GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Contribution to phytochemical study and biological activities of *Artemisia judaïca* from the Algerian Sahara

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GSC Biological and Pharmaceutical Sciences, 2023, 23(01), 137–147

Publication history: Received on 04 March 2023; revised on 10 April 2023; accepted on 13 April 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.23.1.0154

Abstract

This study aims to contribute to the valorisation of the Algerian desert flora, particularly the flora of Ahaggar, by conducting a phytochemical and biological investigation of the essential oil of Artemisia judaïca, a traditional aromatic plant used for various garlics in this region.

After the extraction of the essential oil, chromatographic analysis and evaluations of antimicrobial and antioxidant activities are performed on this plant. The results show a very high yield of essential oil on average (> 3.5 ml per 100 g of dry plant).

The studied essential oil of Artemisia judaïca is characterized by the presence of piperitone (73.65 %). This oil showed strong antifungal and antibacterial activities, probably due to its richness in piperitone. The evaluation of the antioxidant activity of the essential oil gave an IC_{50} value of 2.77± 0.05 mg/ml. The β-carotene bleaching test confirmed the trapping power of this essential oil.

Keywords: *Artemisia judaïca*; Essential oil; GC-MS; Antimicrobial activity; Antioxidant activity

1. Introduction

Artemisia judaïca subsp. sahariensis L. is a Saharo-Sindian species that is widely distributed in the central Sahara, Western Sahara, Libya, Chad, Egypt, and Palestine [1-3]. In Algeria, it grows in the Sahara and the sandy beds of the Ahaggar wadis [4]. It is a perennial, highly branched shrub that forms large, very dense, bluish-green tufts, ranging from 60 to 80 centimeters in height and up to 1.50 meters tall. The stems are more or less woody, the leaves are small, highly divided into lobes and obtuse, and they are covered with a silvery down [4].

Artemisia judaïca enjoys a great reputation throughout the Sahelian Africa for its numerous medicinal and aromatic virtues; in fact, the drug is used in various forms to treat digestive disorders and the flu, and is also anti-diarrheal and vermifugal. This sagebrush is also used as a flavoring and condiment [5,6]. An infusion of its leaves is relaxing and helps to induce sleep. It is the basis of the medicine known as "semen-contra of Barbarie" [7].

The importance of this plant in traditional medicinal use in Ahaggar has led us to carry out a phytochemical study of this species in order to quantify its essential oil, identify the chemical constituents of this oil, and finally study its antimicrobial and antioxidant potential.

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2. Material and methods

2.1. Materials

2.1.1. Plant material

The plant material consisted of the aerial parts of *Artemisia judaïca*, which were harvested from flowering branches in Oued Tamarghit (35 km North of Tamanrasset) during the months of September and October (over several years).

Immediately after harvesting, the samples were dried in the shade in a well-ventilated, warm, and dry place at a temperature not exceeding 30 °C.

2.1.2. Microbial material

Table 1 Tested reference bacterial and fungal strains

Bacterial strains.			
Strains	**N° ATCC**	**Forms**	**Class**
Staphylococcus aureus	6538	CG+ Aerobic-anaerobic facultative	*Micrococcaceae*
Bacillus subtilis	9372	BG+ Strict aerobics	*Bacillaceae*
Pseudomonas aerugenosa	9027	BG- Strict aerobics	*Pseudomonadaceae*
Escherichia coli	4157	BG- Aerobic-anaerobic facultative	*Enterobacteriaceae*
Enterococcus faecium	6569	BG+ anaerobic facultative	*Enterococcaceae*
Fungal strains.			
Candida albicans	24433	Yeast	*Cryptococcaceae*

2.1.3. Physicochemical equipment

Refractometer

The refractometer used for determining the refractive index of the essential oil is a CARL ZEISS model reference 89717.

Apparatus for hydrodistillation

This is a standardized apparatus for the extraction of essential oil that complies with the measurements of the European Pharmacopoeia 8th edition.

Equipment for physicochemical analysis:

The gas chromatograph used in this work is a Hewlett-Packard (HP) Palo-Alto CA, USA (Agilent technologies) 6800 plus.

Injector: The sample is introduced with a microsyringe.

Column:

Type: Hewlett Packard-5MS, non polar.

Dimensions: length 30 m, inner diameter: 0.25 mm, film thickness 0.25 µm.

Stationary phase: 5 % phenyl 95 % dimethylpolysiloxane.

