Eugenol	Methylcinnamate	D-(+)-Limonene

2.4.5. Alkaloids

Are cyclic compounds containing one or more nitrogen atoms in their chemical structure. They are stored in plants as products of various biosynthetic biosynthetic pathways. Some structures are relatively simple, while others are quitecomplex. Alkaloids can be found in all parts of the plant and the part in which the alkaloids accumulate is not always the same.

alkaloids accumulate is not necessarily the part where they are synthesized Harborne,1995). They can be found infamilies of plants, and most alkaloids are soluble in water and alcoholwater and alcohol. (Doncheva,2014).

They are bitter and aperitive, and form the basis of many therapeutic molecules (Marouf. 2007).

2.4.6. Flavonoids

Are natural polyphenolic compounds. They are considered to be almost universal plant pigments. pigments in plants. Structurally, they are generally in free form or in the form of aglycoside. They are found in various plant organs such as roots, stems, wood, leaves, flowers and fruit (Marfak. 2003).

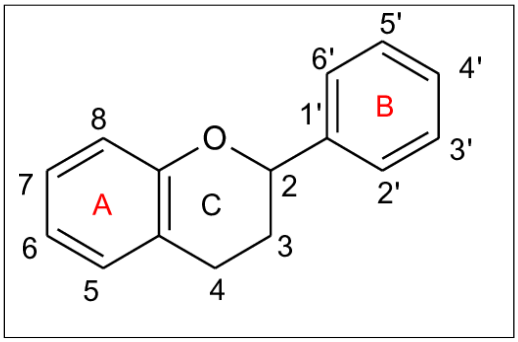


Figure 5 : Basic structure of flavonoids (Harborne, 2000)

2.5. Uses of *Retama sphaerocarpa*

Retama sphaerocarpa, commonly known as needle bush or Spanish broom, has several traditional and potential modern uses, although scientific research on its uses may vary in availability. Here are some common and potential uses:

2.5.1. Traditional medicine

Retama sphaerocarpa has numerous applications in traditional medicine across the Mediterranean and North Africa. It is utilized as a diuretic, a treatment for skin diseases, and an emollient. Additionally, decoctions or infusions of the plant are used to treat respiratory ailments and promote wound healing (Mohammed ***et al***., 2009).

2.5.2. Ornamental plant

Due to its attractive yellow flowers and pinnate leaves, *Retama sphaerocarpa* is cultivated as an ornamental plant in gardens and landscapes in regions with moderate climates. It

enhances the aesthetic appeal of gardens and is commonly used for landscaping purposes (Soussi ***et al***., 2017)

2.5.3. Soil stabilization

In certain regions, *Retama sphaerocarpa* is planted for soil stabilization purposes. Its extensive root system helps prevent erosion in areas prone to soil loss, such as coastal dunes and degraded lands (El-Kammar ***et al.***, 2017).

2.5.4. Honey production

The flowers of *Retama sphaerocarpa* provide a nectar source for bees, and the honey produced from this plant may have unique characteristics depending on the region and environmental conditions (Mulinacci ***et al.***, 2008).

2.5.5. Potential pharmaceutical uses

Some studies have explored the pharmacological properties of *Retama sphaerocarpa* extracts, indicating potential applications in modern medicine. These properties include antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities. However, further research is necessary to fully understand and validate these potential uses. (Tundis ***et al***., 2014).

2.6. Interest s of *Retama sphaerocarpa*

2.6.1. Ecological interest

Retama sphaerocarpa act as ecological dune stabilizers, soil fixers, and revitalizers of semi-arid and arid ecosystems (Allen, 1981). It has the ability to fix nitrogen through its symbiosis with rhizobia at the root level, and its deep root system enables it to access deep water sources. (Guerrouj, 2013).

2.6.2. Pharmacological interest

Retama sphaerocarpa are wild desert shrubs found in Algeria, commonly used as a healing plant with antiseptic, antipyretic, and antidiarrheal effects. In traditional medicine, decoctions of the aerial parts are used to treat diabetes, hypertension, rheumatism, and inflammation. These pharmacological effects are attributed to the presence of certain alkaloids and flavonoids. Additionally, *Retama sphaerocarpa* exhibits antioxidant, antimicrobial, and antifungal activities. (Hammouche-Mokrane, 2017).

