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TOPIC

**Ethnopharmacological survey and study of the
antibacterial activity of *Atriplex halimus* leaves
extracts**

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DEDICATION

*First of all, we thank Almighty **Allah** who has given us the courage, strength and will to accomplish this work.*

I dedicate this work

To my dear father, my hero, the man of my life who has always sacrificed to help and encourage me to move forward in life, my source of love and strength.

To my dear mother, the light of my days, the source of my efforts mom who has been by my side and supported me throughout my life.

To my lovely brothers, who gave me courage, support.

To my dear sister, my soulmate

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I thank all the persons that I could not quote their names here, and who directly or indirectly to the realization of this work.

Selma

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الملخص

أهداف هذا العمل هي الفحص الكيميائي النباتي للعديد من المستقلبات الثانوية وتقييم النشاط المضاد للبكتيريا للمستخلصات الميثانولية والمستخلص المائي لأوراق *Atriplex halimus* L. أظهر الفحص الكيميائي النباتي وجود مواد ذات قيم علاجية عالية (الفلافونويد ، التانين. ، البوليفينول ، الكومارين ، إلخ). يتم تنفيذ اختبار النشاط المضاد للبكتيريا للمستخلصات بطريقة الانتشار على وسط agar على ثلاث سلالات بكتيرية: *Escherichia coli* ATCC2592 ، *Staphylococcus aureus* ATCC25923 ، *Pseudomonas aeruginosa* ATCC27853 ، أظهرت الدراسة أن المستخلصات المختبرة ليس لها نشاط مضاد للبكتيريا المختبرة ، و استناداً إلى الإستبيان حول *Atriplex halimus* L في المسيلة واستخداماتها في الطب التقليدي وجدنا أن لها استخدامات متنوعة لعلاج العديد من الأمراض وخاصة التكريس.

الكلمات المفتاحية: *Atriplex halimus* L , مستخلص مائي , مستخلص ميثانولي , النشاط المضاد للبكتيريا , الإستبيان .

Abstract

The goals of this work are the phytochemical screening of several secondary metabolites and the evaluation of the antibacterial activity of the methanolic extracts and aqueous extract of the leaves of *Atriplex halimus* L. Phytochemical screening revealed the presence of substances with high therapeutic values (flavonoids, tannins, polyphenols, coumarin, etc.). The antibacterial activity of the extracts is carried out by the diffusion method on agar medium on three bacterial strains : *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC2592, *Pseudomonas aeruginosa* ATCC27853, The study shows that the extracts tested have no antibacterial activity against the bacteria tested, and based on ethnopharmacological survey about *Atriplex halimus* L in *M'sila* and it's uses in Traditional medicine we found that it's have a variety uses to treat numerous diseases mainly the kyst.

Keywords: *Atriplex halimus* L, methanolic extract, aqueous extract, antibacterial effect ,ethnopharmacological survey.

Résumé

Les objectifs de ce travail sont le criblage phytochimique de plusieurs métabolites secondaires et l'évaluation de l'activité antibactérienne des extraits méthanoliques et extrait aqueux des feuilles d'*Atriplex halimus* L. Le criblage phytochimique a révélé la présence de substances à hautes valeurs thérapeutiques (flavonoïdes, tanins, polyphénols, coumarine, etc.). L'activité antibactérienne des extraits est réalisée par la méthode de diffusion sur milieu gélosé sur trois souches bactériennes : *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC2592, *Pseudomonas aeruginosa* ATCC27853, L'étude montre que les extraits testés n'ont aucune activité antibactérienne contre les bactéries testées, et sur la base d'une enquête ethnopharmacologique sur *Atriplex halimus* L à M'sila et ses utilisations en médecine traditionnelle, nous avons constaté qu'il a une variété d'utilisations pour traiter des nombreuses maladies , principalement le kyste.

Mots clé : *Atriplex halimus* L, extrait méthanolique, extrait aqueux, effet antibactérien, enquête ethnopharmacologique.

List of abbreviations

AE: Aqueous extract.

A. halimus: *Atriplex halimus*

ATCC: American type culture collection

DMSO: Dimethylsulfoxide

FAO: The Food and Agriculture Organization

GAE: Gallic acid equivalent

GM: Gentamicin

m/m: Mass per mass

ME: Methanolic extract

Na₂CO₃: Sodium carbonate

QE: Quercetin equivalent

R: Correlation coefficient

SD: Standard deviation

UV: Ultraviolet

V/V: Volume per volume.

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Introduction

Introduction

For a long time, man has treated himself with the plants he had at his disposal. Through the centuries, human traditions have developed the knowledge and use of medicinal plants to improve human health (Iserin, 2001).

Most of the plant species have therapeutic virtues, because they contain active principles that act directly on the physiology of the human body. It is used in traditional medicine as well as in modern phytotherapy, and here appears the role of the ethnopharmacological survey which is to discover new remedies from the plant world.

The Algerian flora is rich of several thousands of medicinal species; among this vast natural heritage we find the *Atriplex halimus* known locally by "Guettaf". In traditional medicine, it is widely known for its hypoglycemic and hypolipidemic properties and for the treatment of heart disease and rheumatism (Walker et al., 2014; Nedjimi et al., 2013).

The frequency of Microbial infections has increased in recent years due to the extensive use of chemical antibacterial and antifungal agents in human medication, which leads to the selection of resistant microbial strains. On the other hand, However, there is a concern about the adverse effects of synthetic molecules for the control intended to fight bacterial infections, so it seems important to find an alternative to the use of conventional antibiotics. Herbal remedies are an alternative in primary care systems and thus, a promising avenue for the development of traditionally improved medicines (Benbrinis, 2012).

To our knowledge, few studies are carried out on the biological activities and a few ethnopharmacological survey about this plant. In this context, we are interested in the study of *Atriplex halimus* to evaluate their antibacterial activity and conduct an ethnopharmacological survey to have an overview about the uses of the plant.

This research work is articulated on two great parts:

- ✓ Part of bibliographic synthesis which is devoted to generalities on the plant *Atriplex halimus*, and on the antimicrobial activity.
- ✓ Experimental part which contains the material and the methods used in our work, an ethnopharmacological survey, the results obtained and their discussion. Finally, a conclusion gives a general idea of the present work.

Bibliographic part

Chapter I

Atriplex halimus L.

Chapitre I. *Atriplex halimus* L.

I.1. Family *Chenopodiaceae* (*Amarantaceae*)

Chenopodiaceae are widely distributed in temperate and subtropical saline habitats, especially in the littoral regions of the especially in the littoral regions of the Mediterranean Sea, the Caspian Sea and the Red Sea, the arid steppes of central and eastern Asia, the margins of the Sahara Desert, the alkaline grasslands of the United States, in the Karoo in southern Africa, in Australia, and in the Argentine Pampas. They also grow as herbaceous plants on salt-rich soils in inhabited areas, especially in the presence of water runoff and rough terrain (Mulas, 2004).

I.2. Generalities on *Atriplex*

Atriplex is a shrubby plant, belonging to the family *Amarantaceae* which includes about 417 species in the Mediterranean basin (Le Houerou, 1992).

The species *A. halimus* (Mediterranean saltbush) is a halophytic shrub widely distributed in arid and semi-arid regions of the Mediterranean basin and east of Saudi Arabia, at altitudes below 900 m. It grows on a variety of soils, from fine to fine to coarse texture, with varying degrees of salinity. (Walker et al., 2014).

I.3. Systematic of the species

According to Quézel and Santa (1962) the classification of *A. halimus* is as follows:

Kingdom : Vegetable

Planting : Phanerogams or Spermaphytes

Sub-branch : Angiosperms

Class : Dicotyledons

Subclass : Preasteridae

Order : Caryophyllades

Sub-order : Chenopodiales

Family : *Amaranthaceae*

Genus : *Atriplex*

Species : *Atriplex halimus* L

I.4. Geographical distribution of *Atriplex halimus*

I.4.1. In the world

Atriplexes are located in temperate, Mediterranean and subtropical zones, between 20 and 50° of altitude North and South (Le Houérou, 1992).

In the world, Atriplexes are found from Alaska to Patagonia, from Brittany to Siberia and from Norway to South Africa (Franclet et Le Houérou, 1971).

The species is spontaneous within a relatively vast area encompassing the countries of northern Africa and the near and middle east from the the Canaries to Iran. In Europe, the *Atriplex halimus* is present in Bulgaria in addition to the Mediterranean area.

In North Africa the genus *Atriplex* includes 15 spontaneous species, 2 naturalized species and 2 introduced species. These species are divided into 9 perennial species, one biannual species and 9 annual species. Among the most used species in Northern Africa is *Atriplex halimus* this species has also been the subject of specific research in the semi-arid environments of southern Europe (Papanastasis, 2000).

Table 01: Numerical distribution of *Atriplex* species in the world (Le Houérou, 1992).

