Chemical characterization and biological activity assessment of *Atriplex halimus* essential oil: exploring new avenues in pharmacology and medicine

Caracterização química e avaliação da atividade biológica do óleo essencial de *Atriplex halimus*: explorar novos caminhos na farmacologia e na medicina

Caracterización química y evaluación de la actividad biológica del aceite esencial de *Atriplex halimus*: exploración de nuevas vías en farmacología y medicina

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ABSTRACT
The plant *Atriplex halimus* was the subject of the investigation. 99 volatile compounds were found in the essential oil after being analyzed by gas chromatography-mass spectrometry (GC-MS). The majority of these were E-anethole (13.9%), phytone (9.5%), carvone (6.0%), carvacrol (4.2%) and geranylacetone (3.0%). To assess the antioxidant potential of the essential oil, the 1,1-diphenyl-2-picrylhydrazyl (1,1-DPPH) and Ferric Reducing Antioxidant Power (FRAP) tests were performed. The results showed that *Atriplex halimus* essential oil has significant antioxidant potential as well as the ability to reduce iron, as indicated by absorbance (A). At a concentration of 3 mg/ml, the essential oil reduces the DPPH radical by a maximum of 51.12%. The concentration needed to reduce the DPPH radical by 50%, was determined to be 28.20 mg/ml. The essential oil did, exhibit stronger antioxidant activity at lower concentrations than ascorbic acid, but not at higher concentrations. The Minimum Inhibitory Concentration (MIC) dilution method was used to test the antibacterial effects of *Atriplex halimus* essential oils against nine different bacterial strains.
With an MIC range of 2.6 to 10.41 mg/ml and higher activity compared to gentamicin against some bacterial strains, the results demonstrated that the oils have significant antibacterial activity.

Keywords: *Atriplex halimus*, flora, characterization, essential oil.

RESUMO
A central *Atriplex halimus* foi objeto da investigação. Foram encontrados 99 compostos voláteis no óleo essencial após análise por cromatografia gasosa-espectrometria de massa (CG-EM). A maioria deles era E-anetol (13,9%), fitona (9,5%), carvona (6,0%), carvacrol (4,2%) e geranilacetona (3,0%). Para avaliar o potencial antioxidante do óleo essencial foram realizados os testes 1,1-difenil-2-picrilhidrazil (1,1-DPPH) e Poder Antioxidante Redutor Férrico (FRAP). Os resultados mostraram que o óleo essencial de *Atriplex halimus* apresenta um potencial antioxidante significativo, bem como a capacidade de reduzir o ferro, como indicado pela absorbância (A). Na concentração de 3 mg/ml, o óleo essencial reduz o radical DPPH no máximo em 51,12%. A concentração necessária para reduzir o radical DPPH em 50% foi determinada em 28,20 mg/ml. O óleo essencial exibiu uma atividade antioxidante mais forte a concentrações mais baixas do que o ácido ascórbico, mas não a concentrações mais elevadas. O método de diluição da Concentração Inibitória Mínima (CIM) foi utilizado para testar os efeitos antibacterianos dos óleos essenciais de *Atriplex halimus* contra nove estirpes bacterianas diferentes.
Com um intervalo de CIM de 2,6 a 10,41 mg/ml e uma maior atividade em comparação com a gentamicina contra algumas estirpes bacterianas, os resultados demonstraram que os óleos apresentam uma atividade antibacteriana significativa.

Palavras-chave: *Atriplex halimus*, flora, caracterização, óleo essencial.

RESUMEN
La planta *Atriplex halimus* fue objeto de la investigación. Se encontraron 99 compuestos volátiles en el aceite esencial después de ser analizado mediante cromatografía de gases-espectrometría de masas (GC-MS). La mayoría de ellos fueron E-anetol (13,9%), fitona (9,5%), carvona (6,0%), carvacrol (4,2%) y geranilacetona (3,0%). Para evaluar el potencial antioxidante del aceite esencial se realizaron las pruebas de 1,1-difenil-2-picrilhidrazilo (1,1-DPPH) y de poder antioxidante reductor férrico (FRAP). Los resultados mostraron que el aceite esencial de *Atriplex halimus* tiene un potencial antioxidante significativo, así como la capacidad de reducir el hierro, como lo indica la absorbancia (A). A una concentración de 3 mg/ml, el aceite esencial reduce el radical DPPH hasta un máximo de un 51,12%. Se determinó que la concentración necesaria para reducir el radical DPPH en un 50% era 28,20 mg/ml. El aceite esencial exhibió una actividad antioxidante más fuerte en concentraciones más bajas que el ácido ascórbico, pero no en concentraciones más altas. Se utilizó

el método de dilución de Concentración Inhibitoria Mínima (CIM) para probar los efectos antibacterianos de los aceites esenciales *Atriplex halimus* contra nueve cepas bacterianas diferentes. Con un rango de CIM de 2,6 a 10,41 mg/ml y una mayor actividad en comparación con la gentamicina contra algunas cepas bacterianas, los resultados demostraron que los aceites tienen una actividad antibacteriana significativa.

