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Theme

Extraction, identification and determination of the biological activities of certain active extracts of wild plants *(Peganum harmala, Artemisia campestris, Opuntia, Picris hieracioides)* in the region of Djelfa - Algeria.

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بسم الله الرحمن الرحـــيم

﴿سبحانك لا علم لنا إلا ما علمتنا

إنك أنت العليم الحكيم﴾

الآية 32 من سورة البقرة

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Yahia, Rym, and Rabab

Dedication

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Yahia, Rym, and Rabab

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Abbreviation List

AA: Antiradical Activity

ARr: Artemisia campestris Roots

ARf: Artemisia campestris Leaves

BHA: Butylated Hydroxyanisole

BHT: Butylated Hydroxytoluene

CMI: Minimum Inhibitory Concentration

CFS: Cefsulodine

CI50: Inhibitory Concentration at 50%

DPPH: 2,2-Diphenyl-1-Picrylhydrazyl

Mg EAG/g Ps: Milligram of Gallic Acid Equivalent per gram of Plant Dry Weight

Mg EQ/g Ps: Milligram of Quercetin Equivalent per gram of Plant Dry Weight

MH: Mueller Hinton Agar

NADP: Nicotinamide Adenine Dinucleotide Phosphate

O2*-: Superoxide Radical

*OH: Hydroxyl Radical

ONOO: Peroxynitrite

OP: Opuntia Plant

OX: Oxacillin

P: Penicillin

PCf: Picris hieracioides Leaves

PCr: Picris hieracioides Roots

PHf: Peganum harmala Leaves

PHr: Peganum harmala Roots

ROS: Reactive Oxygen Species

SOD: Superoxide Dismutase

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Introduction

Introduction

Throughout history, herbs and plants have assumed a crucial role in promoting human health and overall well-being, spanning numerous millennia. Plant-based remedies have been utilized in traditional medicine systems to address various health conditions.

The region of Djelfa in Algeria is widely recognized for its abundant biodiversity, encompassing a diverse array of spontaneous plant species. The objective of this thesis is to extract, identify, and evaluate the biological activities of active extracts derived from four indigenous plant species: Peganum harmala, Opuntia ficus-indica, and Picris hieracioides, which were collected from Messâad, and Artemisia campestris from M'liliha.

The selection of these plants is predicated upon their historical utilization as medicinal plants and the identification of biologically active compounds in prior research endeavors.

The findings of this study will enhance the scholarly comprehension of these indigenous floras and furnish significant insights into their prospective utilization as reservoirs of novel pharmaceutical compounds.

The present study encompasses four main sections: theoretical background, experimental methodology, results, and discussion.

The theoretical background section provides a comprehensive overview of the historical significance of plant-based remedies in traditional medicine systems, highlighting their enduring relevance in contemporary medical practices.

The experimental methodology section outlines the specific steps involved in extracting and isolating the biologically active compounds from the four indigenous plant species.

The results and discussion section will present the findings of the study and offer insights into the potential utilization of these plants as sources of novel pharmaceutical compounds.

Chapter one

Bibliographic Study

Chapter one Bibliographic Study

I.1 Artemisia campestris.L plant

Artemisia campestris.L is a perennial subshrub with a lifespan of multiple years, and it can attain a height ranging from 30 to 150 cm. The plant exhibits a distinctive growth pattern with branching, ascending stems that form a panicle-like structure. These stems are typically characterized by a reddish-brown hue and a smooth, glabrous texture. Notably, the lower part of the stems becomes lignified or woody, while the upper parts remain herbaceous (El Bahri and Chemli, 1997).

The leaves of *Artemisia campestris* are typically green or green-brown in color. They are intricately divided into fine, narrow strips, a characteristic that distinguishes them within the genus *Artemisia*. These leaves are further classified as doubly divided, with the upper leaves being sessile (attached directly to the stem) and the lower leaves featuring petioles **(fig 01)** (Boudjouref, 2011).

When it comes to its reproductive structures, *Artemisia campestris* produces small, yellowish flowers that are densely clustered within numerous ovoid capitula. The capitula consist of two key parts: the involucre and the receptacle, both of which are characterized by their smooth, glabrous surfaces. Within the capitula, the central flowers can exhibit two different sexual conditions—they may be sterile or hermaphroditic, meaning they possess both male and female reproductive organs. In contrast, the flowers located at the periphery of the capitula are generally unisexual and exclusively female. The stamens of the plant's flowers are distinguished by their anthers, which are extended into pointed tips. The flowering period of *Artemisia campestris* typically spans from the month of August to October, making it an autumn-blooming species (Boudjouref, 2011).

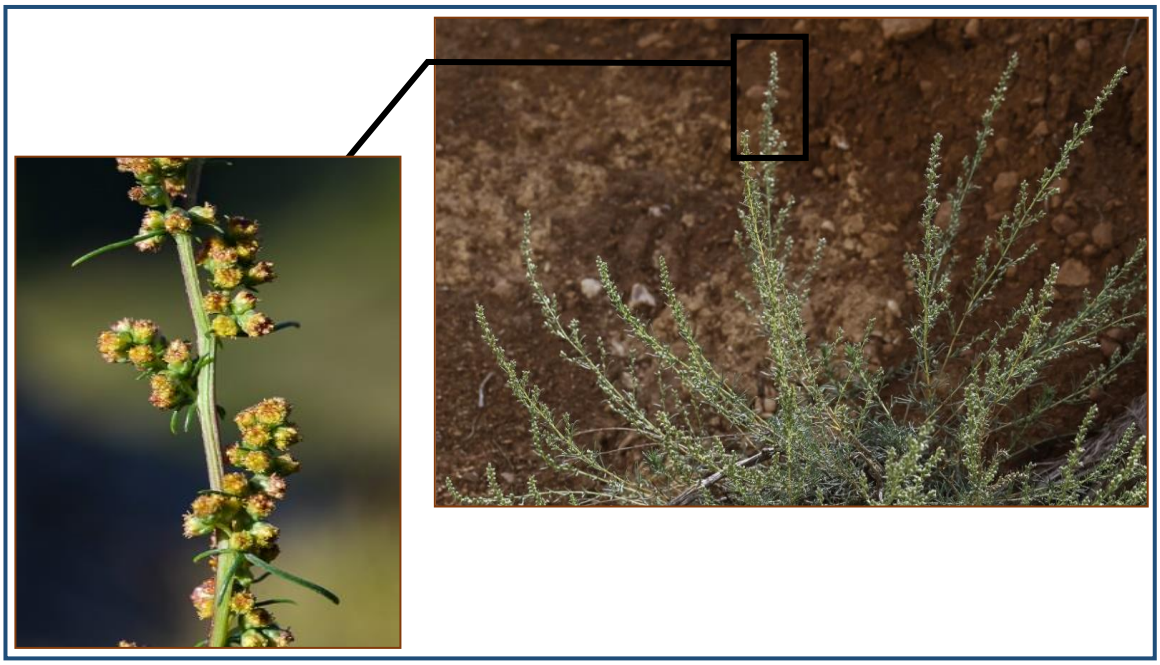


Fig 01: *Artemisia campestris. L* picture

I.1.2 Systematique classification:

Accordiong to Linnaeus, C. (1758) the classification is as follows:

- ✓ **Kingdom:** *Plantae*
- ✓ **Class:** *Equisetopsida*
- ✓ **Subclass***: Magnoliidae*
- ✓ **Superorder:** *Asteranae*
- ✓ **Order:** *Asterales*
- ✓ **Family:** *Asteraceae*
- ✓ **Genus:** *Artemisia*
- ✓ **Species:** *Artemisia campestris L.*

I.1.3 Traditional uses

Throughout history, *Artemisia campestris*, a botanical species of significance, has found application in diverse medicinal contexts, contributing to its enduring relevance. Traditional wisdom has attributed several therapeutic uses to this plant:

Digestive Ailments: *Artemisia campestris* has enjoyed a long-standing reputation as an effective remedy for digestive disorders, encompassing afflictions like bloating, flatulence, and indigestion. Its purported carminative and antispasmodic attributes have been harnessed to ameliorate these gastrointestinal discomforts. (Khan, & Abourashed, 2010)

Dermatological Conditions: Traditional knowledge has also harnessed *Artemisia campestris* in the treatment of dermatological maladies, including eczema, psoriasis, and other inflammatory skin disorders. Its believed anti-inflammatory and antimicrobial characteristics have been employed to alleviate and protect the skin (Bhat. R & Karim. A, 2010).

Respiratory Issues: In addressing respiratory afflictions like coughs, colds, and bronchitis, Artemisia campestris has been historically utilized. It is postulated to possess expectorant and anti-inflammatory properties, which contribute to the alleviation of these respiratory symptoms. (Nair, A. V., & Alagarsamy, V. 2012)

Menstrual Challenges: For women's health, this plant has been employed to alleviate menstrual problems, including menstrual cramps, irregular periods, and menopause-related symptoms. Its perceived hormone-regulating properties have been utilized to mitigate these discomforts (Bown, D. (2001).

Anthelmintic Effects: Furthermore, *Artemisia campestris* has traditionally functioned as a natural anthelmintic remedy to combat parasitic infections, such as roundworm and tapeworm infestations. Its reputed antiparasitic properties facilitate the expulsion of these parasites from the body (Duke, J. A. 2002).

I.2 *Peganum harmala*

Peganum harmala belongs to the family *Zygophyllaceae*, and it is often referred to as "Syrian rue," "African rAue," or "*Harmal.*" It contains β-carbolines such as harmaline, harmine, harmalol, harmol, tetrahydroharmine, and the quinazoline derivatives vasicinone and deoxyvasicinone. it possess therapeutic properties and used in traditional medicine and pharmaceutical industries (Mashayekhi, *and AL*. 2007).

A perennial herb belongs to the family *Nitrariaceae*. A bushy plant grows to a height of 60-100 cm and has woody stems and branches. The leaves are blue-green and feathery, with a pinnate arrangement.

The plant produces small, inconspicuous flowers that are typically greenish-yellow in color and arranged in clusters. Capsule-like fruits that contain numerous small, black seeds follow the flowers **(fig 02).**

Peganum harmala is well adapted to arid and semi-arid environments and is commonly found growing in rocky and sandy soils. It is widely distributed in the Mediterranean region, Central Asia, and the Indian subcontinent (L. López-Sáez, *and al.* 2010).

I.2.1 Systematics of the Plant:

According to (Shahrajabian, *and al*.2021) the classification of *peganum harmala* is as follows:

- ✓ **Kingdom*:*** *Plantae*
- ✓ **Phylum*:*** *Magnoliophyta*
- ✓ **Class*:*** *Magnoliopsida*
- ✓ **Order*:*** *Sapindales*
- ✓ **Family*:*** *Zygophyllaceae*
- ✓ **Genus*:*** *Peganum*
- ✓ **Species*:*** *P. harmala*
- ✓ **Subspecies:** *P. harmala*

Fig 2: *Peganum harmala plant*

I.2.2 Traditional usages

Asthma, Insomnia, Tapeworm Infection, Menstrual Disorder it regulates and relieves difficult and painful menstruation but must be avoided during pregnancy.

The Harmal is effective in relieving remittent and chronic fevers, also in chronic malaria. It reduces weakness, body pains, and loss of appetite.

Laryngitis, Head Lice ,Maintain Blood Pressure, depression, stress, anxiety disorder or social anxiety ,Stomach Illnesses, epilepsy, palsy, dementia, and amnesia It also reduces inflammation of the brain, Blood Purifier ,helps Kidney Function, Improve Digestion, Cooling Effects, increases lactation. (Saeidnia, Sand al 2016)

I.3 Opuntia ficus-indica (L.):

The genus *Opuntia* belongs to the *Cactaceae Opuntia,* presents high adaptation capacity to extreme environmental conditions (high temperature, drought, UV radiation), and is distributed in arid and semiarid regions.

I.3.1 ficus-indica (L.):

Opuntia ficus-indica, commonly referred to as the prickly pear cactus, is a versatile and resilient plant species known for its ability to thrive in diverse environmental conditions. It is characterized by its distinctive flattened stems, which are covered in sharp spines and glochids, small hair-like structures that can cause irritation upon contact. The plant's vibrant and showy flowers can range in color from yellow to red, and its edible fruit, often referred to as prickly pear or cactus pear is popular for its sweet and juicy flesh, **(fig 03, 04)** (Cota-Sánchez, J. H., *and al* 2015).

The botanical characteristics of Opuntia ficus-indica make it well suited to arid and semi-arid regions, where it is known for its ability to efficiently utilize water and nutrients. Its drought resistance and adaptability have made it an important plant in regions with challenging environmental conditions (Le Houérou, H. N. 1996).

I.3.2 Systematics of the Plant:

According to (Apak et al., 2018) the classification of *opuntia* is as follows

- ✓ ***Kingdom:*** *Plantae*
- ✓ ***Subkingdom:*** *Tracheobionta*
- ✓ ***Super division:*** *Spermatophyta*
- ✓ ***Division:*** *Magnoliophyta*
- ✓ ***Class:*** *Magnoliopsida*
- ✓ ***Subclass:*** *Caryophyllidae*
- ✓ ***Order:*** *Caryophyllales*
- ✓ ***Family****: Cactaceae*
- ✓ ***Subfamily****: Opuntioideae*
- ✓ ***Tribe:*** *Opuntieae*
- ✓ ***Genus:*** *Opuntia*
- ✓ **Species:** *O. ficus-indica (.L)*

  Species (edible): there exist various names (INE, 1994; USDA 2016): *ficus indica, albicarpa, megacantha, undulata,*

Fig 03 : *Opuntia ficus-indica* paddle

Fig 04: *Opuntia ficus-indica* plant

I.3.3 The traditional uses of *opuntia*

Opuntia ficus-indica, commonly known as the prickly pear cactus, has a rich history of traditional and pharmaceutical uses.

