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Research Article

PHYTOCHEMICAL SCREENING AND ANTI-BACTERIAL ACTIVITY OF METHANOLIC EXTRACTS OF THE AERIAL PARTS OF *Atriplex halimus* L., FROM BISKRA (ALGERIA)

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ABSTRACT. The objectives assigned to the present study are the phytochemical screening of several secondary metabolites and the evaluation of the antibacterial activity of the methanolic extracts of the aerial parts 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 vis-à-vis sixteen bacterial strains, chosen according to the traditional use of this species in Algeria: *Staphylococcus* MRSA, *Staphylococcus aureus*, *Staphylococcus* ATCC 00, *Streptococcus* Sp, *Enterococci* Sp, *Enterococci feacalis* ATCC 12, *Salmonella, E. coli* ESBL +, *Klebsiell*a pn Marseille, *Kp*C+, *Kp* ETP R / IMP R, *Serratia* Sp, *Serratia environmental*, *Pseudomonas aerogenosa* ATCC 53, *Pseudomonas* VIM 2, *Bacillus*. The study shows a remarkable antibacterial activity against Gram+ bacteria compared to Gram- bacteria.

Keywords: *Atriplex halimus L., Biskra, aerial part, methanolic extract, antibacterial effect*

INTRODUCTION

Today, and despite the advances made in medicine, many people resort to plants for treatment, either because of inaccessibility to drugs prescribed by modern medicine, or because these plants have given very encouraging therapeutic results and with lesser side effects noticed during their use, or because they are less aggressive and less harmful for the body[1].The search for new active pharmacological molecules via the screening of natural sources has led to the discovery of a large number of useful drugs that are beginning to play a major role in the treatment of many human diseases [2].

The flora of Algeria is rich of several thousand medicinal species, among this vast natural heritage, our choice was on the *Atriplex halimus* L., commonly called "Guettaf" which is a shrub native of North Africa where it is very abundant it also extends to the Mediterranean coastal areas of Europe and the gypso-saline inland areas of Spain. It is present in regions where the ecological imbalance is accentuated and where the phenomenon of desertification takes alarming dimensions.

It constitutes, during drought and seasonal seals, a forage preferred by Camelidae and particularly sheep and goats. However, overgrazing, climatic constraints and the lack of rational rangeland management have led to a severe degradation of their stands. A species renowned for the nutritional and energetic value of its tender leaves rich in dietary fiber (cellulose), proteins, vitamins (B and C) and mineral salts (sodium,

calcium, potassium, magnesium, phosphorus), not only for livestock, but also as food for nomads and the local steppe population [3].

In addition, it is ranked among the most used plants by the steppe population to treat hyperglycemia [4]. In Algeria it is widely used in therapy mainly for the treatment of different types of cysts "ovarian, uterine and breast cysts".

The aim of our study is to evaluate the antibacterial activity of the methanolic extracts of the aerial parts of the *Atriplex halimus* L. harvested from the region of Biskra (Algeria).

MATERIALS AND METHODS

Plant Material

The species *Atriplex halimus* L. was harvested in the region of Biskra (southeastern Algeria) in December 2018.

The identification of the plant was done with the key to determining the flora of Quezel and Santa [5]. Specimens were kept at the Laboratory of Cryptogamy and Medical Botany, Department of Pharmacy, Faculty of Medicine Annaba-Algeria.

Preparation of Methanolic Extracts

Dry aerial parts (stem and leaf) of *Atriplex halimus* L. have been ground and stored in glass bottles, hermetically sealed at low temperatures. 10 g of the vegetable powder was macerated in 100 ml of methanol with stirring for 24 hours at a temperature of 25 ± 2° C. The extract obtained was filtered and evaporated to dryness under reduced pressure at 50° C on a rotavapor. The dry residue is taken up in 3 ml of methanol and stored at -18 ° C until it is used [6].

The yield of the methanolic extracts was calculated by the following formula: R (%) = (M/M_0) ×100. With: R (%): yield expressed in%; M: mass in grams of the resulting dry extract; M_0: mass in grams of the plant material to be treated.