Detector:

The detector used is a mass detector or Mass Spectrometer (MS):

It is a HP (Agilent technologies) model MSD 5973, with a triple quadrupole mass filter (QQQ).

2.2. Methods

2.2.1. Extraction and measurement

The essential oil was extracted by hydrodistillation, and the measurement was carried out directly on the apparatus by reading the volume on the graduated tube.

Table 2 Operating conditions

Dry weight (g)	Solvent (ml)	Duration of extraction
20	Water 500	03h 45m

2.2.2. Physical indices

Refractive indice

The refractive index at 20 ° C and density are determined by methods conforming to the AFNOR standards, 2011 [8].

Relative density:

It is the ratio of the mass of a certain volume of an essential oil at 20°C to the mass of an equal volume of distilled water at 20 °C [8].

2.2.3. Separation by gas chromatography/mass spectrometry (GC/MS)

Operational conditions for GC/MS analysis:

Injector: split-splitless set at 250 °C, injection mode: Split 50:1, injected volume: 0.2 µl

Analysis mode: Scan (from 34 to 450)

Solvent used: Hexane, Solvent delay: 4 min

Interface temperature: 280 °C

Oven temperature: programmed at 60 °C (8 min), at a rate of 2 °C/min up to 250 °C, for 10 minutes.

Carrier gas: Helium purity: N 6, Carrier gas flow rate: 0.5 ml/min.

2.2.4. Biological methods:

Method for evaluating antimicrobial activity

We adopted the agar diffusion method using sterile cellulose disks impregnated with essential oil, called aromatograms, [9].

Determination of minimum inhibitory concentration (MIC): The MICs of essential oils were determined using the method reported by Remmal et al. [10] and Satrani et al. [11]. The MIC is defined as the lowest concentration of an antimicrobial agent that can visibly inhibit the growth of a microorganism after 24 hours for bacteria and 48 hours for yeasts.

Determination of minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC): The MBC is the lowest concentration of the essential oil capable of killing microorganisms after 24 hours of incubation for bacteria and 48 hours for yeasts (called MFC in this case) to obtain the destruction of 99.99 % of the initial inoculum and leaving a percentage of surviving bacteria < 0.01% of the starting inoculum.

Antioxidant activity

The methods chosen to measure the antioxidant activity of our essential oils are as follows:

- DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay
  - Principle

The antioxidant activity of the essential oil of *Artemisia judaïca* as well as that of a standard antioxidant, Butylated Hydroxy Toluene (BHT), against the DPPH radical was evaluated using a spectrophotometer by monitoring the reduction of this radical which is accompanied by a color change from violet (DPPH•) to yellow (DPPH-H), measurable at 517 nm.

  - Preparation of DPPH solution [12]

The DPPH solution is prepared by solubilizing 4 mg of DPPH in 100 ml of methanol.

Extracts to be tested are prepared as follows:

The essential oil, obtained by hydrodistillation, has a density of 0.93, therefore, we have a mother solution with a concentration of 930 mg/ml.

From this solution, dilutions are prepared in absolute methanol to obtain different concentrations from 10 to 930 mg/ml.

50 µL of extract solutions are added to 1250 µL of the methanolic solution of DPPH (0.004 g/l).

The mixture is left in the dark for 30 min and the decoloration compared to the negative control containing only the DPPH solution is measured at 517 nm.

The absorbance reading is taken against a blank prepared for each concentration.

The positive control is represented by a solution of a standard antioxidant; BHT, whose absorbance was measured under the same conditions as the samples. The test is repeated three times for each concentration. The results were expressed as percentage of inhibition (I %).

  - Expression of results

The antioxidant capacity of our samples was expressed as percentage inhibition of the DPPH radical according to the following equation **: AA (%) = (A0-A /A0) x 100 ;** (AA=The antioxidant activity (%); A0= The optical density of the blank at 517 nm; A= The optical density of the diluted extract at 517 nm).

The EC50 values were determined graphically by linear regression. The EC50 values expressed in mg/ml represent the effective concentration of the antioxidant extract needed to trap and reduce 50 % of DPPH moles in methanol solution [13,14].

- Beta-carotene bleaching test by linoleic acid
  - Principle

In this test, the antioxidant capacity of the essential oil is determined by measuring the inhibition of the oxidative degradation of beta-carotene (decolorization) by the oxidation products of linoleic acid according to the method described by Kartal and colleagues, 2007 [15].