Traditionally, various cultures have utilized its fruits and pads for both food and medicinal purposes. The pads are consumed as a source of nutrition, while the fruits are enjoyed fresh or processed into jams, jellies, and beverages (Stintzing, F. C., & Carle, R. 2007).

In traditional medicine, the cactus has been used to treat various ailments, such as digestive issues and skin conditions, owing to its perceived healing properties. In the pharmaceutical industry, *Opuntia ficus-indica* is valued for its potential health benefits. Its extracts, especially from the pads and fruits, are used in the production of dietary supplements, cosmetics, and skincare products due to their antioxidant, anti-inflammatory, and moisturizing properties. The cactus is being studied for its potential role in managing conditions like diabetes and obesity, making it an intriguing candidate for pharmaceutical research and development (Feugang, J. M., *and al* 2006).

I.4 Picris hieracioides:

Picris hieracioides, also known as *Helminthotheca hieracioides*, is a plant belonging to the *Asteraceae* family (Bottine, 2011). This annual herb typically exhibits a robust, erect, and branching stem, measuring 3-10 decimeters in height. The stem is covered in bristles, giving it a rough texture. The leaves of *Picris hieracioides* are oblong in shape and are characterized by their rough and bristly texture. The basal leaves can be either entire or loosely sinuate and narrow into petioles, while the upper leaves are cordate-embracing with two rounded lobes **(fig 05).**

The plant's involucre consists of outer leaflets that are broadly ovate-cordate and pointed, and inner leaflets that are narrow and taper into a long point. *Picris hieracioides* produces yellowish or reddish slightly compressed achenes that are transversely wrinkled and terminate abruptly in a hair-like beak of the same length as the achene. The aigrette, a feathery structure, is white, and the plant bears yellow flowers (Sofowora, 2010).

I.4.1 Systematic of the plant:

According to (OMS, 2003) the classification of this plant is as follows:

- ✓ **Kingdom:** *Plantae*
- ✓ **Division:** *Magnoliophyta*
- ✓ **Class:** *Magnoliopsida*
- ✓ **Superorder:** *Asteranae*
- ✓ **Order:** *Asterales*
- ✓ **Family:** *Asteraceae*
- ✓ **Genus:** *Picris*
- ✓ **Species:** *Picris hieracioides*

Common names: *Picride fausse-vipérine, picride vipérine, picris fausse vipérine, helminthie fausse vipérine.*
Scientific name: *Helminthotheca echioides.*

Fig 05: picture of *Picris hieracioides*

I.4.2 Traditional use of *Helminthotheca echioides*

Helminthotheca echioides is used as a traditional remedy in some regions of Kabylie, especially in OUADHIAS, where it is called "Lahlafa". In this region, the *picride* is used as feed for livestock and is highly recommended for gastric problems. It is also considered an edible plant, and the leaves of this plant are consumed in salads. It is also recommended for wound healing. treating cold, ulcers, hemorrhoids, cough, bronchitis, etc (BENALI, 2016).

I.5 Phytochemicals

Phytochemicals are compounds that are synthesized by plants to help protect them from environmental stresses such as UV radiation, pests, and pathogens (Pandey & Rizvi, 2009). These compounds can have a range of properties and functions, including acting as pigments, contributing to plant flavor and aroma, and serving as defense compounds (Harborne, 1991).

For example, flavonoids, which are a class of phytochemicals, serve as pigments that give many fruits and vegetables their vibrant colors. They also contribute to the flavor and aroma of some plants, such as the bitterness of grapefruit and the astringency of tea. In addition to these functions, flavonoids have been shown to have antioxidant properties and may help protect against chronic diseases such as cardiovascular disease, cancer, and neurodegenerative diseases (Pérez-Jiménez et al., 2010).

Another example of a phytochemical with potential health benefits is curcumin, which is found in turmeric. Curcumin has been shown to have anti-inflammatory and antioxidant properties, and may also have anti-cancer effects (Gupta et al., 2013).

Overall, phytochemicals are an important aspect of plant biology and contribute to the nutritional and medicinal value of many plant-based foods. By consuming a diverse array of fruits, vegetables, herbs, and spices, we can benefit from the health-promoting properties of these compounds (Pandey & Rizvi, 2009).

Alkaloids are nitrogen-containing compounds that have been shown to have pharmacological activities, including analgesic, anti-inflammatory, and anti-tumor effects. They are found in various plant species, such as coffee, tea, and poppy (Pandey, and al. 2009).

Flavonoids are water-soluble compounds that have antioxidant properties and have been associated with a lower risk of chronic diseases, such as cardiovascular disease, cancer, and neurodegenerative diseases. Flavonoids are abundant in fruits, vegetables, tea, and wine (Harborne, J. B. 1991).

Terpenoids, also known as isoprenoids, are a large and diverse class of compounds that are responsible for the aroma and flavor of many plants. They have been shown to have anti-inflammatory, antimicrobial, and anti-tumor effects. Terpenoids are found in many plant species, including herbs, spices, fruits, and vegetables (Pérez-Jiménez, and al. 2010).

Phenolic compounds are aromatic compounds that have been shown to have antioxidant properties and anti-inflammatory effects. Phenolic compounds are found in many plant-based foods, such as fruits, vegetables, whole grains, and nuts, and have been associated with a lower risk of chronic diseases (Ghosh, D., & Konishi, T. 2007).

I.5.1 Secondary Metabolites

Secondary metabolites are complex organic molecules synthesized and accumulated in small quantities by autotrophic plants. They are mainly divided into three major families: polyphenols, terpenes, and alkaloids (Lutge et al., 2002; Abderrazak et Joël., 2007).

I.5.2 Polyphenols

Polyphenols are products of secondary metabolism in plants, characterized by the presence of at least one benzene ring directly linked to at least one free hydroxyl group or engaged in another function such as ether, ester, glycoside, etc. (Bruneton, 1999), (Lugasi et al., 2003). In fact, phenolic compounds constitute the largest and most widely distributed group in the plant kingdom, with over 8000 known phenolic structures (Lugasi et and., 2003). The main classes of phenolic compounds are: phenolic acids (caffeic acid, hydroxycinnamic acid, chlorogenic acid), flavonoids which represent more than half of the polyphenols, tannins, and coumarins (King et Young, 1999; Tapiero et al., 2002). Polyphenols are present in all parts of higher plants: roots, stems, leaves, flowers, fruits (Boizot and Charpentier, 2006).

I.5.3 Flavonoids

The term flavonoid refers to a wide range of natural compounds belonging to the polyphenol family (Seyoum et al., 2006). They are considered almost universal pigments in plants, often responsible for the coloration of flowers, fruits, and sometimes leaves. In their natural state, flavonoids are most often found in the form of glycosides (Ghestem et al., 2001), (Bruneton, 1999). From a structural point of view, flavonoids can be classified into several classes of molecules, with over 6400 structures identified (Harborne and Williams, 2000).

a- Structure and classification:

Flavonoids are benzo-y-pyran derivatives (Skerget et al., 2005). Their basic structure is that of a 15-carbon diphenyl propane (C6-C3-C6), consisting of two aromatic rings referred to as rings A and B, connected by an oxygen-containing heterocycle referred to as ring C (Dacosta, 2003).

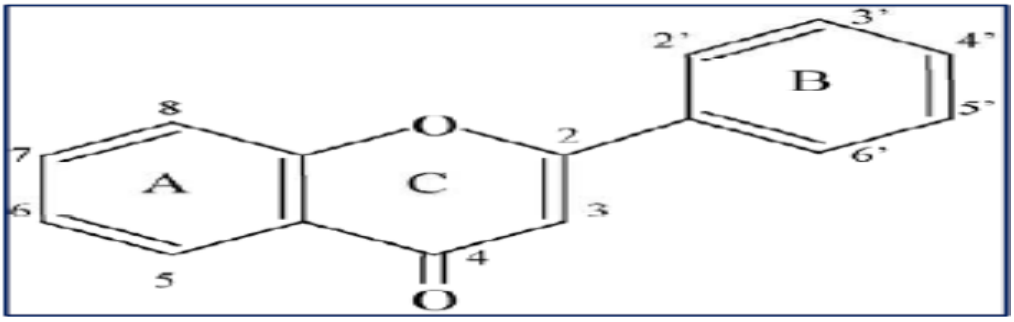


Fig 06: Basic structure of flavonoids (Di Carlo et al., 1999).

In general, flavonoids can be found either in a free state, in which case they are called aglycones, or in the form of C- or O-glycosides, where they are linked to sugars such as glucose, rhamnose, or arabinose. They can also exist as monomers or oligomers (Dacosta, 2003).

Flavonoids can be subdivided into several classes, with the most important ones being flavones, isoflavandiols, flavanols, flavondiols, aurones, chalcones, and anthocyanins (Effendi et al., 2008).

I.6 Oxidative Stress

VI.1 Definition

Oxidative stress is defined as the imbalance between the generation of reactive oxygen species and the body's capacity to neutralize and repair oxidative damage (Boyd and al., 2003).

I.6.1 Free Radicals

A free radical is defined as any molecule that possesses one or more unpaired electrons (Jacques and André, 2004). This molecule is highly unstable and quickly reacts with other components, attempting to capture the necessary electron to acquire stability. A chain reaction begins when a free radical attacks the nearest stable molecule, taking its electron, and the attacked molecule itself becomes a free radical (Martinez-Cayuela, 1995).

I.6.2 Reactive Oxygen Species

Among the most interesting radical species are reactive oxygen species (ROS), which are free radicals derived from the oxygen molecule by adding an electron. The main reactive oxygen species include the superoxide radical (O2 *-), the hydroxyl radical (*OH), nitric oxide (NO*), as well as certain non-radical reactive oxygen derivatives with significant toxicity, such as hydrogen peroxide (H2O2) and peroxynitrite (ONOO-) (Jacques et André, 2004; Gutteridge, 1993)

I.6.3 Superoxide radical

The main source of the superoxide radical is undoubtedly the mitochondrial respiratory chain. This system allows the production of the superoxide radical by adding an electron to molecular oxygen, and this reaction is catalyzed by mitochondrial cytochrome oxidase:

I.6.4 The hydroxyl radical

The hydroxyl radical (·OH) is a highly reactive radical species. It is mainly formed during reactions of metal ions with hydrogen peroxide, which are described as Fenton reactions:

$$M_n^+ + H2O_2 \longrightarrow M_{(n+1)}^+ + OH + OH$$

Iron can also catalyze the conversion of the superoxide anion in the presence of hydrogen peroxide, producing hydroxyl radicals according to the Haber-Weiss reaction. This reaction is relatively slow and less common than the previous one in living tissues (Jacques et André, 2004)

$$O_2\cdot^- + H_2O_2 \longrightarrow O_2 + OH\cdot + OH^-$$

I.7 Antioxidants

Antioxidants are a group of molecules capable of directly inhibiting the production, limiting the propagation, or destroying reactive oxygen species. They can act by reducing or dismutating these species, trapping them to form a stable compound, sequestering free iron, or generating glutathione.

Within cells, there are two lines of defense of unequal power to detoxify the cell:

I.7.1 Endogenous antioxidants

The human body has an enzymatic system composed mainly of three enzymes: superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). These enzymes have complementary actions on the radical cascade involving superoxide and hydrogen peroxide, ultimately leading to the formation of water and molecular oxygen. (Marfak, 2003).

I.7.2 Exogenous antioxidants

Many molecules found in our diet, such as vitamins, nutrients, and natural compounds, are considered antioxidants.

I.8 Antimicrobial activity

I.8.1 General Information

Bacteria are unicellular microorganisms classified as prokaryotes because they lack a nuclear membrane. This characteristic distinguishes them from other unicellular organisms classified as eukaryotes (fungi, algae, protozoa). They are divided into true bacteria (Bacteria) and primitive bacteria (Archaea). All bacteria encountered in pathology belong to Bacteria. Bacteria generally have a diameter of less than 1µm. They can be observed under a light microscope, in a fresh state or after staining. Their shape can be spherical (cocci), rod-shaped (bacilli), curved (vibrions), or spiral (spirochetes). The details of their structure are only visible under electron microscopy (Nauciel and Vildé, 2005).

I.8.2 Bacterial culture

Complex media based on extracts or enzymatic hydrolysates of meat are usually used to culture bacteria. These media can be liquid (broths) or solid. Solidification of the media is achieved by adding agar, an extract from algae that melts at boiling temperature and solidifies at temperatures below 40°C. In liquid media, bacteria disperse freely, and their multiplication is usually indicated by a homogenous turbidity. On a solid medium, when the quantity of bacteria is low, each bacterium can multiply in place until it forms a visible cluster of bacteria called a colony (If the bacterial density is too high in the inoculated sample, the colonies become confluent and form a continuous layer). The use of solid media allows for the enumeration of viable bacteria in a sample (Nauciel and Vildé, 2005).

I.9 Description of the studied bacteria's

1) Escherichia coli

Escherichia coli is a gram-negative bacillus, non-sporulated, facultative anaerobe, usually mobile due to flagella. Its length ranges from 2 to 6 µm, while its width is 1.1 to 1.5 µm. E. coli bacteria are part of the aerobic microbial flora in the human and animal digestive tracts. Some strains can cause specific infections in humans or certain animal species, such as spontaneous infections of the digestive or urinary tracts, or neonatal meningitis. Other strains that belong to the commensal flora can cause various opportunistic infections, especially in individuals with weakened immune defenses **(fig 07)**.

