



Phytochemical Screening and Antibacterial Activity of Some Extracts from *Atriplex halimus* Growing in Algerian Sahara

A. Berreghioua¹, L. Ziane¹

10.18805/ajdfr.DRF-367

ABSTRACT

Background: Natural products possess interesting biological activities, which attracted several researchers to their elucidation to provide knowledge that will lead to the advancement medicine. *Chenopodaceae* is an important plant family in the world; it contains a number of phytochemicals and has been used to treat a wide range of human diseases. The aim of this work was to carry out qualitative analyses of secondary metabolites and an antimicrobial investigation on the crude extracts from *Atriplex halimus* (*Chenopodaceae*) growing in Algerian Sahara.

Methods: In this laboratory investigation during 2021-2022, phytochemical screening based on staining and precipitation tests was carried out using some solvents of different polarities. The antibacterial assay was carried out by the disc diffusion method on solid medium, using seven bacterial strains chosen for their high pathogenicity.

Result: The secondary metabolites detected in the various extracts were essentially flavonoids, tannins, saponins and terpenoids. The antibacterial investigation made it possible to know that the aqueous extract from *Atriplex halimus* showed a significant inhibition against all bacteria tested. Diethyl ether and chloroform extracts had only one activity against *Enterococcus faecalis* and *Listeria monocytogenes*, respectively, whereas the methanolic extract exhibited the highest inhibition of *Pseudomonas aeruginosa*.

Key words: Antimicrobial, *Atriplex halimus*, *chenopodiaceae*, Metabolites, Sahara, Screening.

INTRODUCTION

For centuries, our ancestors used plants to relieve pain, heal ailments and heal wounds. From generation to generation, they have passed on their knowledge and experiences, trying, whenever possible, to put them in writing. Thus, even today, despite the progress of pharmacology, the therapeutic use of medicinal plants is present in certain countries of the world, especially in developing countries, in the absence of a modern medical system. According to the World Health Organization (WHO), it is estimated that up to four billion people, which represents 80% of the world's population, rely on herbal medicinal products as their primary source of healthcare in the developing world. Traditional medical practices that involve the use of herbs are viewed as an integral part of the culture in those communities (Bandaranayake, 2006; Ekor, 2013; Al-Senosi, 2018; Rouchem *et al.*, 2023).

Historically, plants have provided a source of inspiration for novel compounds belonging to different molecular families; that have various properties for humans. These molecules possess interesting biological activities, which attracted several researchers to their elucidation to provide knowledge that will lead to the advancement medicine. Nearly, all cultures and civilizations from ancient times to the present day have depended fully or partially on herbal medicine because of its effectiveness, affordability, availability, low toxicity and acceptability (Berreghioua *et al.*, 2013).

Saharan plants are known for their resistance to various stress factors. They contain several nutrients and phytochemicals and have been used to treat many human

¹Laboratory of Chemistry and Science Environment, Faculty of Exact Sciences, Tahri Mohammed University, 08000, Bechar, Algeria.

Corresponding Author: A. Berreghioua, Laboratory of Chemistry and Science Environment, Faculty of Exact Sciences, Tahri Mohammed University, 08000, Bechar, Algeria.

Email: berghioua.abdelaziz@univ-bechar.dz

How to cite this article: Berreghioua, A. and Ziane, L. (2024). Phytochemical Screening and Antibacterial Activity of Some Extracts from *Atriplex halimus* Growing in Algerian Sahara. Asian Journal of Dairy and Food Research. DOI: 10.18805/ajdfr.DRF-367.

Submitted: 28-10-2023 **Accepted:** 14-05-2024 **Online:** 21-06-2024

diseases (Kiehlbauch *et al.*, 2000; Skandrani *et al.*, 2007; Benhammou *et al.*, 2009; Djellouli *et al.*, 2015; Vaou *et al.*, 2021; Bendifallah, 2023).

Recently, researchers have reported the antimicrobial activity of traditional medicinal plants worldwide. Examining a wider range of *Chenopodiaceae* species for the production of antimicrobial metabolites is of great interest. Given these considerations and the aim of valorizing Saharan medicinal plants growing in Algeria and following our phytochemical works (Berreghioua *et al.*, 2013; Berreghioua *et al.*, 2015; Berreghioua and cheriti, 2018; Bennaceur *et al.*, 2021), it was considered of interest to carry out a phytochemical screening and antibacterial investigation of *Atriplex halimus* (*A. halimus*) extracts.

A. halimus L. (syn. *Atriplex capensis* Mog., *Chenopodium halimus* Thunb. and *Schizotheca halimus* Fourr.) is a perennial, halophytic and nitrophilous shrub. Its variety of

common names reflects its wide geographical distribution. It grows under semi-arid and arid conditions throughout Eurasia: from the Atlantic coasts, through the Mediterranean basin countries and into the Middle East. It was reported that *A. halimus* was one of the dominant halophytes at the edge of an Algerian chott, together with *Atriplex canescens* (Pursh) Nutt., *Chenopodium album* L. and *Salvia fruticosa* (Khaznadar *et al.*, 2009). It exists as two sub-species: subsp. *halimus* in semi-arid, non-saline zones and subsp. *schweinfurthii* generally occurs in arid and/or saline areas (Le Houérou, 1992). This plant is known for its ecological and economic benefits, for its use as fodder and for its tolerance of salinity and aridity. It is considered among the plant species that make best use of the water in saline soils; because of its high vacuolar osmotic pressure due to high salt concentrations.