  - Technique

The absorbance of the control at 517 nm (AT) and the absorbance of the sample at 517 nm (Ae) were determined. The EC50 values were graphically determined by linear regression. The EC50 values expressed in mg/ml represent the effective concentration of the antioxidant extract required to trap and reduce 50 % of DPPH moles dissolved in methanol [13,14].

- Beta-carotene bleaching test by linoleic acid
  - Principle

In this test, the antioxidant capacity of the essential oil is determined by measuring the inhibition of the oxidative degradation of beta-carotene (decolorization) by the linoleic acid oxidation products, according to the method described by Kartal and colleagues in 2007 [15].

The β-carotene/linoleic acid emulsion is prepared by solubilizing 0.5 mg of β-carotene in 1 ml of chloroform, adding 25 µL of linoleic acid and 200 mg of Tween 40. The chloroform is completely evaporated, and then 100 ml of oxygen-Saturated distilled water is added. The resulting emulsion is vigorously shaken.

350 µL of the essential oil or reference antioxidants (BHT) solubilized in methanol (2 mg/ml) are added to 2.5 ml of the above emulsion [16]. The decolorization kinetics of the emulsion in the presence and absence of antioxidant (negative control in which the sample is replaced by 350 µl of methanol) are followed at 490 nm at regular time intervals for 48 hours. The percentage of the relative antioxidant activity of the extracts (AA%) is calculated according to the following equation:

$$AA\% = Abs\ t=24h\ (sample)/\ Abs\ t=24h\ (BHT)\ x100$$

3. Results

3.1. Physical indices

Table 3 Efficiency, refractive index and relative density

Plante	Average yield (ml /100 gps)	IR_n^{x20}	Density g/m
Artemisia judaïca subsp. sahariensis	3.4 ± 0.2	1.4815 ± 0.0005	0.9315

3.2. Gas chromatography-mass spectrometry (CPG-SM)

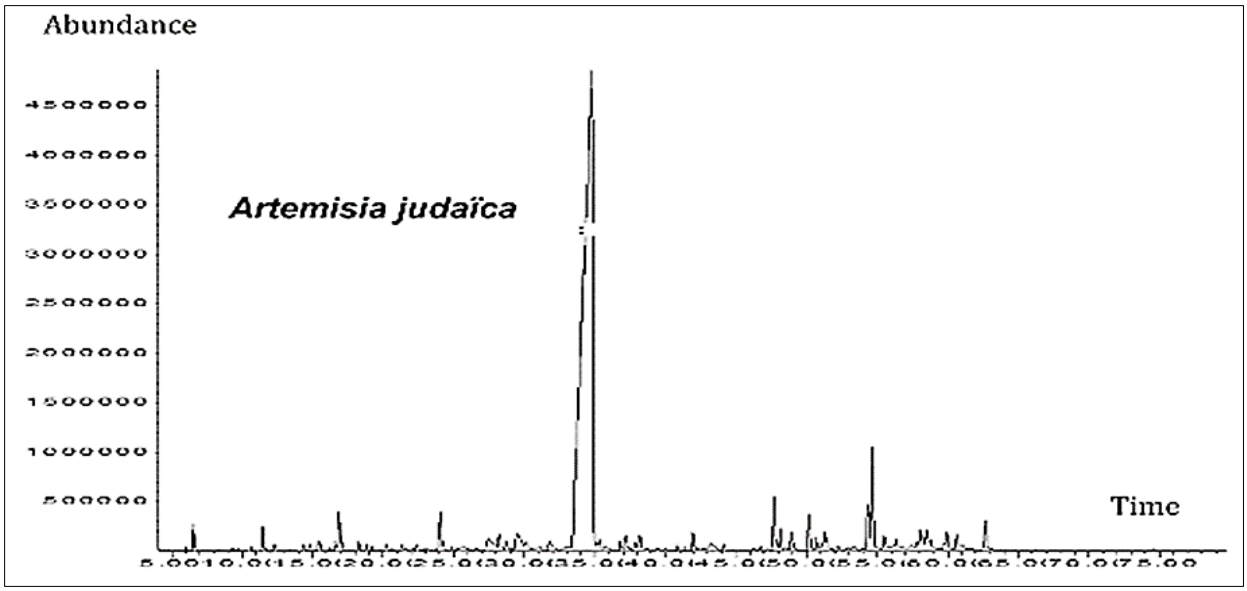


Figure 1 Chromatographic profile of the essential oil of *Artemisi judaïca*

Fifty-four compounds were enumerated of which four were not identified.