2) Staphylococcus aureus

Staphylococcus aureus is a gram-positive cocci, spherical in shape, with a diameter of 0.8 to 1 µm. They are found in pairs (diplococci) or small clusters (grape-like clusters). These bacteria are non-motile, non-sporulated, and usually lack a capsule. Many strains of Staphylococcus aureus produce a golden yellow pigment. S. aureus is known to cause meningitis, osteomyelitis, and diarrhea **(Fig 08).**

3) Pseudomonas aeruginosa

Pseudomonas aeruginosa is a gram-negative bacillus. These slender bacteria are 1.5 to 3 µm long and 0.5 to 0.8 µm wide. They are mobile due to a polar monotrichous flagellation, giving them a "swarming" appearance. P. aeruginosa does not form spores or spheroplasts. It is responsible for 10% of all nosocomial infections, ranking third after E. coli and S. aureus. However, it ranks first for lower respiratory tract infections and third for urinary tract infections **(*Fig 09)*.**

4) Proteus mirabilis

Proteus mirabilis is a Gram-negative, facultatively anaerobic, rod-shaped bacterium. It shows swarming motility and urease activity. P. mirabilis causes 90% of all Proteus infections in humans. It is widely distributed in soil and water .Proteus mirabilis can migrate across the surface of solid media or devices using a type of cooperative group motility called swarming. Proteus mirabilis is most frequently associated with infections of the urinary tract, especially in complicated or catheter-associated urinary tract infections **(fig 10).**

5) Streptococcus pneumoniae

Streptococcus pneumoniae are lancet-shaped, gram-positive, facultative anaerobic bacteria with more than 100 known serotypes. Most *S. pneumoniae* serotypes can cause disease, but only a minority of serotypes produce the majority of pneumococcal infections **(fig 11)**.

6) Klebsiella pneumoniae

Klebsiella pneumonia is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It appears as a mucoid lactose fermenter on MacConkey agar **(fig 12)**.

Although found in the normal flora of the mouth, skin, and intestines,it can cause destructive changes to human and animal lungs if aspirated, specifically to the alveoli resulting in bloody, brownish or yellow colored jelly-like sputum.

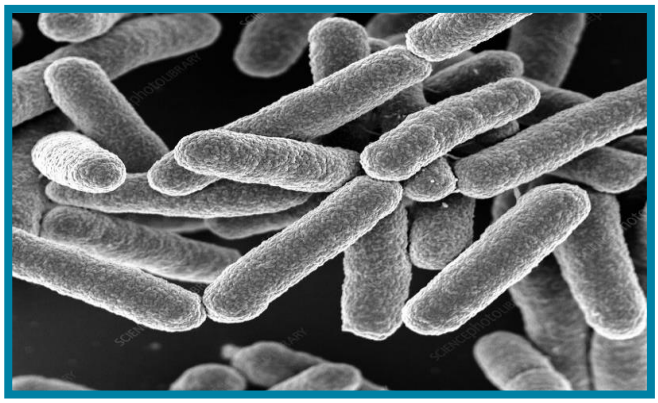


Fig 07: *Escherichia coli observed under electronic microscope*

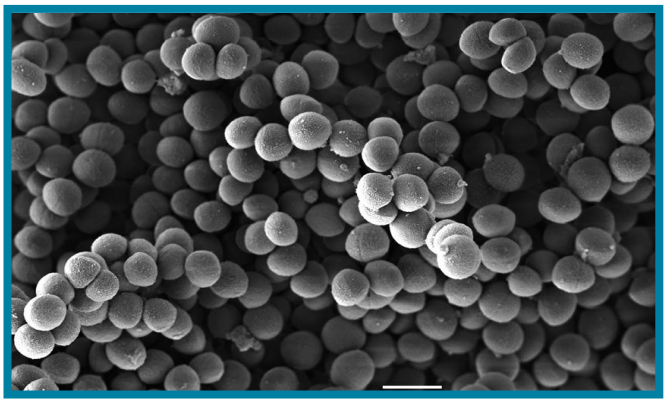


Fig 08: *Staphylococcus aureus* observed under an *electronic microscope*

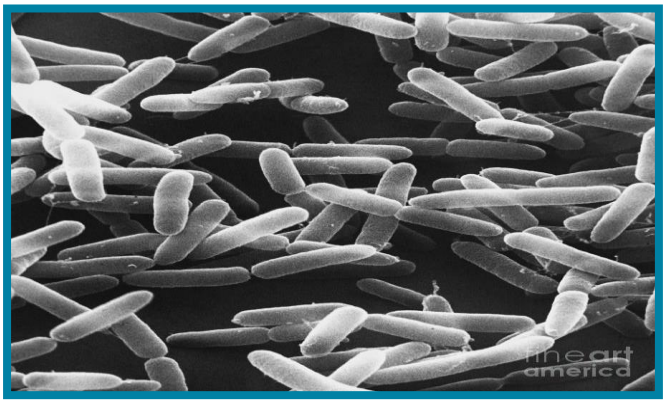


Fig 09: *Pseudomonas aeruginosa* observed under an electronic microscope

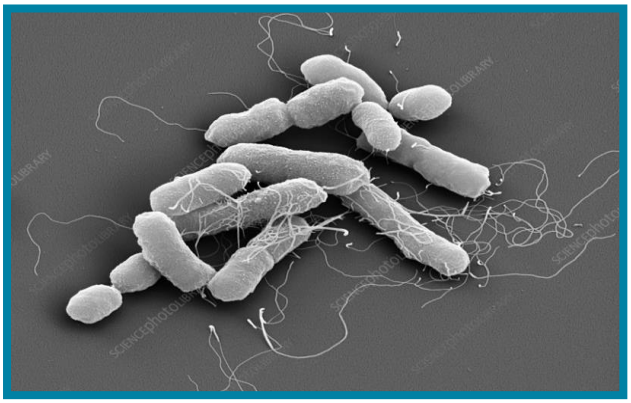


Fig 10: *Proteus mirabilis observed under an electronic microscope*

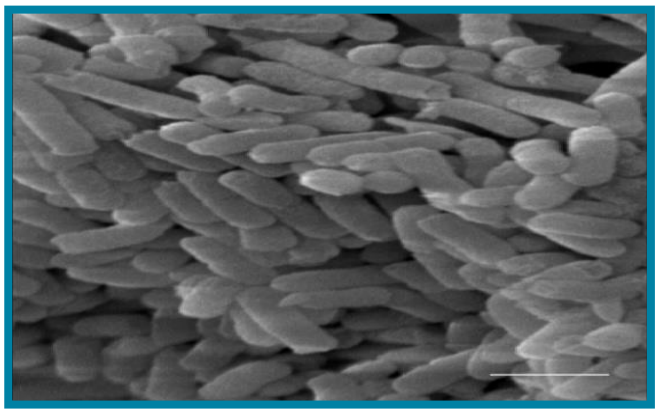


Fig 12: *Klebsiella pneumonia observed under an* electronic microscope

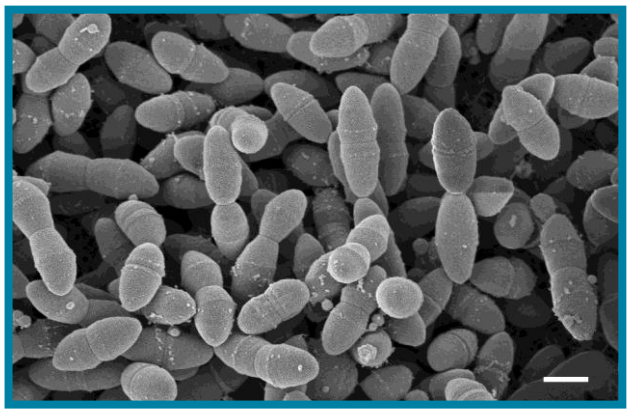


Fig 11: *Streptococcus pneumoniae observed under an electronic microscope*

Chapter two

Experimental study

Chapter II: Materials and Methods

Within this chapter, we expound the equipment employed, and delineate the requisite methodologies. Furthermore, we furnish an exhaustive account of the process of harvesting the four plant specimens subjected to testing. Additionally, we delve into the extraction and maceration procedures that were employed, as well as the techniques utilized for quantifying biological activities.

II.1 vegetal Material:

Survey area:

The Djelfa region, located in Algeria, is widely recognized for its dry climate and challenging natural conditions. El M'lilha and Mesaad, two towns situated in Djelfa, have similar ecological issues marked by a limited presence of vegetation resulting from the prevailing arid conditions and increasing desertification. These regions are located in the transitional zone between the Saharan and sub-Saharan ecosystems, where they often face challenges related to limited water supplies and the gradual growth of dry landscapes.

El M'liliha is geographically situated in the northern portion of the Djelfa area. The region is characterized by a semi-arid environment, which presents challenges in maintaining a significant vegetative canopy. Within this specific geographical location, the prevalent vegetation consists mostly of robust plant species that have adapted to arid environmental circumstances. The presence of greenery is generally focused in oasis and cultivated regions, where agricultural activities can take advantage of the limited water resources that are accessible.

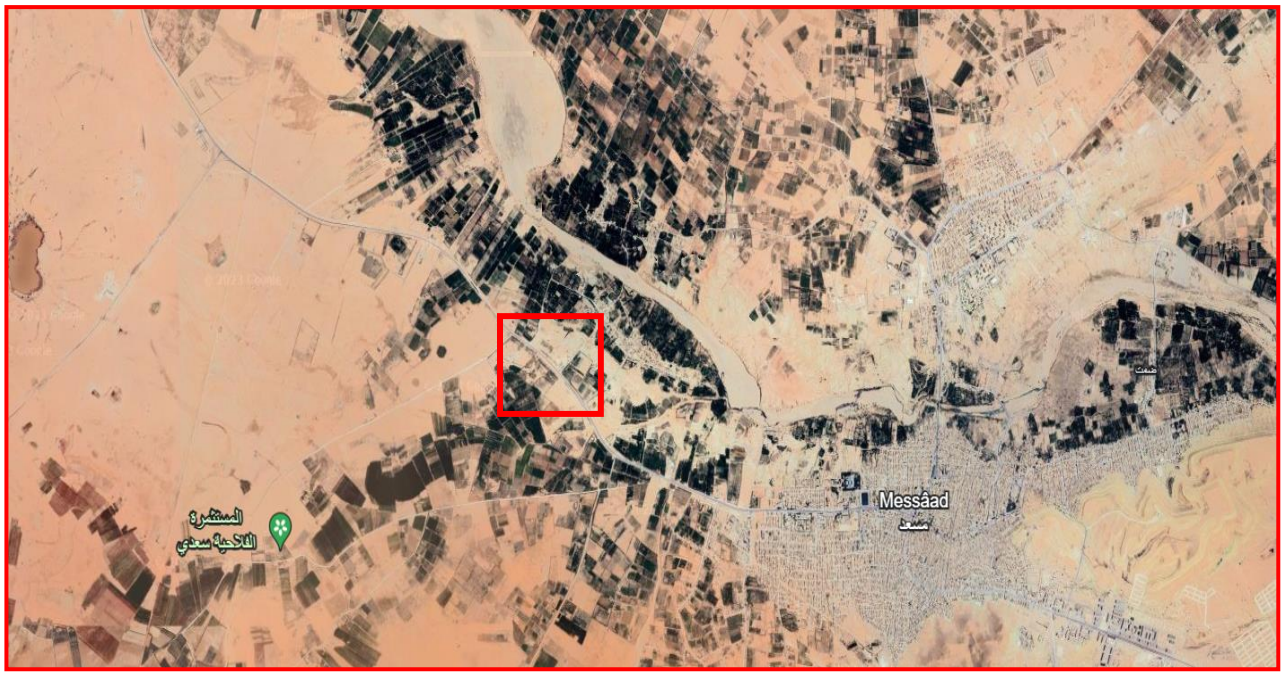
Mesaad, akin to El M'liliha, confronts challenging dry environmental circumstances and a scarcity of water supplies. The indigenous flora in this region is limited, primarily consisting of arid-adapted shrubs and resilient plant species. The existence of vegetation in Mesaad is predominantly ascribed to agricultural activities, particularly the production of resilient crops like barley and dates, which are able to withstand arid conditions.

The plants *peganum harmala*, *Picric hieracioides, Opuntia ficus-indica* collected from messad with the altitude 34,1691274 and longitude of 3.4450588 **(fig 13)**.

However *Artemisia Campestris* was harvested from M’liliha **(fig 14).**

Both *Artemisia Campestris* and *Peganum Harmala* were collected in January of 2023, While the *Opuntia* and *Picris Heraciodes* were collected in march 2023

The aerial part and the root part of these plants were dried in the shade before use were dried before being utilized.



, **Fig 13:** location of the harvesting site of the plants *p. harmala, p. hieracioides and Opuntia*

Fig 14: location of the harvesting site of *Artemisia campestris*

II.2 Laboratory equipment:

- ✓ Spectrophotometer (*spectronic20genesys TM*).
- ✓ Incubator.
- ✓ Rotary steamer (*Büchi*)
- ✓ Precision scale.
- ✓ Agitator.
- ✓ Autoclave.
- ✓ Grinder (*Bomann*).
- ✓ Precision balance
- ✓ Fridge
- ✓ Ultrasonic bath *(Bandlex)*

II.2.1 Small material:

- ✓ Bunsen burner
- ✓ Vortex
- ✓ Platinum handle
- ✓ Metal clip
- ✓ Micropipette
- ✓ Caliper

II.2.2 Glassware:

- ✓ Screw tubes
- ✓ Sterile petri dishes
- ✓ Pasteur pipette
- ✓ Bottle with cap
- ✓ Sterile swabs
- ✓ Beakers
- ✓ Test tube

II.2.3 Microbial strains used:

The used microbial strains were extracted from the laboratory of Mohad Abd Elkader Hospital in Djelfa, and they are as follows:

- ✓ *Escherichia coli*
- ✓ *Pseudomonas aeruginosa*
- ✓ *Staphylococcus aureus*
- ✓ *Proteus mirabilis*
- ✓ *Streptococcus pneumonia*
- ✓ *Klebsiella pneumonia*

II.3 Extraction

II.3.1 Extraction by solvents:

We employed the successive maceration extraction method using a mixture of ethanol and distilled water as the solvent.