Country or region	number of species	Country or region	number of species
United States	110	California (Mexico)	25
Australia	75	North Africa	22
Mediterranean Basin	50	Texas	20
Europe	40	South Africa	20
Former USSR	36	Iran	20
Near East	36	Syria	18
Mexico	35	Palestine & Jordan	17
Argentina	35	Algeria & Tunisia	17
California	32	Bolivia & Peru	16

I.4.2. In Algeria

In Algeria, the *Atriplex* is spontaneous in the semi-arid and arid bioclimatic stages, (Tébessa, Batna, M'sila, Boussaâda, Biskra, Djelfa, Tiaret, Saida ...).

Quezel and Santa (1962) counted in Algeria 13 native species including 5 perennial and 8 annual. Le Houérou (1992) added to this list two naturalized species: *Atriplex semibacata* R.Br Perennial species and *Atriplex injlata* F.V Muell: Annual species.

Table 02: Distribution of the different species of *Atriplex* in Algeria (Quezel and Santa, 1962).

Species	Name	Location
Annual (Differs generally by the shape of the of the leaves, the habit and the fruiting valves)	<i>A. Chenopodioides</i> Batt.	Bouhanifia (Mascara) (rare)
	<i>A. littoralis</i> L.	Around Algiers (rare).
	<i>A. hastata</i> L.	Quite common in the Tell and very rare elsewhere
	<i>A. patula</i> L.	Quite common in the Tell and very rare in Aflou.
	<i>A. tatarica</i> L.	Annaba and Setif (very rare)
	<i>A. rosea</i> L.	Biskra and oran (very rare)
	<i>A. dimorphostegia</i>	Northern Sahara (rare).
	<i>A. tornabeni</i> Tineo.	Sahel of Algiers (very rare).
Perennials (Generally differ by the shape of the leaves, the size of the shrub, the port of the stems and the aspect of the perianth).	<i>A. portulacoides</i> L.	Quite common in the Tell
	<i>A. halimus</i> L.	Common in all Algeria.
	<i>A. mol</i> / <i>A. coriacea</i> Forsk.	Biskra (very rare).
	<i>A. glauca</i> L f. lis Des.	Common in Algeria

I.5. Vernacular name

It is known in French: arroche halime or pourpier de mer and in Arabic name: G'ttaf (L'egttef l'malih) in Algeria, chenane in Morocco, and saltbuch in English (Medjekal and Bousseboua, 2016).

I.6. Botanical description of *Atriplex halimus*

Atriplex halimus is a halophyte shrub from 1 to 3 m high, very branchy, forming tufts that can reach 1 to 3 m in diameter. It is characterized by the presence of stems, leaves and flowers (Tab.3). Also presenting a photosynthesis in C4, this species contains two subspecies (figure 2) subsp. *halimus* and subsp. *schweinfurthii*. The distribution area of subsp. *halimus* extends from semi-arid to humid areas easily identifiable thanks to its upright on the other hand, the subspecies *schweinfurthii*; widespread in arid and desert areas presents a tangled bushy habit.



Figure 01: *Atriplex halimus* L (Ziarnek, 2017)

The natural populations of *Atriplex halimus* in the Algerian steppe regions almost all belong to the subspecies *schweinfurthii* (Nedjimi et al.,2013).

Table 03: Description of *Atriplex halimus* (Quèzel and Santa, 1962)

Part of the plant	Description
-Stems	Erect and upright, woody
- Leaves	Large, 2-5 cm, usually twice as long as wide long or oval-obtuse. Fruiting valves leathery, rounded-reniform, smooth.
- Flowers	Yellowish, in elongated spikes forming a terminal panicle naked or a little leafy at the base

I.6.1 *Atriplex halimus schweinfurthii*

A. Schweinfurthii is a tetraploid and occupies arid and saline sites (Walker et al., 2014). It is widespread in arid and desert areas, has a bushy and tangled habit with bare fruiting branches tangled with fruiting branches naked at the top, strongly lignified and pointed is characterized by flowering branches without leaves (Nedjimi et al., 2006).

I.6.2 *Atriplex halimus*

Generally, is the most leafy and occurs on the semi-arid and humid coastal areas. Is a native forage shrub that tolerates arid conditions well.



Figure 02: Plants of *Atriplex halimus*: subspecies *schweinfurthii* (left) and *halimus* (right)

(Walker et al., 2014).

I.7. Uses of the plant

In human nutrition, *A. halimus* is a shrub known for the nutritional and energetic value of its tender leaves, not only for livestock, but also as food for nomads and the local steppe population. Indeed, in spring, in several regions in Algeria (Djelfa) and Tunisia (Gabes), the young shoots of guettaf are consumed by the man, by preparing it like spinach (Nedjimi et al., 2013).

In economics, the plantation of *Atriplex* appears to be one of the best ways to rehabilitate desert areas and restore them to production. It can provide sources of fodder with good nutritional value during dry seasons, and periods of scarcity of grazing resources. In addition, it can contribute to the valorization of marginal and degraded soils and to the improvement of the vegetal and animal productions in several (Le Houreau, 1992).

In traditional medicine, the steppe population uses *A. halimus* for therapeutic purposes mainly to treat hyperglycemia in diabetic patients. (Nedjimi et al., 2013). It is also used to treat inflammation of the urinary tract (cystitis), urinary tract (cystitis), ovarian and breast cystitis and urinary lithiasis (Emam, 2011).

Chapter II

Antimicrobial Activities of phenolic compounds

II.1. Naturally occurring antimicrobial substances

The large-scale and sometimes inappropriate prescription of antibiotics to treat microbial infections has led to the selection of multi-resistant strains, hence the importance of directing to orient research towards the discovery of new pathways that constitute a source of new drugs new drugs based on plant extracts (Cowan, 1999).

Essential oils and Polyphenols, especially flavonoids and tannins, are known to be toxic to microorganisms. The mechanism of toxicity may be related to the inhibition of hydrolytic enzymes (proteases and carbohydrases) or other interactions to inactivate microbial adhesins, transport and cell envelope proteins (Harrar, 2012).

II.2. Antimicrobial activity of phenolic compounds

The activity of these substances towards microorganisms can be exerted according to extremely diverse modalities. Some exert their activity by oxidizing or denaturing bacterial proteins (Shiota et *al.*, 2004); others have a more specific power by altering membrane structures or inactivating compounds or essential functions of the cell.

II.2.1 Action mode of phenolic acids

Medicinal plants containing phenolic acids have been shown to be effective against bacteria. Phenolic acids (cinnamic acid, coumaric acid, caffeic acid and ferulic acid) have shown good activity against Gram-positive bacteria and only weak activity against Gram-negative bacteria (*Escherichia coli*, *Salmonella enterica*) at high concentrations (Dehimat, 2014).

II.2.2 Action mode of quinones

Quinones can irreversibly complex with nucleophilic amino acids of proteins, often leading to the inactivation of these proteins and the loss of their functions. For this reason, the antimicrobial effects of quinones are high and the likely targets in the microbial cell are: surface-exposed adhesins, cell wall polypeptides and membrane enzymes. Quinones can also render substrates unavailable to microorganisms (Cowan, 1999).

II.2.3 Action mode of coumarins

The association of hydroxycinnamic acid with coumarins seems to have an inhibitory effect on Gram positive bacteria. According to Cottiglia et al., (2001), some compounds belonging to this class show an activity against *Staphylococcus aureus*, *Bacillus lentus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

II.2.4 Action mode of flavonoids

Flavonoids are effective antimicrobial substances against a wide range of microorganisms. Their activity is probably due to their ability to complex with extracellular proteins and to complex with the bacterial wall (Dehimat, 2014).

II.2.5 Action mode of tanins

Tannins prevent substrates from passing through the wall, decreasing its permeability by forming complexes with the proteins of the cell wall (Dehimat, 2014).

II.3. Description of the bacteria studied

II.3.1 *Escherichia coli*

Escherichia coli is a gram-negative bacillus of non-spore-forming, anaerobic type facultative, generally motile by means of flagella, its length varies from 2 to 6 μm , while its width is 1.1 to 1.5 μm . Bacteria belonging to the species *Escherichia coli* constitute the most of the aerobic microbial flora of the digestive tract of humans and many animals. Some strains are virulent, capable of causing spontaneous infections of the digestive or urinary tract or even meningitis in humans or certain animal species, urinary tract infections or neonatal meningitis. Other strains belonging to the commensal flora can be responsible for various opportunistic infections, especially in subjects with weakened immune defenses (Bensaadi and Guemmouda, 2017).

II.3.2. *Staphylococcus aureus*

Bacteria of the genus *Staphylococcus* are Gram-positive cocci, grouped in clusters of grapes, immobile, non-spore forming, catalase positive and oxidase negative. Among the 27 species of the genus currently listed, the main are *Staphylococcus aureus*, *S.epidermidis* and *S.saprophyticus*. The species *Staphylococcus aureus* will be taken as a type for description (Bensaadi and Guemmouda, 2017).