In Algeria, *Atriplex* is spontaneous in the semi-arid and arid bioclimatic stages, with the largest areas corresponding to the so-called steppe zones (Batna, Biskra, Boussaâda, Djelfa, Saïda, M'sila, Tébessa, Tiaret) (Chikhi, 2014). The *halimus* plant has been used for thousands of years as a traditional remedy for many ailments. Although ineffective in some cases, such as the use of decoctions to treat syphilis (Rolleston, 1942), they continue to be used and in recent years, the pharmacological basis for some of their effects has been elucidated. Ashes from burning plants can be used as alkali to make soap, while twigs can be burned to obtain antacid powder (Uphof, 1968). The leaves (in decoction) are used to treat heart disease and rheumatism (Said *et al.*, 2008; Chikhi *et al.*, 2014; Ziane *et al.*, 2023). The product ("Glucoselevel") that combines extracts from the leaves of *Urtica dioica* L., *Olea europea* L., *Juglans regia* L. and *A. halimus* has an antidiabetic effect (Said *et al.*, 2008). *A. halimus* is also a source of vitamins (A, C, D) and mineral salts (Calcium: 21.5 (\pm 3.7) g/kg Phosphate: 1.92 (\pm 0.3) g/kg, Magnesium: 20.3 (\pm 4.3) g/kg, Sélénium: 22 (\pm 8) g/kg, Zinc: 103 (\pm 27) g/kg, Manganèse: 395 (\pm 49) g/kg) (Martínez-Fernández and Walker, 2012; Mateos-Naranjo *et al.*, 2013).

A traditional medical practices survey carried out in Western Algeria revealed that the ashes of *A. halimus* L., which are absorbed in water, are used to treat stomach acid. The infusion of the leaves is used in the treatment of kidney pain, lithiasis, cardiovascular disease, hypertension and gastric acidity (Chikhi *et al.*, 2014; Ziane *et al.*, 2023). An acute toxicity study on an aqueous extract of *A. halimus* leaves conducted in experimental rats demonstrated the extract's non-toxic nature. Until the end of the trial period, no mortality or adverse reactions were observed with the chosen doses (1000, 2500 and 3000 mg/kg body weight) (Chikhi *et al.*, 2014; Zeghib *et al.*, 2021). The main natural beds of *Atriplex* are: *A. halimus*, *A. portulocoides*, which are used as fodder by herds, especially sheep and dromedaries (Chikhi, 2014). According to Salem *et al.* (2012), sun-drying and the addition of exogenous dietary enzymes may be beneficial for both intakes of *A. halimus* forage by sheep and its digestibility. It

was demonstrated that the quality of *A. halimus* forage was related to the proportion of twigs and leaves eaten by animals (Otal *et al.*, 2010).

MATERIALS AND METHODS

This study was conducted from February 2021 to January 2022 at the Chemistry Sciences and Environment Laboratory (CSEL), Department of Materials Sciences, Exact Sciences Faculty, University of Bechar; (Algeria). Using the global positioning system, the location of the plant from which the specimens were taken was ascertained. 31°55'24" North, 2°27'51" West, the altitude was roughly 1840 meters.

Vegetal product

Atriplex halimus (Fig 1), known by the common name "Guetaf", is widely distributed in the Algerian Sahara. It was collected during the period of (January-February 2021) from Oued Bechar (north of Bechar, south of Algeria), where we found several plants and herbs (Fig 2). It was identified by several herborists. A voucher specimen is deposited under the number BA 21/1 in the herbarium of CSEL, University of Bechar, Algeria. The aerial part of *A. halimus* (L.) was dried in a dry and shady place at ambient temperature for two weeks. Finely powdered (or small pieces) plant materials were stored in airtight polythene bags protected from sunlight until use.

Microorganisms

Pure cultures of the following microorganisms were used: *Bacillus stearothermophilus* (B.s.) (ATCC 11778), *Enterococcus faecalis* (E.f.) (ATCC 29212), *Escherichia coli* (E.c.) (ATCC 25922), *Klebsiella pneumoniae* (K.p.), *Listeria monocytogenes* (L.m.) (ATCC 19115), *Pseudomonas aeruginosa* (P.a.) (ATCC 27853) and *Staphylococcus aureus* (S.a.) (ATCC 25923), which were obtained from the Pasteur Institute (Algiers, Algeria). The bacteria were maintained by frequent sub-culturing on Mueller Hinton agar plates (pH 7.4) and stored at 4°C.

Preparation of extracts

Using the protocol of Nemlin and Brunel (1995), we have prepared different extracts. The selection of an appropriate solvent is essential because the solvent determines the phytochemicals extracted from the samples and enables the extraction of thermolabile phytochemicals (Bitwell *et al.*, 2023). The extracts were prepared by combining 10 g dried powdered samples in 60 mL of each solvent (distilled water, diethyl ether, ethanol, etc.) under reflux for 1 hour. Subsequently, after filtration by using Whatman filter paper, the extracted solution was separated from the solvent using a rotary evaporator at 80 CmHg pressure to obtain a concentrated extract. For all solvents, the extraction process was repeated three times. The extracts were labeled as aqueous extract, diethyl ether extract, ethanolic extract, etc. After calculating the yield, the extracts were stored in a vial bottle at -20°C to 4°C for phytochemical screening and antibacterial investigation.

Phytochemical screening

The phytochemical analysis was carried out on the obtained extracts using standard procedures to identify the constituents as described by Harborne (1998); Bruneton (1999) and Akinyemi *et al.* (2005).

Test for tannins

- i) To 1 mL of the water extract of each organ was added 1 mL of concentrated HCl. The mixture was boiled for 10 minutes. The formation of a red precipitate soluble in isoamyl alcohol indicated the presence of condensed tannins.
- ii) 1 mL of the water extract of each organ was treated with 2 drops of FeCl_3 (2%) reagent. The appearance of a dark blue precipitate indicated the presence of hydrolysable tannins.

Test for saponins

2 mL of the aqueous solution were filtered and completed to 100 mL with water. In ten test tubes were introduced the following volumes (1, 2,... 10 mL) of the mother solution. Then the final volume was readjusted to 10 mL with distilled water and then stirred in a strong way; the formation of froth indicated the presence of saponins.

Test for starch

To 1 mL of aqueous extract, 10 mL of NaCl saturated solution was added and treated with starch reagent. The appearance of a purplish blue color is a positive test for the presence of starch.

Test for alkaloids

The aqueous extract of each organ of the plant (25 mL) was stirred with 15 mL of 10% HCl on a water bath for 30 minutes. 1 mL of the aqueous layer was treated with two drops of Wagner's reagent. The formation of a brownish precipitate was regarded as evidence for the presence of alkaloids in the extract.