Phytochemical Screening

Phytochemical tests of the powders of the stem and leaf of *Atriplex halimus* L. obtained are made from precipitation reactions or characteristic staining in order to highlight chemical groups that may be present in this species. The tests are carried out according to the protocols described by Diallo and al., and Senhadji et al. [7, 8]

The results are classified according to the appearance in:

Frankly positive reaction: +++;
Positive reaction : ++ ;
Moderately positive reaction: +;
Shady reaction: ± ;
Negative reaction: -.

Antibacterial test

The test of the sensitivity of the bacteria is carried out by the diffusion method in agar medium (the disk method). It is a method similar to that of the antibiogram which consists in determining the sensitivity of a bacterial strain vis-à-vis one or more substances [6].

The antibacterial activity of the methanolic extracts of the aerial parts (stem and leaf) of *Atriplex halimus* L. is evaluated vis-à-vis sixteen bacterial strains, chosen according to the traditional use of this species in Algeria: *Staphylocoque MRSA, Staphylocoque bucco-dentaire, Staphylocoque ATCC 00, Streptocoque Sp, Entérocoque Sp, Entéroccocus feacalis ATCC 12, Salmonelle, E. Coli BLSE +, Klebsiella pn Marseille, KpC + , Kp ETP R/ IMP R, Serratia Sp, Serratia environnemental, Pseudomonas aerogenosa ATCC 53, Pseudomonas VIM 2*, Bacillus. These strains were kindly provided by the Microbiology Laboratory Manager at Annaba Medical School, Algeria.

Preparation of The Inoculum

By taking sixteen tubes that each contains 5 ml of sterile physiological saline. Using a platinum loop, some well isolated colonies are scraped from each of the bacteria, each of which will be discharged into a tube.

For the preparation of the different concentrations of extracts, 2, 5 mg of each freeze-dried extract (methanolic extract of leaf and stem), are introduced into a labeled tube, in which we added 1 ml of dimethylsulfoxide (DMSO), solvent without any antibacterial effect. The tubes are vortexed until complete dissolution of the extract, and the dilutions are prepared to obtain 1/2, ¼ and 1/8 concentrations from the stock solution.

Seeding should be done within 15 minutes after the preparation of the inoculum. In 16 sterile Petri® dishes, 20 ml of agar are poured. After solidification of the medium, the latter is inoculated with 1 ml of bacteria to be studied. Then, it is spread on the surface using a glass rake.

Sterile 5 mm diameter disks prepared in Whatman® n°1 papers are impregnated with a sterile metal forceps in each concentration and placed on the surface of the solidified medium (Mueller-Hinton) at the rate of 6 disks per box (3 discs of leaf, 3 disks of stem). The dishes were incubated for half an hour at room temperature, then for 24 to 48 hours in an oven at 37 ° C.

The reading is carried out by measuring the diameter of the inhibition zone (Ø), which translates into a translucent halo around each disc; the presence or absence of a halo would explain the sensitivity or the resistance of the germs vis-a-vis extracts tested; according to a symbolic notation scale from - to +++ [9, 10].

Table 1. *Sensitivity of microbial strains according to zones of inhibition*

Sensitivity	Inhibition zone
Not sensitive or resistant (-)	Diameter <10 mm
Sensitive (+)	Diameter between 10 to 16 mm
Very sensitive (++)	Diameter between 16 to 25 mm
Extremely sensitive (+++)	Diameter > 25 mm

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening results characterizing some existing secondary metabolites in the leaves and stems of *Atriplex halimus* L. are shown in Table 2.

The phytochemical screening tests shown in Table 2 show the following results:

-The presence of flavonoids, polyphenols, hydrolyzable tannins, anthocyanin, coumarins, reducing compounds, C-glycosides, cardiotonic glycosides, sterols, carotenoids, iridoids and amino acids in the two parts of the plant studied;
- The absence of alkaloids, saponosides and O-glycosides in the leaf;
- A moderately positive reaction of carotenoids, saponosides in the stem.

