ANTIBACTERIAL ACTIVITY AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY STUDIES OF ALGERIAN ATRIPLEX HALIMUS L.

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INTRODUCTION

Atriplex species of the family Amaranthaceae (formerly Chenopodiaceae) are among the few salt-tolerant plants which collected the salt lands in bladder cells situated on leaf surfaces and subsequently excreted by the bursting of these cells [1,2]. Atriplex halimus is an evergreen shrub which can grow 2 m high and 3 m diameter at a medium rate. It is a monococious plant that is pollinated by wind and produces a stamine flower [3]. It is widely distributed in Europe and Northern Africa, including the Sahara in Morocco and Algeria [4] (Fig. 1).

A. halimus is used as fodder reserves and as a supplementary forage resource in arid and semi-arid countries [5]. The ensiling A. halimus as a browse forage showed comparable results to polyethylene glycol (PEG) supplementation and might be easier and might lower feeding cost than daily PEG supplementation [6].

A. halimus plants accumulate large amounts of Cd in their tissues (predominance in roots), suggesting the possibility of their use in decontaminating saline soils polluted by Cd [7].

In Algeria and in particular, the Sahara part, the plant is used by Bedouin for feeding their sheep and goats [4], and in popular folk remedy, it is used to treat diabetes, stomach pains, chest ailments, muscular pain, and intestinal worms and to regulate gallbladder excretions.

Regarding the chemical composition of this species, and to the best of our knowledge, few chemical compositions have been reported in the literature [8,9]. In these papers, authors describe the isolation of some phonolic compounds, including flavonoids. We report herein the chemical composition of the essential oil of A. halimus growing in Algeria, and some carried antibacterial activities.

METHODS

Plant material

The aerial parts (leaves and stems) of A. halimus were collected in March 2016 from the region of Bechar, Algeria and identified. A voucher specimen was deposited at the Herbarium of the Valorization of Resource and Food Security in Semi-arid Areas Laboratory, South West of Algeria, University of Bechar [10,11].

Extraction of essential oil

The dried aerial parts of A. halimus (1 kg) were subjected to hydrodistillation for 5 h in 3 times using a Clevenger-type apparatus, according to the method outlined by the European Pharmacopoeia [12]. The essential oil was then separated from the aqueous layer, dried over anhydrous sodium sulfate. The calculated average of essential oil yield is 0.0475%. The essential oil was stored in sealed vials at low temperature (4°C) until (GC–MS) analysis.

GC–MS analysis

The GC–MS analysis was performed using a Hewlett Packard Agilent 6890 GC system coupled with a 5973C MS. HP-5MS analytical fused silica capillary column (60 m x 0.25 mm x 0.25 μm, Agilent, Santa Clara, CA) was used for chromatographic separations. For both columns, the oven temperature had ramped from 60°C to 250°C (8 min) at 2°C/min isothermal for 10 min. The flow rate of the helium was 0.82 μl/ml to 2.4525 μl/ml. The essential oil revealed the presence of 14 components, the dominant compounds are cadina-1(10), 4-diene (10.69%), germacrene D (9.79%), octane (9.37%), pelargonaldehyde (9.06%), 3-Furancarboxaldehyde (6.87%), β-pinene (2.6%), camphene (2.59%), and myrcene (2.10%).

Identification of components

Individual peaks were identified by comparing their Kovats index relative to n-alkanes (C8-C29) obtained by a non-polar HP-5MS column provided in the literature running at the same conditions used for the
### Table 1: Chemical composition of the essential oil of *Atriplex halimus*

| Peak | RT   | Name of compound                        | Formula | Area (%) | RI     |
|------|------|-----------------------------------------|---------|----------|--------|
| 1.   | 4.713| Octane                                  | C₈H₁₈   | 9.37     | 80,057,554 |
| 2.   | 5.028| 3-Furancarboxaldehyde                   | C₉H₁₂O₂ | 6.87     | 809,964,029 |
| 3.   | 5.628| 2-vinyl-5-methylfuran                   | C₇H₁₀O  | 2.16     | 82,690,648 |
| 4.   | 6.239| 1,1,4-Trimethylcyclohexane               | C₁₃H₂₂O | 3.83     | 84,448,921 |
| 5.   | 11.366|Camphene                                | C₁₇H₃₀ | 2.59     | 93,333,556 |
| 6.   | 12.812|β-Pinene                                | C₁₇H₃₀ | 2.6      | 97,745,163 |
| 7.   | 13.612|Myrcene                                 | C₁₇H₃₀ | 2.1      | 99,079,386 |
| 8.   | 14.881|α-Terpine                               | C₁₇H₃₀ | 3.24     | 10,101,573 |
| 9.   | 21.641|Pelargonialdehyde                       | C₁₅H₂₄O | 9.06    | 11,058,323 |
| 10.  | 25.456|p-Menthan-3-one, cis-p-                  | C₁₅H₂₂O₂ | 2.88    | 11,590,624 |
| 11.  | 32.845|Thiophene,2-{(methylthio)ethynyl]        | C₁₅H₂₄O₂ | 3.11    | 12,647,425 |
| 12.  | 36.051|Unknown                                 |         | 8.29     | 13,117,705 |
| 13.  | 46.991|Germacrene D                            | C₁₇H₃₀ | 9.79     | 14,845,492 |
| 14.  | 48.991|Myristicin                              | C₁₇H₃₀ | 4        | 15,179,931 |
| 15.  | 49.374|Cadina-1(10)4-diene                     | C₁₅H₂₄ | 10.69    | 15,245,626 |
| 16.  | 59.193|Unknown                                 |         | 8.59     | 16,981,887 |
| 17.  | 66.812|Unknown                                 |         | 2.77     | 1,745,191  |