Palabras clave: *Atriplex halimus*, flora, caracterización, aceite esencial.

1 INTRODUCTION

Essential oils are considered a raw material for various sectors such as perfumery, cosmetics, pharmaceuticals and food industry, so knowledge of their chemical composition is an important and necessary step (Aouadj et al., 2023a-b). Several works have been carried out on the chemical analysis of the extracts and essential oils of *Atriplex.*

We noted among others the study of Chaouche et al. (2015) on Phytochemical screening and evaluation of the antioxidant and antibacterial activity of *Atriplex halimus* from two regions of Algeria (Emam, 2011). On the bioactive constituents of Atriplex halimus from Egypt and those fromon (Benhammou- Belyagoubi, 2009) the antioxidant activity of methanolis ectracts and some bioactive compounds of *Atriplex halimus*.

Algeria, with its vast land area from North to South and East to West and its varying climate, has abundant, rich and varied flora (Aouadj et al., 2020 a-e ; Aouadj, 2021; Nasrallah et al., 2020), where numerous aromatic species capable of providing essential oils have been counted (Demarly and Chalbi, 1991; Degdag et al., 2022).

Atriplex halimus is a plant species in the Amaranthaceae family according to phylogenetic classification. It is primarily used as a forage plant. Its persistent foliage, rich in proteins, is widely used as a medicinal plant in traditional pharmacopeia (Aouadj et al., 2020f). Atriplexes are found in temperate, Mediterranean and subtropical zones, between 20 and 50° latitude North and South (Aouadj et al., 2020g-j, Aouadj, 2009). They grow in arid and saline regions (Aouadj et al., 2020k; Benchiha et Aouadj, 2024). *Atriplex halimus* is the most represented indigenous plant in the Mediterranean basin. It grows near the sea, on fixed dunes and scrubland. It is often planted to form hedgerows and sometimes spontaneous. In Algeria, it can be found in several regions including M'sila, Relizane, el Mesrane (Djelfa), ES-Sénia (Oran), Tlemcen, Naâma and Béchar.

The chemical composition of *Atriplex halimus* depends on several parameters such as climate, plant age and season (Abbad et al., 2004). This vegetable matter is very rich in proteins, fibres, mineralsalts (Arslan and Özcan, 2011) (in vitamins A,C, and (Belenkii, 1939 ; Benzie and Strain, 1996) and saponins, alkaloids, flavonoids (Chaouche et al., 2021; Bessaid et al., 2021; Derrab

et al., 2023; Mezouar et al., 2023: Hasnaoui et al., 2023). Recently, studies on the antioxidant activities of medicinal plants have significantly increased due to growing interest in their potential as a source of rich and natural antioxidants (Darughe et al., 2012 ; Demarly and Chalbi, 1991b ; Emam, 2011b ; ESPLIN et al., 1937 ; Haddouchi et al., 2018) as well as in this context (Haddouchi et al., 2018; Henni et al., 2024) confirmed that medicinal plants are easily available and potent source of antioxidants as they contain a mixture of different chemical compounds that may act individually or in synergy to cure disease and improve health.

Objectif of this study is Chemical Characterization and Biological Activity Assessment of *Atriplex halimus* Essential Oil: Exploring New Avenues in Pharmacology and Medicine.

2 METHODOLOGY

2.1 PREPARATION

The essential oils were extracted from the aerial parts (leaves, stems, and flowers) of *Atriplex halimus*, collected in April 2022 in the El Kesdir, Naâma region, using the hydrodistillation technique on a Clevenger-type apparatus. The plant material was dried in the sun and stored for future use. The average yield was around 0.02% of the dry matter weight. Gas chromatography/mass spectrometry (GC/MS) is a powerful analytical technique used to separate, identify, and quantify chemical compounds. In GC, a sample is vaporized and passed through a column packed with a stationary phase, which separates the components of the sample based on their physical and chemical properties. The separated components are then ionized and analyzed by a mass spectrometer, which identifies each component based on its unique mass-to-charge ratio. GC/MS is widely used in various fields.