II.3.3. *Pseudomonas aeruginosa*

They are Gram-negative bacilli, non-spore forming, aerobic, mobile thanks to the presence of 1 to 2 flagella, this type of bacteria synthesizes main types of pigments pyocyanine: blue phenazine, pyoverdine: yellow green, they are bacteria resistant to several antibiotics. *Pseudomonas aeruginosa* is responsible for 16% of cases of nosocomial pneumonia, 12% of urinary tract infections, 8% of infections following surgical wound (Bensaadi and Guemmouda, 2017).

EXPERIMENTAL

PART

Chapter III

MATERIAL AND METHODS

Chapter III. Materials and methods

1. Materials

1.1. Plant material

The aerial part of the plant *A. halimus* was harvested in March 2022 in the wilaya of Biskra. The plant was identified by the application of Pl@ntNet and by Professor Sarri Djamel in the laboratories of the Department of Biology, University Mohamed Boudiaf of M'sila. The leaves of the plant are cleaned, dried in the shade and at room temperature, crushed and sieved to obtain a powder and then stored in the dark until use.

1.2. Equipment and chemicals

Equipment

- Precision balance.
- Oscillator (vortex).
- Rotavapor Buchi (R-210).
- Spectrophotometer.

Chemicals

- Gallic acid (Sigma).
- Folin-Ciocalteu reagent (Sigma Aldrich).
- Sodium carbonate.
- Quercetin (Sigma).
- Aluminium chloride.

1.3. Microbial strains used

1.3.1. The bacteria

The strains used are reference strains from the American type culture collection (ATCC), and were provided by the microbiology laboratory of the Department of Microbiology and Biochemistry where we carried out this part of our work, the strains are kept at 4°C in test tubes containing slant agar.

The bacteria used are shown in Table 4.

Table 04: List of tested bacterial strains.

Strain	Types
<i>Staphylococcus aureus</i> ATCC25923	Gram positive bacteria +
<i>Escherichia coli</i> ATCC2592	Gram negative bacteria -
<i>Pseudomonas aeruginosa</i> ATCC27853	

2. Methods of analysis

2.1. Preparation of extracts

The methanolic and aqueous extracts are prepared following the method described by (Belhattab et al., 2004; Ben Ammar et al., 2007; Dehimat et al., 2021) with some modifications.

2.1.1. Aqueous extract

The aqueous extract of *A. halimus* leaves was obtained by decoction of 100 g of the powder in 1000 ml of distilled water for 30 min under magnetic stirring. The solution was left to stand on the hot plate for 15 min, the mixture was first decanted, and then filtered on Whatman paper (n°3). Aliquots of the filtrate were placed in an oven at 40 °C for 24 h to dry. Finally, the dry extract was collected, weighed and stored at 4°C until use.

**Figure 03:** Dried leaves of *Atriplex halimus*.

2.1.2. Methanolic extract

The methanolic extract of *A. halimus* leaves was prepared from 50 g the powder, it was macerated in 500 ml of 70% (V/V) methanol at room temperature and protected from light for 72h under stirring. After 72h the macerate was recovered and the residues were exhausted again in 70% (V/V) methanol in the dark for 72h. The mixture was first decanted, and then filtered on Whatman paper (n°3). The filtrates obtained were added and evaporated with a rotary evaporator at a temperature of 40°C and then placed in an oven at 40°C for 24 h to dry. Finally, the dry extract was recovered, weighed and stored at 4°C until use.

- **Calculation of the yield**

The yield of the extraction is determined by calculating the ratio between the weight of the dry extract to the weight of the plant material used for the extraction. The yield is expressed as a percentage and is calculated by the following formula:

$$R (\%) = (M0/M1) \times 100$$

Where:

R (%): the yield in %.

M: Mass in grams of the resulting dry extract (P1- P2).

M0: Mass in gram of the starting plant material (dry plant material).

(P1) The weight of the full petri dish after total removal of the solvent.

(P2) The weight of empty dish.

2.2. Quantitative analysis

2.2.1 Determination of total polyphenols

The total phenol content of the extracts was determined spectrophotometrically by the Folin-Ciocalteu method (Yakhlaf et al., 2011).

This assay is based on the quantification of the total concentration of hydroxyl groups present in the extract. The Folin- Ciocalteu reagent consists of an acidic yellow solution containing a polymeric complex of ions in alkaline medium, the Folin-Ciocalteu reagent oxidizes phenols to phenolate ions and partially reduces its heteropolyacids resulting in the formation of a blue complex (Yakhlaf et al., 2011).

According to the protocol described by (Bougandoura et al., 2013). A 200µl amount of the extract is mixed with 1ml of freshly prepared Folin-Ciocalteu reagent (10 times diluted) and 0.8ml of 7.5% sodium carbonate (Na₂CO₃). The whole set was incubated at room temperature for 30

minutes and the reading was taken against a blank using a spectrophotometer at 765nm. Polyphenol concentrations were deduced from the range of the linear calibration curve established with gallic acid. Results are expressed as micrograms of gallic acid equivalent per mg of dry extract ($\mu\text{g GAE/mg}$ of extract).

2.2.2 Determination of total flavonoids

The most used method for the estimation of the flavonoid content in the different extracts is the one described by Lamaison and Carnat (1991) and quoted by Bahorun (1997).

Flavonoids have a free hydroxyl group (OH), in position 5 which is likely to give with the CO group, a colored complex with aluminum chloride. Flavonoids form yellowish complexes by chelation of metals (iron and aluminum). This translates the fact that the metal (Al) loses two electrons to unite with two oxygen atoms of the phenolic molecule acting as electron donors (Ribéreau-Gayon *et al.*, 1972).

According to the protocol described by Lamaison and Carnat (1991), three test tubes were prepared 0.5 ml of *A. halimus* extract was put in each tube, 0.5 ml of absolute methanol was added to each tube, 1 ml of 2% aluminum chloride methanolic solution was also added to each tube. The whole set was incubated at room temperature for 10 min, and the reading was taken using a spectrophotometer at 430 nm. For the calibration range a range of 9 concentrations of quercetin was prepared ranging from 2.5 to 40 $\mu\text{g/ml}$ from a stock solution of concentration 40 $\mu\text{g/ml}$ (400 μg of quercetin dissolved in 10 ml of methanol). Aliquots of 0.2 to 3.0 ml of the quercetin stock solution were introduced into a series of test tubes, the final volume in each tube was made up to 3 ml by addition of absolute methanol, 1 ml of each tube was transferred to another, 1 ml of the 2% aluminum chloride methanolic solution was added, the whole set was incubated at room temperature for 10 min, and the reading was taken at 430nm. All operations are performed in triplicate.

The concentrations of flavonoids contained in the extracts of *A. halimus* extracts are calculated with reference to the calibration curve obtained using quercetin as standard, the results are expressed in μg quercetin equivalent / mg dry extract.

2.3. Evaluation of the antibacterial activity

In order to evaluate the antibacterial activity of the extracts of the plant we used the method of diffusion on disk in agar medium (aromatogram).

The aromatogram is based on the technique used in medical bacteriology called antibiogram (De billerbeck, 2007). The principle of this method is based on the diffusion of the antimicrobial compound in a solid medium, in a Petri dish, from a precise point, with the creation of a concentration gradient after a certain contact time between the product and the microorganism.

The disk diffusion method described by Rasoanaivo and Ratsimamanga-Urveg, (1993) was used with some modifications, to evaluate the antibacterial activity of *A. halimus* extracts.

- **Culture of bacterial strains**

The different bacterial strains are transplanted by the streak method and then incubated in the oven at 37°C for (18-24) hours.

- **Preparation of inocula**

The inoculum is prepared from a pure culture of 18 h / 37°C on medium containing 3 to 5 well isolated and perfectly identical colonies are collected with a platinum loop, then suspended in 9 ml of 0.9% sterile physiological water (Communiqué du CA-SFM, 2012).

- **Standardization of inocula**

From the 18-hour bacterial suspensions, a bacterial suspension is prepared at an absorbance between 0.08 and 0.10 lu at 625 nm equivalent to 10^6 - 10^8 CFU/ml (Communiqué du CA-SFM, 2012).

- **Seeding and depositing the discs**

The Seeding is done by swabbing on Petri dishes, a swab is dipped in the bacterial suspension, then wring it out by pressing firmly on the inner wall of the tube. The swab is rubbed on the entire agar surface, from top to bottom in tight stripes. Sterile 6 mm diameter discs cut in Wattamn paper n°3 are placed on the gel. A volume of 10 µl of extract is deposited (on the discs) with concentrations 500 mg/ml, 1000 mg/ml (Rasoanaivo and Ratsimamanga-Urveg, 1993).

