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# Chemical composition and bilogical effects of essential oil of *Artemisia judaica* an endemic plant from central Sahara of Algeria Hoggar

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## Abstract

The medicinal plants contain many secondary metabolites that are gifted of different biologic activities as antiparasital, antimicrobial or antioxidant proprieties. Essential oil extracted from dried aerial parts of *Artemisia judaica* harvested at central Sahara of Algeria Hoggar, was analysed by gas chromatography (GC-FID) and coupled to mass spectrometry (GC-MS). Thirty one compounds were identified, representing 88.98% of the total oil. The major constituents of essential oil were piperitone 66.17%, ethyl cinnamate isomer 6.11% and E-Longipinane 2.55%. The essential oil was tested for radical-scavenging ability using the stable 2,2-diphenylpicrylhydrazyl (DPPH) radical. Antimicrobial and antileishmanail activities of the oil were evaluated against Gram positive and negative bacteria and tow species of leishmania: Lieshmania *major* and *Leishmania infantum* promastigotes respectively. This preliminary study confirmed in part the use of this plant in Sahara folkloric medicine of Algeria, however further investigations into its active constituents and mechanisms of action are needed.

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## Introduction

Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care (WHO, 1978). It is not simply as a food ingredients but also to threat a plethora of ailments. Recently scientific data are demonstrate for many species and related essential oils medicinal proprieties useful in the prevention of diseases or in the relieve of their symptoms (Tognolini *et al*., 2006). Essential oils are complex mixture of volatile substances generally present at low concentrations (Lucchesi *et al*., 2004), several references on the antimicrobial and antifungal efficiency of essential oils are available in the literature (Viljoen, 2005;Oussalah *et al*., 2007;Yesil Celiktas *et al*., 2007,) and on their antiparasitic properties too (Anthony *et al*., 2005; Vuuren, 2006). *Artemisia judaica* known under the name "Tehere gglé" in tamahaq and Bahetseran in arabic (Sahki and Sahki Boutamine, 2004), belong to the Asteraceae family is a perennial semi shrub appearing some times like a herb densely and ramified with1,5m length, the leaves are small alternate sessile and dissected densely covered with hairs whitish the flowers are grouped in pale yellow discoid hemispherical heads 3mm in diameter surrounded by wolly bract and containing 10 to 20 florets (Ozenda,1983; Benchelah *et al*., 2000; Sahki and Sahki Boutamine, 2004). *Artemisia judaica* is used in Algerian traditional medicine for the treatment of various diseases such as digestive diseases: (vomits), fever, respiratory diseases and Wounds (Ramdane *et al*., 2015). There for the aim of the present work was carried out to study in vitro biological activities of essential oils in addition to evaluate its chemical component by GC-MS, which is the first report to publish it on this species from Hoggar.

## Materials and methods

*Plant material*

The dried aerial parts of *Artemisia judaica* were gathered at flowering stage in 2014 from Hoggar located in the central Sahara of Algeria and identified at the National Institute of Forest Research. The dried plant was stored in the dark place until analysis.

*Obtention of essential oil*

The dried aerial parts of *Artemisia judaica* were subjected to hydrodistillation in a Clevenger-type apparatus for 3 h. The essential oil obtained was dried over anhydrous sodium sulfate and stored in sealed glass vials at 4 °C prior to analysis.

*GC-MS analysis of essential oil*

Essential oil analysis was performed on an Agilent 7890A GC system, coupled to an Agilent 5972C mass spectroscopy detector with electron impact ionization (70 eV). A HP-5 MS capillary column (30 m x 0.25 mm, coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 mm film thickness; Hewlett-Packard, CA, USA) was used. The column temperature was programmed to rise from 60 to 260 °C with a 5 °C/min rate then rise to 340°C with a 40 °C/min rate, the carrier gas was helium N60 with a 0.9 mL/min flow rate; split ratio was 100:1. Scan time and mass range were 1s and 50-550 m/z, respectively. The compound identification was based on mass spectra (compared with Wiley Registry 9th Edition/NIST 2011 edition mass spectral library) and published data (Adams, 2011; Dob and Chelghoum, 2006).

*Antimicrobial activity*

*Bacterial strains*

The antimicrobial activity of essential oil was individually tested against pathogen microorganisms, including *Lesteria monocytogenes* ATCC 19195, *Escherichia coli* ATCC 25922. *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 14 759 and *Staphylococcus aureus* ATCC 25923. Bacterial strains were cultured overnight at 37 C° in Mueller Hinton.

*Well diffusion method*

The agar well diffusion method was employed for the determination of antimicrobial activity of *Artemisia judaica* essential oils (Valgas, 2007). After preparation of bacteria suspension and adjusted it to 0.5 McFarland turbidity standard (1 x $10^8$ CFU/mL) then it was spread on Muller Hinton plates. 20 µL of essential oil were added to each of the wells (6 mm diameter holes cut in the agar gel).