2.2 CPG/MASS SPECTROMETRY COUPLING

The analysis is performed using a Perkin Elmer Autosystem XL chromatographer equipped with an automatic injector and two columns: a polar column (Rtx-Wax 60 m x 0.25 mm internal diameter, film thickness: 0.25 µm) and an apolar column (Rtx-1). These two columns are coupled to a Perkin Elmer TurboMass quadrupole mass detector operating under the chromatographic conditions described in The acquisition of chromatographic data is performed in SCAN mode with a mass range of 35-350 uma and a step of 1 s between scans. The mass spectra are recorded under electron impact (EI) at 70 eV. The use of CPG/mass spectrometry coupling allows for a more comprehensive and accurate analysis of the essential oil.

2.3 EVALUATION OF ANTIOXIDANT ACTIVITY

In our study, the antioxidant activity of essential oils was evaluated using two methods: the DPPH free radical scavenging test and the iron reduction test (FRAP).

2.4 DPPH FREE RADICAL SCAVENGING TEST

The ability of the essential oil and solvent extracts to scavenge free radicals was measured by testing their reactivity with the stable free radical DPPH. DPPH is a chemical compound that has a proton free radical and its bright purple color results in a maximum absorption at 520 nm. When DPPH encounters a proton radical, its purple color quickly fades and this scavenging reaction serves as the basis for measuring antioxidant activity.

2.5 IRON REDUCTION TEST (FRAP)

The total antioxidant capacity of the sample was determined using the Ferric Reducing Antioxidant Power (FRAP) assay, which was developed by Oyaizu in 1986. The assay involved mixing different concentrations of the extracts (0.10 and 0.15 mg/mL) in distilled water with 2.5 mL of a phosphate buffer (200 mM, pH 6.6) and 2.5 mL of potassium. In the test, one milliliter of the extract at different concentrations was mixed with 2.5 mL of a 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL of a solution of potassium ferricyanide K3Fe(CN)6 at 1%. The mixture was incubated in a water bath at 50°C for 20 minutes. After adding 2.5 mL of 10% trichloroacetic acid to stop the reaction, the tubes are centrifuged at 3000 rpm for 10 minutes. Then, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of a freshly prepared 0.1% ferric chloride solution. The absorbance of the reaction mixture was read at 700 nm using a UV-VIS spectrophotometer, with a blank made of distilled water being used to calibrate the device. Ascorbic acid, a standard antioxidant, is used as a positive control, and its absorbance was measured under the same conditions as the samples. An increase in absorbance indicates an increase in the reducing power of the tested extracts.

2.6 EVALUATION OF ANTIBACTERIAL ACTIVITY

In this study, the antibacterial activity of the samples was evaluated against four reference bacteria strains: Enterococcus faecalis (ATCC 49452), Staphylococcus aureus (ATCC 25923), Klebsiella pneumoniae (ATCC 70063), and Salmonella typhimurium (ATCC 13311). The antibacterial activity was tested using two methods: the paper disk diffusion method and the dilution agar method. The minimum inhibitory concentration (MIC) was determined using the dilution agar method. The bacteria strains were obtained from the Laboratory of Antibiotic, Anti-fungal, Physical

Chemistry, Synthesis, and Biological Activity in the Department of Molecular and Cellular Biology at the Faculty of Science at the University of Tlemcen in Algeria.

2.7 PAPER-DISC DIFFUSION METHOD

The paper-disc diffusion method involved using 6mm diameter paper discs saturated with a 500mg/ml solution of the essential oil of *Atriplex halimus* and applying it to the surface of agar plates that were previously seeded with an overnight culture of the bacteria. The plates were then incubated at the appropriate temperature and the diameter of the resulting zone of inhibition was measured in millimeters. The results were classified into different levels of activity based on the size of the inhibition zone, with larger zones indicating a stronger activity. The data was the average of three determinations.

The dilution agar method was used to determine the Minimum Inhibitory Concentration (MIC) of the essential oil. Stock solutions were created by dissolving the extracts in DMSO and serial dilutions were made to obtain concentrations ranging from 0 to 50mg/ml of the extract. Each mixture was added to the Mueller-Hinton agar and after incubation at 37°C for 24 hours, the experiments were performed in triplicate to determine the MIC.

3 RESULTS AND DISCUSSIONS

3.1 CHARACTERIZATION OF ESSENTIAL OILS

In this section, we were interested in determining the chemical profile of *Atriplex halimus* essential oil. The results of the analysis are grouped in the Table 1:

Table 1. Chemical composition of the Essential Oils of *Atriplex halimus* (authors, 2024).