All experiments were done in triplicate; the average diameter of the inhibition zone was calculated allowed to classify the bacteria according to their sensitivity. The antibacterial activity of the extracts is observed by the presence of an inhibition zone around the disc.

3. Ethnopharmacological survey

An ethnopharmacological survey was conducted in the city of M'sila, during a period of 30 days (March 10 to April 10, 2022). The survey is based on a questionnaire previously established, containing mentions of the vernacular name of the plant of *A. halimus* (l'egttef l'malih), the part used, the method of preparation and the duration of treatment.

3.1. The questionnaire

The survey questionnaire is divided into two parts to collect information about women affected by polycystic ovaries and about plants that have important biological effects in the treatment of polycystic ovarian syndrome used by this population available on google form, link of questionnaire:

<https://docs.google.com/forms/d/e/1FAIpQLSdzsWFRwOD8Nf3sjn1jAaSc52CPxXYGDXVIMd2u2VI1MIQ-YA/viewform>.

Chapter IV

RESULTS AND DISCUSSION

Chapter IV. Results and discussion

1. Extraction yields

The yield, appearance and color of the extracts of *A. halimus* are shown in Table 5.

- **The maceration**

The extraction operation by maceration of *A. halimus* leaves in methanol resulted a pasty extract with a yield of 25.81% (m/m).

- **The decoction**

The extraction operation by decoction of *A. halimus* leaves resulted a pasty extract, the yield of this aqueous extract was estimated at 15.04% (m/m).

Table 05: Extraction yields of the plant *Atriplex halimus*.

Extracts	Color of the extract	Aspect	Yield in %
ME	Dark green	Pasty	25.81 %
AE	Blackish brown	Pasty	15.04 %

ME: Methanolic extract / AE: Aqueous extract

We find that the methanolic extract has a higher yield than the aqueous extract, the difference in yield between the extracts is due to the extraction techniques used, which are totally different and the chemical composition that differs from one extract to another.

The comparison of the extraction yields with the bibliography is difficult because different conditions can affect it. Slama et al. 2018 obtained a methanolic extract from leaves with a yield similar to ours (23.43%), also Benhammou et al.2009 obtained (24%), while Ounaissia et al. 2020 obtained a very low yield for the leaves (5.4%).

In general, the extraction yield varies depending on the plant species, environmental conditions, the organ used in the extraction, the harvest period, the drying conditions, the type of solvent used and its polarity (Daoudi et al. 2015).

2. Determination of polyphenols and flavonoids

Phenolic compounds are the products of the secondary metabolism of plants. They are becoming increasingly important, especially because of their beneficial effects on health. Indeed, their role as natural antioxidants and antibiotics is gaining interest in the prevention and treatment of cancer, inflammatory diseases, cardiovascular diseases, and cystic fibrosis (Belščak-Cvitanović *et al.*, 2018). For this reason, the determination of total phenols and flavonoids of *A. halimus* leaves was performed in this study.

The polyphenol content of the different extracts was obtained from a calibration curve established with increasing concentrations of gallic acid (Figure 05). While that of flavonoids was obtained from a calibration curve established with increasing concentrations of quercetin (Figure 06). The results are expressed in μg of gallic acid equivalents per milligram of the plant extract for polyphenols. Concerning the flavonoid content, they are expressed in μg of quercetin equivalent per mg of extract. These results are grouped in Table 06.

2.1. Total polyphenol content

The determination of the total polyphenol content of the different extracts was performed according to the method using the Folin-Ciocalteu reagent. The curve shows a linearity of the absorbance according to the concentrations. The quantities of the corresponding polyphenols of each extract were reported in milligram equivalent of gallic acid and determined by the equation of type: $y = 0,0021 x - 0,0059$ knowing that $R^2 = 0,9988$ (Figure 04).

For polyphenols, the results reveal that the methanolic extract of the leaves is the richest in polyphenols with a content of $24.09 \pm 5.09 \mu\text{g EAG/mg extract}$, followed by the aqueous extract of the leaves $6.14 \pm 3.08 \mu\text{g GAE/mg extract}$. It is noticed that the polyphenol content is different for both extracts. The methanolic extract contains the highest polyphenol content compared to the aqueous extract (Table 06).

In the literature, different results for methanolic extract have been found by Benhammou 2009, Emam 2011. This difference in content can be related not only to the variety, but also to the influences of extraction methods and conditions, the nature of solvents used, environmental factors, assay reagents and type of spectrophotometer used (Medini *et al.*, 2014). In addition, the variability of polyphenol contents varies considerably from one species to another and within the same species, due to factors: genetic (the origin of species), physiological (the degree of maturation of the plant and the organs used) (Belhadj Tahar *et al.*, 2015).

To our knowledge, no study has been done on the polyphenol content of the aqueous extract.

2.2. Total flavonoids content

The determination of the total polyphenol content of the different extracts was performed according to the method using the aluminum trichloride ($AlCl_3$). The quantities of the corresponding polyphenols of each extract were reported in milligram equivalent of quercetin. The content of flavonoids in the two extracts was obtained from the calibration curve which follows an equation of type: $y = 0.0491 x - 0.0285$ knowing that $R^2 = 0.9969$.

The flavonoid content is $4.099 \pm 0.737 \mu\text{g EQ/mg extract}$ in the methanolic extract of the leaves followed by the aqueous extract $3.778 \pm 0.784 \mu\text{g QE/mg extract}$, we notice that the flavonoid content is almost the same for both extracts.

In the literature, the same results for methanolic extract were found by Ben hammou 2009, Emam 2011. To our knowledge, no study has been done on the flavonoids content of the aqueous extract.

Table 06: Polyphenol and flavonoid content of different extracts of *Atriplex halimus*.

Extracts	Polyphenol content ($\mu\text{g GAE/mg E}$)	flavonoid content ($\mu\text{g QE/mg E}$)
ME	24.09 ± 5.09	4.099 ± 0.737
AE	6.14 ± 3.08	3.778 ± 0.784

Data are displayed with the mean \pm standard deviation of three replications.

ME: Methanolic extract / AE: Aqueous extract

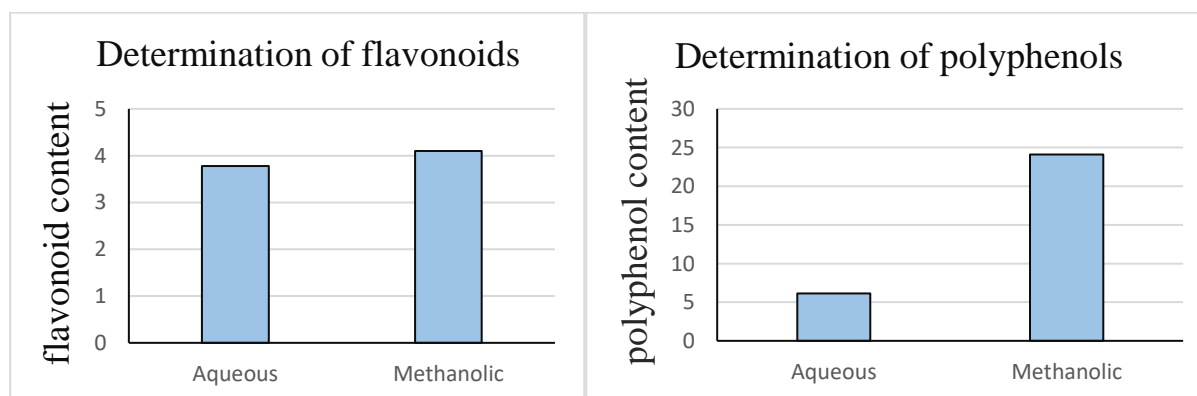


Figure 04: content of polyphenols and flavonoids in the aqueous and methanolic extracts of *Atriplex halimus* leaves.