Test of volatile oils

The residue obtained after evaporation of 20 mL of ethereal solution was dissolved in ethanol and concentrated. A residual aroma revealed a positive test.

Test of free alkaloids

10 mL of the diethyl ether layer was evaporated to dryness. The residue was then dissolved in 1.5 mL of HCl (2%) and treated with two drops of Mayer's reagent. Turbidity and the formation of creamy white precipitate were regarded as evidence for the presence of free alkaloids.

Test of fatty acids

The alkaline aqueous solution was acidified and then extracted with diethyl ether. The ethereal solution is then concentrated to dryness. A positive test is revealed by obtaining a greasy residue.

Test of emodols

To 4 mL of evaporated dry etheric extract, 1 mL of ammonia solution (NH_4OH) was added. A cherish-red color appears

after the addition of Bornträger reactif, which, indicated the presence of emodols (aglycones of anthracenosides in oxidized form).

Test of coumarins

15 mL of 10% HCl was added to 25 mL of ethanolic solution and heated under reflux for 30 min. The residue was extracted with 15 mL of ether in triplicate. 3 mL of the diethyl ether extract was evaporated to dryness in a test tube and the residue was dissolved in hot distilled water. It was then cooled and divided into two portions, one of which was the reference. The other was treated with 0.5 mL of NH_4OH (10%). The occurrence of an intense/fluorescence under UV light ($\lambda_{\text{max}} = 365 \text{ nm}$) is a positive test for the presence of coumarins and derivatives.

Test of flavonoids

5 mL of ethanolic extract were treated with a few drops of concentrated HCl and 0.5 g of magnesium. Color varying from orange to red indicated flavones, red to crimson indicated flavonols and crimson to magenta indicated flavonones.

Test for steroids

To 0.5 g of evaporated ethanolic extract were added 2 mL of acetic anhydride and some drops of H_2SO_4 . The color changed from violet to blue or green in some samples, indicating the presence of steroids.

Test for terpenoids

5 mL of each extract was mixed in 2 mL of chloroform and 3 mL of concentrated H_2SO_4 . A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.

Test for reducing compounds

To 1 mL of the ethanolic extract were added 2 mL of distilled water and 20 drops of Fehling's solution, followed by heating in a test tube on a water bath. A brick red precipitate denotes the presence of reducing compounds.

Test for anthracenosids

To 20 mL of ethanolic extract were added 10 mL of HCl (10%). The mixture was refluxed for 15 minutes. After cooling, the mixture was extracted three times with 10 mL of diethyl ether. After evaporation, 10 mL of ethereal solution were treated with 2 mL of hot water and some drops of NH_4OH (10%). A positive test is revealed by the appearance of red oranges color.

Test for anthraquinones

0.5 g of the studied part of each plant was boiled with 10 mL of sulfuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 mL of chloroform. 1 mL of dilute ammonia was added to the chloroform layer. The resulting solution was observed for color changes (The delicate rose pink color showed the presence of anthraquinones).

Test for cardiac glycosides

The Keller-Killani test was performed to assess the presence of cardiac glycosides. 5 mL of each extract were treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1 mL of concentrated sulfuric acid. A brown ring at the interface indicates a deoxysugar characteristic of cardenolids. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish-blue ring may form just gradually throughout the thin layer indicated the presence of cardiac glycosides.

Antibacterial test

The aerial part of the *Atriplex halimus* was dried at room temperature for two weeks and cut into small pieces before extraction under reflux for four hours (4 hours) with several different polar solvents. The extracts were filtered and evaporated using a rotary evaporator.

Antimicrobial activities were performed using the Müller-Hinton plate diffusion method on solid media; strains were reactivated using a 20-h culture at 37°C and adjusted to 10⁸ CFU/mL. Petri boxes (9 cm in diameter) were filled with 10 ml of the medium Muller Hinton. Bacterial strains were inoculated on the surface of agar plates with radially stained cotton swabs and suspensions of young bacterial cultures prepared according to the Committee of the Laboratory Standards Institute. (CLSI) (Kiehlbauch *et al.*, 2000); since most of the studied extracts are immiscible with water and therefore with the medium of culture, dilution was achieved with a dimethyl sulfoxide solution (DMSO). Diffusion is performed on sterile filter paper discs (diameter 6) placed on inoculated agar surfaces and saturated with 3 µL of each extract; One disc saturated with DMSO was used as a negative control, while chloramphenicol (10 µg/mL) was added to the test as a reference (positive control). Each disc should be gently pressed down before incubation to ensure full contact between the disc and the agar surface. The Petri dish was incubated at 37°C for 24-48 h (Duraffourd, 2002; Toubal *et al.*, 2011) and the diameter of the blockage was measured with a caliper slid around the disk or a ruler, which is held on the back of the inverted petri plate. Each experiment was performed in triplicate.

RESULTS AND DISCUSSION

Phytochemical tests are performed on different extracts prepared from the dried aerial parts using several solvents of different polarities. The presence or absence of certain important compounds in an extract is determined by the color reaction of the compounds with specific chemical reagents, which act as dyes. This procedure is a pre-requisite first step before going for a detailed phytochemical investigation. The results of phytochemical screening of different extracts are reported in Table 1.