Table 2. *Phytochemical Screening Tests*

		Leaf	Stem
Mucilage		++	++
Polyphenols		++	++
Anthocyanins		++	++
Coumarins		++	+
Tannins	Condensed tannins	++	-
	Hydrolyzable tannins	+	++
Flavonoids	Flavonoids	++	++
	Flavonols	++	+
Reducing compounds		++	++
Alkaloids		-	-
Saponosides		-	+ (foam index less than 100)
Anthracene derivatives	Free derivatives	-	-
	O-glycoside	-	-
	C-glycoside	++	++
Cardiotonic glycosides		++	+
Sterols, triterpenes, carotenoids, iridoids	Triterpenes	-	-
	Sterols	++	+
	Carotenoids	+	+
	Iridoids	++	-
	Starch	-	-
	Amino acids	++	+

Extraction Yield

The yield, appearance and color of the methanolic extracts of the stem and leaf of *Atriplex halimus* L. are shown in Table 3.
These results show that:
-The methanolic extract of the leaf is dark green and pasty.
- The yield of the methanolic extract of the stem is the highest, with a percentage of 6.04%, and it is pasty green.

Table 3. *Extraction yield of Atriplex halimus L., by the methanol mixture*

	Extraction Solvent	**Color of the extract**	**Aspect**	**Yield in%**
Stem	Absolute methanol 99%	Green	Pasty	6,04 %
Leaf	Absolute methanol 99%	Dark green	Pasty	5,4 %

Reading Antibiograms

The results of the antibacterial activity are shown in the Table 4.

Table 4. *Inhibition Diameter (mm) of methanolic extracts of Atriplex halimus L.*

	Dilutions of leaf extract			**Dilutions of stem extract**		
	½	**1/4**	**1/8**	**½**	**1/4**	**1/8**
Staphylocoque MRSA	18,2 mm	< 06 mm	27,1 mm	< 06 mm	< 06 mm	<06 mm
	++	-	+++	-	-	-
Staphylocoque bucco-dentaire	< 06 mm	< 06 mm	7,1 mm	7,1 mm	07 mm	07 mm
	-	-	-	-	-	-
Staphylocoque ATCC 00	10,2 mm	11,2 mm	15,2 mm	07 mm	07 mm	07 mm
	+	+	+	-	-	-
Streptocoque Sp	7,1 mm	07 mm	< 06 mm	7,2 mm	8,2 mm	07 mm
	-	-	-	-	-	-
Entérocoque Sp	07 mm	< 06 mm	14,3 mm	< 06 mm	12,1 mm	14,1 mm
	-	-	+	-	+	+
Entéroccocus feacalis ATCC 12	07 mm	11,2 mm	07 mm	14,1 mm	12,1 mm	07 mm
	-	+	-	+	+	-
Salmonelle	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm
	-	-	-	-	-	-
E. Coli BLSE +	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm
	-	-	-	-	-	-
Klebsiella pn Marseille	07 mm	07 mm	07 mm	9,1 mm	7,1 mm	7,1 mm
	-	-	-	-	-	-
KpC +	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm
	-	-	-	-	-	-
Kp ETP R/ IMP R	7,2 mm	07 mm	07 mm	7,2 mm	07 mm	07 mm
	-	-	-	-	-	-
Serratia Sp	07 mm	10,1 mm	12,2 mm	07 mm	07 mm	07 mm
	-	+	+	-	-	-
Serratia environnemental	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm
	-	-	-	-	-	-
Pseudomonas aerogenosa ATCC 53	8,2 mm	6,6 mm	8,4 mm	8,3 mm	07 mm	07 mm
	-	-	-	-	-	-
Pseudomonas VIM 2	7,1 mm	7,1 mm	07 mm	07 mm	7,1 mm	7,1 mm
	-	-	-	-	-	-
Bacillus	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm
	-	-	-	-	-	-

The extraction yields and the characteristics of the extracts depend on the type of solvent used for the extraction and the species. The methanolic extracts of the leaf and stem of *Atriplex halimus* L. are pasty and green or dark green, However, it is difficult to compare these results with those of the bibliography, because the extraction yield is only relative and seems to be related to the extraction methods applied [11], the genetic properties of the species studied [12], at the geographical origin [13] and the conditions for harvesting plant material [14].