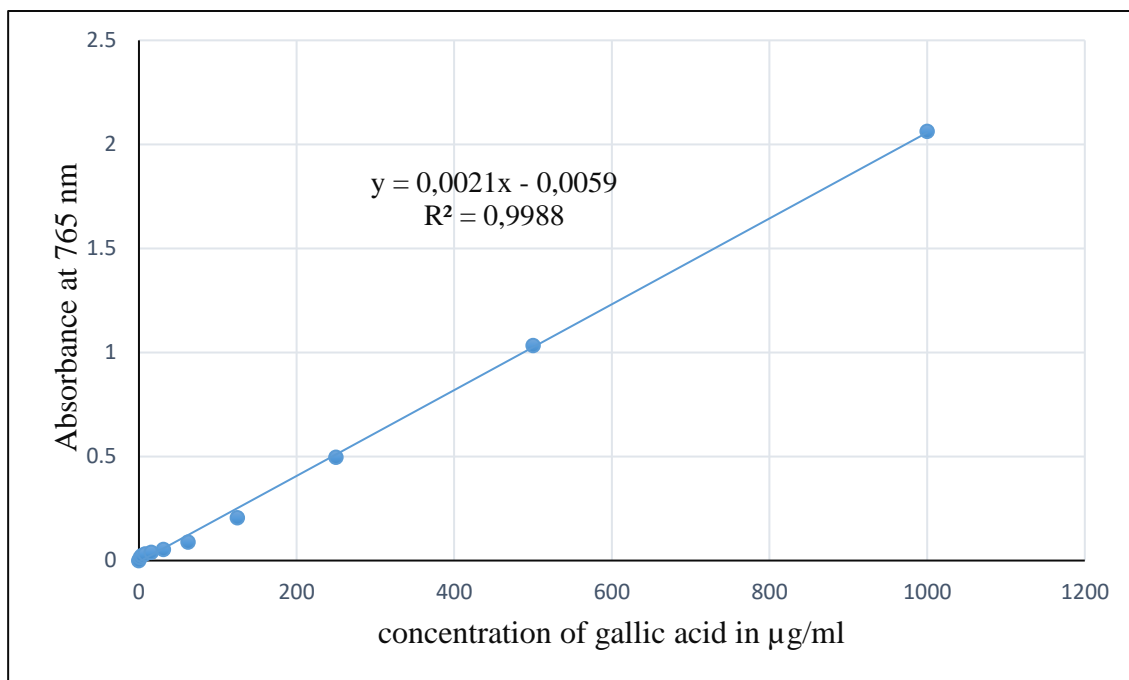


Figure 05: Calibration curve of gallic acid for the determination of total polyphenols.

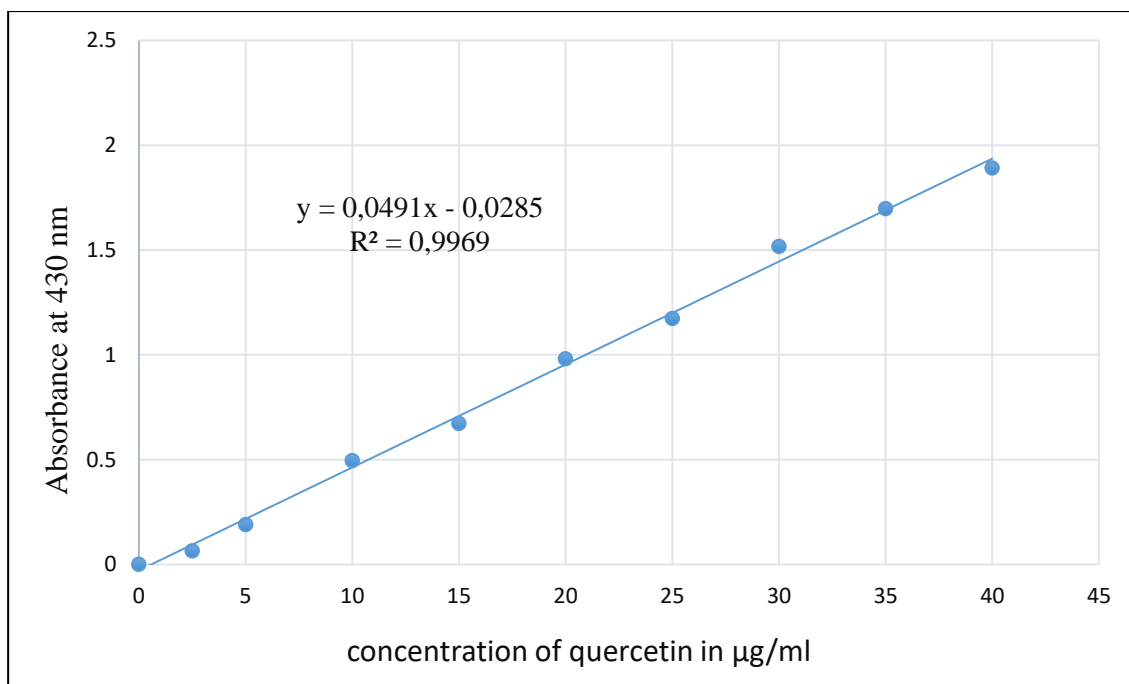
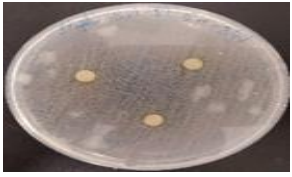


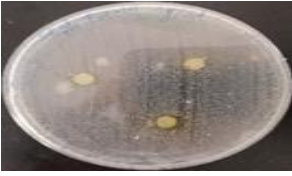
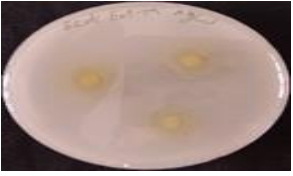

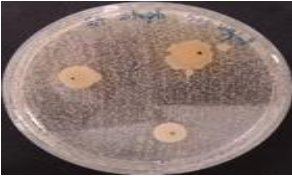
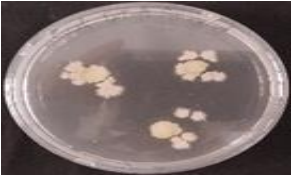

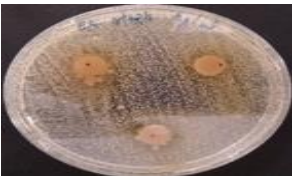
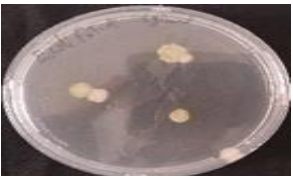


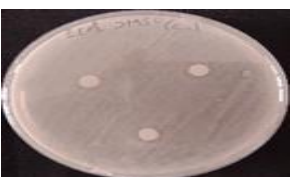



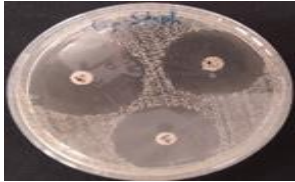
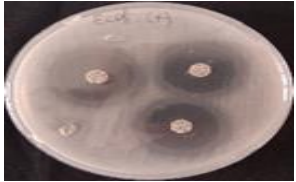
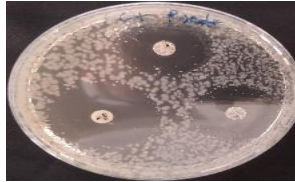
Figure 06: Calibration curve of quercetin for the determination of total flavonoids.

3. Antibacterial activity results

The antibacterial activity of extracts is tested against three bacterial strains by the disk diffusion method, all measurements were made with the unaided eye and a ruler while viewing the back of the Petri dish above a black background and under laboratory light, the results are shown in the following table.

Table 07: The results of the antibacterial activity of *Atriples halimus* extracts.

	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
ME 0.5g/ml	 (-)	 (-)	 (-)
ME 1g/ml	 (-)	 (-)	 (-)
AE 0.5g/ml	 (-)	 (-)	 (-)
AE 1g/ml	 (-)	 (-)	 (-)
DMSO	 (-)	 (-)	 (-)

GM			
	(+) (a \approx 32.6 mm)	(+) (a \approx 25 mm)	(+) (a \approx 27.3 mm)

(+) = Presence of the zone. (–) = Absence of the Zone. **ME** = Methanol Extract. **AE** = Aqueous Extract. **DMSO** = Dimethylsulfoxide is a highly polar, relatively inert, aprotic solvent, and used here as a negative control **GM** = Gentamicin (an antibiotic used to treat several types of bacterial infections, and used here as a positive control for the comparison). (**a** \approx) = Aaverage of Diameters (mm) of the three zones of inhibition.

As it's shown in the previous table, there are no zones of inhibition surrounding the discs except in positive control, which mean that all the tested bacteria have no sensibility for the both tested extracts, so these extracts have no antibacterial effects on the tested bacteria.

It is difficult to compare our results with the bibliography because there is diversity in the results of other similar works, Some of them found a result consistent with our result, like the work of (A.Elbashit et *al.*, 2011), And others found a different result like the study of (Chaouche et *al.* 2021),), The extraction methods used appear to play an important role in this (Markham, K.R.1982), Also the genetic properties of the species studied (Bruneton, J. 1999), The geographical origin (Narayana, K.R et al., 2001) ,And to the conditions of harvesting of the plant material (Lin, J.K., Weng, M.S. 2006), According to Ounaissia et al. (2020) the characteristics of the extracts depend on the type of solvent used for the extraction and the species.

4. Ethnopharmacological survey results

The link to this study was published on social networks and 117 women responded, from 10/03/2022 to 10/04/2022, and the questionnaire results are presented according to the questions asked.