Phytochemical analysis showed the presence of saponins as confirmed by the froth test. The presence of flavonoids was confirmed by a positive reaction in the

presence of HCl and magnesium ribbon in alcoholic and aqueous extracts. Qualitative phytochemical studies of reducing sugars showed a good characteristic color and precipitate in aqueous extract using Fehling's test. In contrast, the study indicated that alkaloids, coumarins and emodols were absent in aqueous, diethyl ether and ethanol extracts. On the other hand, fatty acids were weakly present. Besides, anthraquinones were present in the water extract, whereas, the diethyl ether extract was found to have anthracenosids. Tannins test results showed a positive reaction with ferric chloride (FeCl₃). From this study, we observed well that *A. halimus* is rich in tannins. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003; Dals *et al.*, 2007). Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect, which is important for the treatment of inflamed or ulcerated tissues. These secondary metabolites have various applications due to their

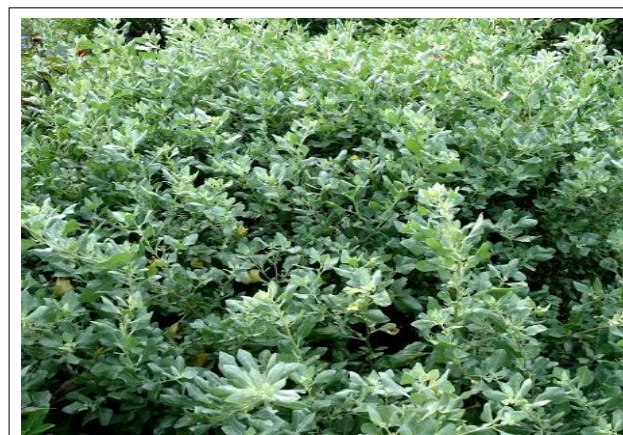
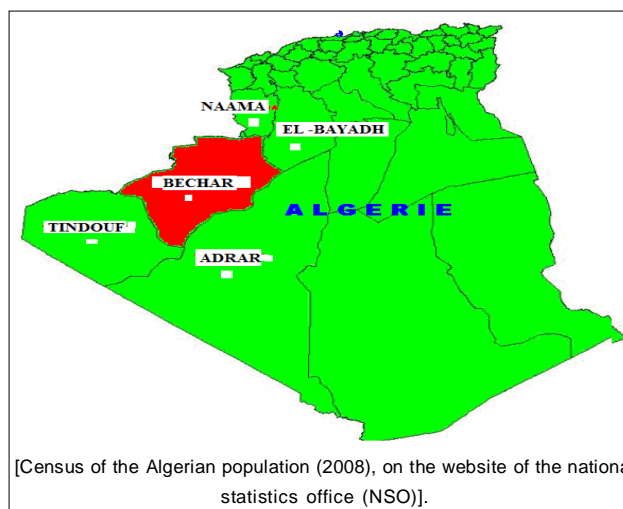


Fig 1: General view of *A. halimus* (in its biotope).



[Census of the Algerian population (2008), on the website of the national statistics office (NSO)].

Fig 2: Geographical location of the South Western Algeria.

antibacterial properties (Parekh and Chanda, 2007). All the extracts produced positive reactions as follows: water extract (tanins, saponins, flavonoids, steroids, terpenoids and cardiac glycosids), diethyl ether extract (tannins, saponins, volatile oils, fatty acids, terpenoids and anthracenosids) and ethanol extract (tannins, flavonoids, saponins and terpenoids). However, the majority of the extracts gave positive results for flavonoids, saponins, terpenoids and tannins. Water, methanol and ethyl acetate solvents are currently used for the extraction of polyphenols and flavonoids; these metabolites are considered as potent antioxidants, anti-inflammatory, antiviral and antibacterial (Bruneton, 1999; Chen and Blumberg, 2008; Berregioua and Cheriti, 2018). These observations therefore support the use of *Atriplex halimus* in herbal remedies. The weight ratio of the extract to the sample weight is known as the extract yield. In our study, the yields ranged from 0.5% to 4.52% (Table 1). Choosing the right solvent for the extraction is crucial because it affects the phytochemicals that are recovered from the samples and makes it possible to extract thermolabile phytochemicals (Bitwell *et al.*, 2023). The two main variables affecting the amount of extracted chemicals are the choice of extraction method and extraction time. The plant species, the organ utilized for extraction, the drying conditions, the amount of metabolites in each species and the type and polarity of the solvent all affect the extraction yield. The sample-to-solvent ratio and temperature are two other variables that impact compound yields (Pham *et al.*, 2019; Nurcholis *et al.*, 2022).

The dosage carried out revealed the total phenol content (TPC) and total flavonoids content (TFC) in the methanol extract was 17.57 ± 0.25 mg gallic acid equivalent (GAE)/g dried extract (DE) and 3.52 ± 0.17 mg quercetin

equivalent (QE)/g DE, respectively. In the butanol extract (68.20 ± 0.03 mg GAE/g DE and 4.39 ± 0.77 mg QE/g DE), in the ethyl acetate (38.80 ± 0.11 mg GAE/g DE and 4.11 ± 0.69 mg QE/mg DE) and in the dichloromethane extract (14.93 ± 0.25 mg GAE/mg DE and 1.91 ± 0.09 mg QE/g DE). In the diethyl ether extract, TPC was 26.40 ± 0.25 mg GAE/mg DE, TFC was 2.12 ± 0.04 mg QE/g DE, tannins content was 0.234 ± 0.25 mg catechine eq. /g DE and phenolic acid content was 5.20 ± 0.15 mg GAE/g DE. A study was carried out on an aqueous extract of the aerial part of the *A. halimus* L. plant from El-Oued (Algerian Sahara) has revealed that TPC was 17.183 mg GAE/g DE, TFC was 4.024mg Q eq/g DE, tannins content was 1.49 mg Catechine eq/g DE) and saponins content was 43.36 mg optical density eq. /g DE (Zeghib *et al.*, 2021). The difference in chemical composition between the same plants being studied, as well as those in another region, can be attributed to various factors that affect the presence, absence and distribution of active ingredients. These factors include climate, soil type, water, altitude, *etc* (Nurcholis *et al.*, 2022).

