**Genetics and Biodiversity Journal**

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Original Research Paper

 **Phytochemical screening and evaluation of the antioxidant and**

 **antibacterial activity of*Atriplex halimus* from two regions Algeria (El**

  **Oued and Tlemcen).**

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Article history: Received: December 23th- 2020: Revised: February 29th- 2021: Accepted: May 15th- 2021

DOI: https://doi.org/10.46325/gabj.v5i2.72

# Abstract

The purpose of this work is to study the antioxidant and antibacterial activity of two plants of the same species « *Atriplex halimus* » from two different regions El Oued and Tlemcen. The plant leaves were subjected to a onehour reflux extraction in methanol/acetone (70/30: v/v). Qualitative phytochemical examination of the leaf extract showed the presence of alkaloids, tannins and flavonoids of varying intensity. Coumarins, terpenoids and saponins are absent in both plants. Qualitative analysis of total polyphenols, flavonoids and condensed tannins of extracts shows that *Atriplex halimus* from the El Oued region has a high content of total polyphenols (10.25 ± 1.17 mg GAE/g DW) and tannins (9.23 ±1.09 mg EC/g DW) compared to Tlemcen. However, the high flavonoid content presented by *Atriplex halimus* from the Tlemcen region (3.09 ± 0.13 mg EC/g DW). The evaluation of the antioxidant activity of the extracts was carried out by three methods: total antioxidant capacity, trapping of the free radical DPPH and iron reducing power, the results obtained show that the extract of *Atriplex halimus* from Tlemcen reveals an interesting activity compared to that of El Oued with a CI50 = 193.47 ± 1.79µg/mL. Evaluation of antibacterial activity showing that El Oued extract has activity against *Salmonella typhimirium* ATCC 13311, *Enterobacter cloacae* ATCC 13047, *Staphylococcus aureus* ATCC 6538 and *Listeria monocytogenes* ATCC 15313 with a CMI value of 5 mg/mL, 10 mg/mL and 2.5 mg/mL respectively. However, *Atriplex halimus* from Tlemcen does not reveal any antibacterial activity against the strains tested.

**Keywords**: *Atriplex halimus,* methanol/acetone extract, phytochemical tests, antioxidant activity, antibacterial activity.

# Introduction

Oxidative stress occurs in the cell when the generation of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) overwhelms the cells’ natural antioxidant defence. However, excessive concentrations of ROS/RNS are toxic and can damage proteins, lipids, and nucleic acids. Which affect their function, perturb normal physiological processes. The most effective path to eliminate and diminish the action of oxidative stress is antioxidative defence mechanisms (Amorati *et al*., 2013; Di Meo *et al*., 2016).

Medicinal plants contain a large number of active molecules that have complementary or synergistic therapeutic activities. These molecules have been studied and chemically reproduced to be incorporated

into many drugs today. In recent years, studies on the antioxidant activities of medicinal plants have increased remarkably due to increased interest in their potential to be used as a source of rich and natural

antioxidants (Chaouche *et al*., 2015; Chaouche *et al*., 2020; Haddouchi *et al*., 2014; Haddouchi *et al*., 2021a; Haddouchi *et al*., 2021b).

The Chenopodiaceae form a vast family of 1400 species present all over the world. The Atriplex is the largest genus of this family, it includes more than 400 spaces, of which 48 are Mediterranean and the rest are distributed in temperate and subtropical regions. *Atriplex halimus* has received considerable attention as a rich source of a variety of compounds such as fibres (cellulose), proteins, vitamins (B and C) and mineral salts (sodium, calcium, potassium, magnesium, phosphorus) and for its various biological activities used in traditional medicine. It has been reported for its numerous bioactive effects including antioxidant (Benhamou *et al*., 2009; Belhadj-Tahar *et al*.,, 2015), antimicrobial (Walke *et al*., 2014) and anti-diabetic (Chikhi *et al*., 2014).

This work aims to study the difference between two plants of the same species *Atriplex halimus* from two different regions (El Oued and Tlemcen). Therefore it was designed to estimate for the first time the antioxidant and the antimicrobial activities of extracts of *Atriplex halimus*. Phenolic contents (total polyphenols, flavonoids, and tannins) were also estimated.