- **Age**

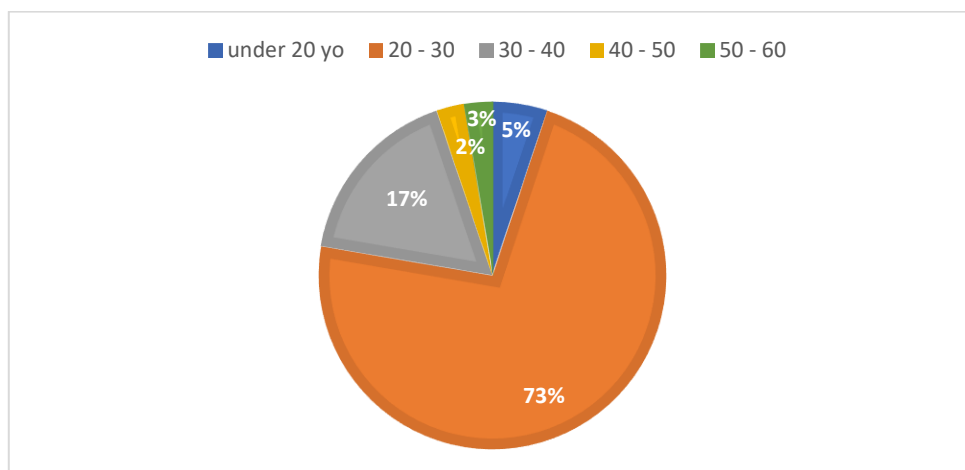


Figure 07: Profile of the age group.

Many age groups participated in this study, but the majority were 20-30 with a percentage of 73%.

- **Family situation**

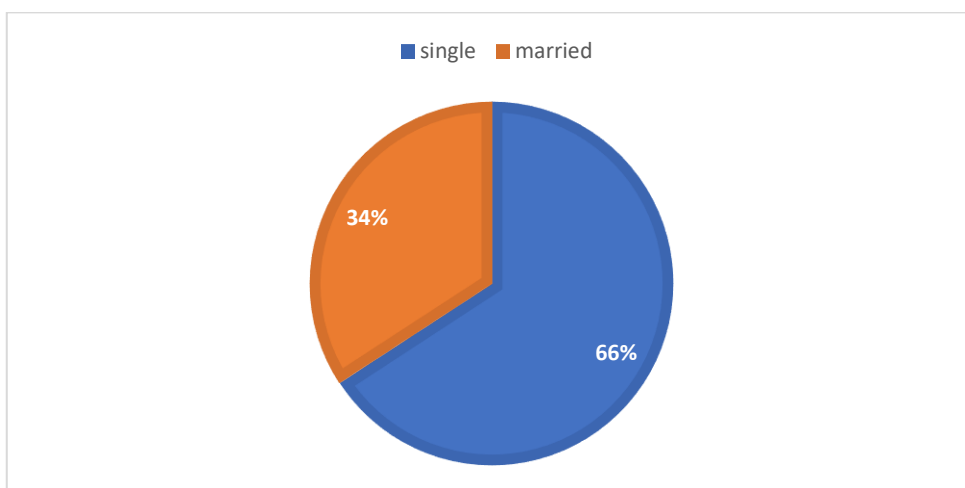


Figure 08: Distribution of the population by family status.

Since the questions are directed to all women, and therefore we will find a difference in terms of family status, and what is meant here is either married or not, the result is shown in figure 8.

- **Region**

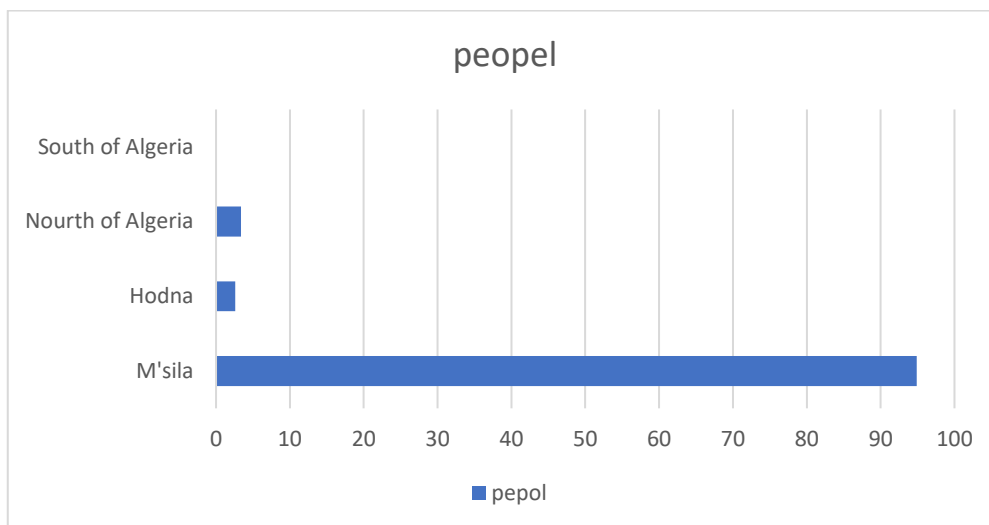


Figure 09: Distribution of the population by region.

The people who answered are from different regions of Algeria, but most of them are from the M'sila region.

- **Have you had fibrocystic disease?**

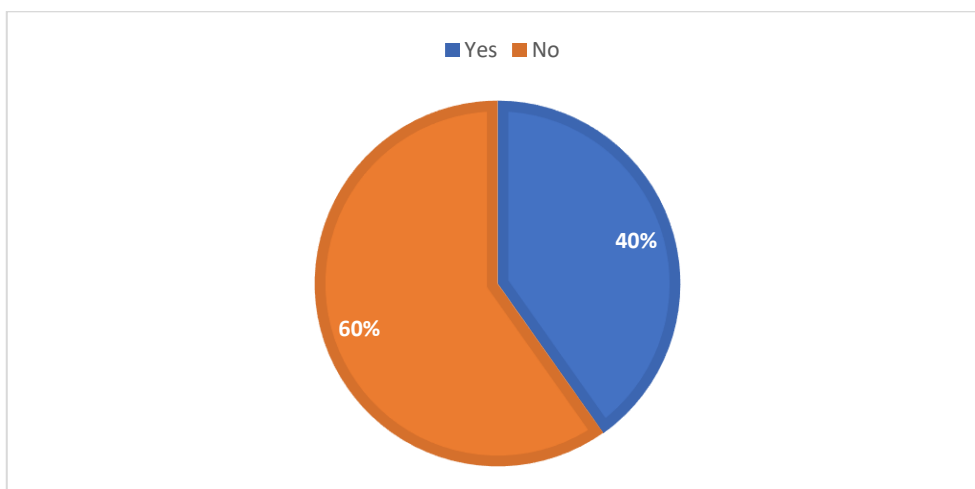


Figure 10: Distribution of women affected by fibrocystic disease.

We found that this disease is relatively common. Almost half of the group of women who answered the questions had this disease.

- **Is it a cyst?**

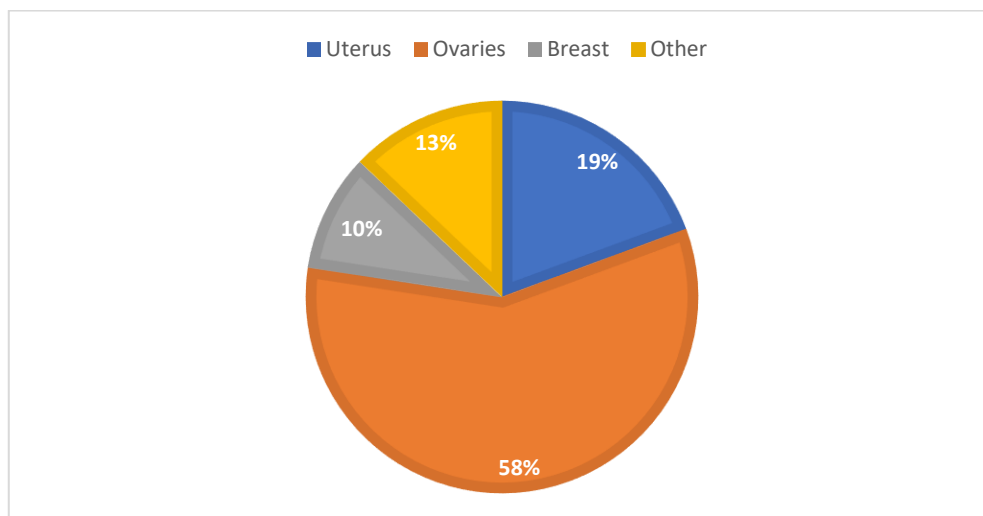


Figure 11: Different types of cysts.

There is a big difference in terms of the type of cyst that affected the studied sample, but it is noticeable that ovarian cysts are more frequent in the groupe of the single women and uterine cysts are more frequent in married women groupe, and this explains that ovarian cysts represent the majority in the attached graphe figure 11.