Number of identified compounds: 17.
Monoterpene hydrocarbons: 23.73%
Oxygenated monoterpene: 15.94%
Sesquiterpene hydrocarbons: 15.94%
Others compounds: 15.94%
Percentage of identified compound: 91.94%

**RI: Retention indices relative to C8-C29 n-alkanes on the HP-5MS column; RT: Retention time**

### Table 2: Antimicrobial activity of essential oils from the aerial parts of *Atriplex halimus*

| Organisms                 | Minimum inhibitory concentration (μl/ml) |
|---------------------------|------------------------------------------|
| *Escherichia coli*        | 1.64                                     |
| *Staphylococcus aureus*   | 2.4525                                   |
| *Bacillus cereus*         | 0.82                                     |

**RESULTS AND DISCUSSION**

**Essential oil analysis**

The yield of the essential oil (light yellow color) obtained by hydrodistillation of *A. halimus* was 0.0475%, and a total of 17 different volatile and semi-volatile compounds were identified (Table 1), distributed by distinct chemical classes: Oxygenated monoterpenes (32.62%), sesquiterpene hydrocarbons (15.94%), monoterpene hydrocarbons (23.73%), and sulfur compounds (3.11%). Nearly 20% of the oil composition is unknown, which might be due to insufficient information about their RI in literature.

The number of components identified in the essential oil of *A. halimus* is 14 which accounted for 91.94% of the total components. Their retention indices and relative percentages are shown in Table 1. The dominant constituents identified in the sample are cadina-1(10)-4-diene (10.69%) [14], germacrene D (9.79%) [15], octane (9.37%) [14], pelargonialdehyde (9.06%) [16], and 3-Furancarboxaldehyde (6.87%) [17]. Other minor constituents of the oil are myristicin (4.00%) [18], 1,1,4-Trimethylcyclohexan (3.83%), α-pinene (3.24%) [19], Thiophene,2-{(methylthio) ethynyl] (3.11%) [18], p-Menthan-3-one, cis-p- (2.88%) [14], β-Pinene (2.60%) [15], camphene (2.59%) [18], 2-vinyl-5-methylfuran (2.16%) [16], and myrcene (2.10%) [18]. These qualitative and quantitative differences in the chemical composition of essential oils could be attributed to several factors such as geographical location, the climatic effects, harvest season, nature of the soil, age of the plant parts, the state of used plant materials (dried or fresh), the part of the plant used, time of collection, and chemotype [20,21].

**Antibacterial activity**

The results obtained for antibacterial activity screening of *A. halimus* essential oil are summarized in Table 2. With the broth dilution method, the MIC values for essential oil of aerial parts were in the range of 0.82–2.4525 μl/ml.

The essential oil of *A. halimus* was found to have moderate to high antimicrobial activity. It showed strong inhibition against *B. cereus* and low activity against *S. aureus*. This antimicrobial activity may be due to the chemical composition of the essential oil, which is rich in oxygenated monoterpenes.

![Fig. 1: Photograph of Atriplex halimus [4]](image-url)

The disk diffusion method was used to screen for antibacterial activities of the crude extracts against the four bacterial strains, namely, *Staphylococcus aureus* (ATCC 29213, positive control: Penicillin), *Escherichia coli* (ATCC 25922, positive control: Gentamycin sulfate injection), and *Bacillus cereus* (ATCC11778). The Petri dishes were inoculated with a loop and incubating at 37°C for 18–24 h. The minimum inhibitory concentrations (MICs) of the extracts were determined using a serial microplate dilution assay against each test bacterial species.

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CONCLUSIONS
This paper presents an interesting analysis of the chemical composition of the aerial parts essential oil of *A. halimus*. Among the 14 components identified, cadina-1(10)4-diene (10.69%), germacrene D (9.79%), octane (9.37%), and pelargonaldehyde (9.06%) were the main components. The antibacterial activity of *A. halimus* oil displayed a significant effect among the different bacterial strains.

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AUTHORS' CONTRIBUTIONS
The author declares that this work was done by the author named in this article.

CONFLICTS OF INTEREST
We declare that we have no conflicts of interest.

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