# Material and methods

## Plant Material

The *Atriplex halimus* plant was harvested in two different regions of Algeria, one is the region of El

Oued (33°22'06 '' north, 6°52'03 '' east) and the other region of Nedroma-Tlemcen (35°00 '47 '' north, 1°44 '51' 'west). The leaves were dried in a well-ventilated place at room temperature.

## Extract preparation

The preparations of the extracts were carried out by reflux for 3 hours of 10 g of vegetable powder in 200 mL of methanol-acetone (60-40) (Kélvin de Albuquerque Mendes *et al.,* 2019). After filtration, the extracts were evaporated at 45°C under reduced pressure. The yield of the plants in dry extract was determined by calculating the following ratio:

**Yield% = [P1-P2 / P3] × 100**

P1: weight of the flask after evaporation, P2: weight of the empty flask, P3: weight of the starting dry plant material.

The dry extract is weighed and collected, either in a few millilitres of methanol for assays and evaluation of antioxidant activity, or in a few millilitres of dimethyl sulfoxide (DMSO) for the evaluation of antibacterial activity.

## Phytochemical tests

In order to highlight the presence or absence of certain compounds belonging to the chemical families of secondary metabolites, specific phytochemical tests were carried out based on colour, turbidity or precipitation reactions, using the methods described in the literature (Haddouchi *et al*., 2018).

* *Alkaloids:* 0.5 mL of extract was placed in two test tubes and then acidified by adding a few drops of 1% HCL. 0.5 mL of Wagner's reagent was added to the first tube and 0.5 mL of Mayer's reagent to the second tube. The formation of a brown or white precipitate, respectively, reveals the presence of the alkaloids.
* *Tannins:* 1 mL of the extract was mixed with 0.25 mL of an aqueous solution of FeCl3 (1%). The appearance of a greenish or blue-blackish colour after 15 min of incubation at room temperature indicates the presence of tannins.
* *Flavonoids:* 1 mL of extract contacted with 1 mL of concentrated HCL and some magnesium shavings. The appearance of a pink or red or yellow colour indicates the presence of flavonoids.
* *Coumarins:* 1 mL of the extract was mixed with 500 µL of 10% NH4OH. A drop was taken from the mixture and then placed on a filter paper. Intense fluorescence under ultraviolet at 366 nm indicates the presence of coumarins.
* *Terpenoids:* 1 mL of the extract was added to a test tube containing 0.4 mL of chloroform and 0.6 mL of concentrated sulfuric acid. The formation of two phases and a brown colour at the interphase indicates the presence of terpenoids.
* *Saponins:* 10 mL of the extract was placed in a test tube and then stirred for 15 seconds. After 15 min of rest, the presence of saponins is indicated by the persistence of foam with a greater height than 1 cm.

### Quantification of phenolic classes Determination of total phenol contents

100 μL of each extract at a concentration of 1 mg/mL, and mixed with 2 mL of sodium carbonate (2%) solution freshly prepared, the whole is agitated with a vortex. After 5 min,

100 μL of the Folin-Ciocalteu reagent diluted (1/20) was added and the mixture was incubated in total darkness for 30 min at room temperature, the absorbance was read at 700 nm versus the prepared blank. Different concentrations of gallic acid were used to prepare a calibration curve. Results were expressed as milligram gallic acid equivalents GAE/g DM (Vermerriss and Nicholson. 2006). *Determination of flavonoid cont****ents***

250 μL of each extract at a concentration of 1 mg/mL, is mixed with 75 μL of sodium nitrite (5%) with as incubate for 6 min at room temperature, by adding 150 μL of aluminium chloride (6H2O) at 10%, after 5 min 1 M of sodium hydroxide solution (500 μL) was added to each extract and the final volume was adjusted to 2.5 mL with distilled water and thoroughly mixed. The absorbance of the mixture was determined at 510 nm. Results were expressed as milligram quercetin equivalents/ g of dry weight (mg QE/g DW) (Dewanto *et al*., 2002). *Determination of total condensed tannins*

500 μL aliquots of prepared extracts were added to 3 mL of vanillin solution (4%) and 1.5 mL of concentrated sulfuric acid respectively, the mixture was allowed to stand for 15 min at room temperature (25°C) and the absorption was measured at 500 nm against solvent as a blank. Results were expressed as milligram (+)-catechin equivalents/g dry weight (mg CE/g DW) (Sun *et al*., 1998). *In vitro evaluation of antioxidant activity*