The amount of solvent was adjusted to match the amount of plant material to be extracted. In our particular case, the mass of plant matter was different, due to the density of each one **(tab 1).**

	Peganum harmala	*Picric hieracioides*	*Opuntia*	*Artemisia campestris*
Leafs (g)	60	36	55	75
Roots (g)	100	45	/	60

***Tab 1*:** quantities of plants material used

Finely, crushed and subjected to extraction with 150 mL of solvent (105 mL ethanol/45 mL water). The extraction process involved continuous stirring at room temperature for 30 minutes to an hour **(fig 15)**. Then, it was left undisturbed for 24 hours and then filtered through Whatman paper.

Fig 15: extracts during stirring at room temperature **(Original, 2023)**

This plant material was subjected to three successive extractions using the same solvent and procedure. The resulting filtrates from each extraction were combined and then concentrated using a *Büchi*-type rotary evaporator to remove ethanol **(fig 16)**.

fig 16: Rotary evaporator (*Buchi*) **(original, 2023)**

fig 17: extracts ready to evaporate **(original, 2023)**

The obtained concentrated extracts were further dried using an *Alpha 1-2 LO plus*-type lyophilizer to eliminate water content **(fig 18)**.

fig 19: Extracts after dry freezing

(original, 2023)

fig 18: Alpha 1-2 LO plus-type lyophilizer

(original, 2023)

II.3.2 Extraction by solvent (ultrasonic)

We utilized identical parameters to those employed in successive maceration extraction, and then we subjected the mixture to acoustic vibrations for a duration of 30 minutes using an ultrasonic bath (*bandelin sonorex*) **(fig 20)**, as an alternative to the conventional 24-hour infusion period.

Fig: 20 ultrasonic bath (bandelin sonorex)

The resulting filtrates from each extraction were combined and then concentrated using a *Büchi*-type rotary evaporator to remove ethanol. The concentrated extracts were further dried using an *Alpha 1-2 LO plus*-type lyophilizer to eliminate water content.

II.3.3 Yield determination

The yield designates the mass of the extract determined after evaporation of the solvent, it is expressed as a percentage compared to the initial mass of the plant subjected to the extraction.

II.4 Quantitative analyzes of extracts

Quantitative determinations of the main groups of secondary metabolites have been carriedout on the extracts.

II.4.1 Total polyphenols dosage:

Principal:

Polyphenols were determined using spectrophotometry according to the Folin-Ciocalteu method (Singleton et al., 1999), this yellow-colored reagent is composed of a mixture of phosphotungstic acid and phosphomolybdic acid. When polyphenols are oxidized, they reduce the Folin-Ciocalteu reagent into a blue-colored complex consisting of tungsten oxide and molybdenum. The intensity of the color is proportional to the levels of oxidized phenolic compounds (Boizot and Charpentier, 2006).

Procedure:

In a test tube, introduce 1.580 ml of distilled water, 20 µl of extract, and 0.1 ml of Folin-Ciocalteu reagent. Agitate vigorously and then, one minute later, add 0.3 ml of sodium carbonate solution (Na2CO3: 7.5%) and incubate in the shade at room temperature for 2 hours. The absorbance is measured at 765 nm against a blank. **(Fig 21)**

A calibration curve is prepared using Gallic acid as the standard, and the results are expressed in milligrams of Gallic acid equivalent per gram of dry plant weight (mg GAE/g DW) **(Fig 22).**

Fig 21: Measuring the absorbance of each extract using a biotech photometer (ultraspec 1000)

(Original, 2023)

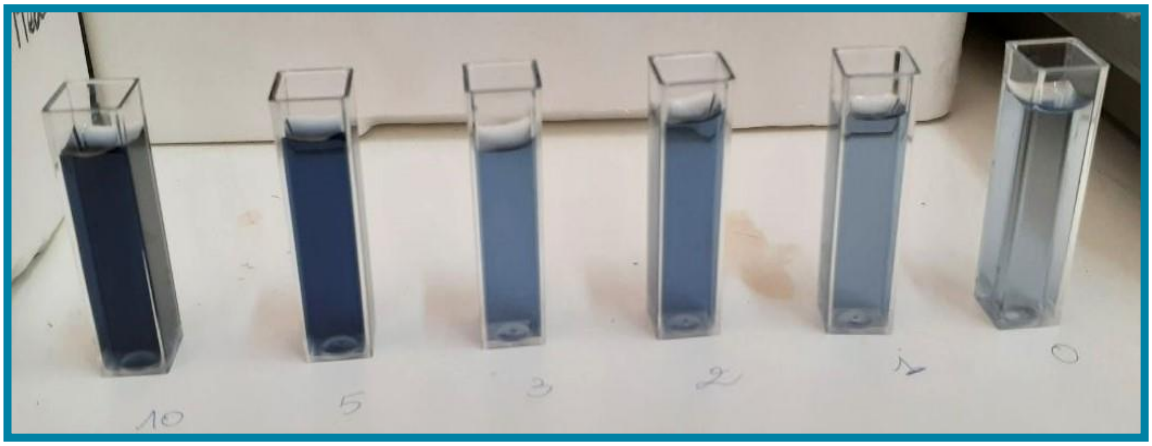


Fig 22: Gallic acid concentrations, which form a calibration curve. **(Original, 2023)**

II.4.2 Flavonoids dosage

The aluminum trichloride AlCl3 method (Kosalec et al., 2004), was employed to quantify flavonoids in our various extracts.

Procedure:

1 ml of the extract solution (prepared in ethanol) was added to 1 ml of 2% AlCl3 in methanol. The mixture was vigorously shaken, and then incubated in the shade at room temperature for 30 minutes. The absorbance was read at 430 nm.

Quantification of flavonoids was carried out based on a calibration curve generated using a standard flavonoid, quercetin. **(Fig 23)**

The flavonoid content is expressed in milligrams of quercetin equivalent per gram of dry plant weight (mg QE/g DW).

Fig 23: Quercitin concentrations, which form a calibration curve. **(Original, 2023)**

II.5 Tests of biological activities:

II.5.1 Antioxidant activity test:

Principle:

DPPH (2, 2-diphényl 1-picrylhydrazyle), a stable violet free radical in solution, exhibits a distinct absorbance within the wavelength range of 512 to 517 nm. This coloration diminishes rapidly upon reduction of DPPH into diphenyl picryl hydrazine through interaction with an antiradical compound, resulting in discoloration. The intensity of this color change is directly proportional to the antioxidant capacity of compounds within the medium to donate protons (Sanchez-Moreno, 2002).

The reaction can be succinctly expressed through the following equation:

$$DPPH^{*} + (AH)_n \longrightarrow DPPH\text{-}H + (A^{*})_n$$

Where: (**AH**) signifies a compound capable of transferring a hydrogen atom to the DPPH radical (violet), thus converting it into diphenyl picryl hydrazine (yellow) (Brand-William et al., 1995)

Experimental Procedure:

A 1 ml solution of ethanolic DPPH (8%) is combined with 1 ml of the extract solution, followed by vigorous agitation. Subsequently, the tubes are incubated in darkness at room temperature for 30 minutes. Methanol serves as the blank solution, while the negative control consists of 1 ml ethanolic DPPH solution and 1 ml ethanol. As for the positive control, it involves a ethanolic solution of a standard antioxidant, such as BHA.

The wavelength of maximum absorption has been previously ascertained, and all readings are conducted at 515 nm.

The antiradical activity is estimated using the following equation:

% Antiradical Activity = (Abs control - Abs sample / Abs control) x 100

Results are presented as the mean of two separate measurements ± standard deviation.

II.2.5 Antimicrobial activity test:

The strains employed to assess the antibacterial activity of our extracts belong to six genera of microorganisms. Specifically, these genera consist of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Streptococcus pneumoniae, Klebsiella pneumoniae,* and *Proteus mirabilis.*

Notably, these strains have been clinically isolated from hospitalized patients at the Djelfa Central Hospital.

a- Strain Conservation:

The strains were preserved at 5°C within sterile tubes containing 10 ml of inclined culture medium (nutrient agar).

b- Culture Media:

In accordance with the methods employed in the assay and the specific strains under investigation, the following culture media were utilized:

- Nutrient agar for the isolation and maintenance of bacterial strains.
- Mueller Hinton agar for studying bacterial sensitivity to our various extracts.

c- Preculture preparation:

The microbial strains designated for testing were cultivated on petri dishes containing nutrient agar. Following an 18-hour incubation at 37°C, microbial suspensions were prepared for each microorganism, with an optical density of 0.5 McFarland, in 5 ml of sterile distilled water.

Fig 24: preparing nutrition agar for the isolation of bacterial strains

d- Antimicrobial Tests:

Sterile Whatman filter paper discs, 6 millimeters in diameter, were impregnated with various solutions of previously dissolved extracts in ethanol. Using sterile forceps, the discs were placed onto the surface of a medium that had been inoculated with a microbial suspension at an optical density of 0.5 McFarland. Following diffusion, the petri dishes were incubated for 18 to 24 hours at 37°C. Post-incubation, the impact of the extracts was manifested by the appearance of a transparent circular zone around the disc, indicating the absence of growth. The larger the diameter of this zone, the more sensitive the strain (Choi et al., 2006).

Ethanol-impregnated discs serving as negative controls.

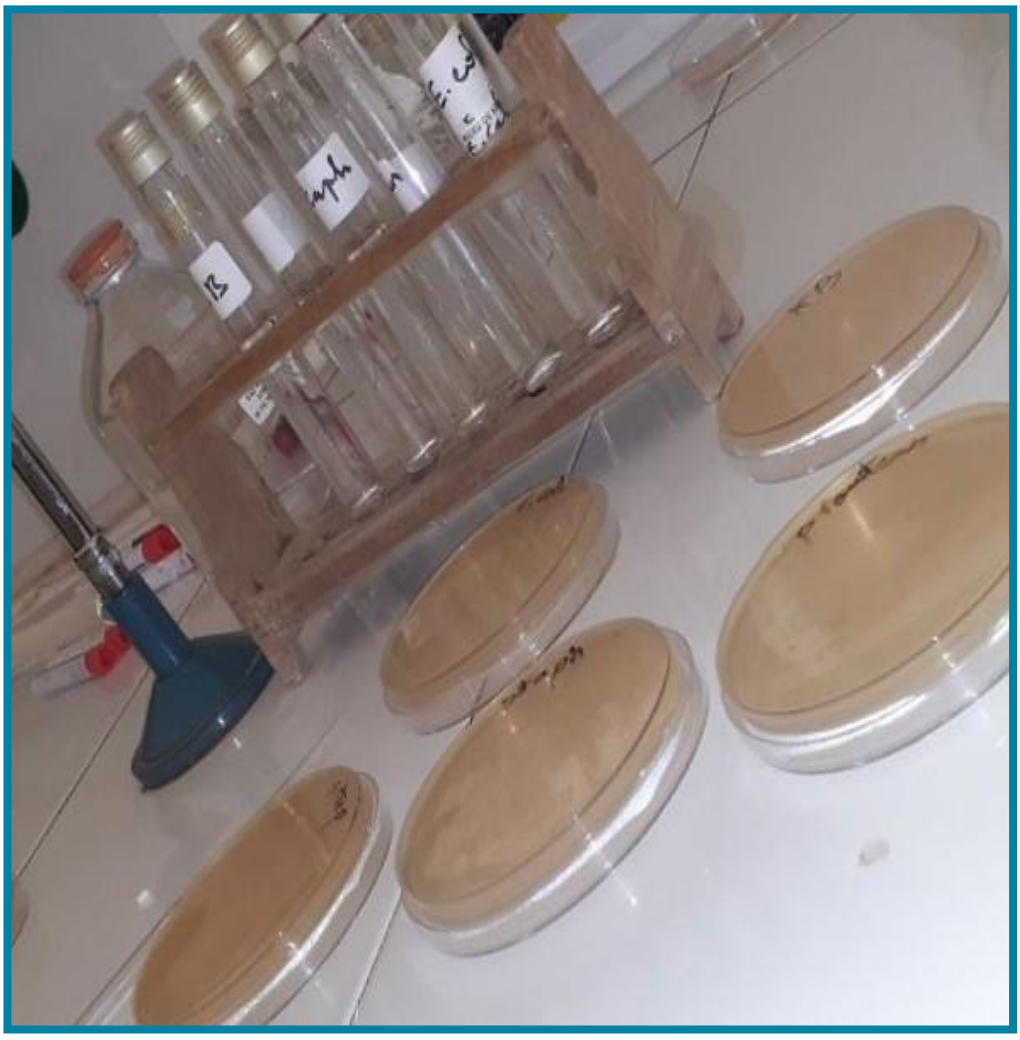


Fig 25: bacterial strains treated with our extracts ready to incubate at 37 Cº

(Original, 2023)

Results

III Results

PeganumHarmala, *Picric hieracioides*, *Opuntia* and *Artemisia campestris*, These are the medicinal plants we used In this research witch contains a huge amount of biological activities such as(polyphenols, flavonoids, tanins…ect) and medical benefits that have been proven by scientists studies before

III.1 Extraction:

a- *successive maceration:*

The preparation of the extracts from the aerial part and roots part of our plant material was carried out by mixing 70% ethanol and 30% water.

The yield of extraction is expressed as a percentage of mass of extract relative to the mass of the fresh plant (**Tab. II**).