2.6.3. Economic interest

The shoots are considered excellent fodder, and the wood is also used for heating. They are rich in fiber, with an average length of 1.93 mm. (Bahi, 1991).

The seeds contain substances like lectins and proteins that trigger allergic reactions, serving as a defense mechanism against insects. This characteristic allows for the production of biopesticides. (El-Shazly, 1996).

2.7. Preparation of the extract

Plant material of *Retama sphaerocarpa* aerial parts was harvested in November 2023 from Bakkaria, Tébessa city, Algeria. Plant identification was conducted at the laboratory of plant biology and physiology at the faculty of exact sciences and natural and life sciences in university of Tébessa. The aerial plant parts were dried for two months at room temperature in the shade, then ground using an electric grinder and sieved to obtain a fine powder. The powders were packed in bags and stored until needed.

In a 1000 mL capacity beaker, a mixture comprising 500 mL of ethanol and 500 mL of distilled water was prepared, followed by thorough agitation. Subsequently, plant powder (*R. Sphaerocarpa*) was introduced into the solution, and the amalgamation was subjected to agitator for 40 minutes. Following complete homogenization, the solution was refrigerated for 24 hours. It was then subjected to filtration using filter paper into another receptacle to isolate the liquid fraction. Post-filtration, the solution was transferred to a rotary evaporator set at 45°C for further processing. The resultant extract was stored in an electric evaporator apparatus to obtain the final extract (Merghem ***et al.***, 1995).

The yield of plant extract is the ratio between the weight of the extract and the weight of the dry matter of the plant, evaluated from 4 extractions, it is expressed as a percentage and calculated by the following formula: $Y = (We/Wp) \times 100$

Y = yield of the extract in%

We = Weight of the extract in g

Wp = Weight of the dry matter of the plant in g

Figure 6 : Steps of extraction

2.8. Phytochemical analysis

To identify the different groups of chemical compounds present in a plant, chemical screening was carried out. Colorimetry and gravimetry were the two main methods used to identify these groups of substances in solution. The alkaloids, flavonoids, saponins, anthocyanins, leucoanthocyanins, tannins, terpenes and sterols contained in the plant were identified using the methods described by Harborne (1998):

2.8.1. Tannins

Non-hydrolysable catechic tannins were identified using Stiasny's reagent. 5 ml of the infusion was evaporated to dryness. After adding 15 ml of Stiasny's reagent to the residue, the mixture was kept in a water bath at 80°C for 30 min. The observation of an orange precipitate characterises the catechic tannins.

Gallic tannins, which are hydrolysable, were identified by adding FeCl3. We filtered the previous solution and saturated it with sodium acetate. The addition of 3 drops of 2% FeCl3 produced an intense blue-black hue, indicating the presence of gallic tannins.

2.8.2. Leucoanthocyanins

Leucoanthocyanins are revealed by adding 04 ml of hydrochloric alcohol (Ethanol/pure HCl: 3-1) to 05 ml of infusion. After a few minutes heating in a water bath at 50°C, the appearance of a red colour (cherry red) is characteristic of their presence.

2.8.3. Saponins

A 2% decoctate is prepared with 2 g of powder in 100 ml of boiling water and boiled for 30 minutes. the final volume being made up to 10 ml with distilled water. Each tube was shaken for 15 seconds and then left to stand for 15 minutes in an upright position. A persistent foam height superior to 1 cm indicates the presence of saponins

2.8.4. Flavonoids

10 g of the powder was macerated in 150 ml of 1% HCl for 24 h. NaOH was added to 10 ml of the filtrate to make the solution basic. The appearance of a light yellow colour in the upper part of the tube after 3 hours indicates the presence of flavonoids.

2.8.5. Alkaloids

The presence of alkaloids is established by salt precipitation and revelation using Mayer's reagent. To 5 g of powder add 50 ml of 1% HCl. After macerating for 3 hours at room temperature, the macerate was filtered. Take 1 ml of filtrate and add 5 drops of reagent. The presence of alkaloids is shown by the appearance of a white precipitate.

2.8.6. Terpenes and steroids

To identify steroids and polyterpenes, we used the Liebermann reagent. 5g of leaf powder was macerated in 20ml of petroleum ether, filtered and then evaporated to dryness in a sand bath at 90°C. The residue was hot triturated in 1 ml acetic anhydride. We added 0.5 ml of concentrated sulphuric acid to the triturate. The appearance of a purple and violet ring at interphase, turning blue and then green, indicates a positive reaction.