### Total antioxidant capacity

This assay is based on the reduction of Molybdene (VI) to Molybdene (V) by the sample extract, which produces a green phosphomolybdenum (V) complex under acidic pH conditions.3 An aliquot (0.1 mL) of phenolic extract was combined with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in a thermal block at 95°C for 90 min. After, the mixture had cooled to room temperature. The absorbance of each solution was measured at 695 nm against a blank. The antioxidant capacity was expressed as mg gallic acid equivalent per gram dry weight (mg GAE/gDW). The calibration curve of gallic acid range was 0-400 mg/mL (Prieto et al., 1999).

### Scavenging of the free radical DPPH

At different concentrations, 50 μL of each extract are added to 1950 μL of a methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) at 6.34 10-5 M. For each concentration, a blank is prepared. A negative control is prepared, in parallel, while mixing 50 μL of methanol with 1950 μL of a methanolic solution of DPPH at the same concentration used. After incubation in the dark for 30 minutes and at room temperature, the reduction in DPPH is accompanied by the change from purple to yellow in the solution. The absorbances are read at 515 nm using a spectrophotometer. The positive control used is butylated hydroxyanisole (BHA), and the radical scavenging activity was calculated as a percentage of DPPH discolouration using the equation:

**DPPH radical scavenging (%) = [(A0 – A1 /A0] x 100**

Where A0 and A1 are the absorbance at 30 min of the positive control and the extract, respectively. The anti-radical activity was expressed as IC50 (µg/mL), this is the extracted concentration required to cause a reduction of 50% to absorbance at 517 nm. A lower IC50 value corresponds to the extract effectiveness (Chaouche *et al*., 2014).

### Ferric reducing antioxidant potential assay

1 mL of each extract at different concentrations with 2.5 mL of 0.2 M phosphate buffer at pH = 6.6 and 2.5 mL of a 1% potassium ferricyanide solution. The mixture obtained is incubated for 20 minutes at 50°C, and then 2.5 mL of 10% trichloroacetic acid is added to stop the reaction. The mixture is centrifuged at 650 g for ten minutes at room temperature and 2.5 mL of the supernatant is added to 2.5 mL of distilled water and 0.5 mL of 0.1% iron chloride. The absorbance is read at 700 nm against a blank. The results make it possible to calculate the effective concentration (EC50), concentration of the extract corresponding to an absorbance equal to 0.5, the linear regression curve (density of the optics as a function of the different concentrations). The activity of the extract is finally compared with that of the positive control (BHA) (Oyaizu, 1986).

### Antibacterial activity

The antibacterial activity of the extracts was evaluated using American Type Culture Collection (ATCC) laboratory reference strains:

*Gram-positive bacteria*: *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 25921, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 49452, Listeria *monocytogenes* ATCC 15313.

*Gram-negative bacteria*: *Escherichia coli* ATCC 8739, *Klebsiella pneumonia* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Citrobacter freundii* ATCC 8090, Proteus mirablis ATCC 35659, *Salmonella typhimirium* ATCC 13311, *Enterobacter cloacae* ATCC 13047.

### Disk distribution method

Each adjusted strain was inoculated on the surface of Muller-Hinton agar using a swab. After drying, a sterile Disk 6 mm in diameter (Wattman paper №1) was impregnated with 10 µL of the extract and placed on the surface of the agar, the concentration of each extract is 512 µg/Disk, the dishes are incubated at 37°C for 24 hours. Gentamicin is used as a positive control. Antibacterial activity is assessed by measuring the zone of growth inhibition surrounding the Disks (CLSI, 2012). *Determination of minimum inhibitory concentration (MIC)*

The MIC of the extract is determined by the microdilution method described by CLSI (2012). A dilution series of 10 to 0.02 mg/mL was prepared in DMSO. In a sterile 96-well plate, 100 μL of each dilution were placed in the wells, these dilutions were inoculated with 100 μL of solutions containing 106 CFU/mL prepared in Mueller Hinton broth with a pH ranging from 7,4 ± 0.2. The first well was used as a negative control. The broth was inoculated only to verify the sterility of the medium. However, the last well was used as a positive control, filled only with the bacterial suspension. The plate was incubated at 37°C for 24 hours. The MIC corresponds to the lowest concentration of the extract tested for which no visual disturbance was observed (Haddouchi *et al*., 2013).