Extract		**Aspect**	**Color**	**Yield**
Peganum harmala	Leafs	Sticky paste	Green	23.16%
	Roots	powder	Brown	7.9%
Picric hieracioides	Leafs	Sticky paste	Green	16.66%
	Roots	Pasty	Brown	6.22%
Artemisia campestris	Leafs	pasty	Brown	10.13%
	Roots	Powder	Brown	4.16%
Opuntia	Leafs	Powder	Dark Green	6.22%

TAB II: Aspects, colors and yield of extracts.

b- **Maceration by ultrasonic vibrations:**

The preparation of the extracts from the aerial part and roots part of our plant material was carried out by mixing 70% ethanol and 30% water and subjected to ultrasonic vibrations using ultrasonic bath (*bandelinsonorex*),

The yield of extraction is expressed as a percentage of mass of extract relative to the mass of the fresh plant (**Tab. III**)

Extract		**Aspect**	**Color**	**Yield**
Peganum harmala	Leafs	Stickypaste	Green	15.18%
	Roots	Powder	Brown	5.3%
Picric hieracioides	Leafs	Stickypaste	Green	9.42%
	Roots	Pasty	Brown	5.32%
Artemisia campestris	Leafs	Pasty	Brown	8.17%
	Roots	Powder	Brown	4.87%
Opuntia	Leafs	Powder	Dark Green	4.79%

TAB III: Aspects, colors and yield of extracts (US)

III.2 Quantitative study results

III.2.1: Determination of Total Polyphenols

The quantification of total polyphenols was conducted using the Folin-Ciocalteu method. Gallic acid was employed as the standard compound. The absorbance measurements were performed at a wavelength of 765 nm. The acquired results were graphically depicted on a calibration curve with the equation:

Y = 0.0023x + 0.0605, R^2 = 0.990.

The quantity of polyphenols was expressed in milligrams of gallic acid equivalent per milligram of dry weight of the extract (mg CAE/mg DW)**(fig 26)**. Based on the calibration curve, the concentration of total polyphenols are as follows in (**fig 27**), (**fig 28**).

III.2.2: Determination of Flavonoids:

The determination of flavonoids was carried out using the aluminum trichloride (AlCl3) method, with quercetin employed as the standard compound. The absorbance readings were taken at a wavelength of 430 nm. The experimental outcomes were depicted on a calibration curve with the equation:

$Y = 0.031x + 0.100$, $R^2 = 0.995$.

The content of flavonoids was reported in milligrams of quercetin equivalent per milligram of dry weight of the extract (mg QE/mg DW). (**Fig 29**)

The concentration of total flavonoids are as follows in (**Fig 30**), **(Fig 31)**.

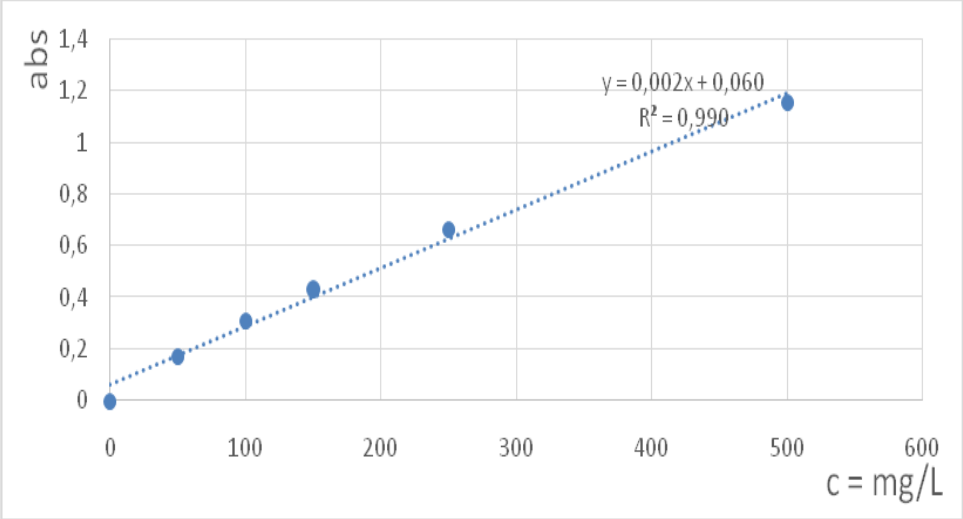


Fig.26: Gallic acid calibration curve

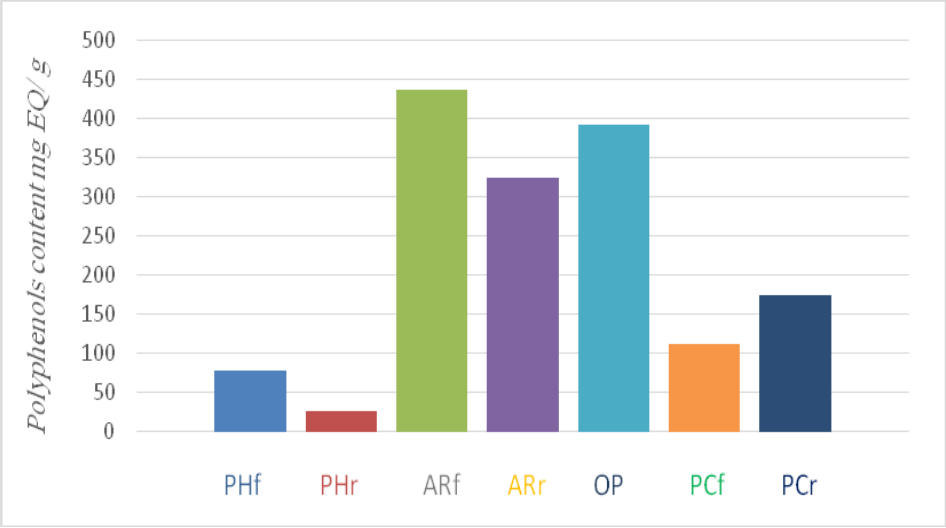


Fig.27: *Evaluation of total polyphenols in extracts (maceration).*

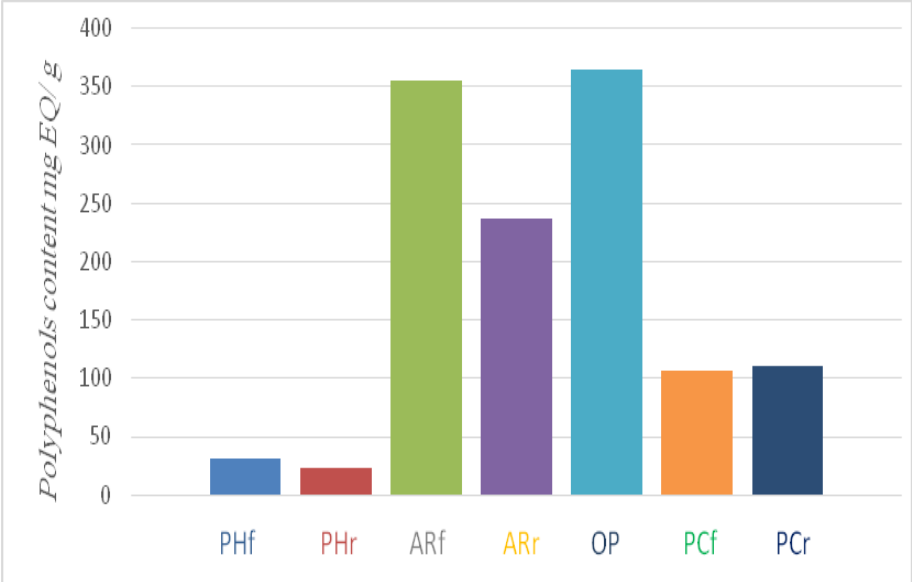


Fig.28: *Evaluation of total polyphenols in extracts(Ultrasonic)*

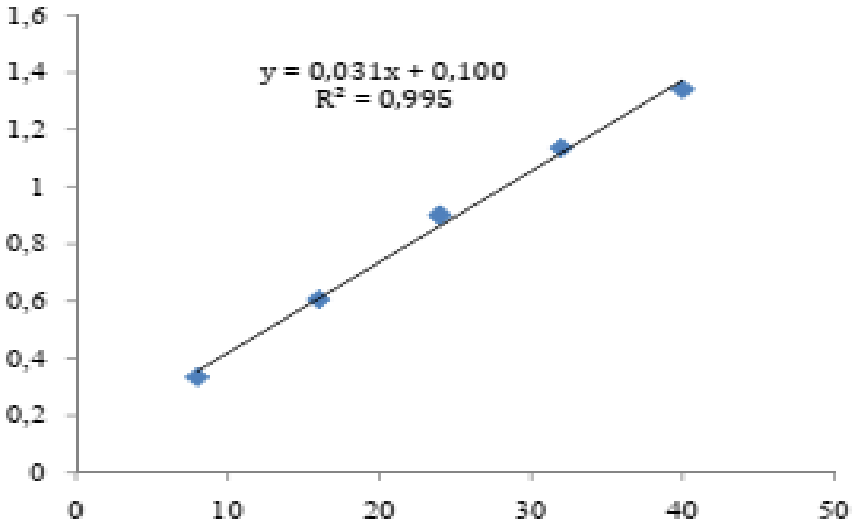


Fig 29: *quercetin calibration curve*

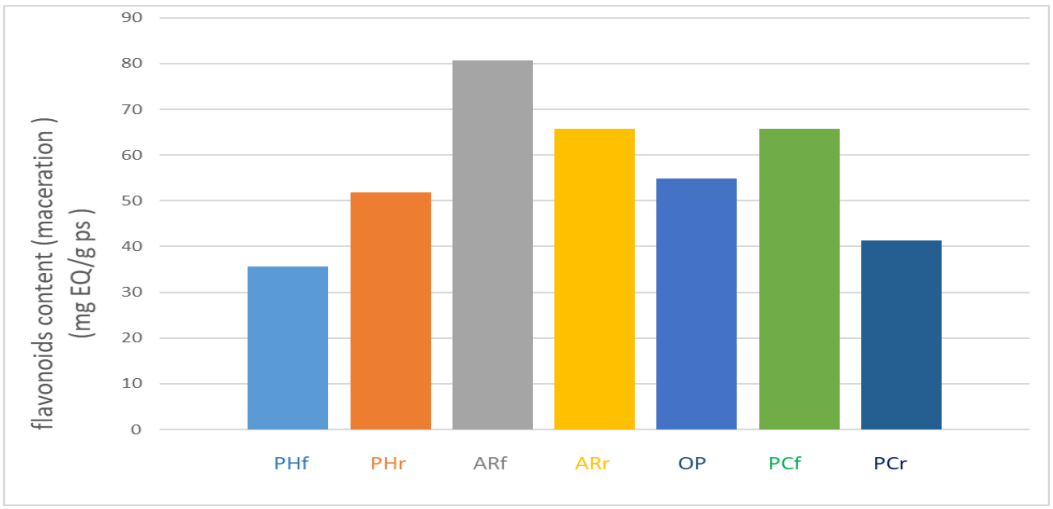


Fig 30: *Evaluation of total flavonoids in extracts (maceration)*

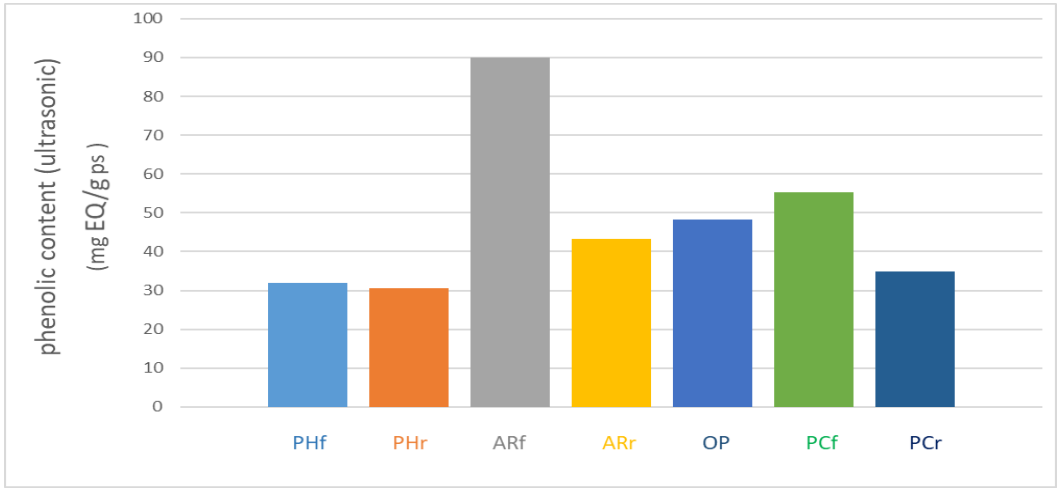


Fig 31: *Evaluation of total flavonoids in extracts (ultrasonic)*

III.3 Biological test results:

III.3.1 Antioxidant activity:

The antioxidant activity of the extracts was evaluated in vitro by the DPPH free radical reduction method.

The antiradical activities of the extracts and of the BHA positive control were determined by the DPPH method.