Table 4 Chemical composition of the essential oil of *Artemisia judaïca*

N°	Identified compounds	TR	IR	IR réf.	%
01	α-pinene	10.02	932	932	0.04
02	2(5H)-furanone,5,5-dimethyl-	11.42	955	952	0.14
03	2,3 dehydro-1,8-cineole	13.52	990	991	0.07
04	benzene,1,2,4-trimethyl-	13.90	996	994	0.05
05	yomogi Alcool	14.49	1006	1000	0.19
06	cyclohexane, (1-methylpropyl)-	15.73	1022	-	0.12
07	m-cymene	15.95	1025	1023	0.31
08	dl-limonene	16.16	1028	1030	0.11
09	4-methyl-4-vinyl butyrolactone	17.25	1043	1041	0.25
10	3-octen-2-one	17.89	1053	1055	0.11
11	γ-terpinene	18.30	1059	1060	0.04
12	artemisia alcool	20.35	1086	1085	0.14
13	filifolone	21.45	1103	1109	0.12
14	Linalool	21.67	1104	1104	0.04
15	α-Isophorone	22.85	1122	1222	0.19
16	chrysanthenone	23.09	1125	1125	0.79
17	Isopulégol	24.43	1144	1146	0.21
18	Camphre	24.65	1147	1145	0.10
19	Not Identified	26.26	1169	-	1.09
20	terpinen-4-ol	27.08	1180	1180	0.35
21	α-terpineol	28.26	1196	1195	1.23
22	p-cymen-8-ol	28.99	1207	-	0.36
23	nordavanone	30.73	1232	1228	0.52
24	cis-carveol	31.59	1245	1247	0.15
25	pipéritone	32.03	1251	1251	73.65
26	phellandral	34.27	1282	1281	0.47
27	Isopiperitenone	34.76	1288	1285	0.20
28	2-hydroxypiperitone	35.71	1304	1305	0.31
29	3-hexenyl caproate	36.04	1310	-	0.34
30	carvacrol	36.37	1314	1314	0.27
31	ethyl dihydrocinnamate	38.88	1351	1350	0.05
32	acetate de geranyle	39.82	1365	1365	0.14
33	α-copaene	40.31	1373	1374	0.05
34	Not Identified	40.78	1380	-	0.44
35	cis-Jasmone	42.03	1399	1399	0.42

36	methyl cinnamate	41.28	1388	-	0.21
37	davana furane	43.06	1416	1419	0.12
38	limonene dioxyde 1	45.65	1459	1467	0.13
39	ethyl cinnamate	46.50	1471	1467	1.41
40	γ- muurolene	46.75	1476	1477	0.06
41	germacrene-D	47.00	1480	1480	0.43
42	bicyclogermacrene	47.30	1485	1486	0.11
43	davana ether	47.85	1494	1497	1.58
44	Not Identified	49.56	-	-	0.40
45	Not Identified	50.19	-	-	0.78
46	(+) spathulenol	53.06	1582	1582	1.26
47	cis-davanone	53.55	1590	1592	2.84
48	4HDMMCHP*	54.34	1604	-	0.49
49	isospathulenol	56.46	1642	1640	0.22
50	τ-cadinol	56.66	1645	1650	0.41
51	Jasmonate de methyl	57.03	1652	1655	0.37
52	β-eudesmol	57.18	1654	-	0.51
53	α-cadinol	57.42	1659	1660	0.34
54	2,6,10-trimethyl-cis-7,10-oxido-do decadien -3e,11- dien-2-ol-5-one	60.26	1710	-	0.15
Total (%)					94.88

*4HDMMCHP : 4-(1-Hydroperoxy-2,2-dimethyl-6-methylene-cyclohexyl)-pent-3-en-2-one; TR : time retention, IR : indice of retention, IR réf. : indice of retention of reference (litterature)

3.3. Testing for antimicrobial activity

The antimicrobial activity of the essential oil of *Artemisia judaïca* is variable from one species to another, (Table 4), indeed this essential oil has a very strong activity on *Staphylococcus aureus* ATCC 6538 but especially on *Candida albicans* ATCC 24433, it also has a strong activity on *Enterococcus faeci*um ATCC 6569, *Escherichia coli* ATCC 4157 and *Bacillus subtilis* ATCC 9372 and a moderate activity on *Pseudomonas subtilisa* ATCC 9027