2.8.7. Quinones

Moisten 5 g of crushed plant material with a few drops of HCl. Macerate the plant material for one hour or 24 hours in a closed Erlen Meyer containing 10 ml petroleum ether. After filtration, 2 ml of filtrate is stirred with 2 ml of 10% NaOH. A red to violet coloration appeared in the presence of quinones.

2.9. Antioxidant activity

The most frequently mentioned characteristic of polyphenols, and of plant phenolic compounds in general, is undoubtedly their recognised ability to trap reactive oxygen species (ROS). This so-called antioxidant capacity is frequently cited as being the key property underlying the prevention and/or reduction of chronic diseases linked to oxidative stress and age-related disorders such as cardiovascular disease (e.g. atherosclerosis), carcinogenesis, neurodegeneration (e.g. Alzheimer's disease), as well as skin deterioration, by dietary plant polyphenols and others (Quideau, 2011).

2.9.1. Free radicals

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable andhighly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants. The most important oxygen-containing free radicals in many disease states are hydroxyl radical, superoxide anion radical, hydrogenperoxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical. These are highly reactive species, capable in the nucleus, and in the membranes of cells of damaging biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids. Free radicals attack important macromolecules leading to cell damage and homeostatic disruption. Targets of free radicalsinclude all kinds of molecules in the body. Among them, lipids, nucleic acids, and proteins are the major targets (Lobo, 2010).

2.9.2. Antioxidant activity by measuring anti-free radical activity on DPPH

The use of this radical as a reagent is based on the fact that as soon as the DPPH radical reacts with an anti-radical compound, the latter releases hydrogen after reduction, thus leading to a change in color (from dark purple to light yellow. Anti-radical activity can therefore be visualised very quickly, simply following a change in color of the radical (DPPH) (Fig 10) (Meriane, 2018).

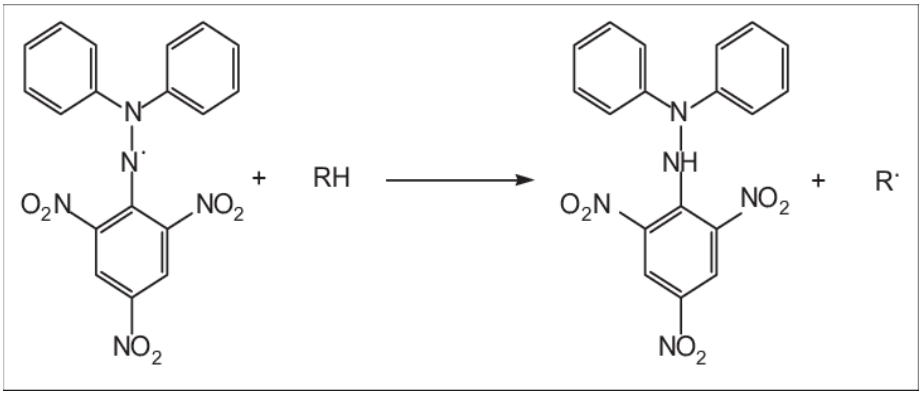


Figure 7 : Structure of DPPH and its reduction by an antioxidant (Kunal, 2012)

2.9.3. Dosage method

In our study, this test was evaluated according to the protocol applied by Kuramasamy ***et al*** (2007). Briefly, 1 ml of a methanolic solution of DPPH (0.2 mM) was mixed with 1ml of different dilutions of plant extracts (0-1 mg/ml). The mixture obtained was then kept protected from light at room temperature for 30 minutes. The absorbance was then measured at 517 nm against a control consisting of 1ml of the DPPH solution and 1ml of methanol. The samples, ascorbic acid and the control were prepared under the same operating conditions. The decrease in absorbance is measured with a spectrophotometer and the % PI (percentage inhibition) is calculated according to the formula below:

$$IP\% = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

2.10. Antibacterial activity

To illustrate in vitro the antibacterial activity of *Retama sphaerocarpa* extract, we employed the microdilution method to ascertain the minimum inhibitory concentration (MIC) values.

2.10.1. Bacterial strains tested

The antibacterial activity of *Retama sphaerocarpa* extract was assessed against six bacterial strains: three Gram-negative and three Gram-positive. The Gram-negative bacteria included four clinical *Enterobacteria* isolated from hospital patients, while the Gram-positive bacteria comprised three strains of *Staphylococcus*.