The results are estimated in the following charts :

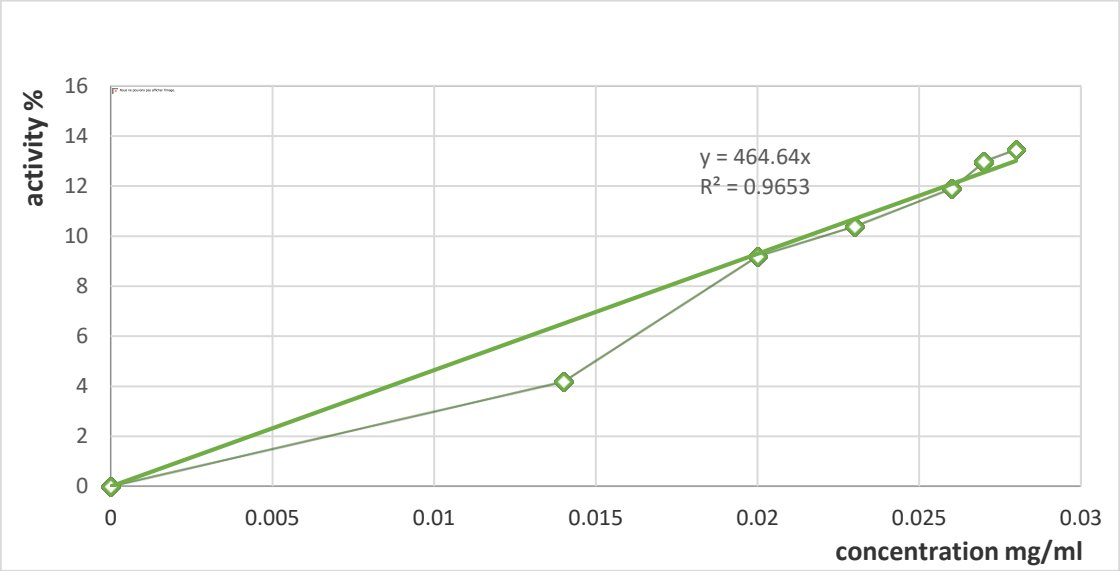


Fig 32: activity curve of *opuntia* extract in inhibiting the free radical DPPH in terms of concentrations.

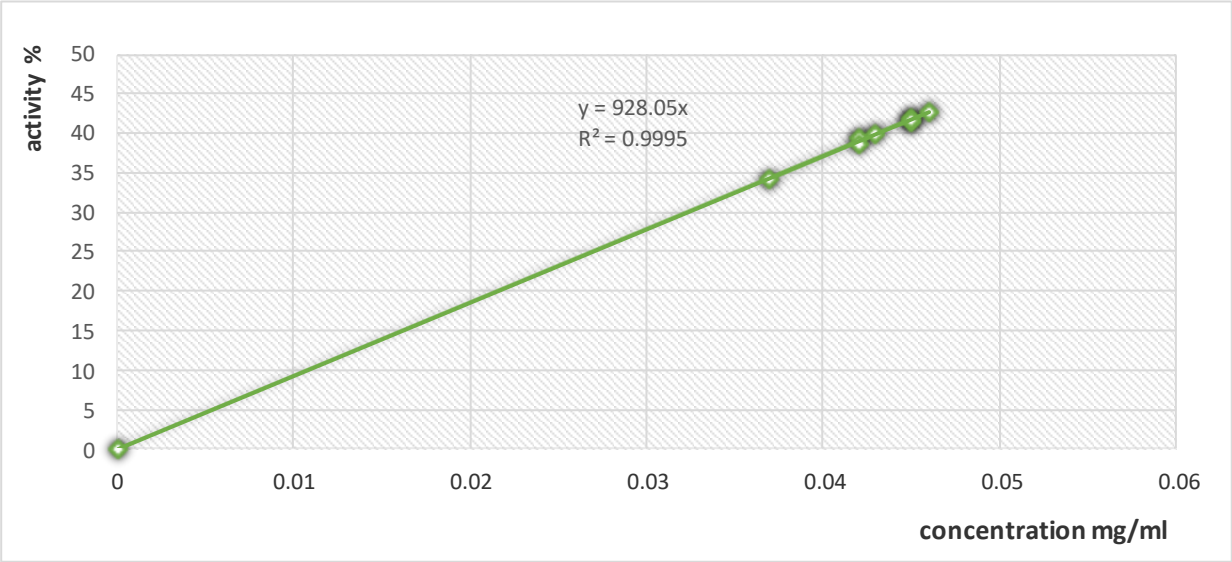


Fig 33: activity curve of *Picric hieracioides* leafs extract in inhibiting the free radical in terms of concentration

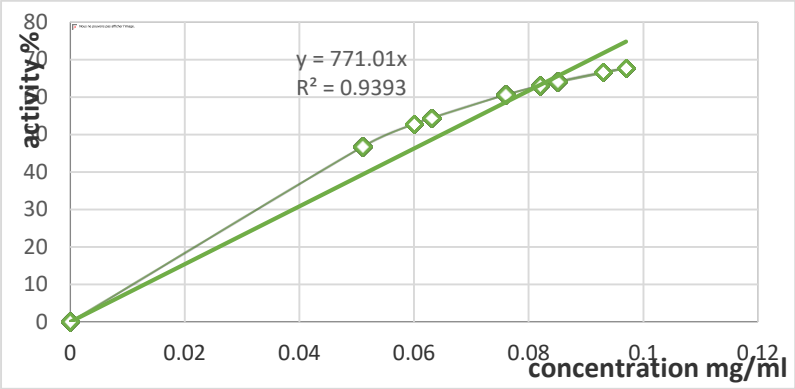


Fig 34: activity curve of *Picric hieracioides* roots extract in inhibiting the free radical in terms of concentration

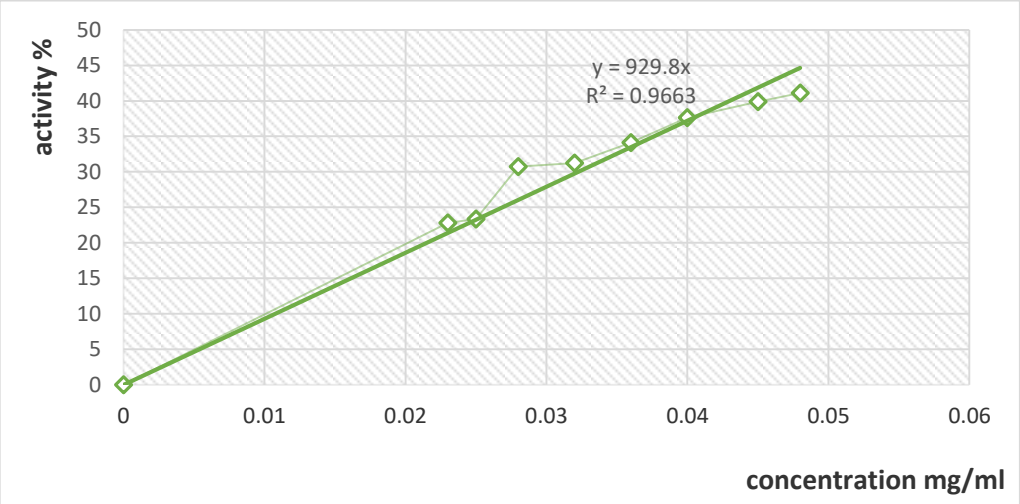


Fig 35: activity curve of *peganum harmala* leafs extract in inhibiting the free radical in terms of Concentration

.

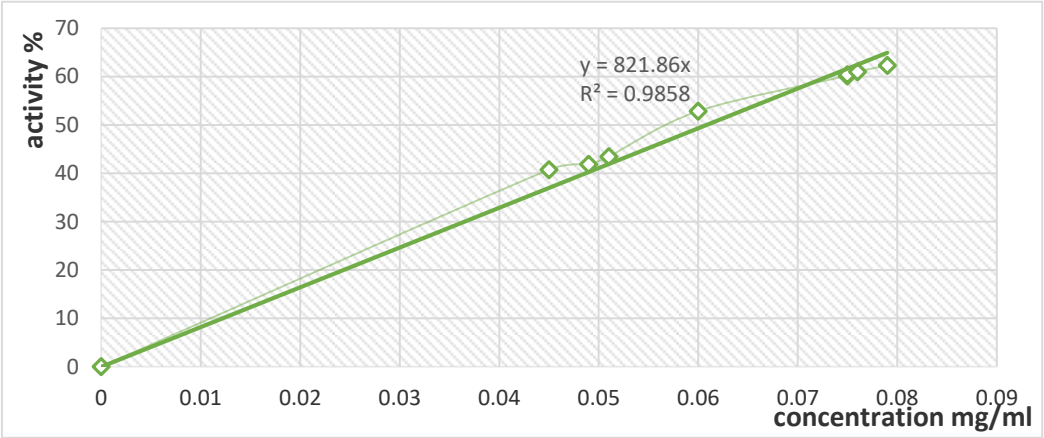


Fig 36: activity curve of *peganum harmala* roots extract in inhibiting the free radical in terms ofConcentration.

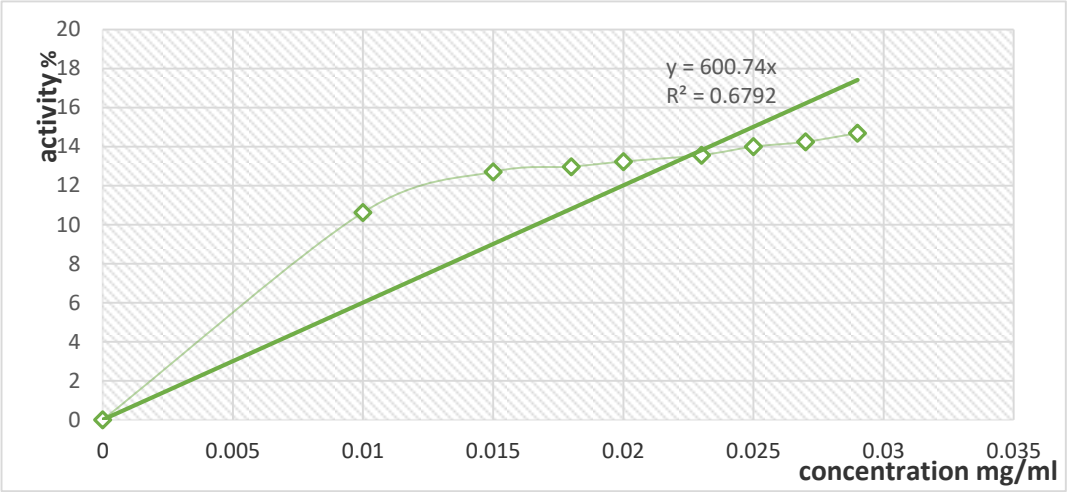


Fig 37: activity curve of *Artemisia campestris* leafs extract in inhibiting the free radical in terms ofConcentration.

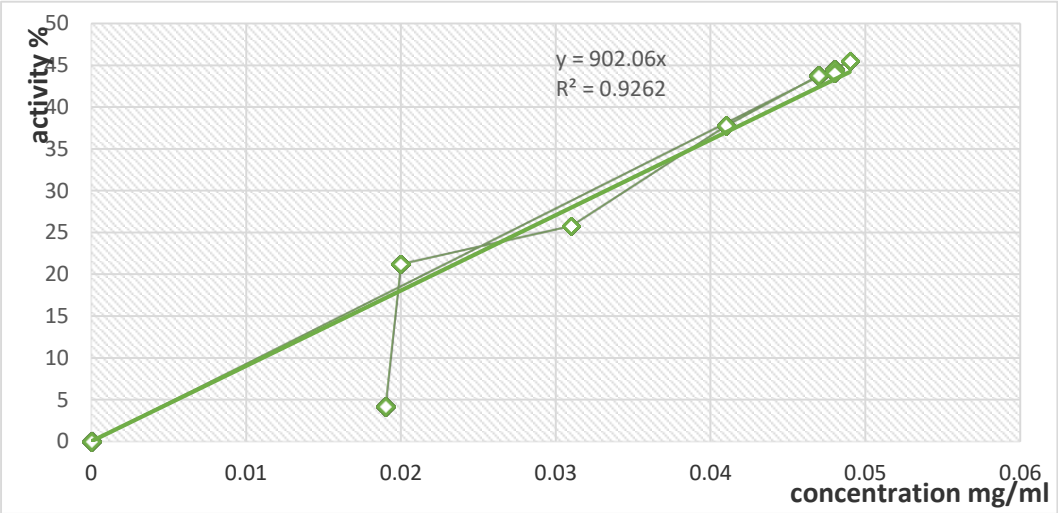


Fig 38: activity curve of *Artemisia campestris* roots extract in inhibiting the free radical in terms of Concentration.

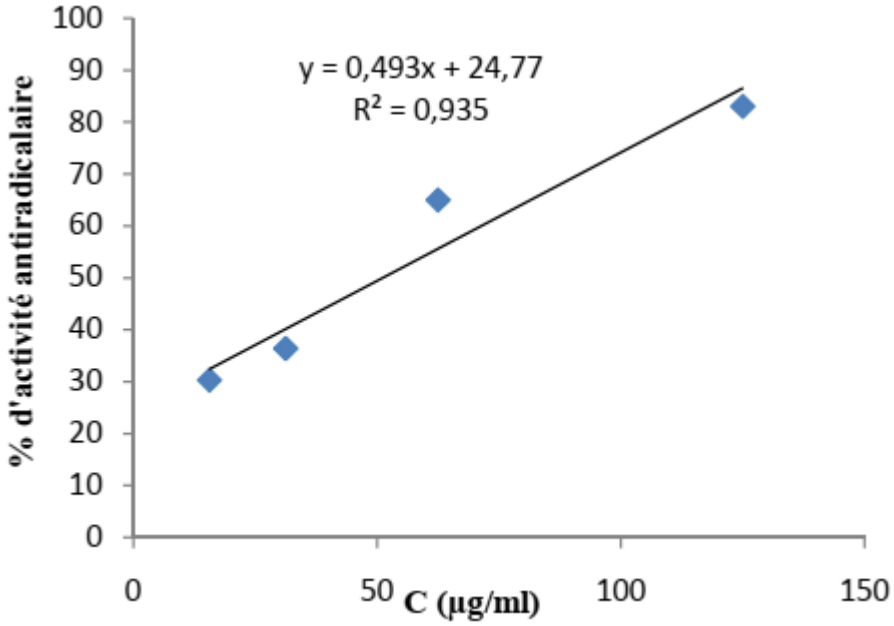


Fig 39: activity curve of slandered BHA (ascorbic acid) in inhibiting the free radical in terms of Concentration.