- **Has it been treated?**

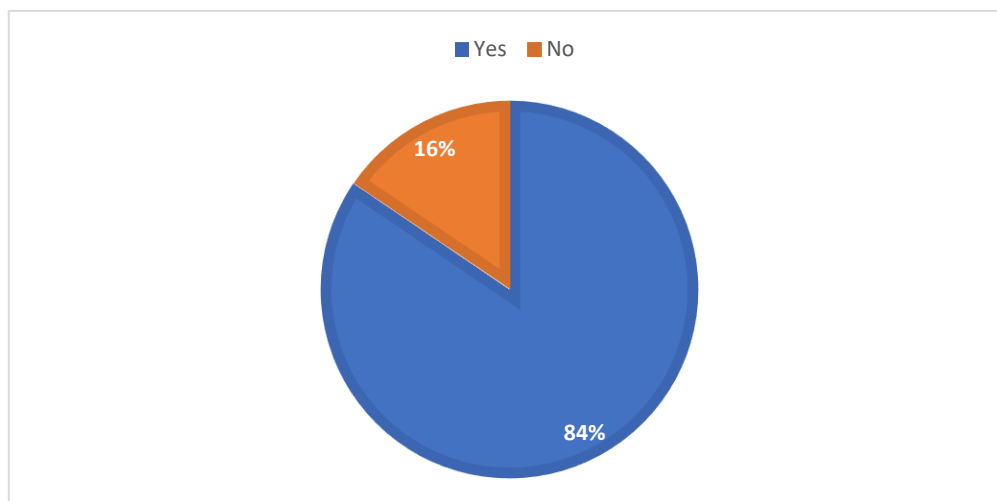


Figure 12: Treatment of fibrocystic disease.

It turns out that the majority of women are cured of this disease, and this indicates that recovery from it is very possible.

- **Is the use of natural herbs for medicine acceptable to you?**

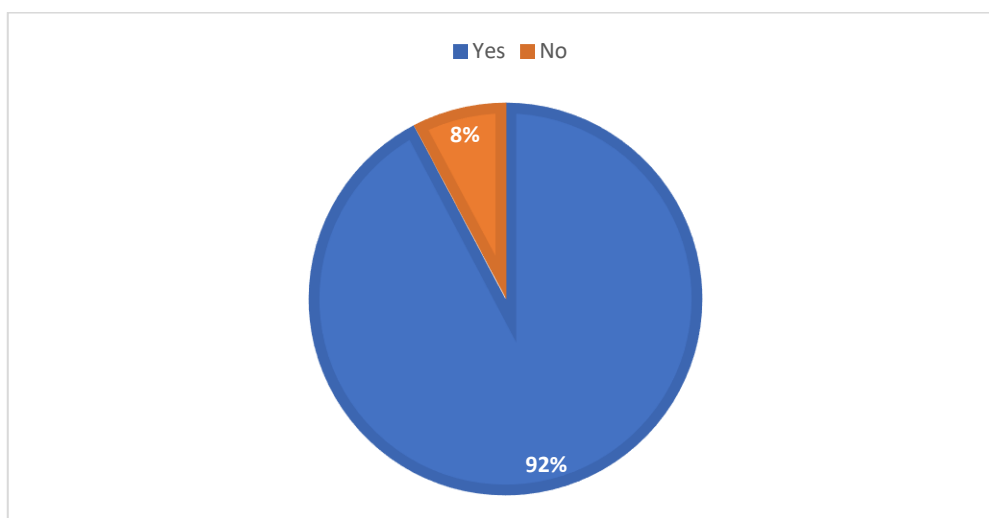


Figure 13: Confidence and satisfaction of the population.

The majority of people showed a great acceptance of medicinal herbs, and this is evidence that it is common in the studied society.

- **What did you use as a treatment?**

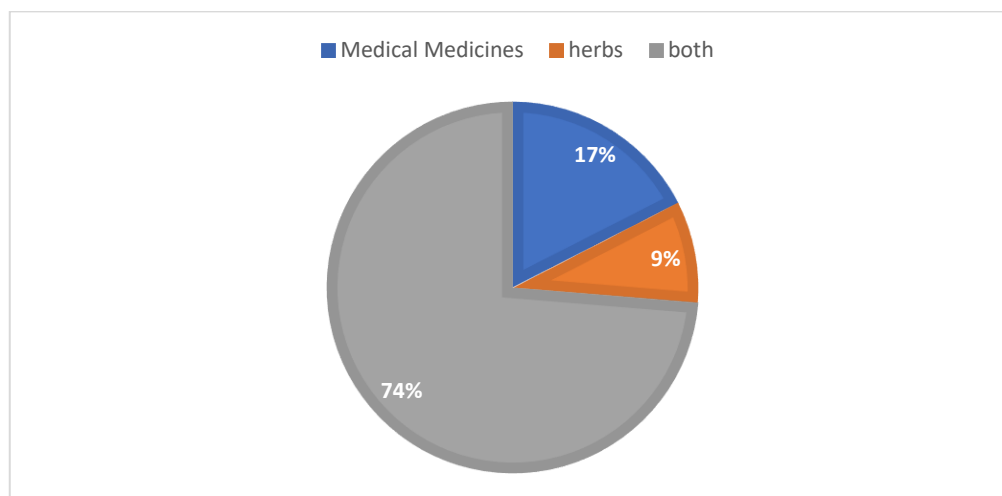


Figure 14: The treatment used.

Based on the graph, 74% of people use both synthetic and natural herbs to treat disease.

- **Do you know the gettaf plant?**

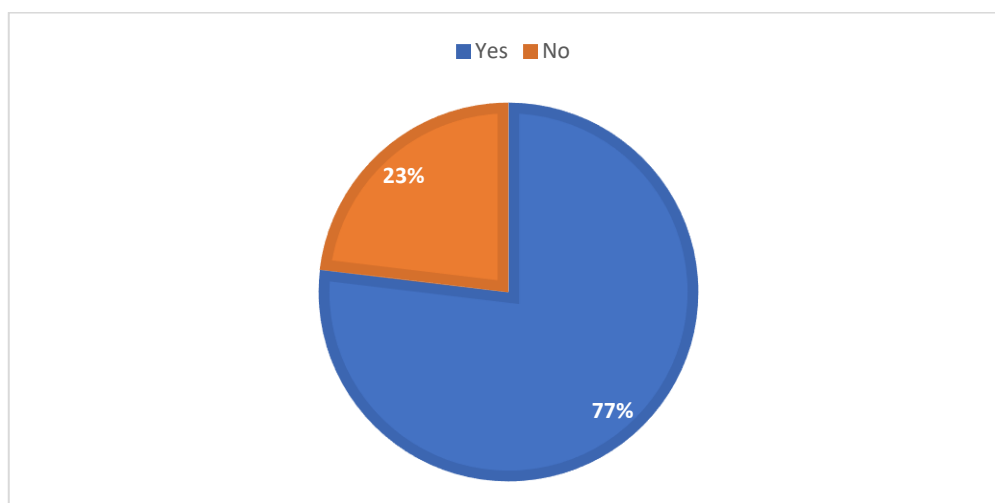


Figure 15: Percentages of populations that know the gettaf plant.

As it is clear, this plant is very well known in the studied sample.

- **Have you used gettaf for treatment before?**

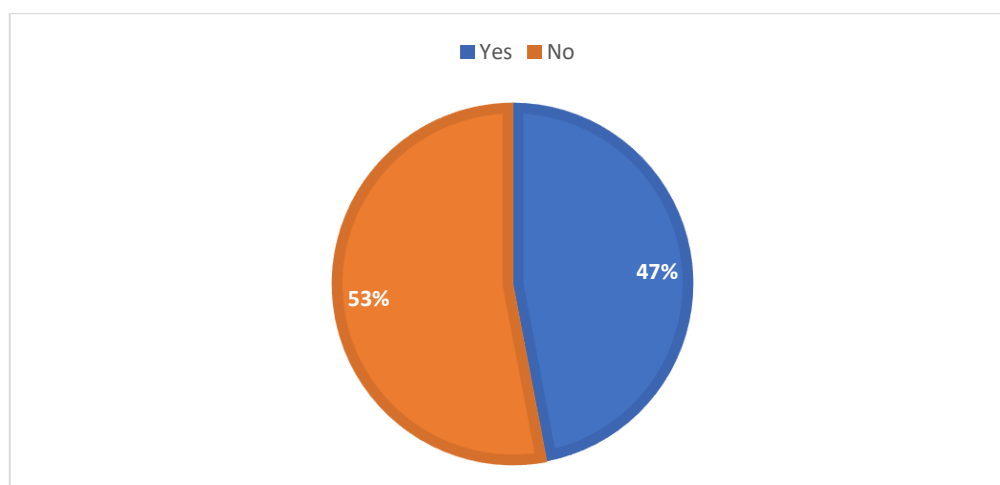


Figure 16: People used *Atriplex halimus*.