2.10.3. Preparation of pre-cultures

The bacterial strains were cultured on petri dishes containing nutrient agar and incubated at 37°C for 24 hours to obtain fresh bacterial cultures.

2.10.4. Preparation of the bacterial inoculum

Prepare a suspension of the bacterial strain from a 24-hour pure culture in 5 ml of sterile physiological water, adjusting the density to 0.5 McFarland standard (108 CFU/ml) using isolated colonies.

2.10.5. Minimum inhibitory concentration (MIC)

The MIC is determined by the liquid microdilution technique, using a sterile 96-well microplate (8 × 12 wells). A concentration range ranging from 20 to 0.01 µl. 180 µl of sterile Mueller Hinton Broth (MHB) is added to well 1. Then place 100µl of sterile BMH in wells 2 to 10. A series of dilutions of 2 (or ½-fold dilutions) was made extemporaneously in Mueller Hinton broth from the stock solution in well1, by transferring 100µl from well to well until well 10 (the 100µl from the last well to be discarded). The contents of the well should be mixed well.

Finally, place 5µl of the inoculum previously diluted 1:100 (approximately 106 CFU/ml) in each well to give a final concentration of 5. 105 CFU/ml. The microplate is covered and incubated at 37°C for 24 hours (Mann and Markham, 1998). The MIC is the lowest concentration of extract at which no cloudiness is observed (Eloff, 1998 ; Eucast, 2003).

2.10.6. Minimum bactericidal concentration (MBC)

To determine the lowest concentration that can inhibit 99.99% of germs, the minimum inhibitory concentration (MIC) test results were relied on. After identifying the MIC, the content of wells with concentrations equal to or greater than the MIC on Petri dishes

containing MH agar medium was looked for. The plates were observed after 24 h of incubation at 37 °C to determine the antibacterial effect. If the CMB/CMI ratio is less than or equal to 4, it will be considered bactericidal, while a BMC/MIC ratio of 4 or more indicates a bacteriostatic effect (Rachidatou ***et al***., 2024).

Results and discussion

3.1. Results

3.1.1. Yield of *Retama sphaerocarpa* hydro-ethanolic extract

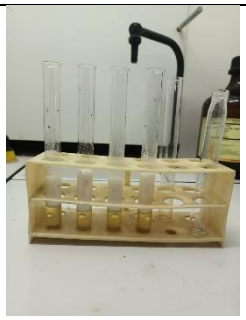
The extraction of the aerial part of the plant was prepared following the protocol described by Merghem ***et al*** (1995). The yield of the aerial part of *Retama sphaerocarpa* hydro-ethanolic extract is 23.18%

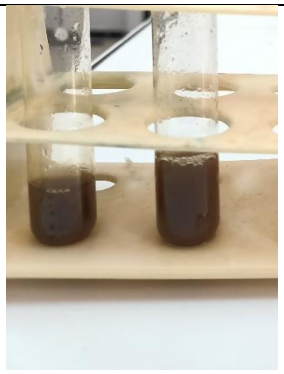
3.1.2. Phytochimical scrinning of the aerial part of *Retama sphaerocarpa*

The results of the phytochemical analysis are presented in Table 3. The '+' sign indicates the presence of the group of chemical compounds and the '-' sign a negative reaction.

The results indicate that the the aerial part of *Retama Sphaerocarpa* contain flavonoids, catechic and gallic tannins, steroids, saponins and alkaloids, and no leucoanthocyanins, terpenes and quinones.

Table 2 : The results of the phytochemical scrinning of the aerial part of *Retama sphaerocarpa*.

Secondary metabolites	**Results**	**Observation**		**Results**	**Secondary metabolites**
Flavonoids		+	+		**Sponins**
Tannins catechic		+	+		**Alkaloids**

Tannins gallic		+	–		**Leucoantho-cyanins**
Quinones		–	– +		**Terpenes** **Steroids**

3.1.3. Antioxydant activity

By following the protocol described by Kuramasamy (2007), we calculated the Ec50% for ascorbic acid and *R. sphaerocarpa* extracts and we obtained these results.