The Ic_{50} value for each extract:

Extracts	Peganum harmala leafs	*peganum harmala* roots	*Artemisia campestris* leafs	*Artemisia campestris* roots	*Picric hieracioides* leafs	*Picric hieracioides* roots	Opuntia
Ic_{50} (µg/ml)	**31.96**	**51.485**	**12.65**	**24.845**	**36.385**	**57.235**	**8.81**
Ic_{50} of the stranded BHA	**56.5**						

Tab. III: The Ic50 value for each extract.

III.3.2 Antimicrobial activity:

The disc method made it possible to determine the action of the plant extracts dissolved in ethanol on the different strains, this results in the appearance of a zone of inhibition around the disc of paper previously impregnated with the extract as a witness to the absence of bacterial growth in this area.

The diameter of the zone of inhibition differs from one bacterium to another and from one extract to another. The variation in the antimicrobial activity of the extracts explains the variations in their chemical compositions.

All the extracts reacted positively on at least one of the microbial strains tested, which confirms that the plants are endowed with antimicrobial properties.

The results of extracts activity with strains are listed in the following table:

	PHf	**PHr**	**ARr**	**ARf**	**PCf**	**PCr**	**OP**
Streptococcus pneumonia	S	S	S	S	R	S	R
Pseudomonas aeruginosa	S	Very S	Very S	S	S	S	R
Staphylococcus aureus	very S	S	S	S	R	Very S	R
Escherichia coli	very S	S	S	S	R	S	S
Klebsiella pneumonia	S	S	S	S	R	Very S	S
Proteus mirabilis	S	R	S	ExtrS	Very S	Very S	S
Extr S: Extremely Sensitive *Very S: Very sensitive* *S: Sensitive* *R: resist*							

Table IV: Sensitivities of microbial strains to extracts.

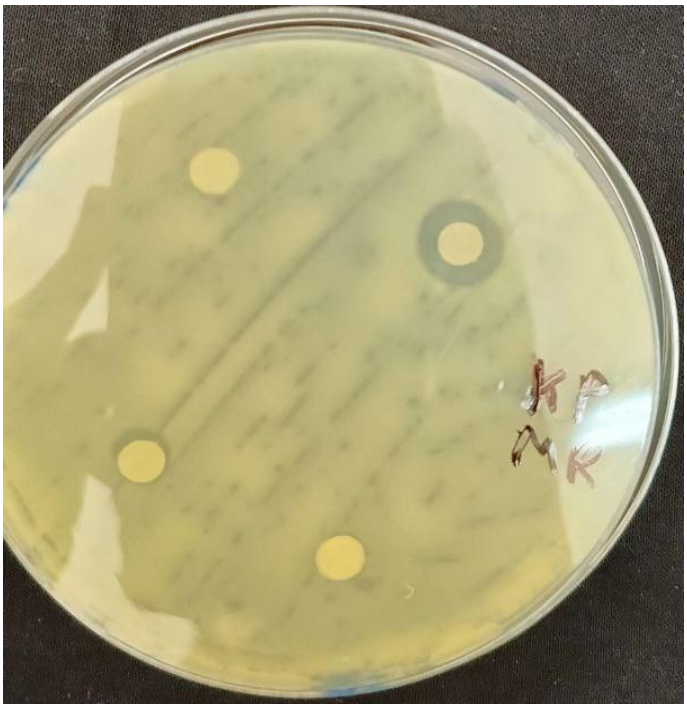


Fig 40: *Klebsiella pneumonia : PCr extract*

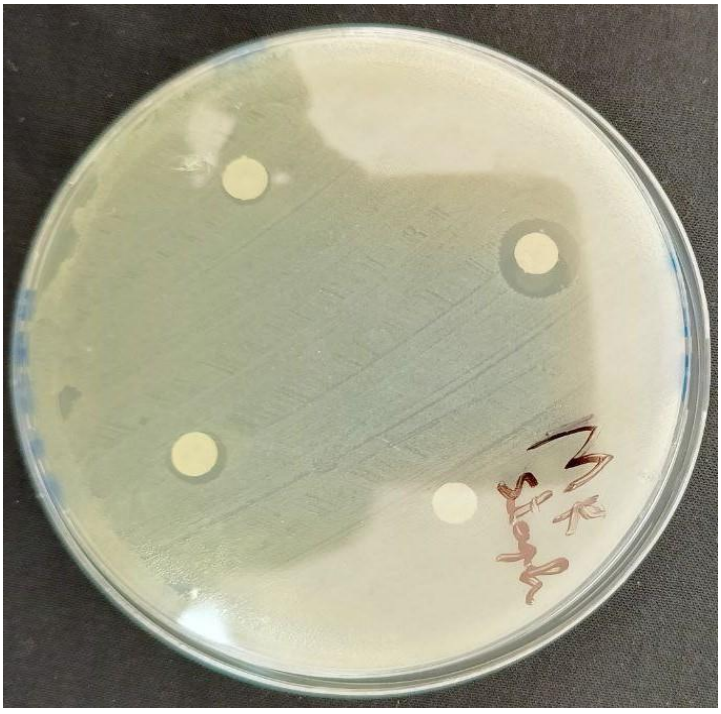


Fig : 41 *Staphylococcus aureus: PCr extract*

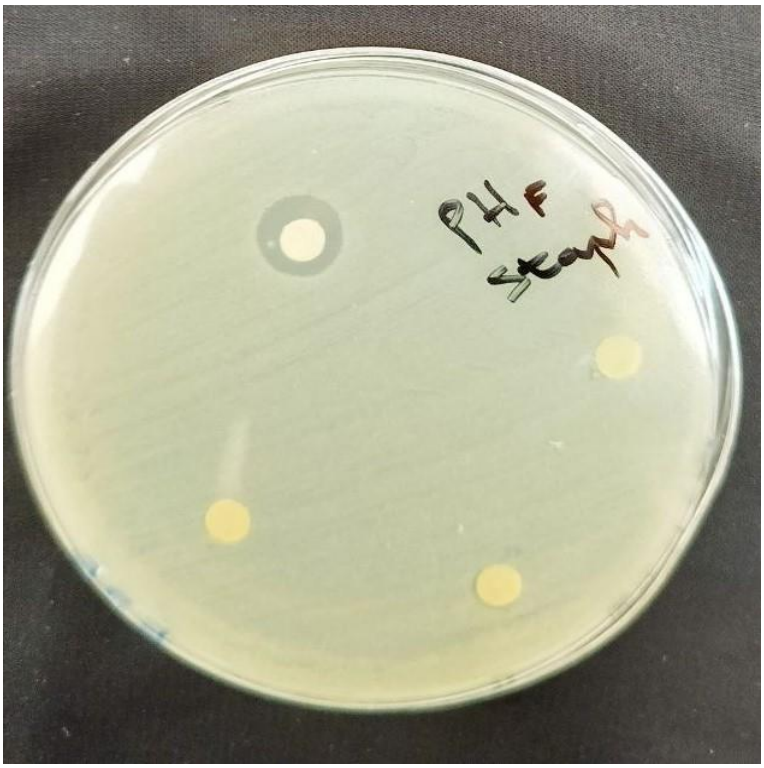


Fig 42 : *Staphylococcus aureus :PHf extract*

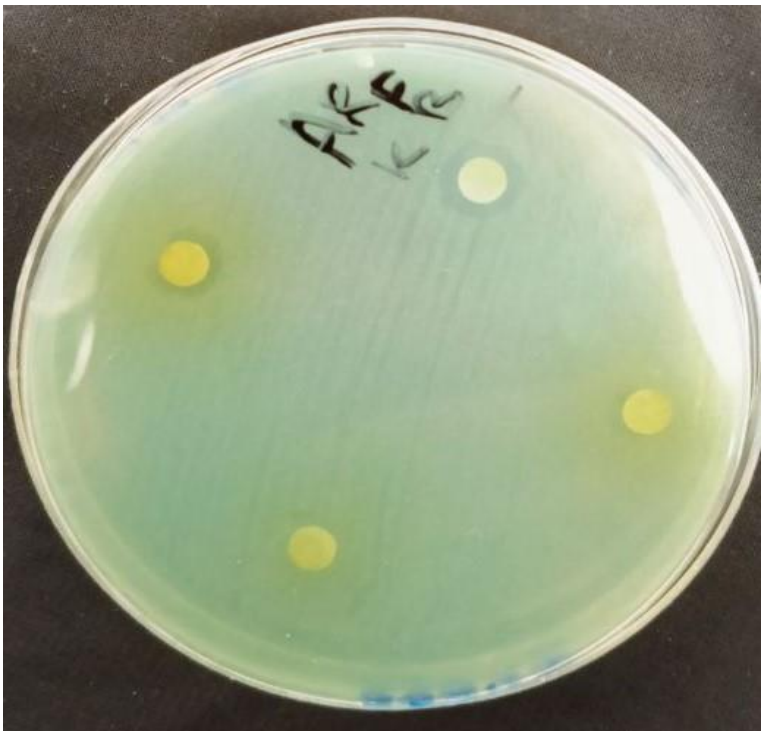


Fig 43: *Klebsiella pneumonia: ARf extract*

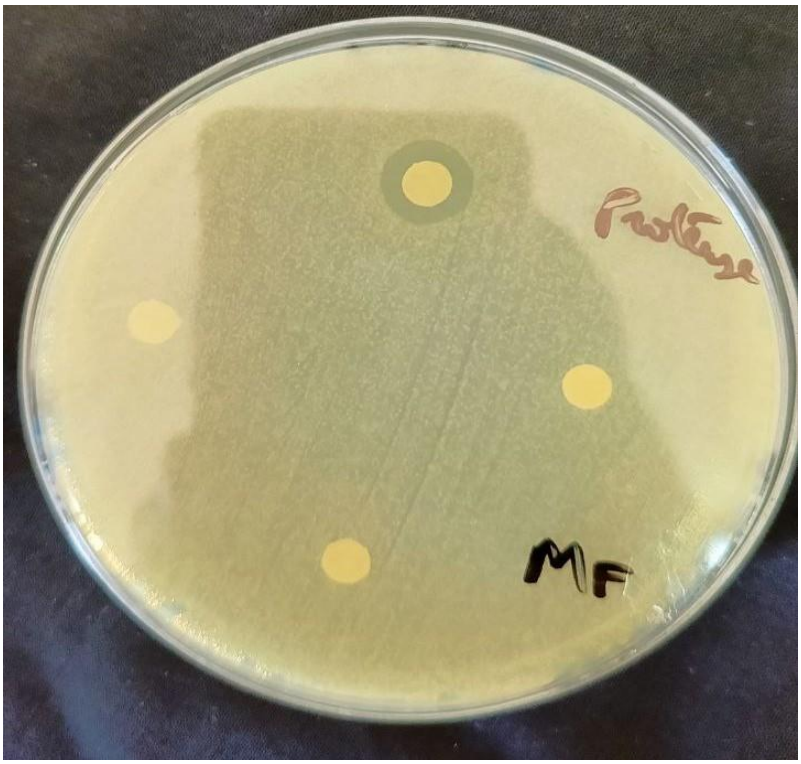


Fig 44: *Proteus mirabilis: PCf extract*

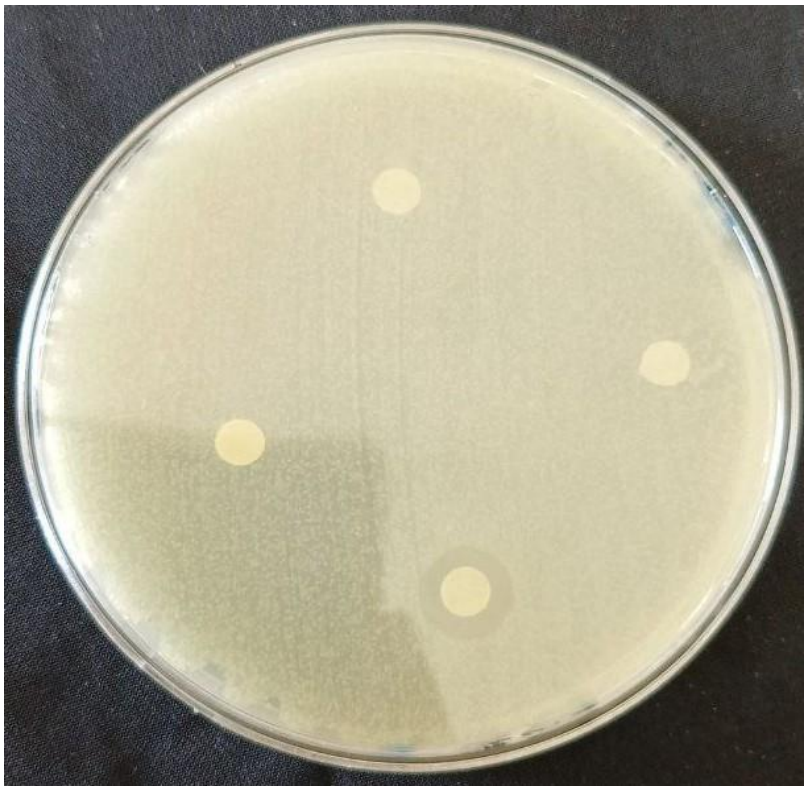


Fig 45 : *Staphylococcus aureus :PHf extract*

Discussion

Discussion

IV.1 Extraction:

IV.1.1 Successive Maceration:

Successive maceration is a traditional method of extracting compounds from plant materials. It involves multiple cycles of soaking the plant material in a solvent, usually at room temperature, and then separating the solvent from the plant material to obtain the desired extract. This method relies on the principle of diffusion, where the solvent gradually penetrates the plant material to dissolve the target compounds.

Prior, R and al, (2005). In their study titled "Extraction methods of natural antioxidants from edible plant materials," the authors discuss the successive maceration technique as one of the conventional methods for extracting antioxidants from plant materials.