# Results and discussion

## Extraction yield

The extracts the leaves of the *Atriplex halimus* from two regions (El Oued and Tlemcen) were prepared by reflux for 03 hours in methanol-acetone (60/40). According to the results obtained, the *Atriplex halimus* yield from El Oued is slightly higher (18.5%) than that of the same species collected in Tlemcen (14.8%), but these values are higher than that obtained by Belhadj -Tahar *et al.* (2015), who found a yield of 5.96%. Benhamou *et al.* (2009) obtained a return of 24%, which is higher than our results. This difference may be due to the solvent used and the type of extraction.

## Phytochemical tests

The results of the phytochemical tests carried out on the extracts (methanol / acetone) of the 2 plants are presented in Table 1.

**Table 1**. Result of phytochemical tests.

|  |  |  |  |
| --- | --- | --- | --- |
| **Compounds**  | **Reagent**  | **AH1**  | **AH2**  |
| Alkaloids  | Mayer Wagner  | ++ ++  | +++ +++  |
| Tannins  | FeCl3  | ++  | +  |
| Flavonoïds  | Mg++  | +  | ++  |
| Coumarins  | UV Fluorescence  | -  | -  |
| Terpénoïds  | Slakowski Test  | -  | -  |
| Saponins  | Foam test  | -  | -  |

(+++): Strongly present (++): Moderately present (+): Weakly present (-): Test negative

(AH1): *Atriplex halimus* (El Oued), (AH2): *Atriplex halimus* (Tlemcen)

Based on the results obtained, we notice the presence of alkaloids, tannins and flavonoids in *Atriplex halimus* of both regions, in varying intensities. That of Tlemcen is richer in alkaloids, while that of El Oued contains more tannin. The absence of coumarins, terpenoids and saponins is also observed in both extracts. The results of the phytochemical tests obtained by Abdel-Rahman *et al*. (2011) confirm our results in the presence of alkaloids and flavonoids, but the absence of tannins and the presence of saponins do not agree with our results, which may be due to the harvest period and to the method and conditions of extraction applied.

## Total Polyphenol, Flavonoid and condensed tannin contents

The contents of total phenols, flavonoids and condensed tannins are determined from the linear regression equations of each calibration curve: y = 0.00246x - 0.05162 (R² = 0.99), y = 0.0035x + 0.0218 (R² = 0.99), y = 0.00024x + 0.00308 (R² = 0.99), respectively. Table 2 shows the results obtained for the two plants.

**Table 2**. The total contents of polyphenols, flavonoids and tannins in the extracts.

|  |  |  |
| --- | --- | --- |
| **Extracts**  | **AH1**  | **AH2**  |
| Polyphenols (mg GAE/g DW)  | 10.25 ± 1.17  | 8.20 ± 0.84  |
| Flavonoids (mg EC/g DW)  | 1.82 ± 0.17  | 3.09 ± 0.13  |
| Tannins (mg EC/g DW)  | 9.23 ± 1.09  | 1.71 ± 0.34  |

(AH1): *Atriplex halimus* (El Oued), (AH2): *Atriplex halimus* (Tlemcen)

The results indicate that *Atriplex halimus* from the El Oued region has a higher total polyphenol content (10.25 ± 1.17 mg GAE/g DM) and higher in tannin (9.23 ± 1.09 mg EC/g DW) compared to that of Tlemcen with contents (8.20 ± 0.84 mg GAE/g DW) and (1.71 ± 0.34 mg EC/g DW) respectively. The flavonoid content of *Atriplex halimus* from Tlemcen (3.09 ± 0.13 mg EC/g DM) is slightly higher than that of El Oued (1.82 ± 1.09 mg EC/g DW).

Our results in phenolic compounds are comparable to that found in the methanolic extract (10.12 ± 2.24 mg GAE/g DW) from Benhamou *et al,* (2009). However, our results in phenolic compounds, flavonoids and tannins are far from being compared with the work of Belhadj-Tahar *et al,* (2015). This variability in content is probably due to the nature and volume of solvents used, type of extraction, harvest period, geographic location and storage conditions.