The inoculated plates were incubated at 37°C for 24 hours. Antibacterial activities were determined by measurement of the inhibition zone diameter (mm) around each well.

*Antioxidant activity*

*Scavenging free radical DPPH assay*

The Scavenging free radical DPPH of essential oil and ascorbic acid as standard was measured by the method of Rai *el al*, 2006. The reaction mixture consisting of 2 mL DPPH solution (100 µM) in methanol and 0.1 mL of essential oil (at different concentrations) was incubated in dark for 30min. After, absorbance was measured at 517. The scavenging activity of the DPPH radical (in percentage) was calculated by the following equation: Scavenging activity % = $A_o$- A1/ $A_o$. Where $A_o$ is the absorbance of the control reaction and $A_1$ is the absorbance in the presence of essential oil.

*Anti-leishmanial activity*

Promastigote forms of Leishmania *major* and *Leishmania infantum* promastigotes ($2\times10^5$ parasite/ml) strain were cultured in 96-well plates. Culture (RPMI-1640 medium (Gibco) containing 100µg of streptomycin/ml and 100U penicillin/ml supplemented with 10% of fetal bovine serum (FBS) and treated with serial dilutions of the essential oil (Asghari, 2014). Plates were then incubated at 27°C for 72 h. The antileishmanial activity was expressed as the $IC_{50}$ (50% inhibitory concentration).

*Cytotoxicity Assay and Selectivity Index*

A suspension of murine macrophagic cells (Raw264.7) was placed in RPMI-1640 medium supplemented with 10% FBS, in the presence of antibacterial and antifungal solution (Gibco) and incubated at 37°C with 5% CO2 (after approximately 24 h). 100µl of different concentrations of essential oil were added in the plate containing$10^5$cells/well. Also a negative control with cells was prepared. Plates were incubated for 72 h in the same conditions described above. The results were obtained by an ELISA reader (in optical density (OD) at 490 nm (Essyd, 2015)

*Statictical analysis*

The results were given as mean SD for at least three replicates. The $IC_{50}$ (DPPH) values were calculated by linear regression analysis.

**Results and discussion**

*Essential oil composition*

The essential oil was found to be Yellow with 0.96 % to 1.7% (w/w) (Table 1).

Several studies have showed different yields of *Artemisia judaica* oil in different regions, the one of Ilizi (Algeria) 0.70% (Dob and Chelghoum, 2006), Egypt 1.4% (El-Massry *et al.*, 2002), Libya 0.62% (Janaćković *et al.* ,2015), Jordan 1.62% which is more close to our results (Abu-Darwish *et al.*, 2016). In total thirty one components were identified in the essential oil extracted from *Artemisia judaica* which representing for 88.98 % of total oil listed in table 1 in order of their elution.

The major component was piperitone (66.17%), ethyl cinnamate isomer (6.11%) E-longipinane (2.55%) and spathulenol (2.34%). The abundant compounds in this Asteraceae species oil were oxygenated monoterpenes (70.44%) followed by oxygenated sesquiterpenes (6.46%), monoterpenes hydrocarbons represent the less percentage with 0.58% (figure 1). Different variations were apparent, in the essential oil composition and quantities of this plant in many localities, from Illizi (Algeria) is in majority composed of piperitone (61.9%) terpin-4-ol (4.6%) and bornyl acetate (3.0%) (Dob and Chelghoum, 2006).

The main components of essential oil investigated by Abu-Darwish *et al.*, 2016 in Jordan were Piperitone-camphor ethyl cinnamate, chrysanthenone, piperitone oxide with 30.4, 16.1, 11.7, 6.7, 3.9 % respectively similar to those identified in the same plant in Egypt as has been mentioned by the same authors. In contrary the species from Libya contains piperitone, chrysanthenone, cis-chrysanthenyl acetate and cis-chrysanthenol as major compounds (Abu-Darwish *et al.*, 2016).

Variation between chemical compositions of essential oil of *Artemisia judaica* showed in literature reports, depending on many differences climatical, seasonal, geographical and geological (Tepe *et al.,* 2005; Boulanouar *et al*., 2013).