The results of the *in vitro* screening of different extracts of *Atriplex halimus* tested against pathogenic bacteria (Table 2) showed that the methanol extract was most active against *P. aeruginosa*. This last has the reputation of being in general, very resistant to all sorts of antimicrobial agents and antibiotics. It is the cause of a number of diseases affecting humans and animals (Adegoke and Ojo, 1982; Miyake *et al.*, 1991; Couto *et al.*, 1995; Berregioua and Cheriti, 2018; Rouchem *et al.*, 2023). The aqueous extract had a significant inhibitory effect on all bacteria tested; its highest activity is against *E. faecalis* and *P. aeruginosa*. The activity seems to be due to the presence of polyphenolic compounds and antimicrobial agents in the aqueous

Table 1: Phytochemical analysis and yields of extracts from *A. halimus*.

| | W | De | E | C | A | M | Pe | D | H | Ac |
|--------------------|-----|------|------|-----|------|------|------|------|------|------|
| Yield (%) | 4.4 | 1.02 | 3.29 | 0.5 | 1.38 | 4.52 | 0.48 | 0.69 | 0.52 | 3.98 |
| Alkaloids salts | | - | | | | | | - | | |
| Anthracenosids | | + | ... | ... | ... | | | + | | |
| Anthraquinons | + | - | ... | ... | ... | + | | - | | + |
| Cardiac glycosids | + | - | + | - | - | + | | - | | + |
| Coumarins | - | - | - | + | + | + | | - | | |
| Emodols | - | - | - | | | | | - | | |
| Fatty acids | | + | - | | | | | + | | |
| Flavonoids | + | - | + | + | + | + | | - | | + |
| Free alkaloids | - | - | - | - | - | - | | - | | - |
| Reducing compounds | + | + | + | + | + | + | | + | | + |
| Saponins | + | + | + | - | - | + | | + | | + |
| Steroids | + | - | + | | | + | | - | | + |
| Tanins | + | + | + | + | + | + | | + | | + |
| Terpenoids | + | + | + | + | + | + | | + | | + |
| Volatile oils | - | + | - | | | - | | + | | - |

A: Ethyl acetate, Ac: Acetone, C: Chloroform, D: Dichloromethane, De: Diethyl ether, E: Ethanol, H: Hexane, M: Methanol, Pe: Petroleum ether, W: Water.

extract (Belboukhari *et al.*, 2011). The growth of *P. aeruginosa* was inhibited by ethanol, but it was not inhibited by ethyl acetate, cyclohexane, diethyl ether, chloroform or hexane extracts of the aerial part of *A. halimus*. Infection caused by *P. aeruginosa* is difficult to treat with conventional antibiotics (Berregioua *et al.*, 2013). The results show that *Staphylococcus aureus*, a gram-positive bacterium, is more sensitive to ethyl acetate extract compared to the two other strains, *P. aeruginosa* and *E. coli*, gram-negative bacterium, which have almost the same level of sensitivity. The ethyl acetate extract of aerial parts had a significant inhibitory effect on all bacteria tested, its highest activity was against *E. coli*. We observed that the dichloromethane extract was active, but not against *S. aureus*, *E. faecalis* and *L. monocytogenes*. However, the chloroform extract exhibited the highest inhibition of *S. aureus*. In spite of advances in medical science, epidemiology and the discovery of new

antibiotics, *S. aureus* infections still present considerable morbidity and mortality (Couto *et al.*, 1995). According to Ibiri (2005), *S. aureus* has the reputation of being in general, very resistant to all sorts of antimicrobial agents and antibiotics. It is established that the inhibition of this bacterium by antimicrobial agents; require considerable concentrations. *S. aureus* is the cause of a number of diseases affecting humans and animals (Adegoke and Ojo, 1982; Miyake *et al.*, 1991; Couto *et al.*, 1995, Rouchem *et al.*, 2023).

The crude extract of methanol showed an important activity for *K. pneumoniae* and *P. aeruginosa*, this last has the reputation to be in general very resistant to all sorts of antimicrobial agents and antibiotics (Berregioua and Cheriti, 2018). The aqueous extract had a significant inhibitory effect on all bacteria tested, the activity seems to be due to the presence of polyphenolic compounds and

Table 2: Zone of inhibition (mm).

| | | 3 µl/disk | | | | | | |
|--------|----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Solv. | Sol. | <i>E.c.</i> | <i>S.a.</i> | <i>K.p.</i> | <i>P.a.</i> | <i>B.s.</i> | <i>E.f.</i> | <i>L.m.</i> |
| Chlor. | 10 µg/ml | 35 | 15 | 15 | 28 | 25 | 30 | 15 |
| W | Cs | 12 | 13 | 11 | 15 | 12 | 16 | 12 |
| | 1/4 | 08 | 08 | 06 | 06 | 06 | 06 | 06 |
| | 1/10 | 06 | 06 | 00 | 06 | 06 | 06 | 06 |
| M | Cs | 06 | 06 | 16 | 06 | 10 | 09 | 08 |
| | 1/4 | 06 | 06 | 11 | 06 | 06 | 06 | 06 |
| | 1/10 | 06 | 06 | 00 | 06 | 06 | 06 | 06 |
| E | Cs | 12 | 06 | 12 | 06 | 10 | 08 | 06 |
| | 1/4 | 06 | 06 | 11 | 06 | 06 | 06 | 06 |
| | 1/10 | 06 | 06 | 00 | 06 | 06 | 06 | 06 |
| A | Cs | 12 | 06 | 12 | 06 | 10 | 08 | 06 |
| | 1/4 | 11 | 10 | 06 | 06 | 06 | 06 | 06 |
| | 1/10 | 06 | 06 | 06 | 06 | 06 | 06 | 06 |
| C | Cs | 08 | 15 | 08 | 10 | - | - | - |
| | 1/4 | 06 | 06 | 06 | 06 | 06 | 06 | 06 |
| | 1/10 | 06 | 06 | 06 | 06 | 06 | 06 | 06 |
| D | Cs | 20 | 06 | 18 | 17 | 08 | 06 | 06 |
| | 1/4 | 12 | 06 | 07 | 10 | 06 | 06 | 06 |
| | 1/10 | 06 | 06 | 06 | 06 | 06 | 06 | 06 |
| H | Cs | 06 | 06 | 06 | 06 | 06 | 06 | 06 |
| | 1/4 | 06 | 06 | 06 | 06 | 06 | 06 | 06 |
| | 1/10 | 06 | 06 | 06 | 06 | 06 | 06 | 06 |
| Pe | CS | 06 | 06 | 06 | 06 | 06 | 06 | 06 |
| | 1/4 | 06 | 06 | 06 | 06 | 06 | 06 | 06 |
| | 1/10 | 06 | 06 | 06 | 06 | 06 | 06 | 06 |
| De | Cs | 06 | 12 | 15 | 10 | 09 | 13 | 06 |
| | 1/4 | 06 | 08 | 06 | 06 | 06 | 06 | 06 |
| | 1/10 | 06 | 06 | 06 | 06 | 06 | 06 | 06 |
| Ac | Cs | 06 | 12 | 10 | 15 | 15 | 12 | 10 |
| | 1/4 | 06 | 06 | 06 | 06 | 06 | 06 | 06 |
| | 1/10 | 06 | 06 | 06 | 06 | 06 | 06 | 06 |