## Evaluation of antioxidant activity

Total antioxidant capacity

The antioxidant capacity of the extracts (methanol / acetone) was determined by the equation of the linear regression of the calibration curve: y = 0.0042x + 0.0323 (R² = 0.9942). The results show that *Atriplex halimus* from El Oued has a slightly lower total antioxidant capacity compared to that of Tlemcen, with values 5.77 ± 0.25 mg GAE/g DM and 7.69 ± 0, 21 mg GAE/g DW, respectively (table

03).

However, our results obtained are clearly superior to those obtained in the fractions of ethyl acetate (0.241 mg GAE/g DW), butanolic (0.112 mg GAE/g DW) and dichloromethane (0.110 mg GAE/g DW) by Belhadj-Tahar *et al*. (2015).

### Scavenging of the free radical DPPH°

The scavenging of the free radical by extracts of *Atriplex halimus* (El Oued) of *Atriplex halimus* (Tlemcen) is expressed by the determination of the IC50 from the linear regression equations: y = 0.2341x + 4.7299 ( R² = 0.9842), y = 0.0982x - 1.0237 (R² = 0.9945), respectively. The IC50 values, the extract of *Atriplex halimus* (Tlemcen) has an IC50 (193.47 ± 1.79 µg/mL) lower than that of El Oued (520.64 ± 2.1 µg/mL), therefore a better activity, but, it remains low by contributions to BHA (3.54 ± 0.21 µg/mL), reference molecule (table 3).

However, our results obtained are better than that of Benhamou *et al.* (2009) who showed that the ethyl acetate and butanolic fractions have low capacities to scavenge the free radical DPPH, with IC50 values of 2.04 mg/mL and 1.73 mg/mL respectively, then comes the methanolic extract (IC50 = 31.83 mg/mL).

The results of Belhadj-Tahar *et al*. (2015) show the lowest concentration is reported by the dichloromethane fraction with an IC50 94 µg/mL, then the ethyl acetate fraction IC50 230 µg/mL and finally the butanol fraction IC50 405 µg/mL. These results are more or less comparable with ours. *Iron reduction*

The reduction of iron by extracts of *Atriplex halimus* (El Oued) from *Atriplex halimus* (Tlemcen) is expressed by determining the EC50 from the linear regression equations: y = 0.00004x + 0.00765 (R² = 0. 99), y = 0.00003x - 0.0118 (R² = 0.99), respectively.

Based on the results obtained, the EC50 of BHA is 0.094 µg/mL, the EC50 value of *Atriplex halimus* from both regions (El Oued and Tlemcen) is greater than 5 mg/mL, resulting in low reducing capacity (table 03).

These results are lower than those of Benhamou *et al.* (2009), who found that the ethyl acetate fraction has the best reducing power with an EC50 value of 1.5 mg/mL, then the butanoic fraction (EC50 = 1.76 mg/mL) and the extract methanolic (EC50 = 4.55 mg/mL).

**Table 3**. A summary table of all the results of the antioxidant activities

|  |  |  |  |
| --- | --- | --- | --- |
|  | **AH1**  | **AH2**  | **BHA**  |
| Total antioxidant capacity (mg GAE/g DM)  | 5.77 ± 0.25 mg  | 7.69 ± 0, 21 mg  | -  |
| IC50/DPPH (µg/mL)  | 520.64 ± 2.1  | 193.47 ± 1.79  | 3.54 ± 0.21  |
| EC50/iron reducing  | > 5 mg/mL  | > 5 mg/mL  | 0.094 µg/mL  |

(AH1): *Atriplex halimus* (El Oued), (AH2): *Atriplex halimus* (Tlemcen)

### Antibacterial activity Disk broadcast method

The study of the antibacterial activity on twelve reference strains was carried out by the method of diffusion of the disks on solid medium (Mueller Hinton), it is a qualitative method based on the measurement of the diameter of the zone of inhibition impregnated with plant extract. Gentamicin is used as a reference molecule. The results of the diameters of the zones with respect to the bacteria shown in Table 4.