IV.1.2 Ultrasonic Maceration:

Ultrasonic maceration is a modern extraction technique that utilizes high-frequency ultrasound waves to enhance the extraction process. Ultrasonic waves create cavitation bubbles in the solvent, which implode near the plant material's surface, facilitating the release of compounds from the plant cells. This method is known for its efficiency and reduced extraction time compared to traditional methods.

The paper "An overview of the ultrasonically assisted extraction of bioactive principles from herbs" by Vinatoru, (2001) provides insights into the principles and applications of ultrasonic extraction, emphasizing its advantages in improving extraction efficiency.

IV.1.3 Comparative Analysis:

Extraction Efficiency: Successive maceration is a relatively slower process as it relies on diffusion alone. It may require longer extraction times to achieve complete compound extraction.

Ultrasonic maceration significantly improves extraction efficiency due to the cavitation effect, leading to faster and more complete extraction of target compounds.

Quality of Extracts: Successive maceration is generally considered gentler on heat-sensitive compounds, which may be advantageous for preserving the quality of certain bioactive compounds.

Ultrasonic maceration, while efficient, may generate heat due to cavitation, potentially affecting the stability of heat-sensitive compounds.

Solvent Consumption: Successive maceration may require larger volumes of solvent due to the need for multiple extraction cycles.

Ultrasonic maceration often requires smaller volumes of solvent, making it more environmentally friendly and cost-effective.

Energy Consumption: Successive maceration is energy-efficient as it typically operates at room temperature.

Ultrasonic maceration consumes more energy due to the ultrasonic equipment but offers faster extraction times.

In conclusion, while successive maceration is a traditional and gentler extraction method, ultrasonic maceration stands out for its efficiency and reduced extraction time. The choice between these methods should depend on the specific objectives of the extraction process, the nature of the plant material, and the desired quality of the extract. Researchers often select the method that aligns best with their goals and available resources.

IV.2 Quantitative analyzes of the extracts

The quantitative study of raw extracts by means of spectrophotometric assays, aimed to determine the latency of total polyphenols, flavonoids. The main reason for choosing these substances lies in the fact that the majority of the antioxidant and antimicrobial properties of plants are attributed to them.

Polyphenols are estimated by several methods, such as the Prussian blue method (Graham, 1992), but the most widely used is that of Folin-Ciocalteu. This reagent consists of a mixture of phosphotungstic acid and phosphomolybdic acid, it is reduced by phenols to a mixture of blue oxides of tungsten and molybdenum (Boizot and Charpentier, 2006).
In the presence of polyphenols, the Folin-Ciocalteu complex changes its color from yellow to blue, which makes it possible to measure the intensity of the color at a wavelength of 765 nm (Huang et al., 2005).

The results of the dosage of total polyphenols show that the extract of the aerial part of Artemisia campestris represents the richest extract with: 435.86 mg EAC/g of extract, followed by the extract of the root part of Artemisia campestris 323.69 mg EAC/g extract.
The peganum harmala leaf extract with 77.39 mg EAC/g of extract and 23.26 mg
EAC/g root part represents the fraction, which contains the lowest polyphenol content.

In a study made on eleven medicinal plants including, Djeridane et al., (2006) determined the content of total polyphenols of the aerial part of a 70% (v/v) ethanolic extract, they found that the content of polyphenols total is 20.38 mg EAG/g Ps, this content is relatively high. In another study,

Djeridane et al. (2007) measured the total polyphenols in an ethanolic extract (80%), the content found was 103.4 mg EAG/g

Ps, this result is relatively very high, it is 5 times higher to that found in the previous study. This content can reach more than 450 mg EAG/g of extract when the extraction is carried out with a 50% alcoholic solution (Akrout et al., 2011).

This difference in the contents can be explained by the environmental, climatic conditions and collection period as well as by the genetic factors and the experimental conditions.

The quantitative determination of flavonoids is carried out by the aluminum trichloride method, this one is the most used, it is based on the formation of a flavonoid-aluminum ion complex having a maximum absorbance at 430 nm. Quercetin is used as a standard, the results of the flavonoid assay are in the range of 95.7 and 2.60 mg EQ/g extract.
Djeridane et al, (2006) and (2007), determined the concentration of flavonoids in two 70% and 80% (v/v) ethanol extracts. This content was estimated at 7.46 and 5 mg RE/g Ps in the two extracts respectively.

However, Akrout et al (2011) estimated this value at 56.31 mg RE/g of extract when the extraction is carried out with a 50% alcoholic solution.

While Saoudi et al., (2010) found a content of 131.89 mg EQ/g of aqueous leaf extract.

The total polyphenol content of the extracts are relatively identical to those found by other authors on plants of the same species obtained from other sites (Algeria and Tunisia). While the flavonoid content of the species studied seems slightly lower than those found by other authors for the same species (Saoudi et al., 2010).

IV.2 Antioxidant activity

IV.2.1 DPPH free radical reduction method

The DPPH test was utilized to assess the antiradical activity of various extracts. This technique is commonly employed for its rapidity in yielding results and for evaluating the antioxidant properties of compounds found in plant extracts (Yi et al., 2008).

Among the various extracts examined, the extract derived from the roots of Picric hieracioides exhibits the highest level of activity, as indicated by its IC50 value of 57.235 (µg/ml). This is closely followed by the extract obtained from the roots of peganum harmala, which demonstrates an IC50 value of 51.485 (µg/ml). Conversely, the remaining extracts exhibit lower antiradical capacities in comparison to the IC50 value of BHA. Furthermore, the antioxidant capacity, as determined by the DPPH test, is relatively modest for all extracts.

The antioxidant activities of three different extracts obtained from Artemisia campestris were investigated by Akrout et al. (2011). The investigation resulted in the determination of an IC50 value of 2,053 mg/mL for the 50% ethanol extract. When considering the comparison, it becomes evident that this particular number is significantly lower in relation to the IC50 value acquired from our extract, which was measured at 24.845 µg/ml.

Moreover, Lopes-Lutz et al. (2008) supported the findings in their own comprehensive investigation that included multiple Artemisia species. The findings of their study aligned with the prevailing understanding that the plants under investigation demonstrate a very low level of antioxidant activity.

In accordance with this assertion, Kang et al. (2003) put up a theory positing that plant extracts containing polar molecules have a propensity to demonstrate heightened levels of activity in scavenging free radicals.

Several academic studies have emphasized the association between the inhibitory ability of botanical substances on the DPPH radical and their chemical composition. Furthermore, these investigations have emphasized the crucial significance of phenolic components in the antioxidant effectiveness of botanical extracts. DEBOUBA et al. have expounded upon the correlation between the antioxidant properties of phenolic compounds and the number of hydroxyl groups connected to the aromatic ring in their study conducted in 2012. In addition, the study by ZHENG et al. (2010) encompassed a thorough investigation into the inhibitory properties of 13 distinct flavonoids on DPPH radicals. This research provided valuable insights into the crucial role played by the quantity and arrangement of hydroxyl groups in determining the inhibitory activity. According to Ben Salama (2012), the optimal inhibitory activity on DPPH radicals is attributed to the presence of a hydroxyl group at C3 and the ortho-dihydroxyl structure.

IV.3 Antimicrobial activity

Plants contain many compounds with an action antimicrobial, these constituents include phenolics, flavonoids, oils essential and triterpenoids (Rojas et al., 1992), the antimicrobial power of plant extracts East depending on their chemical composition.
Very little research has focused on the study of antimicrobial activity (Ben Sassi et al., 2007; Naili et al., 2010).

All extracts were found to be active against all bacterial strains tested but with different degrees (**Tab VI**).

Naili et al (2010) studied the antibacterial activity of the methanolic extract of leaves of A. campestris. They used several strains including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, the results obtained in this study showed that this extract has an inhibitory effect on all the bacteria studied.

Conclusion

Conclusion

Nowadays, a large number of aromatic and medicinal plants have very important biological properties which find many applications in various fields, namely in medicine, pharmacy, cosmetology and agriculture. This renewed interest comes on the one hand from the fact that medicinal plants represent an inexhaustible source of bioactive substances, and on the other hand, the side effects induced by the drugs worry the users who turn to less aggressive care for the body. In the present work, different aspects have been studied some photochemical properties and antioxidant and antimicrobial activities of crude extracts.

The extraction of the aerial and root part of these plants has made it possible to obtain yields which differ according to these plants used and the method of extraction, therefore the content of phenolic compounds, flavonoids are relatively different.

The antioxidant activity of the different extracts was evaluated by the DPPH free radical reduction method. In the DPPH free radical reduction test, the scavenging activity is high in the polar extract.

The antimicrobial activity was determined on six bacterial strains, according to the disk diffusion method. The results indicate that the extracts have antimicrobial activity on all the strains tested except the *opuntia* extract, which has no activity on the strains*: Streptococcus pneumonia*, *Pseudomonas aeruginosa*, and Staphylococcus aureus. Following these results, it would therefore be interesting to extend the range of antioxidant and antimicrobial tests as well as the isolation and characterization of the active compounds in the different extracts in order to identify the different molecules responsible for the different biological activities. of these plants.

All of these results obtained in vitro constitute only a first step in the search for biologically active substances of natural origin, an in vivo study is desirable, to obtain a more in-depth view of the antioxidant and antimicrobial activities of the extracts of these plants.

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Abstract
There are numerous medicinal plants in the region of djelfa in Algeria, including Peganum harmala, Artemisia campestris, Opuntia, and Picris hieracioides. Traditional medicine employs these plants due to their medicinal properties. Algeria's diverse plant flora contains numerous medicinal plants, such as Peganum harmala, Artemisia campestris, Opuntia, and Picris hieracioides. Traditional medicine has utilized these plants for their therapeutic potential. High yields of organic extracts were obtained from Peganum harmala leaf extract and Artemisia campestris roots. Utilizing the Folin-Ciocalteu method, the total phenolic content was determined. The concentration was highest in the aerial portion of Artemisia campestris (435.86 mg EAC/g of extract), followed by the root portion (323.69 mg EAC/g of extract). The AlCl3 method was used to determine flavonoid content, with assay results ranging from 95.70 to 2.60 mg EQ/g of extract. The leaf extract of Picric hieracioides exhibited the highest antiradical activity at 45.07%, surpassing all other extracts. Overall, however, the antioxidant capacity was only moderate. Using disk diffusion assays, antimicrobial activity was evaluated against six bacterial strains. The extracts showed sensitivity against all tested microorganisms, but Opuntia extract did not show any effect on Streptococcus pneumonia, Pseudomonas aeruginosa, or Staphylococcus aureus.

Résumé
Il existe de nombreuses plantes médicinales dans la région de djelfa en Algérie, y compris Peganum harmala, Artemisia campestris, Opuntia, et Picris hieracioides. La médecine traditionnelle emploie ces plantes en raison de leurs propriétés médicinales. La flore végétale diversifiée de l'Algérie contient de nombreuses plantes médicinales, telles que Peganum harmala, Artemisia campestris, Opuntia et Picris hieracioides. La médecine traditionnelle a utilisé ces plantes pour leur potentiel thérapeutique. Des rendements élevés d'extraits organiques ont été obtenus à partir de l'extrait de feuilles de Peganum harmala et des racines d'Artemisia campestris. En utilisant la méthode Folin-Ciocalteu, la teneur phénolique totale a été déterminée. Les concentrations les plus élevées ont été observées dans la portion aérienne d'Artemisia campestris (435,86 mg d'ACE/g d'extrait), suivie de la partie de racine (323,69 mg de ACE/G d' extrait). La méthode AlCl3 a été utilisée pour déterminer la teneur en flavonoïdes, avec des résultats d'essai allant de 95,70 à 2,60 mg EQ/g d'extrait. L'extrait de feuille de Picric hieracioides a montré la plus grande activité antiradiqualaire à 45.07%, dépassant tous les autres extraits. Dans l'ensemble, cependant, la capacité antioxydante n'était que modérée. En utilisant des essais de diffusion des disques, l'activité antimicrobienne a été évaluée contre six souches bactériennes. Les extraits ont montré une sensibilité à l'égard de tous les micro-organismes testés, mais l'extrait d'Opuntia ne montre aucun effet sur Streptococcus pneumonia, Pseudomonas aeruginosa ou Staphylococcus aureus.

ملخص
هناك العديد من النباتات الطبية في منطقة الجلفة في الجزائر، بما في ذلك نبات بيجانوم هارمالا، وأرتيميسيا كامبيستريس، وأوبونتيا، وبيكريس هيراسيويدز. يستخدم الطب التقليدي هذه النباتات بسبب خصائصها الطبية. تم الحصول على إنتاجية عالية من المستخلصات العضوية من مستخلص أوراق بيجانوم هارمالا وجذورأرتيميسيا كامبيستريس .تم تحديد المحتوى الفينولي الكلي باستخدام طريقة فولين-سيوكالتو وكان التركيز الأعلى في أوراق الارتيميسيا كامبيستريس (435.86 ملجم/EAC جم من المستخلص)، يليه الجزء الجذري (323.69 ملجم/EAC جم من المستخلص) .تم استخدام طريقة ثلاثي كلوري الالمنيوم لتحديد محتوى الفلافونويد، حيث تراوحت نتائج الفحص من 95.70 إلى 2.60 ملجم مكافئ/جم من المستخلص. أظهر مستخلص أوراق المرير الصقري أعلى نشاط مضاد للجذور بنسبة 45.07%، متجاوزًا جميع المستخلصات الأخرى. ومع ذلك، بشكل عام، كانت قدرة مضادات الأكسدة معتدلة نسبيًا. تم تقييم النشاط المضاد للميكروبات ضد ستة سلالات بكتيرية. أظهرت المستخلصات حساسية ضد جميع الكائنات الحية الدقيقة التي تم اختبارها، الا مستخلص الصبار الهندي لم يظهر أي تأثير على بكتيريا المكورات الرئوية, المكورات العنقودية و بسودوموناس اريجانوزا.