**Table 1.** Chemical composition of essential oil of *Artemisia judaica*.

| N° | Compounds | Retention time | % |
|---|---|---|---|
| 1 | p-Cymene | 9.11 | 0.48±0.03 |
| 2 | β-Pinene | 9.25 | 0.10±0.00 |
| 3 | 1,8-cineole | 9.30 | 0.14±0.021 |
| 4 | 3-Hepten-2-one, 4-methyl | 9.59 | 0.09±0.013 |
| 5 | Spiro[2.4]heptane-5-methanol, 5-hydroxy- | 9.98 | 0.34±0.01 |
| 6 | 1-Cyclopentene-1-methanol, 2-methyl-5-(1-methylethyl) | 10.60 | 0.33±0.05 |
| 7 | Trans-p-2-Menthen-1-ol | 12.319 | 0.28±0.01 |
| 8 | Cis-p-Menth-2-en-1-ol | 12.936 | 0.24±0.05 |
| 9 | 2,5-Furandione, 3-(1,1-dimethylethyl) | 13.277 | 0.17±0.11 |
| 10 | Terpinen-4-ol | 14.26 | 0.19±0.04 |
| 11 | Thujone | 14.49 | 0.09±0.00 |
| 12 | p-Cymen-8-ol | 14.615 | 0.36±0.01 |
| 13 | α-Terpineol | 14.798 | 0.34±0.01 |
| 14 | Piperitone | 17.485 | 66.17±1.84 |
| 15 | Thymol | 18.15 | 0.69±1.036 |
| 16 | Diosphenol | 18.645 | 0.44±0.26 |
| 17 | Carvacrol | 19.066 | 0.14±0.02 |
| 18 | Ethyl cinnamate | 21.21 | 1.70±0.24 |
| 19 | 1-(1,3-Dimethyl-3-cyclohexen-1-yl)ethanone | 22.26 | 0.35±0.01 |
| 20 | Ethyl cinnamate isomer | 23.48 | 6.11±0.35 |
| 21 | γ-Muurolene | 23.78 | 0.19±0.03 |
| 22 | β-copaene | 23.88 | 0.32±0.02 |
| 23 | Davana ether | 24.13 | 0.70±0.04 |
| 24 | γ-Elemene | 24.24 | 0.28±0.06 |
| 25 | E-Longipinane | 24.584 | 2.55±0.11 |
| 26 | 2-Ethyl-3-methoxy-2-cyclopentenone | 24.818 | 0.47±0.02 |
| 27 | Z-Longipinane | 25.00 | 1.31±0.04 |
| 28 | 6-epi-shyobunol | 25.357 | 0.15±0.01 |
| 29 | Spathulenol | 26.03 | 2.34±0.07 |
| 30 | Davanone | 26.188 | 0.42±0.00 |
| 31 | β-Eudesmol | 27.516 | 1.54±0.25 |
| Oil Yield (% w/w) | 0.96 % to 1.7% | | |
| Total 88.98 % | | | |

*The antioxidant activity*

The potential antioxidant activity of the *Artemisia judaica* essential oil was evaluated on the basis to reduce the free stable radical DPPH. In this assay essential oil has demonstrated a good activity to scavenging and decolorate the radical DPPH with an $IC_{50}$= 5.61 mg/ml but this value was high then that expressed by ascorbic acid standard with an $IC_{50}$ =5.86µg/ml. Very little has been given about antioxidant activity of essential oil of this species to the exception the study of El-Massry *et al* (2002).

**Table 2.** Effect of *Artemisia judaica* essential oil on Gram positive and negative bacteria

| Bacteria strains | *Bacillus cereus* | *Listeria monocytogenes* | *Staphylococcus aureus* | *Escherichia coli* | *Pseudomonas aeruginosa* |
|---|---|---|---|---|---|
| Zone inhibition diameters (mm) | 10 | 22 | NA | NA | NA |

NA: not active.

Previous report has demonstrated that methanol and aqueous extracts of Jordanian *Artemisia judaica* exhibited low antioxidant activity as measured by DPPH assay (Al-Mustafa and Al-Thunibat, 2008). However, finding demonstrated by El-Sayed *et al* (2013) and Bakr (2014) on Egyptian and Saudian *Artemisia judaica* showed higher antioxidant effect than standards.

**Table 3.** Antileishmanial activity of essential oil of *Artemisia judica* and amphotricin B.

| | IC50 *L. infantum* (µg/ml)± *SD* | IC50 *L. major* (µg/ml)±*SD* | LC50 Raw264.7 (µg/ml)±*SD* | SI *L. infantum* | SI *L. major* |
|---|---|---|---|---|---|
| Essnetial oil of *Artemisia.judaica* activity | 91.45±1.22 | 76.23±0.43 | 237.98±1.61 | 2.6 | 3.12 |
| Amphotricin B | 0.22 ± 0.09 | 0.80 ± 0.18 | 9.23 ± 0.13 | 11.53 | 41.95 |

*Antimicrobial activity*

Antimicrobial well susceptibility test was selected as a preliminary procedure for screening the antibacterial efficacy of essential oil on Gram positive and Gram negative bacteria, and the results, are reported in table 2. The most interesting results were obtained with the essential oil in fact, on *Listeria monocytogenes* bacteria tested which was clearly sensitive to the oil, demonstrated by the presence of large inhibition zones 22 mm. and moderate activity on *Bacillus cereus* with an inhibition zone of 10 mm. However *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* were resistant.