N°	volatile compound a	RIab	RIac	(S)d	Identificatione
1	Heptanal	882	875	0.1	RI, MS
2	Benzaldéhyde	941	937	0.3	RI, MS
3	Octen-3-one	964	959	0.1	RI, MS
4	6-méthyl Hept-5-èn-2-one	978	969	0.1	RI, MS
5	Octanal	980	986	0.1	RI, MS
6	Isovalérated' isobutyl	993	990	0.1	RI, MS
7	Decane	1000	999	0.2	RI, MS
8	Phénylacétaldéhyde	1012	1017	0.2	RI, MS
9	Limonène	1025	1027	0.2	RI, MS
10	(Z)-β-Ocimene	1029	1031	0.1	RI, MS
11	(E)-2-Octenal	1034	1038	0.2	RI, MS
12	(E)-β-Ocimène	1041	1040	0.2	RI, MS
13	M-Tolualdehyde	1053	1047	0.2	RI, MS
14	Nonan-2-one	1070	1062	0.2	RI, MS
15	Nonanal	1086	1088	0.5	RI, MS
16	Linalol	1087	1090	0.4	RI, MS
17	Undecan	1100	1099	0.2	RI, MS
18	2-Octanol, acétate	1125	1120	0.1	RI, MS

19	Camphor	1123	1124	0.2	RI, MS
20	1,2-Dihydrolinalool	1130	1130	0.1	RI. MS, Ref1
21	(E)-2-Nonenal	1139	1139	0.2	RI, MS
22	4-methyl Acétophénone	1156	1153	0.5	RI. MS, Ref1
23	Terpinen-4-ol	1164	1163	0.3	RI, MS
24	salicylate de méthyle	1169	1165	0.3	RI, MS
25	Decan-2-one	1176	1173	0.3	RI, MS
26	Estragole	1175	1176	0.1	RI, MS
27	Decanal	1180	1178	0.3	RI, MS
28	Safranal	1182	1188	0.6	RI, MS
29	(E-E)-2,4-Nonadiénal	1188	1191	0.1	RI. MS.
30	β-Cyclocitral	1197	1199	0.2	RI. MS
31	Dodecane	1200	1200	0.3	RI, MS
32	Carvone	1214	1217	6	RI, MS
33	Cuminaldéhyde	1217	1222	1.5	RI, MS
34	acétate de myrtényle	1313	1310	0.2	RI. MS, Ref1
35	Pipéritone		1315	0.1	RI, MS
36	cis-Anéthol	1230	1230	0.4	RI. MS, Ref1
37	Piperitenone	1232	1232	0.3	RI, MS
38	(E)-2-Decenal	1236	1240	0.4	RI, MS
39	Linalyl-acetate	1239	1243	0.2	RI, MS
40	Geranial	1244	1245	1.1	RI, MS
41	α-Terpinen-7-al	1257	1261	0.8	RI, MS
42	E-Anéthol	1262	1270	13.9	RI, MS
43	Undecan-2-one	1273	1271	2.7	RI, MS
44	Vitispirane B	1271	1272	1.2	RI, MS
45	Carvacrol	1278	1274	4.2	RI, MS
46	(E-E)-2,4-Decadienal	1291	1291	0.6	RI, MS
47	Tridecane	1300	1300	0.6	RI, MS
48	Piperitenone	1318	1318	0.3	RI, MS
49	Eugénol	1131	1332	0.2	RI, MS
50	α-Terpinyl acétate	1335	1334	0.2	RI. MS
51	1,2-dihydro-1,1,6- triméthyl -naphthalène	1339	1340	0.3	RI, MS
52	(E)-2-Undecanal	1345	1346	0.2	RI, MS
53	(E)-anéthol époxyde	1347	1350	0.2	RI. MS. Ref1
54	α-Longipinene	1359	1360	0.2	RI, MS
55	1-Undecanol	1359	1363	0.6	RI. MS, Ref2
56	(E)-β-Damascenone	1363	1366	1.3	RI, MS
57	Isoledene	1373	1380	0.6	RI, MS
58	Dodecan-2-one	1381	1386	0.7	RI. MS, Ref1
59	Dodecanal	1389	1390	0.5	RI, MS
60	Tetradecane	1400	1400	0.9	RI, MS
61	α-Ionone	1409	1408	1.2	RI. MS, Ref1
62	γ-Caryophyllène	1421	1415	0.6	RI, MS
63	(E)-β-Caryophyllène	1424	1423	0.7	RI, MS
64	Acétone de Géranyl	1429	1426	3.0	RI, MS
65	α-Humulene	1455	1450	0.2	RI, MS
66	β-Ionone	1468	1461	1.4	RI, MS
67	α-Curcumène	1473	1471	0.4	RI, MS
68	γ –Humulène	1483	1484	0.5	RI, MS
69	Bicyclogermacrène	1494	1494	0.6	RI, MS
70	Pentadecane	1500	1501	0.7	RI, MS
71	γ –Cadinene	1507	1504	0.6	RI, MS
72	t-Cadinene	1520	1514	0.5	RI, MS
73	(Z)-Nerolidol	1522	1520	0.3	RI. MS, Ref1
74	E-Nerolidol	1553	1554	0.5	RI, MS
75	Spathulenol	1572	1564	0.6	RI, MS