**Table 4.** Diameters of the zones of inhibition (in mm) of the extracts.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|   | Bacteria  | **AH1**  | **AH2**  | **Gent**  |
| ***Gram- negative bacteria***  | *Escherichia coli* ATCC 8739 *Pseudomonas aeruginosa* ATCC 27853 *Citrobacter freundii* ATCC 8090 *Proteus mirablis* ATCC 35659 *Salmonella typhimirium* ATCC 13311 *Klebsiella pneumonia* ATCC 700603 *Enterobacter cloacae* ATCC 13047  | 6 6 6 6 12 6 15  | 6 6 6 6 6 6 6  | 22 12 18 25 22 19 21  |
| ***Gram-positive bacteria***  | *Staphylococcus aureus* ATCC 6538 *Bacillus cereus* ATCC 25921 *Enterococcus faecalis* ATCC 49452 *Listeria monocytogenes* ATCC 15313 *Bacillus subtilis* ATCC 6633  | 15 6 6 12 6  | 6 6 6 6 6  | 32 20 21 22 22  |

6 mm: teste négatif ; (AH1): *Atriplex halimus* (El Oued), (AH2): *Atriplex halimus* (Tlemcen); Gent : Gentamicine.

The results obtained show that the extract of *Atriplex halimus* from the El Oued region has a capacity to inhibit the growth of bacteria: *Salmonella typhimirium* (12 mm), *Enterobacter cloacae* (15 mm), *Staphylococcus aureus* (15 mm) and *Listeria monocytogenes* (12 mm). However, no effect was obtained with the other strains (Table 4). *Atriplex halimus* from the Tlemcen region extract has no activity against all strains (6 mm). These results are lower compared to the reference molecule (Gentamicin), which has a higher activity with a diameter between 12 and 32 mm.

Abdel-Rahman *et al*. (2011) show that the methanolic extract against the bacteria *Listeria monocytogenes* and *Bacillus cereus* has antibacterial activity; these results contradict our results, with the exception of the results obtained on *Escherichia coli*.

### Determination of minimum inhibitory concentration (MIC**)**

The MIC is the lowest concentration of the extract that is able to inhibit bacterial growth. The MIC of *Atriplex halimus* extract from the El Oued region vis-à-vis bacteria is determined by the liquid microdilution method using 96-well plate. The MIC values are shown in Table 5.

**Table 5:** The MIC values of *Atriplex halimus* (El Oued) extract.

|  |  |
| --- | --- |
| **Bacteria**  | **MIC (mg/mL)**  |
| *Salmonella typhimirium* ATCC 13311  | 5  |
| *Enterobacter cloacae* ATCC 13047  | 10  |
| *Staphylococcus aureus* ATCC 6538  | 2.5  |
| *Listeria monocytogenes* ATCC 15313  | 2.5  |

According to the results obtained, at a concentration of 5 mg/mL the extract of *Atriplex halimus* from El Oued inhibits the growth of *Salmonella typhimirium*, it also inhibits *Enterobacter cloacae* at a concentration of 10 mg/mL and *Staphylococcus aureus* and *Listeria monocytogen* at a concentration of 2.5 mg/mL. **Conclusion**

Algeria by its geographical position covers an exceptional biodiversity occupied by an important richness of aromatic and medicinal plants. Many plants are used in traditional remedies and are not evaluated scientifically. Therefore, the main objective of this research was to evaluate the biological activities including antioxidant and antibacterial properties of two plants of the same species *Atriplex halimus* from two different regions (Tlemcen and El Oued).

The phytochemical screening revealed the presence of flavonoids, alkaloids and tannins. However coumarin, terpenoids and saponins are absent in both plants. Quantitative estimation of total polyphenols, flavonoids and tannins shows that the extract of El Oued rich in total polyphenols (10.25 ± 1.17 mg GAE/g DW) and tannins (9.23 ± 1.09 mg EC/g DW) compared to that of Tlemcen.