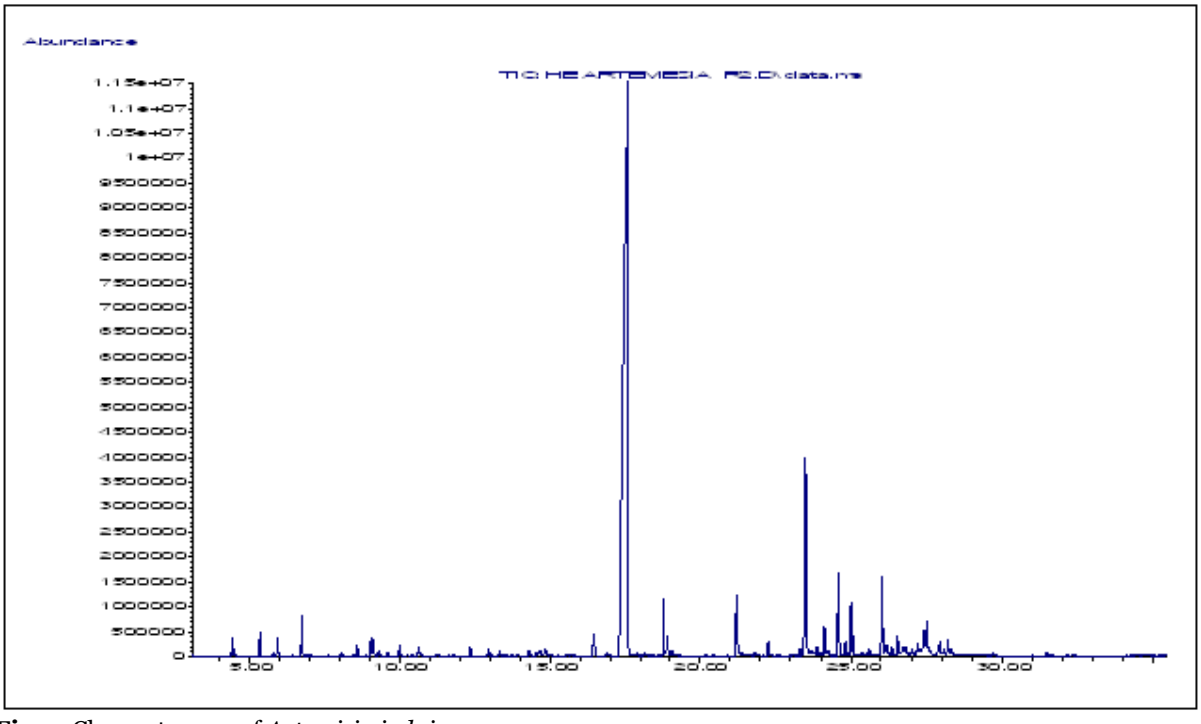


**Fig. 1.** Chromatogram of *Artemisia judaica*.

The interesting activity of oil may be attributed to the major component (Bakkali *et al*., 2008). According to Nakatsu *et al*., 2000 significant role of minor compounds in biological activity was observed where a mixture of all compounds found in the essential oil of Thyme showed increased biological activity in comparison to the biological activity of the six major compounds when investigated independently.

*Antileishmanial activity*

Essential oil exerted prominent antileishmanial activity when tested against promastigotes of *Leishmania major* and *Leishmania infantum* with $IC_{50}$ values of 76.23±0.43µg/ml and 91.45±1.22 respectively whereas it was lower to the standard amphotericin B with $IC_{50}$ 0.80 ± 0.18 and 0.22 ± 0.09 in the same order (Table 3). Our results demonstrated, for the first time the antiparasital activity of *Artemisia judaica* essential oil. The results of cytotoxic activity showed no cytotoxic activity of the essential oil on macrophage cells. This finding corroborates an earlier report by Abu-Darwish *et al* (2016), who reported a no-cytotoxic activity of this plant from Jordan.

## Conclusion

It is very important to characterize essential oils of plants which are used for many years in the prevention of diseases or in the relieve of purposes. This study is about the characterization of essential oil of an endemic *Artemisia judaica* from central Sahara of Algeria, the essential oil was characterised by the presence of oxygenated monoterpenes (70.44%) with high content of piperitone (66.17 %). The essential oil was a potent scavenging agent for the stable radical DPPH and has a strong activity on *Lesteria monocytogens*, the species has showed a good antileishmanial activity on the promastigote form of the parasite but further studies would therefore be needed to evaluate the *in vivo* and clinical responses.

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