Table 5 Antimicrobial activity of *Artemisia judaïca* essential oil

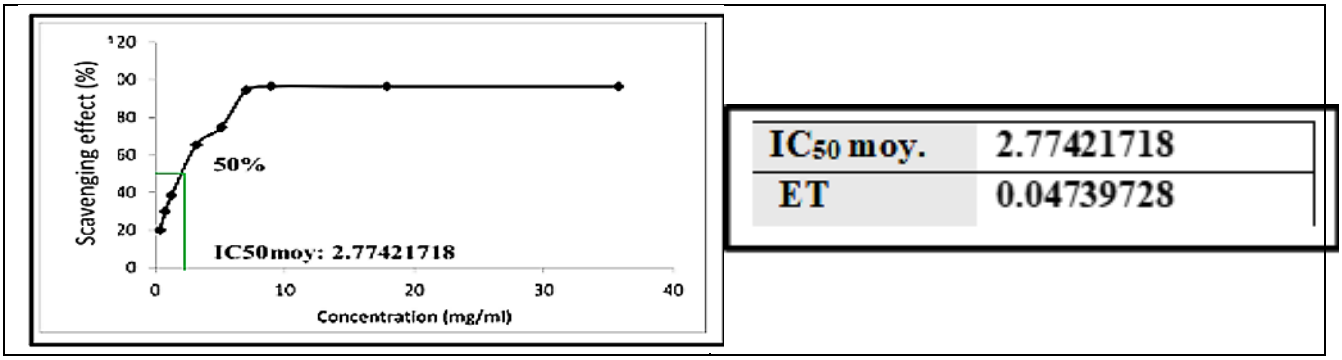
Microorganism	**Qualitative test**			**Quantitative test**		
	Diamter of inhibition (mm)			**Essential oil**		
	Essential oil	**Control (-)**	**Gentamycin**	CMI (%)	CMB (%)	CMF (%)
P. aeruginosa ATCC 9027	16	**-**	**37**	0.50	2.00	-
E. coli ATCC 4157	24	**-**	25	0.125	0.50	-
B. subtilis ATCC 9372	20		**34**	0.50	2.00	-
S. aureus ATCC 6538	26	**-**	**36**	0.06	0.50	-
E. faecium ATCC 6569	25	**-**	**31**	0.29	1.16	-
C. albicans ATCC 24433	**35**	**-**	25	≤ 0.03	-	0.06

Contrôle (-): Distilled water ; **Genta**. : Gentamycin (20 mg/ml) ; **T.** : test.

3.4. Antioxidant activity

3.4.1. DPPH free radical scavenging test

The results obtained are as follows:



IC_{50} moy.	2.77421718
ET	0.04739728

Figure 2 Percentage inhibition of DPPH with increasing concentrations and IC_{50} of *Artemisia judaïca* essential oil

3.4.2. β-carotene/linoleic acid bleaching test

The results obtained are presented in the following figure:

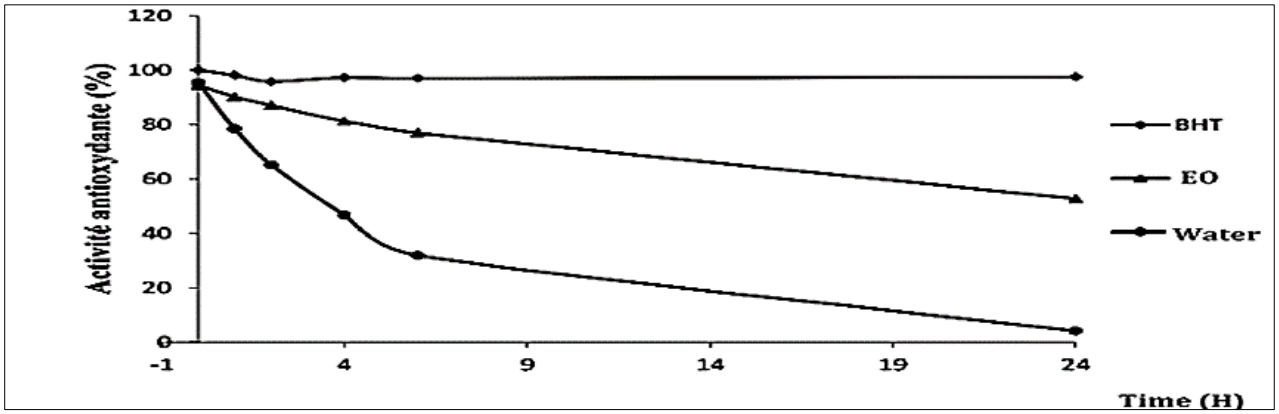


Figure 3 Bleaching kinetics of β-carotene in the presence of essential oil, water and BHT for 24 hours