This curve shows the interaction of ascorbic acid and *R. sphaerocarpa* extracts with DPPH, which acts as a free radical in this experiment. After calculation, the EC50 is as below:

Antioxydant	**EC50**
Ascorbic acid	**12.37 µg/mL**
R. sphaerocarpa	**29.48 µg/mL**

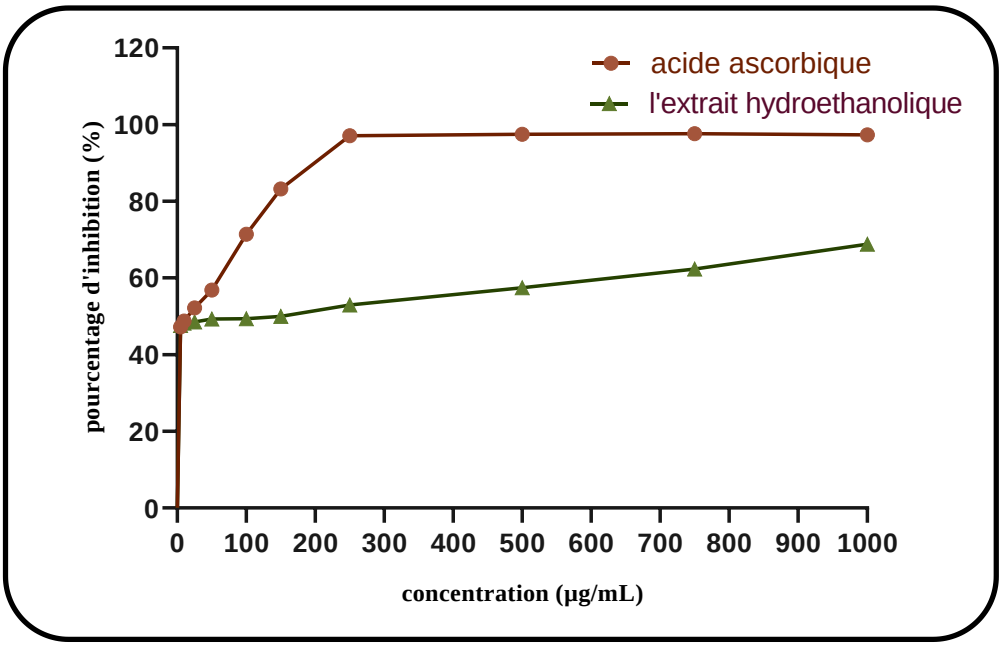


Figure 8 : Percentage of DPPH radical efficacy as a function of *Retama Sphaerocarpa* extract concentation

3.1.4. Antibacterial activity

3.1.4.1. Evaluation of the antibacterial activity of *Retama Sphaerocarpa* hydro-ethanolic extract using the microdilution method

This antibacterial activity was also studied using the liquid microdilution technique on sterile microplates (96 wells). This is a quantitative technique used to determine the range of concentrations that effectively inhibit bacterial growth.

3.1.4.2. Determination of the Minimum Inhibitory Concentration (MIC) and Bactericidal Concentration (BMC)

After 24 hours incubation of the microplates, we noted the appearance of a clear appearance in some wells, and in others a deposit (in some cases a cloudiness) indicating bacterial growth.

In the present study we carried out a cascade dilution in MHB medium in the presence of DMSO in order to obtain a dilution range of *Retama sphaerocarpa* hydro-ethanolic extract, from 280 mg/ml to 0.14 mg/ml, as shown in the table.

The microplate results obtained in our study are summarised in Table 4.

Table 3 : Effect of different concentrations of *Retama sphaerocarpa* hydro-ethanolic extract on bacterial growth.

Well / Concentration (mg/mL) / Strains	1	2	3	4	5	6	7	8	9	10	11	12
	280	140	70	35	17.5	8.75	4.37	2.18	1.09	0.54	0.27	0.14
Staphylococcoceae (s1)	-	-	-	+	+	+	+	+	+	+	+	+
Staphylococcoceae (s2)	-	-	+	+	+	+	+	+	+	+	+	+
Entérobacteriaceae (214)	-	-	-	+	+	+	+	+	+	+	+	+
Entérobacteriaceae (357)	-	-	+	+	+	+	+	+	+	+	+	+
Entérobacteriaceae (9)	+	+	+	+	+	+	+	+	+	+	+	+
Staphylococcoceae (s3)	-	-	-	-	+	+	+	+	+	+	+	+
Indicator +	+	+	+	+	+	+	+	+	+	+	+	+
Indicator -	-	-	-	-	-	-	-	-	-	-	-	-

- : No deposits + : Deposits present

* Strains 153, 214 and 357 are Gram-negative multi-resistant pathogenic bacteria.