76	Tridecanol	1580	1586	1.5	RI, MS
77	Caryophyllène oxyde	1578	1569	0.1	RI, MS
78	β-copaen-4α-ol	1575	1575	0.3	RI, MS
79	Tetradecanal	1592	1590	0.7	RI, MS
80	Hexadecane	1600	1600	0.1	RI. MS
81	Ar-Tumerone	1643	1639	1.1	RI. MS, Ref1
82	α-Tumerone	1649	1645	0.2	RI. MS, Ref1
83	Pentadecanal	1693	1689	1.2	RI, MS
84	Hexadecanal	1782	1780	0.2	RI, MS
85	Octadecane	1800	1801	0.2	RI. MS, Ref1
86	Phytone	1823	1836	9.5	RI, MS
87	Nonadecene	1888	1875	2.2	RI. MS, Ref2
88	(E-E)-Farnesyl acétone	1896	1894	1.2	RI. MS, Ref2
89	Nonadecane	1900	1901	0.8	RI. MS
90	Eicosane	2000	2001	0.2	RI. MS
91	(Z)-phytol	2080	2072	0.3	RI, MS
92	(E)-Phytol	2114	2102	1.2	RI, MS
93	Docosane	2200	2199	0.2	RI. MS
94	n-Tricosane	2300	2299	0.9	RI. MS
95	n-Tetracosane	2400	2398	0.3	RI. MS
96	Pentacosane	2500	2500	1.5	RI. MS
97	Hexacosane	2600	2599	0.2	RI. MS
98	n-Heptacosane	2700	2701	1.7	RI. MS
99	n-Octacosane	2800	2799	0.1	RI. MS
% d'identification		/	/	8 5.1	/
Monoterpènes hydrocarbonés		/	/	0.5	/
Monoterpènes oxygénés		/	/	20.9	/
Sesquiterpènes hydrocarbonés		/	/	4.9	/
Sesquiterpènes oxygénés		/	/	3.1	/
Diterpènes		/	/	12.2	/
Nor-terpènique		/	/	3.9	/
Hydrocarbonés non terpénique		/	/	11.6	/
Arylpropanoïdes		/	/	14.8	/
Oxygénés non terpénique		/	/	13.2	/

a.The elution order is given on apolar column (Rtx-1). B. RILit : retention indices from the literature on apolar column referenced from Harie, (1947) and Kelley and (Norlyn, 1979) .c RIa : retention indices on apolar column Rtx-1. RI: retention indices; MS: mass spectrum in electron impact mode; d percentages of compounds. S: El Kesdir Station, Naama. Ref1, compounds identified from the data of Kelley and Norlyn, (1979) ; Ref2, compounds identified from NIST data (Harie, 1947 ; Kelley and Norlyn, 1979).

The literature search reveals that no studies have been published to date on the chemical identification of the essential oil of *Atriplex halimus*. Our investigations reveal that the essential oil of the aerial part (leaves and stems) consists of 155 compounds, of 99 volatile compounds that were are identified, representing 85.1% of the total composition of the essential oil. The main compounds are (Table 1): E-anethol (13.9%), phytone (9.5%), carvone (6.0%), carvacrol (4.2%), and geranyl acetone (3.0%).

This essential oil is proportionally composed of: oxygenated monoterpenes (20.9%), arylpropanoides (14.8%), non-terpene oxygenates (13.2%), diterpenes (12.2%), non-terpene hydrocarbons (11.6%), hydrocarbon sesquiterpenes (4.9%), oxygenated sesquiterpenes (3.1%), and monoterpene hydrocarbons (0.5%). The most important oxygenated monoterpenes are: carvone

(6%), carvacrol (4.2%) and geranyl acetone (3.0%). On the other hand, monoterpene hydrocarbons are present in quite small quantities, approximately (0.5%). Furthermore, Ar-tumerone (1.1%) and E-(B)-caryophyllene (0.7%) are the predominant compounds of oxygenated and hydrocarbon sesquiterpenes.

For the other classes, the constituents present in high concentrations are:

- Arylpropanoides (14.8%): E-anethol (13.9%)
- Non-terpene oxygenates: undecan-2-one (2.7%)
- Diterpenes: phytone (9.5%)
- Non-terpene hydrocarbons: nonadecene (2.2%)

Through the obtained results shown in the table 1 we find some of these compounds, such as linalool, limonene, and eugenol, are commonly used in aromatherapy and are believed to have medicinal properties, For example; Linalool, Limonene, and Eugenol are terpenes, which are a class of volatile organic compounds contribute to their distinct fragrances. These compounds have been used for centuries in traditional medicine and are believed to have various health benefits.