The strain sensitivity test showed the presence of some antibacterial activity. According to Table 4, the most sensitive germ is the *Staphylococcus MRSA* with diameters of 27,1 and 18,2 mm for the dilutions of the leaf extract 1/8 and ½ respectively; followed by *Staphylococcus ATCC00* with diameters ranging from 10,2 to 15,2 mm for the range of dilutions of leaf extract ½ to 1/8. For *Enterococcus sp*. and *Enterococcus faecalis ATCC12*, antibacterial activity was observed for dilutions of the stem extract. In addition, leaf extracts and the stem of the *Atriplex halimus* have no activity against gram-negative germs except *Serratia* sp. where we noticed some activity.

Recalling that *Atriplex halimus* contains flavonoids, saponins, tannins, triterpenoids and other phenolic compounds, these compounds having known antibacterial properties, their presence could therefore explain the observed microbial properties.

The difference in the structure of the bacterial wall plays an important role in the susceptibility of bacteria [15, 16]. According to several authors, Gram-bacteria have an outer membrane made of lipopolysaccharides (LPS) which limits the diffusion of hydrophobic compounds [17, 16, 18]. In addition, the periplasm contains enzymes capable of destroying foreign molecules introduced from outside [19], which makes these bacteria generally less sensitive to plant extracts than Gram + bacteria [15, 20, 21]. Moreover, the two methanolic extracts of *Atriplex halimus* L. seem to be more active against Gram + than Gram-. The lipophilic ends of lipoteichoic acids in the wall of Gram + bacteria facilitate the penetration of hydrophobic compounds [22], such as tannins that can reach the cytoplasmic membrane, and disrupt the motive force of proton, active transport and coagulation of cellular contents [16].

The literature reports that the antibacterial activity may be related to tannins which are active compounds of several medicinal plants. They form irreversible complexes with proline-rich proteins, which would result in the inhibition of cell wall protein synthesis. This property has been able to explain the mechanisms of action of plant extracts [23]. They are also effective inhibitors of many hydrolytic enzymes such as the pectolytic enzymes used by phytopathogens [24].

Min et al. (2008) [25] suggest that the source of tannins influences antimicrobial activity. Tannins and gallic acid showed in vitro activity against *B. subtilis, S. aureus and E. coli. K. pneumoniae, L. monocytogenes and H. pylori.* Phenolic compounds, particularly ellagitannins, are strong inhibitory compounds of the genus Staphylococcus. It has been reported that new gallotannins and ellagitannins isolated from the *Punica granatum* fruit crust are the main components responsible for the antimicrobial action [26]. It has been suggested that gallotannins of *Galla chinensis* are effective antibacterial agents [16].

The glycosylated flavonols isolated from the aerial parts of *Brunfelsia grandiflora* and some of these structures are known to be responsible for the antibacterial activity [27]. This confirms the work of Rojas et al., 1992 and Marjorie, 1999, [28, 29], which showed that flavonoids, triterpenoids and tannins, as well as other phenolic compounds

or free hydroxyl groups, are classified as very active antibiotic compounds. In addition, it has been shown that the mechanism of toxicity of flavonoids to microorganisms is either by deprivation of metal ions such as iron, or by non-specific interactions such as the establishment of hydrogen bridges with the cell wall proteins of microorganisms (adhesins) or enzymes [12].

CONCLUSION

The diffusion method on agar medium made it possible to demonstrate the antibacterial power of the methanolic extracts of the leaf and the stem of the *Atriplex halimus* L. from Biskra, Algeria, vis-à-vis the sixteen bacterial strains tested. The leaf extract showed antibacterial activity against Gram-positive bacteria as Gram-negative bacteria.

Following these results, it would be interesting to extend the range of antimicrobial tests to other microbial agents to confirm their effectiveness. As it is essential to look for new effective antibacterial substances with broad spectrum of action. All these results obtained is only a first step in the search for biologically active substances of natural origin. A chemical analysis is desirable to obtain a more in-depth view of the qualitative and quantitative composition of these extracts studied in order to highlight the therapeutic effect of this medicinal species.

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