A: Ethyl acetate, Ac: Acetone, C: Chloroform, Chlor: chloramphenicol, Cs: Concentred solution, D: Dichloromethane, De: Diethyl ether, E: Ethanol, H: Hexane, M: Methanol, Pe: Petroleum ether, Sol: Solutions, Solv: Solvents, W: Water.

antimicrobial agents (Belboukhari *et al.*, 2011). The aqueous extract showed an highest activity against *E. faecalis* and *P. aeruginosa*, Infection caused by *p. aeruginosa* is difficult to treat with conventional antibiotics (Cheriti *et al.*, 2005). Water, methanol and ethyl acetate solvents are currently being used for the extraction of polyphenols and flavonoids. The polyphenols are considered potent antioxidants, anti-inflammatory, antiviral and antibacterial (Bruneton, 1999; Chen and Blumberg, 2008). Our present antimicrobial evaluation showed that hexane and petroleum ether extracts had no activity for all bacteria tested. It is noted that the majority of extracts from *A. halimus* can inhibit the growth of some bacteria that cause different human diseases (Abdel Rahman *et al.*, 2011; Bachanti *et al.*, 2018). The ethanol extracts from the aerial parts of *A. halimus*, containing alkaloids, steroids, flavonoids and glycosides, showed antibacterial activity against a variety of pathogenic gram-positive and gram-negative bacteria (Abdel Rahman *et al.*, 2011). Endophytic fungi isolated from *A. halimus* has antibacterial effects against antibiotic-resistant bacterial species (Peláez *et al.*, 1998; Dahou *et al.*, 2021; Radja *et al.*, 2022).

CONCLUSION

The phytochemical screening, based on laboratory tests, allowed us to characterize the family of chemical compounds that exist in the plant we studied.

Based on the results of the antibacterial activity test, it seems that some of the extracts of this plant were active to varying degrees, which is related to the interaction between the content of the extracts and the antibacterial agents in the extraction solvents. This probably explains the use of this plant in traditional medicine for many human ailments for generations. The plant studied here can be considered as a possible source of useful drugs. Further chemical and pharmacological studies should be performed to isolate and identify bioactive compounds from extracts of interest. Lastly, just like other human-use medications, herbal medicines must now be governed by a drug regulatory system in every nation on the planet to guarantee that they meet the necessary requirements for efficacy and quality.

ACKNOWLEDGEMENT

We gratefully acknowledge the Gen-eral Directorate of Scientific Research and Technological Development (DGRSDT) for its unwavering encouragement.

Author contributions

Concept: Berreghioua, Ziane; **Design:** Berreghioua; **Control:** Berreghioua, Ziane; **Sources:** Berreghioua, Ziane; **Materials:** C.S.E.L.; **Data Collection and Processing:** Berreghioua, Ziane; **Analysis and/or Interpretation:** Berreghioua, Ziane; **Literature Review:** Berreghioua; **Manuscript Writing:** Berreghioua; **Critical Review:** Berreghioua.

Ethics committee approval

The authors declare that ethics committee approval is not required for this study.

Conflict of interest

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

REFERENCES

- Abdel, R.S.M., Abd-Ellatif, S.A., Deraz, S.F., Khalil, A.A. (2011). Antibacterial activity of some wild medicinal plants collected from the western Mediterranean coast, Egypt: natural alternatives for infectious disease treatment. *African Journal of Biotechnology*. 10: 10733-10743.
- Adegoke, G.O. and Ojo, M.O. (1982). Biochemical characterization of staphylococci isolated from goats. *Veterinary Microbiology*. 7(5): 463-470. doi: 10.1016/0378-1135(82)90063-3.
- Al-Senosi, N.K. Abou-Eisha, A., Ahmad, E.S. (2018). *In vitro* antiproliferation effect of *Atriplex halimus* L. crude extract on human cell lines by induction of apoptosis and G2/M phase arrest. *Egyptian Academic Journal of Biological Sciences. Physiology and Molecular Biology*. 10(1): 115-126. doi: 10.21608/EAJBSC.2018.13660
- Akinyemi, K.O., Oladapo, O., Okwara, C.E., Ibe, C.C., Fasure, K.A. (2005). Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. *BMC Complementary Alternative Medicine*. 5: 6. doi: 10.1186/1472-6882-5-6.
- Bachanti, P., Vij, S., Patil, M.R., Bajad, D.N., Adil, S., Kalyankar, S.D. (2018). Antimicrobial activity of casein fermentate of probiotic *Lactobacillus* spp. *Asian Journal of Dairy and Food Research*. 37(3): 175-181. doi: 10.18805/ajdfr.DR-1206.
- Bandaranayake, W.M. (2006). Quality Control, Screening, Toxicity and Regulation of Herbal Drugs, In: *Modern Phytomedicine*. Wiley-VCH and Co. KGaA, Weinheim. pp. 25-57. doi: 10.1002/9783527609987.ch2.
- Bendifallah, L. (2023). Biocidal Activity of Plant Extracts: The Case of Algeria, In: *Antimicrobial Research and One Health in Africa*. Springer International Publishing, pp. 305-315. doi: 10.1007/978-3-031-23796-6-13.
- Bennaceur, S., Berreghioua, A., Bennamoun, L., Mulet, A., Draoui, B., Abid, M., Carcel, J.A. (2021). Effect of ultrasound on henna leaves drying and extraction of lawsone: Experimental and modeling study. *Energies*. 14: 1329. doi: 10.3390/en14051329.
- Berreghioua, A., Cheriti, A., Belboukhari, N. (2013). Preliminary phytochemical investigation of antibacterial extracts from *Moricandia arvensis* growing in Algerian Sahara. *Natural products: An Indian Journal*. 9(8): 322-325.
- Berreghioua, A., Cheriti, A., Belboukhari, N. (2015). Flavone and flavone glucuronide from *Zilla macroptera* L. (Brassicaceae) growing in Algerian Sahara. *Der Pharma Chemica*. 7(8): 197-201. <http://derpharmachemica.com/archive.html>.
- Berreghioua, A. and Cheriti, A. (2018). Phytochemical investigation of the medicinal plant *Moricandia arvensis* L. from Algerian Sahara. *Asian Journal of Pharmaceutical and Clinical Research*. 11(5): 450-453. doi: 10.22159/ajpcr.2018.v11i6.23822.