Linalool is often used as a mild sedative and anxiolytic. It is also have anti-inflammatory and analgesic properties. Limonene has been shown to have anti-inflammatory, antifungal, and antibacterial properties. It is also believed to have potential for use as a natural remedy for anxiety and stress.

Eugenol is used in dental care as a local anesthetic and antiseptic. It is also believed to have anti-inflammatory and analgesic properties, making it useful for managing pain and inflammation in conditions like arthritis. However, it is important to note that the effects of these compounds on human health may vary depending on the dose, duration of exposure, and individual response. Additionally, some of these compounds may be toxic in high doses, so it is always important to use caution when using substances that have not been fully tested for safety. In addition, some of these compounds, such as camphor, eucalyptol and salicylic acid, have been used in traditional medicine to treat various ailments. Camphor has been used for centuries in traditional medicine as a treatment for a wide range of conditions, including pain relief and insect bites. Salicylic acid is used to treat a variety of ailments, including pain relief, fever and infections. With regard to eucalyptol, it is used as an antiseptic and decongestant. For example, relieving symptoms of colds, flu and sinusitis by inhaling steam from eucalyptus oil.

Monoterpene hydrocarbons and oxygenated monoterpenes have been shown to possess antimicrobial and antioxidant properties. In addition, hydrocarbon sesquiterpenes and oxidized sesquiterpenes may exhibit bactericidal and antioxidant properties. Diterpenes exhibit antimicrobial properties, while norepinephrines can act as antiseptics and non-terpene hydrocarbons act as

bactericides. Moreover, arylpropanoids may exhibit antioxidant properties. It is important to consider that the efficacy of a compound as an antibiotic, antioxidant, or bactericide depends on various factors, including dose, biological target, and route of administration. Hence, thorough testing should always be done to evaluate the efficacy of these compounds before using them for medicinal purposes.

3.2 ANTIOXIDANT ACTIVITY

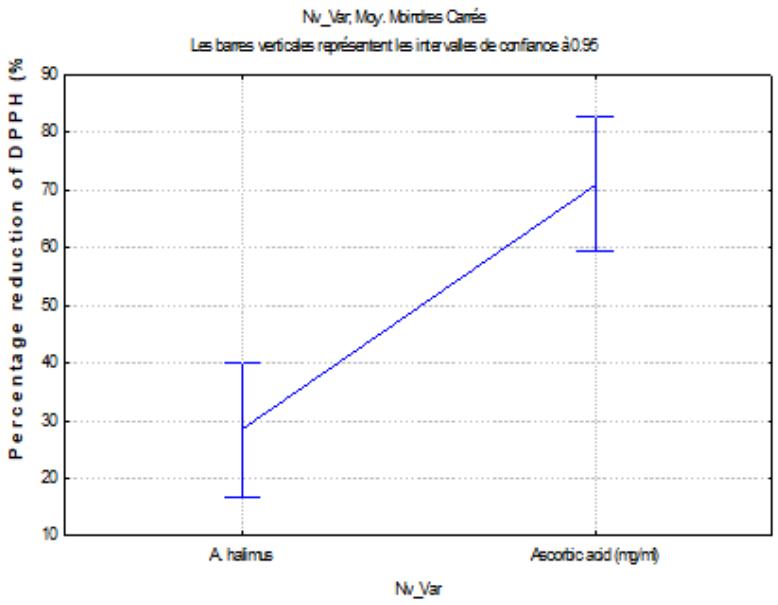
3.2.1 DPPH Free Radical Scavenging Test

The results in figure 1 and the table 2 show the evaluation of the ability of the essential oil of *Atriplex halimus* to reduce the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. The concentration of the oil was varied from 0.2 to 3 mg/ml, and the percentage of DPPH reduction was measured.

Table 2. DPPH° Radical Reduction Test by Essential Oils (authors, 2024).

Samples (mg/ml)	Percentage of reduction of DPPH (%)	IC50a(mg/ml)
Atriplex halimus essential oil		
0.2	9.3	
0.5	13.71	
1	26.72	
1.5	40.2	28.20
3	51.12	
Concentration-Ascorbic_Acid (mg/ml)	/	/
0.04	39.40	
0.05	51.03	
0.06	68.57	0.048
0.08	97.84	
0.2	98.36	

Figure 1: DPPH° radical reduction test by essential oils (authors, 2024).



aIC50: Concentration that provides 50% inhibition of DPPH°
A: Absorbency of essential oil.