* Strains s1, s2 and s3 are Gram-positive multi-resistant pathogenic bacteria.

According to the table 4, the growth of different strains of bacteria was influenced by the concentrations of *Retama sphaerocarpa* hydro-ethanolic extract.

The wells showed no deposits and a clear appearance, indicating total inhibition of bacterial growth. On the other hand, the wells showing microbial growth indicate that the *Retama sphaerocarpa* hydro-ethanolic extract has not effect on bacterial growth.

The microplate method enabled us to determine the minimum inhibitory and bactericidal concentration values when reading through the bacterial growth disorders. The results are shown in table (4).

sTable 4 : MIC and MBC of *Retama sphaerocarpa* hydro-ethanolic extract tested on the six bacterial strains studied expressed in mg/ml

Strains	MIC	MBC	MBC / MIC
***Staphylococcoceae* (s1)**	70	/	/
***Staphylococcoceae* (s2)**	140	/	/
***Entérobacteriaceae* (214)**	70	140	2
***Entérobacteriaceae* (357)**	140	/	/
***Entérobacteriaceae* (9)**	/	/	/
***Staphylococcoceae* (s3)**	35	70	2

3.1.4.3. Nature of the antibacterial activity of *Retama sphaerocarpa* hydro-ethanolic extract

The nature of the antibacterial activity of *Retama sphaerocarpa* hydro-ethanolic extract against the six bacterial strains studied was determined from the 280 mg/ml to 0.14 mg/ml dilution boxes showing visible growth inhibition. The results are presented in Table 5.

Table 5 : Nature of the activity of *Retama sphaerocarpa* hydro-ethanolic extract against the six strains tested.

bacterial strains	Nature of activity
***Staphylococcoceae* (s1)**	Bacteriostatic
***Staphylococcoceae* (s2)**	Bacteriostatic
***Entérobacteriaceae* (214)**	Bactericidal
***Entérobacteriaceae* (357)**	Bacteriostatic
***Entérobacteriaceae* (9)**	/
***Staphylococcoceae* (s3)**	Bactericidal

3.2. Discussion

3.2.1. Extract yield

According to the results obtained, we found that the extraction yield was 23.18%. this yield is superior than that obtained in the same region by Bouabida et Dris (2022) where they found The yields of methanolic extracts from dried leaves of *R. graveolens*, *R. montana*, and were 19.14% and 22.58% respectively.

The yield of plant extraction is influenced by various parameters, which can significantly impact the efficiency and quality of the extracted compounds. Here are the primary factors:

*** Solvent Type:** The choice of solvent is crucial as different solvents have vary in polarities, which affect the solubility of plant compounds. Polar solvents (like water and ethanol) are more effective for extracting polar compounds, while non-polar solvents (like hexane) are better for non-polar compounds (Stalikas, 2007).

*** Extraction Time:** The duration of the extraction process can affect the yield. Longer extraction times can lead to higher yields, but excessively long times may result in the degradation of sensitive compounds (Proestos & Komaitis. 2008).

*** Temperature Higher:** temperatures generally increase the solubility of compounds and the rate of extraction. However, high temperatures can also degrade thermo-labilecompounds (Wang & Weller. 2006).

*** Particle Size:** Smaller particle sizes increase the surface area for solvent interaction, thus improving the extraction efficiency. However, too fine a powder can lead to difficulties in filtration and solvent recovery (Azmir. 2013).

*** Solvent-to-Solid Ratio:** The ratio of solvent to plant material affects the extraction yield . A higher solvent-to-solid ratio typically increases the yield up to a certain point, after which no significant increase is observed**.** (Liu ***et al***., 2004).

*** Agitation or stirring** can enhance the contact between the solvent and the plant material, thus improving the extraction yield**.** (Dai & Mumper. 2010).

3.2.2. Phytochimical scrinning

According to the results obtained in our study, *Retama sphaerocarpa* plant is rich in many secondary Metabolites (flavonoids, Tannins, Alkaloids, Terpenoids, Saponins), and has no or few secondary Metabolites like (quinones, leucoanthoyanes) .