- Benhammou, N., Bekkara, A.F., Panovska, T.K. (2009). Antioxidant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus*. *Comptes Rendus de Chimie*. 12(12) : 1259-1266. doi: 10.1016/j.crci.2009.02.004.
- Belboukhari, M., Cheriti, A., Belboukhari, N. (2011). Total phenolic content and *in vitro* antioxydant activity of extracts from the endemic medicinal plant *warionia saharae*. *Natural Products: An Indian Journal*. 7(3): 147-150.
- Bitwell, C., Indra, S., Luke, C., Kakoma, M.K. (2023). A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. *Scientific African Journal*. 19: e01585. doi: 10.1016/j.sciaf.2023.e01585.
- Bruneton, J. (1999). *Pharmacognosy-phytochemistry-medicinal plants* (3rd Edn.). Lavoisier, Paris. pp. 199-388.
- Census of the Algerian population, (2008), on the website of the national statistics office (NSO). Recensement Général De La Population et De L'Habitat 2008.
- Chen, C.Y.O. and Blumberg, J.B. (2008). Are the Age-related Changes in Flavonoid Bioavailability? In: *Phytochemicals Aging and Health*. CRC Press/Taylor-francis group, New York. pp.19-31.
- Cheriti, A., Belboukhari, N., Hacini, S. (2005). Medicinal plants from the Bechar region: Flavonoid content and antibacterial evaluation. International seminar on the use of medicinal plants in arid zones. Ouargla. Algeria.
- Chikhi, I. (2014). Chemical composition and biological activities of extracts of five aromatic and medicinal plants from the Algerian ouset. PhD Thesis. Aboubeker belkaid University, Tlemcen, Algeria.
- Chikhi, I., Allali, H., Dib, M.E.A., Medjdoub, H., Tabti, B. (2014). Antidiabetic activity of aqueous leaf extract of *Atriplex halimus* L. (Chenopodiaceae) in streptozotocin-induced diabetic rats. *Asian Pacific Journal of Tropical Diseases*. 4(3): 181-184. doi: 10.1016/S2222-1808(14)60501-6.
- Couto, I., Melo-Cristino, J., Fernandez, M. L., Garcida, T., Serrano, N., Salgado, M.J., Torres-Pereira, A., Sanchez, I.S., De Lencastre, H. (1995). Unusually large number of methicillin resistant *Staphylococcus aureus* clones in a portuguese hospital. *Journal of Clinical Microbiology*. 33(8): 2032-2035. doi: 10.1128/jcm.33.8.2032-2035.1995.
- Dahou, A.A., Bekada, A., Homrani, A. (2021). Identification of a *Lactococcus lactis* isolated from a fresh local cheese of the western Algerian steppe. *Asian Journal of Dairy and Food Research*. 40(1): 40-44. doi: 10.18805/ajdfr.DR-208.
- Dals, D., Limem, I., Ben Mansour, H., Chibani, J., Ghedira, L.C., Ghedira, K. (2007). Study of the antibacterial and antioxidant activities of different extracts from the leaves of *Moricandia arvensis*. *Review of Arid Regions*. 1: 262-269.
- Dharmananda, S. (2003). Gallnuts and the uses of tannins in Chinese medicine. *Traditional Medicine Proceedings*, Portland, Oregon.
- Djellouli, M., Benmehdi, H., Mammeri, S., Moussaoui, A., Ziane, L., Hamidi, N. (2015). Chemical constituents in the essential oil of the endemic plant *Cotula cinerea* (Del.) from the southwest of Algeria. *Asian Pacific Journal of Tropical Biomedicine*. 5 (10): 930-932. doi: 10.1016/j.apjtb.2015.06.007.
- Duraffourd, C. and Lopraz, J.C. (2002). *Clinical treaty of phytotherapy*. Masson, Paris. pp.352.
- Ekor, M. (2013). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*. 4: 177. doi: 10.3389/fphar.2013.00177.
- Harborne, J.A. (1998). *Phytochemical Methods* (3rd Edn.). Chapman and Hall, London. pp. 302.
- Ibiri, P.M.C. (2005). Microbiological sanitation of air and ventilation systems using essential oils. PhD thesis. Federal polytechnic school, EPFL. Lausanne, Suisse.
- Khaznadar, M., Vogiatzakis, I.N., Griffiths, G.H. (2009). Land degradation and vegetation distribution in chott El Beida wetland, Algeria. *Journal of Arid Environment*. 73(3): 369-377. doi: 10.1016/j.jaridenv.2008.09.026.
- Kiehlbauch, J.A., Hannet, G.E., Salfinger, M., Archinal, W., Monserrat, C., Carlyn, C. (2000). Use of the national committee for clinical laboratory standars guidelines for disk diffusion susceptibility testing in New York state laboratories. *Journal of Clinical Microbiology*. 38(9): 3341-3348. doi: 10.1128/JCM.38.9.3341-3348.2000.
- Le Houérou, H.H. (1992). The role of saltbushes (*Atriplex* spp.) in arid land rehabilitation in the Mediterranean Basin: A review. *Agroforestry Systems*. 18: 107-148. doi: 10.1007/BF00 115408.
- Martínez-Fernández, D. and Walker, D.J. (2012). The effects of soil amendments on the growth of *Atriplex halimus* and *Bituminaria bituminosa* in heavy metal contaminated soils. *Water, Air and Soil Pollution*. 223(1): 63-72. doi: 10.1007/s11270-011-0839-0.
- Mateos-Naranjo, E. andrades-Moreno, L., Cambrollé, J., Perez-Martin, A. (2013). Assessing the effect of copper on growth, copper accumulation and physiological responses of grazing species *Atriplex halimus*: Ecotoxicological implications. *Ecotoxicology and Environmental Safety*. 90: 136-142. doi: 10.1016/j.ecoenv.2012.12.020.
- Miyake, Y., Iwai, T., Sugai, M., Miura, K., Suginaka, H. (1991). Incidence and characterization of *Staphylococcus aureus* from the tongues of children. *Journal of Dental Research*. 70(7): 1045-1047. doi: 10.1177/002203459107 00070501.
- Nemlin, J. and Brunel, J.F. (1995). *Practical work on medical subjects* (3rd Edn.). National university of Ivory Coast. pp. 47.
- Nurcholis, W., Alfadzrin, R., Izzati, N., Arianti, R., Vinnai, B.Á., Sabri, F., Kristóf, E., Artika, I.M. (2022). Effects of methods and durations of extraction on total flavonoid and phenolic contents and antioxidant activity of Java cardamom (*Amomum compactum* Soland Ex Maton) fruit. *Plants*. 11(17): 2221. doi: 10.3390/ plants11172221.
- Otal, J., Orengo, J., Quiles, A., Hevia, M.L., Fuentes, F. (2010). Characterization of edible biomass of *Atriplex halimus* L. and its effect on feed and water intakes and on blood mineral profile in non-pregnant Manchega-breed sheep. *Small Ruminant Research*. 91(2-3): 208-214. doi: 10.1016/j.smallrumres.2010.03.016.
- Parekh, J. and Chanda, S. (2007). In vitro antibacterial activity of the crude methanol extract of *Wood fordia fruticosa* Kurz. flower (*Lythraceae*). *Brazilian Journal of Microbiology*. 38(2). doi: 10.1590/S1517-83822007000200004.