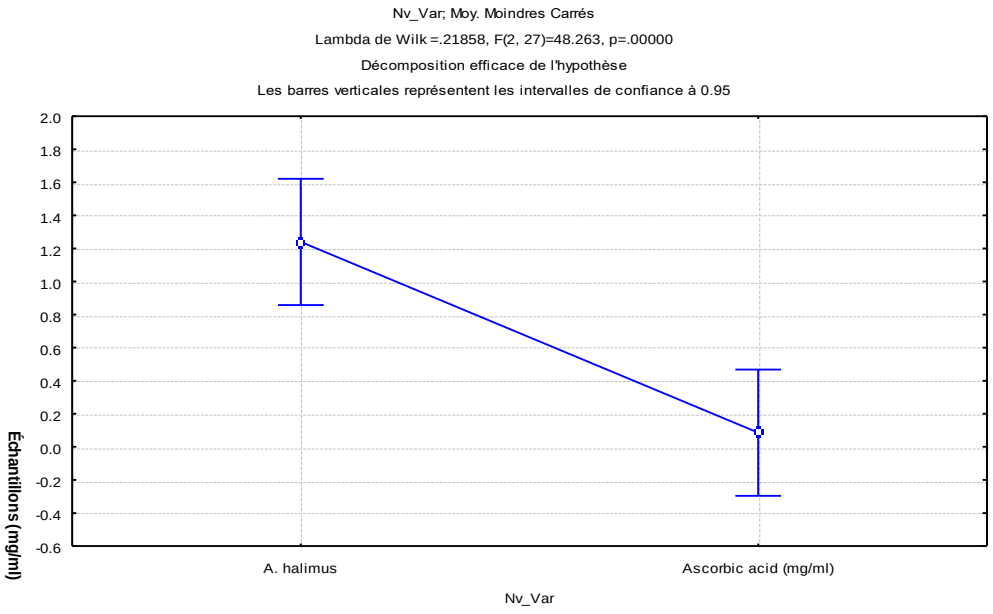
At a concentration of 0.2 mg/mL, the essential oil shows a 9.3% reduction in DPPH roots. It increased to 13.71% at a concentration of 0.5 mg/ml, and DPPH radicals increased with increasing concentration until it reached a maximum of 51.12% at a concentration of 3 mg/ml. The IC50 value, which is the oil concentration required to reduce the DPPH radical by 50%, was calculated to be 28.20 mg/mL. This indicates that a relatively high concentration of oil is required to achieve a significant reduction of DPPH radicals. Compared to essential oil, ascorbic acid showed higher DPPH radical scavenging activity. The ascorbic acid concentration required to reduce DPPH by 50% was 0.048 mg/mL, which is much lower than the IC value of the essential oil. However, it is important to note that ascorbic acid is a known powerful antioxidant, so this result was expected. The test result of the iron-lowering antioxidant capacity (FRAP) by the essential oils of the studied plant is compiled in Table 3 and figure 2 below:

Table 3. Test of Fer reduction by *Atriplex halimus* essential oils (authors, 2024).

Concentration (mg/ml)	1.5	1	0.75	0.50
A (*Atriplex halimus*)	0.097	0.053	0.039	0.014
A (Ascorbic Acid)	0.633	0.533	0.481	0.246

A: The absorbance of the essential oil.

Figure 2: Iron reduction test by the different essential oils (authors, 2024).



Results of the FRAP method confirm the antioxidant capability of *Atriplex halimus* essential oil. The reduction in iron concentration, which varied from 0.50 to 1.5 mg/mL, is evidence that the essential oil of *Atriplex halimus* can, in fact, lower iron levels. It also has a higher reducing capability than ascorbic acid at lower doses. Ascorbic acid, however, has a strong potential to decrease iron at

larger quantities. *Atriplex halimus* essential oil might be thought of as a potential antioxidant by lowering oxidized iron species, according to the data.

This study's findings suggest that *Atriplex halimus* essential oil possesses antioxidant capabilities and can be used as a natural source of these compounds.

According to Table 4, *Atriplex halimus* essential oils have antibacterial properties against various bacterial strains including *Escherichia coli, Citrobacter freundii, Pseudomonas aeruginosa, Acinetobacter baumanni, Bacillus cerius, Listeria monocytogenes, Salmonella typhimurium and Enterococcus faecalis.* The MIC (Minimum Inhibitory Concentration) of essential oils was calculated by the dilution method.

Tab 4. The results of the antibacterial activity of the essential oils of *Atriplex halimus (*authors, 2024).