-Flavonoids test, The color of the solution changed to yellow, indicating their presence , Our results are consistent with the findings by Ben zarouga (2014) and Hadj Moussa (2011) in *Retama retama*.

-The presence of Alkaloids was confirmed by the appearance of a white precipitate. These results are consistent with those by Ben zarouga (2014) and Hadj Moussa (2011) in *Retama retama*, and according to Ngussan (2009) have a role in rheumatism, analgesic, antispasmodic, and anticancer.

-As for the Tanins test, it was found that *Retama sphaerocarpa* contains Tanins catechic, Tanins galique by changing the color of the solution to dark green The same results obtained in the research by Ben zarouga (2014) and Hadj Moussa (2011) in *Retama retama*, its role is to stop bleeding and fight infection (Makhloufi. 2013).

-As for the saponins test, the appearance of a persistent moss more than 1cm in height is evidence of their presence. Our results are in line with the results obtained by Ben zarouga (2014) and Hadj Moussa (2011) in *Retama retama***,** their properties are analgesic and anti-inflammatory (Roux and Catier. 2007).

-The presence of steroids was confirmed by changing the color of the solution to purple. These results are consistent with the results obtained by Ben zarouga (2014) and Hadj Moussa (2011) in *Retama retama*, The properties of steroids are antibacterial and cardiotonic (Saad. 2017 ; Makhloufi. 2013).

-For the test of quinones and leucoanthoyanes, the results were negative, the color of the solution did not change to red, indicating their absence in *Retama sphaerocarpa*, our results agree with the results of obtained by Ben zarouga (2014) and Hadj Moussa (2011) in *Retama retama*,

There are several parameters that control how secondary substances vary from plant to plant, including For example:

***Genetic Factors:** Species and Genotype: Different plant species and even different genotypes within a species can produce distinct secondary metabolites. The genetic makeup controls the enzymatic pathways that synthesize these compounds .

*** Gene Expression:** Variations in the expression of genes involved in metabolic pathways can lead to differences in metabolite profiles. Regulatory genes play a crucial role in controlling these pathways. (Mithöfer & Boland. 2012)

*** Environmental Factors:** Climate and Weather: Temperature, light, humidity, and rainfall can significantly influence the synthesis of secondary metabolites. For instance, high light intensity can enhance the production of flavonoids, which act as UV protectants .

*** Soil Composition:** The availability of nutrients and minerals in the soil can affect the biosynthesis of secondary metabolites. For example, nitrogen availability can impact alkaloid production .Water Availability: Water stress can lead to the accumulation of certain secondary metabolites like tannins and phenolics, which help plants cope with drought conditions. (Hartmann. 2007)

*** Biotic Interactions:** Herbivory and Pathogens: Plants often produce secondary metabolites as a defense mechanism against herbivores and pathogens. For example, the

production of phytoalexins increases in response to pathogen attack .Symbiotic Relationships: Interactions with symbiotic organisms such as mycorrhizal fungi or nitrogen-fixing bacteria can influence the production of secondary metabolites. (Treutter, 2010)

*** Developmental Stage:** The production of secondary metabolites can vary depending on the plant's developmental stage. Seedlings, mature plants, and senescing plants may all exhibit different metabolite profiles .Tissue Specificity: Different parts of the plant (roots, leaves, flowers, seeds) can produce different secondary metabolites, reflecting their specific roles and requirements (Bryant. 1983)

*** Ecological and Evolutionary Factors:** Over evolutionary time, plants adapt to their environments, leading to the development of unique secondary metabolites that provide competitive advantages .Geographic Variation: Plants from different geographical regions often have distinct secondary metabolite profiles due to varying environmental pressures and evolutionary histories**.** (Wink. 2003)

*** Human Influence:** Farming practices, including the use of fertilizers, pesticides, and crop management techniques, can influence the secondary metabolite composition of plants . Selective Breeding and Genetic Modification: Humans have selectively bred plants for specific traits, including the production of certain secondary metabolites. Genetic engineering can also introduce or enhance pathways for desirable metabolites. (Neilson. 2013)

3.2.3. Antioxydant activity of *Retama sphaerocarpa* hydro-ethanolic extract

The results obtained when testing the antioxidant activity of *Retama sphaerocarpa* extract showed that the percentage of free radical inhibition increases with the concentration of the plant extract and the concentration of ascorbic acid,

The EC50 value indicates the concentration of the antioxidant needed to reduce the initial concentration of free radicals by 50%. A lower EC50 value signifies a more potent antioxidant, as a smaller amount is required to achieve the same effect. This value is used to compare the efficacy of different antioxidants in various experiments (Rita. 2019).