- Pham, T.N., Lam, T., Nguyen, M., Le, T., Quoc, T.T., Sang, V.T. (2019). Effect of various factors on extraction efficiency of total anthocyanins from Butterfly pea (*Clitoria ternatea* L. Flowers) in Southern Vietnam. IOP Conference Series: Materials Science and Engineering. 544 012013. doi: 10.1088/1757-899X/544/1/012013.
- Peláez, F., Collado, J., Arenal, F., Basilio, A., Cabello, A., Díez Matas, M.T., García, J.B., González Del Val, A., González, V., Gorrochategui, J., Hernández, P., Martín, I., Platas, G., Vicente, F. (1998). Endophytic fungi from plants living on gypsum soils as a source of secondary metabolites with antimicrobial activity. Mycology Research Laboratories. 102(6): 755-761. doi: 10.1017/S0953756297005662.
- Rolleston, J.D. (1942). The folk-lore of venereal disease. British Journal of Venereal Diseases. 18(1-2): 1-13. doi: 10.1136/sti.18.1-2.1.
- Radja, D.S., Homrani, M., Dahou, A.A., Haddad, A., Rechidi, N., Homrani, A. (2022). Identification and antibacterial activity of *Lactobacillus* isolated from different raw honey in mostaganem province, Algeria. Asian Journal of Dairy and Food Research. 41(4): 468-473. doi: 10.18805/ajdfr.DRF-264.
- Roucham, Z. and Cheriti, A. (2023). Antifungal activity of the extracts of two medicinal plants from South Western Algeria: *Ephedra alata* and *Bubonium graveolens*. Asian Journal of Dairy and Food Research. doi: 10.18805/ajdfr.DRF-312.
- Said, O., Fulder, S., Khalil, K., Azaizah, H., Kassis, E., Said, B. (2008). Maintaining a physiological blood glucose level with 'Glucoselevel', a combination of four antidiabetes plants used in the traditional arab herbal medicine. Evidence-Based Complementary and Alternative Medicine. 5(4): 421-428. doi: 10.1093/ecam/nem047.
- Salem, A.Z.M., Hassan, A.A., Khalil, M.S., Gado, H.M., Alersy, H., Simbaya, J. (2012). Effects of sun-drying and exogenous enzymes on nutrient intake, digestibility and nitrogen utilization in sheep fed *Atriplex halimus* foliages. Animal Feed Science and Technology. 171(2-4): 128-135. doi: 10.1016/j.anifeedsci.2011.10.011.
- Skandrani, I., Ben Sghaier, M., Neffati, A., Boubaker, J., Bouhlel, I., Kilan, S., Mahmoud, A., Ghedira, K., Chekir-Ghedira, L. (2007). Antigenotoxic and free radical scavenging activities of extracts from *Moricandia arvensis*. Drug and Chemical Toxicology. 30(4): 361-382. doi: 10.1080/01480540701522494.
- Toubal, O., Djahoudi, A., Bouzabata, A. (2011). Preliminary studies and antimicrobial evaluation of the aerial parts of *Genista numidica* ssp. Journal of Life Sciences. 5(11): 954-959.
- Uphof, J.C.T.H. (1968). Useful species: Dictionary of economic plants (2nd Edn.). Stechert-Hafner, New York. pp.591
- Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and futures perspectives. Microorganisms. 9(10): 2041. doi: 10.3390/microorganisms9102041.
- Zeghib, K., Boutlelis, D.A., Menai, S., Debouba, M. (2021). Protective effect of *Atriplex halimus* extract against benzene-induced haematotoxicity in rats. Ukrainian Biochemical Journal. 93(4): 66-76. doi: 10.15407/ubj93.04.066.
- Ziane, L., Berregioua, A., Djellouli, M. (2023). Chemical characterization of the flavonol glycosides constituents, Total phenolic and flavonoid quantification of bioactive extracts from the leaves of *Atriplex halimus*. Aditum Journal of Clinical and Biomedical Research. 6(2). doi: 02.2023/1.1098.