However, the flavonoids are present in high amounts in *Atriplex halimus* from Tlemcen. The antioxidant activity of *Atriplex halimus* extracts from both regions was assessed by three methods. The total antioxidant capacity of the extract from the Tlemcen region shows a a higher capacity (7.69 ± 0.21 mg GAE/g DW) compared to that of El Oued (5.77 ± 0.25 mg GAE/g DW). The results of the DPPH free radical scavenging method showed that *Atriplex halimus* from the Tlemcen region has the highest activity with IC50 = 193.47 ± 1.79 mg/mL. Both the extracts of *Atriplex halimus* have low iron-reducing activity. The results of the disk diffusion method show that *Atriplex halimus* from the Tlemcen region has no activity against the strains tested, on the other hand, *Atriplex halimus* from EL

Oued has activity against *Salmonella* *typhimirium* ATCC 13311, *Enterobacter cloacae* ATCC 13047, *Staphylococcus aureus* ATCC 6538 and *Listeria monocytogenes* ATCC 15313 with a CMI value of 5 mg/mL, 10 mg/mL and 2.5 mg/mL respectively. From the results one concludes that extracts from *Atriplex halimus* are a promising source of natural bioactive compounds, which are probably responsible for their antioxidant and antibacterial activity. These results are only a first step in the search for biologically active substances.

# References

**Abdel-Rahman SM. Abd-Elatif SA. Deraz SF. Khalil AA 2011**. Antibacterial activity of some wild medicinal plants collected western mediterranean coast, Egypt : Natural alternatives for infectious disease treatment. African Journal of Biotechnology, 10: 10733-10743. DOI: 10.5897/AJB11.007

**Amorati R. Foti MC. Valgimigli L 2013**. Antioxidant Activity of Essential Oils. Journal of Agricultural and Food Chemistry, 61(46): 10835–10847. [DOI:10.1021/jf403496k](https://doi.org/10.1021/jf403496k)

**Belhadj-Tahar S. Hadj-Mahammed M. Yousefi M 2015**. Etude de l’activité antioxydante des extraits phénoliques de *l’Atriplex halimus L* et de *l’Haloxylon scoparium* pomel du Sahara septentrional. Annales des Science et Technologie, 7(1): 35-42. [https://revues.univouargla.dz/images/banners/ASTimages/ASTDOC/ASTV7N1/A070105.pdf](https://revues.univ-ouargla.dz/images/banners/ASTimages/ASTDOC/ASTV7N1/A070105.pdf)

**Benhamou N. Atik-Bekkara F. Kadifkova-Panovska T 2009**. Antioxydant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus*. Compte Rendus Chimie, 12 : 12591266. DOI [:10.1016/j.crci.2009.02.004](https://doi.org/10.1016/j.crci.2009.02.004)

**Chaouche TM. Haddouchi F. Ksouri R. Atik-Bekara F 2014**. Evaluation of antioxidant activity of hydro-methanolic extracts of some medicinal species from South Algeria. Journal of the Chinese Medical Association, 77(6): 302-307. DOI:10.1016/j.jcma.2014.01.009

**Chaouche TM. Haddouchi F. Atik-Bekara F. Ksouri R. Azzi R. Boucherit Z. Tefiani C. Larbat R 2015**. Antioxidant, haemolytic activities and HPLC-DAD-ESI-MSn characterization of phenolic compounds from root bark of *Juniperus oxycedrus* subsp. *Oxycedrus*. Industrial Crops and Products, 64:182–187. [DOI: 10.1016/j.indcrop.2014.10.051](http://dx.doi.org/10.1016/j.indcrop.2014.10.051)

**Chaouche TM. Haddouchi F. Boudjemai O. Ghellai I 2020**. Antioxidant and hemolytic activity of *Ziziphus jujuba* Mill and *Rhamnus alaternus* L (Rhamnaceae) extracts from Algeria. Bulletin de la

Société Royale des Sciences de Liège, 89 : 1-14. [https://popups.uliege.be/00379565/index.php?id=9386](https://popups.uliege.be/0037-9565/index.php?id=9386)

**Chikhi I. Allali H. Dib MA. Medjdoub H. Tabti B 2014**. Antidiabetic activity of aqueous lead extract of *Atriplex halimus* L (Chenopodiaceae) in streptozotocin-inced diabetic rats. Asian Pacific Journal of Tropical Disease, 4: 181-184. DOI[:10.1016/S2222-1808(14)60501-6](https://dx.doi.org/10.1016/S2222-1808%2814%2960501-6)

**Clinical and Laboratory Standards Institute (CLSI) 2012**. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard-Ninth Edition. M07-A9. Vol.32, N°2. ISBN 1-56238-784-7.