The EC50 value of ascorbic acid was 12.37ug/mL and the EC 50 value of *R. sphaerocarpa* extract was 29.48ug/ mL, which proves the effectiveness of *R. sphaerocarpa* extract against oxidative stress. The more effective an antioxidant compound is, the lower its EC50 value is. Antioxidant compounds work by donating an electron to free radicals and neutralizing them, preventing oxidative stress, and have high reactivity with free radicals like Dpph, so they can achieve the desired effect at a lower concentration (Molyneux. 2004). Our Ec50 value at both

ascorbic acid 12.37ug/ul and *R. sphaerocarpa* extract 29.48ug/ul shows a Lower than Ec50 obtained in the research of (Halima, Aisha 2020) where their Ec50 was estimated (141.31ug/ mL, 688.74ug/ mL) in the leafs of *Retama sphaerocarpa*

Based on all our results and research, we can say that *Retama sphaerocarpa* has great antioxidant activity because it contains flavonoids and tannins that reduce Dpph due to their ability to release hydrogen, it is possible that the polyphenols in the extract are responsible for the antioxidant action (Potter and Champ, 1986).

3.2.4. Antibacterial activity

In this study, the antibacterial activity was tested against 6 strains of bacteria, 3 gram positive and 3 gram negative. The efficacy of *Retama sphaerocarpa* hydro-ethanolic extract was tested against these bacteria and the evaluation showed the following results.

CMI 70 mg/ml for both s1 and p214, CMI between 140 mg/mL for s2 and 357, CMI was estimated to be 35 mg/mL for s3. Our results superior for the values obtained by Guenfissi and laifaoui (2012). Where The CMI value for *E. coli, P. aeruginosa, B. sbtillis, V. cholerea, sp. Salmonello* is 20 mg/ml and for SARM is 5 mg/ml, and S. aureus MIC = 1.25 mg/ml.

The ability of *Retama sphaerocarpa* plant extract to inhibit bacterial growth or directly kill bacteria can be attributed to its chemical composition, specifically its rich content of bioactive compounds. *Retama sphaerocarpa*, also known as white broom, contains various phytochemicals such as alkaloids, flavonoids, saponins, tannins, and phenolic compounds, among others (Elansary ***et al***., 2019). These bioactive compounds have been reported to possess antimicrobial properties. For example, alkaloids and flavonoids are known for their antimicrobial activity against a wide range of bacterial strains. They can disrupt bacterial cell membranes, interfere with essential enzymes, and inhibit bacterial DNA replication, ultimately leading to bacterial growth inhibition or cell death (Bouzabarta ***et al***., 2017). Based on this study and through the results obtained in the antibacterial activity test of the hydroethanolic extract of *Retama sphaerocarpa* twigs, we can say that it has effective antibacterial activity due to the secondary metabolism it contain.

Conclusion

As part of our research into the biological activities of *Retama sphaerocarpa* ethanolic extract, we evaluated its antioxidant and antibacterial activities and the knowledge of its constituents at the end of this study we were able to conclude.

The yield of ethanol extraction was 23.18%.

The results also showed that the plant is rich in many secondary metabolites such as flavonoids, tannins, alkaloids, saponins and steroidal saponins.

Results showed antioxidant activity against free radicals compared to ascorbic acid, which is the preferred antioxidant. The Dpph test led to the conclusion that the percentage of inhibition was higher in ascorbic acid compared to the extract, the EC50 of ascorbic acid was 12.37µg/ml and that of the plant extract was 29.48µg/ml.

The antibacterial activity of *Retama sphaerocarpa* shows an effect on 3 strains of Gram-negative bacteria and 3 strains of Gram-positive bacteria.

The results of our work are just a first step in the search for a natural remedy with antioxidant and antibacterial properties. Looking ahead, it will be important to conduct further research on a wide range of bacterial strains, identify the active ingredients responsible for these activities and determine their mode of action.

Finally, as a conclusion to our research, we can say that *Retama sphaerocarpa* has many biological advantages as it acts as an antioxidant and antibacterial due to the secondary metabolites it contain.

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