Strains	Dilution Method MIC (mg/ml)
Atriplex halimus	
Escherichia coli ATCC 25922	5.2± 0.21
CitrobacterfreundiiATCC 8090	10.41
Pseudomonas aeruginosaATCC 27853	2.6 ± *0.26*
Proteus mirabilis ATCC 35659	10,41±*1.01*
AcinetobacterbaumanniATCC 19606	5.2 ± *0.1*
Bacillus ceriusATCC 10876	5.2 ± *0.1*
Listeria monocytogenesATCC 15313	10.41±*1.4*
Salmonella typhimurium ATCC 13311	5.2
Enterococcusfaecalis ATCC 49452	10.3± *1.1*
Gentamycin	
Escherichia coli ATCC 25922	0.39
Citrobacterfreundii ATCC 8090	0.19
Pseudomonas aeruginosa ATCC 27853	0.78
Proteus mirabilis ATCC 35659	0.19
Acinetobacterbaumanni ATCC 19606	0.78
Bacillus cerius ATCC 10876	0.19
Listeria monocytogenes ATCC 15313	3.12
Salmonella typhimuriumATCC 13311	0.78
Klebsiella pneumonia ATCC 70063	3.12
Staphylococcus aureu ATCC 25923	0.19
Enterococcusfaecalis ATCC 49452	3.12

The results confirmed that the essential oils of *Atriplex halimus* exhibit strong antibacterial activity against several bacterial strains. *Atriplex halimus* essential oils have higher antibacterial activity than the antibiotic gentamicin against certain bacterial strains including *Salmonella*

typhimurium, Citrobacter freundii, Pseudomonas aeruginosa, Proteus mirabilis, Acinetobacter baumanni and *Escherichia coli.*

Even though the inhibitory action of organic extracts on *Pseudomonas aeruginosa* is less potent than that of reference antibiotics, these extracts nevertheless have antibacterial activity since they are crude extracts rather than pure products. (Sanogo and al., 2006).The outcomes highlight the essential oils of *Atriplex halimus* potential as organic antibacterial agents.

The antibacterial and antioxidant activities of *Atriplex halimus* essential oil have been demonstrated whose antibacterial activity has been evaluated against various bacterial strains including *Escherichia coli, Citrobacter freundii, Pseudomonas aeruginosa, Proteus mirabilis, Acinetobacter baumanni, Bacillus cerius, Listeria monocytogenes, Salmonella typhimurium* and *Enterococcus faecalis,* with results showing significant antibacterial activity and minimum inhibitory concentration. . (MIC) ranging from 2.6 to 10.41 mg/ml. *Atriplex halimus* essential oil has been shown to have more effective antibacterial action against particular strains of bacteria than the common antibiotic gentamicin.

Essential oils similar to *Atriplex halimus* have antioxidant and antibacterial effects. Numerous studies have shown that a number of plant essential oils have antioxidant and possibly antimicrobial properties. The research presented in the article emphasizes the potential antibacterial and antioxidant activities of certain essential oils. A study by König (2021) found that certain bacterial strains, such as Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa, were highly sensitive to the antibacterial effects of cinnamon, clove, and thyme essential oils. In a study by LE Houérou (1992) it was also found that tea tree essential oil has potent antibacterial action against methicillin-resistant *Staphylococcus aureus*. Regarding antioxidant activity, Rosemary and oregano essential oils were found to have strong antioxidant activity according to a study by (Murbach et al., 2014; Oyaizu , 1986 : Özcan and Derya , 2011 ; Roselló et al., 2015).

Another study (Sanogo et al., 2006) found that basil and mint essential oils also had strong antioxidant activity. These findings demonstrate the potential uses of essential oils for various purposes, including as natural alternatives for antibacterial and antioxidant agents.

It is crucial to remember that the extraction process, storage conditions, and chemical makeup of the oil can all affect the antibacterial and antioxidant activities of essential oils. Additionally, additional research is required to establish the effectiveness and safety of essential oils in a variety of applications, despite the fact that they have potential as antibacterial and antioxidant agents.

In conclusion, *Atriplex halimus* essential oil, as well as other essential oils, can be considered as potential natural agents for both antibacterial and antioxidant applications due to their significant

biological activity. Further research is needed to fully understand the mechanisms of action and potential clinical applications.

4 CONCLUSION

In summary, the investigation into the antioxidant and antibacterial properties of *Atriplex halimus* essential oil yielded encouraging findings. The substantial free radical scavenging activity revealed by the study's findings suggested that the essential oil has significant antioxidant qualities. Furthermore, it was discovered that the essential oil had strong antibacterial properties against a number of bacterial species, including *Escherichia coli, Citrobacter freundii, Pseudomonas aeruginosa*, and others. When compared to the reference antibiotic gentamicin, the essential oil showed greater effectiveness against several types of bacteria. The minimum inhibitory concentration values varied from 2.6 to 10.41 mg/ml.

These results underscore the value of researching natural products for any potential health benefits and point to the potential of *Atriplex halimus* essential oil as a natural antibacterial agent.

In future studies, we will try to develop new natural antibacterial, antifungal agents, essential oil of medicinal plant of the Algerian flora.

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