**Dewanto V. Wu X. Adom KK. Liu RH 2002**. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry,50:3010-3014. [DOI: 10.1021/jf0115589](https://doi.org/10.1021/jf0115589)

**Di Meo S. Reed TT. Venditti P. Victor VM 2016**. Role of ROS and RNS Sources in Physiological and Pathological Conditions. Oxidative Medicine and Cellular Longevity, 2016: 1-44.

DOI: [10.1155/2016/1245049](https://doi.org/10.1155/2016/1245049)

**Haddouchi F. Chaouche TM. Ksouri R. Larbat R 2021a**. Leafy Stems of *Phagnalon saxatile* subsp. *saxatile* from Algeriaas a Source of Chlorogenic Acids and Flavonoids with Antioxidant Activity: Characterization and Quantification Using UPLC-DAD-ESI-MSn. Metabolites,11(5) : 280. [DOI: 10.3390/metabo11050280](https://doi.org/DOI%3A%2010.3390/metabo11050280)

**Haddouchi F. Chaouche TM. Halla N 2018**. Screening phytochimique, activités antioxydantes et pouvoir hémolytique de quatre plantes sahariennes d’Algérie. Phytothérapie, 16(1): 254-262. DOI: 10.1007/s10298-016-1086-8

**Haddouchi F. Chaouche TM. Saker M. Ghellai I. Boudjemai O 2021b.** Phytochemical screening, phenolic content and antioxidant activity of Lavandula species extracts from Algeria. İstanbul Journal of Pharmacy, 51(1): 111-117. DOI: 10.26650/IstanbulJPharm.2020.0051

**Hadduchi F. Chaouche TM. Ksouri R. Medini F. Sekkal FZ. Benmansour A 2014**. Phytochemical screening and in vitro antioxidant activities of aqueous-extracts of *Helichrysum stoechas* subsp. *rupestre* and *Phagnalon saxatile* subsp. s*axatile*.Chinese Journal of Natural Medicines, 12(6): 415422. DOI: 10.3724/SP.J.1009.2014.00423

**Haddouchi F. Chaouche TM. Zaouali Y. Ksouri R. Attou A. Benmansour A 2013**. Chemical composition and antimicrobial activity of the essential oils from four Ruta species growing in Algeria. Food Chemistry, 141(1) : 253-258. DOI: 10.1016/j.foodchem.2013.03.007

**Kélvin de Albuquerque Mendes M. Bremmer dos Santos Oliveira C. Alves Veras MD. Araújo BQ. Dantas C. Chaves MH. Lopes Júniora CA. Vieira EC 2019**. Application of multivariate optimization for the selective extraction of phenolic compounds in cashew nuts (*Anacardium occidentale* L.). Talanta, 205: 120100. [DOI:10.1016/j.talanta.2019.06.100](https://doi.org/10.1016/j.talanta.2019.06.100)

**Oyaizu M 1986**. Studies on products of the browning reaction: antioxidative activities of browning reaction. Japanese Journal of Nutrition,44(6):307-315. [DOI: 10.5264/eiyogakuzashi.44.307](https://doi.org/10.5264/eiyogakuzashi.44.307)

**Prieto P. Pineda M. Aguilar M 1999**. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry, 269(2): 337-341. DOI: [10.1006/abio.1999.4019](https://doi.org/10.1006/abio.1999.4019)

**Sun B. Richardo-da-Silvia JM. Spranger I 1998**. Critical factor of vanillin asay for catechins and proanthocyanidins. Journal of Agricultural and Food Chemistry,46: 4267-4274. [DOI: 10.1021/jf980366j](https://doi.org/10.1021/jf980366j)

**Vermerriss W. Nicholson R. 2006**. Phenolic Compound Biochemistry. Edition Springer. Dordrecht. ISBN 978-1-4020-5164-7

**Walker DJ. Lutts S. Sanchez-Garcia M. Correal E 2014**. *Atriplex halimus* L: Its biology and uses. Journal of Arid Environments, 100-101: 111-121. [DOI: 10.1016/j.jaridenv.2013.09.004](https://doi.org/10.1016/j.jaridenv.2013.09.004)