47% of people used it for treatment as a medicinal herb.

- **In what disease was it used for?**

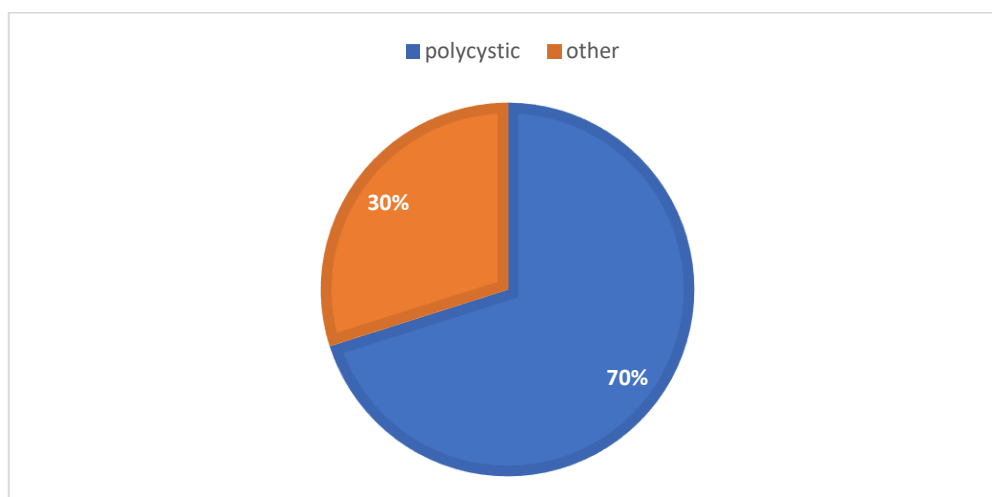


Figure 17: The reason herbal use of *Atriplex halimus* by the population.

Polycystic treatment is the first reason for using this medicinal plant.

- **What part of the plant is used?**

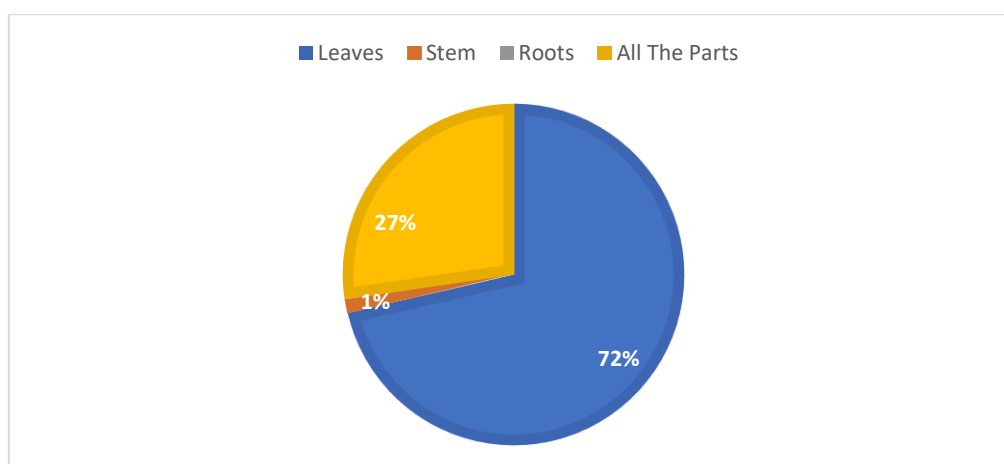


Figure 18: Distribution of plants according to the parts used.

72% using the leaves, 27% using all the parts together, only 1% using the stem and no one using the roots.

- **How to use the plant?**

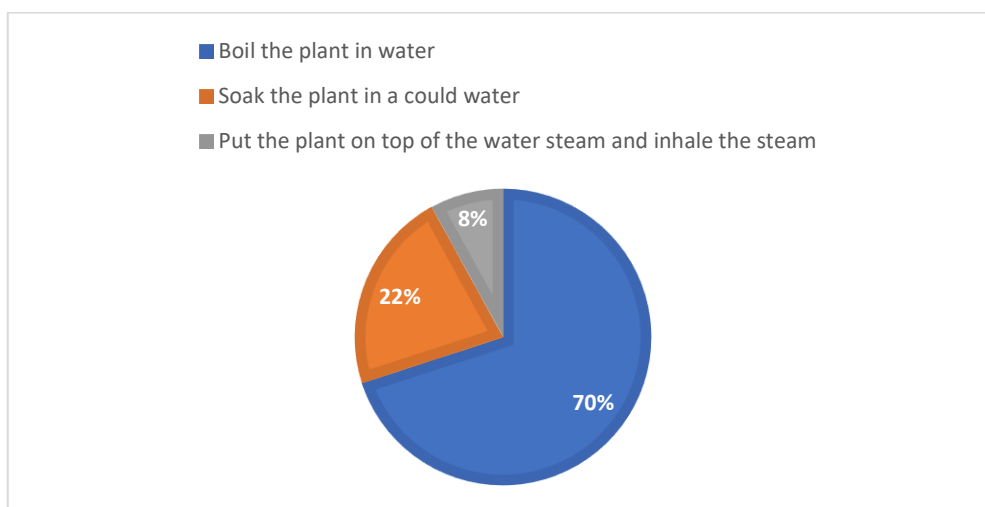


Figure 19: How the plant is used.

There are many methods of consumption, but the most common is boiling the plant in water.

- **Did you have any negative effects after using the plant?**

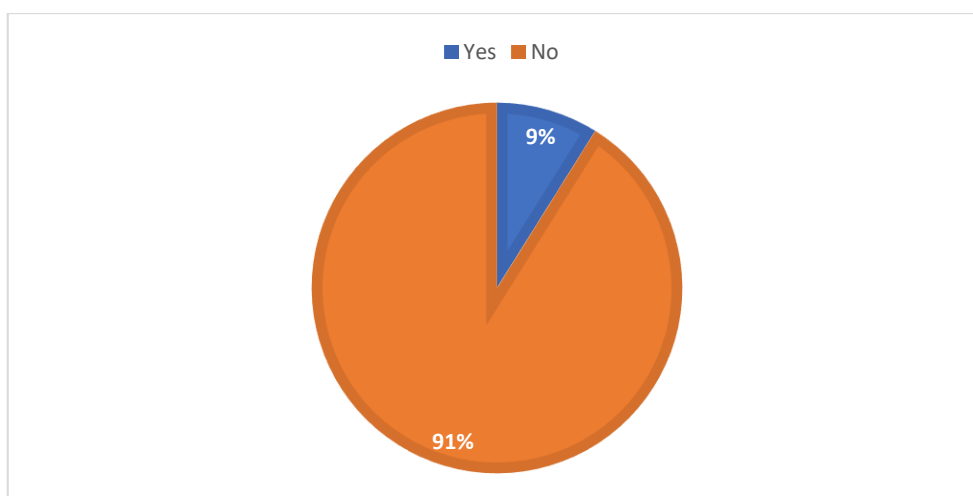


Figure 20: Undesirable effects.

The negative effects of this plant are very low.

- **What was the effects?**

The people who found that this plant had negative effects, all agreed that it was high blood pressure, Because the plant contains a very high percentage of salt.

- **If another plant is used to treat cyst, please write its name?**

This other plants used for cyst treatment by the women who answered: *Peganum* (el-harmell), *Origanum majorana* (el-bardakouche).

CONCLUSION

Conclusion

The use of medicinal plants is today the most widespread form of medicine throughout the world. It is considered as a source of raw materials essential for the discovery of new molecules necessary for the development of future medicines. Among these plants *Atriplex halimus* L. which is a living plant in the region of wilaya of M'sila and used in traditional medicine. The study of this plant allowed to have important results.

The photochemical screening of this plant revealed its content in polyphenols, flavonoids, tannins, and coumarins. Moreover, the spectrophotometric determination of the aqueous and methanolic extract of this plant shows that the methanolic extract of *Atriplex halimus* leaves is richer in polyphenols and flavonoids than the aqueous extract.

The results show that none of the extracts had an antibacterial effect on the three bacterial strains. This is a negative result compared to the literature, which we explained by several factors: genetic (the origin of species), physiological (the degree of maturation of the plant and the organs used), environmental conditions, content of flavonoids and polyphenols.

The conclusion of this study showed that *Artiplex* known under the vernacular name l'egttef l'malih is used to treat polycystic (70%), precisely polycystic ovary (58%), in herbal tea form (70%) using the leaves of the plant (72%).

Our results remain preliminary and open the door for further studies, since *Atriplex halimus* is a rather important reservoir of secondary metabolites with particular therapeutic and pharmacological characteristics. We suggest as perspectives to identify the bioactive molecule of this plant responsible for the anti-fibrocystic activity and also the antibacterial activity, and to understand the molecular mechanisms behind these beneficial effects in vitro and in vivo.

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