The antioxidant activity of Judean mugwort essential oil compared to that of BHT and water for 24 hours gives the following profile:

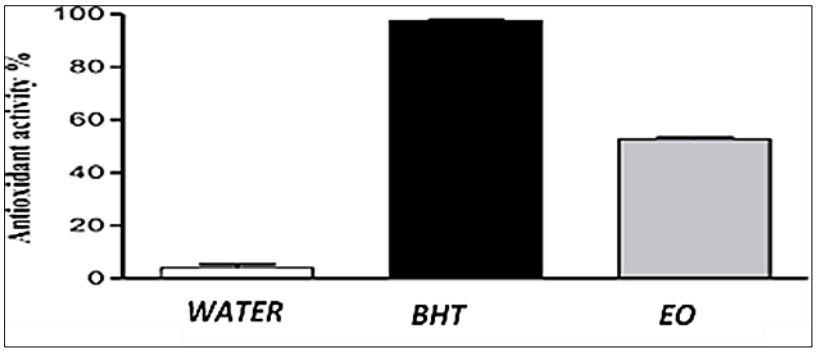


Figure 4 Antioxidant activity of *Artemisia judaïca* essential oil compared to BHT and water

4. Discussion

4.1. Yield and Physical Indices

4.1.1. Yield

The yield of essential oil of *Artemisia judaïca* from the Hoggar is very high; it is estimated at 3.4 ml per 100 g of dry plant weight; this considerable content has already been estimated at 2.45 ml % [17].

This yield is also variable from one region to another, it is clearly higher than that of the essential oils of the species harvested in In Amenas (Algeria) (0.70 % DM) [18] and in different regions in Egypt where it varies from 0.4 % to 1.4 % [19], the climate and the geographical area may be the reason for this difference.

4.1.2. Physical clues:

The refractive index cannot be used for the identification of the essential oil of the plant, as several essential oils can have the same refractive indices. Determining the refractive index for an essential oil essentially verifies if it meets established purity standards. The refractive index of the essential oil of *Artemisia judaïca* is low (1.4815), but it still falls within the recognized standards, which generally vary for aromatic plants from 1.4400 to 1.5700 [20]. This index reflects its refraction of light, which could favor its use in cosmetic products. The refractive index essentially varies with the content of monoterpenes and oxygenated derivatives; a high content of monoterpenes will result in a high index [21].

Density: the relative density of the essential oil of *Artemisia judaïca* (0.9315) is lighter than that of water and meets AFNOR standards (0.90-0.94) [22].

4.2. GC/MS results

GC/MS revealed the presence of fifty-four compounds, which represent a total of 94.88% of the compounds, of which four compounds were not identified. The essential oil of *Artemisia judaïca* is rich in monoterpenes (83.52%), of which 82.90% are oxygenated monoterpenes (OM) and 00.62% are hydrocarbon monoterpenes (HM). Sesquiterpenes represent 07.91%, of which 07.31% are oxygenated sesquiterpenes (OSQ) and only 0.60% are hydrogenated sesquiterpenes (HSQ). Other compounds may exist, but they represent only 03.45%. The high content of oxygenated monoterpenes in this essential oil is mainly due to the ketone compounds it contains, the main one being a cyclic ketone, piperitone (73.65%), and its two isomers, 2-hydroxypiperitone (0.31%) and isopiperitone (0.20 %), hence their high toxicity already reported. This result is higher than that in the literature for the same species from other regions of Algeria. Piperitone is accompanied by other minor ketones, chrysanthenone (0.79%), α-isophorone (0.19%), camphor (0.10%), etc. Among the monoterpenols, the presence of α-terpineol (1.23%) and terpinen-4ol (0.35%) can be mentioned. Other phenols (carvacrol (0.27%), isopulegol (0.22%)) are present, but this low content has no impact on the general properties of this plant.

The sesquiterpenes, which are present in relatively low amounts, are essentially oxygenated in nature: cis-davanone (2.84%), davana-ether (1.58%), spathulenol (1.26%), α and β-cadinol (0.92%), among others; these terpenes contribute to the pharmacological properties of the plant.

Santonin was reported to be present at an average of 0.431% by Gherib and Belarbi, 1968 [17], but our results do not mention this substance in the essential oil of this species. Other compounds have been isolated, including aromatic hydrocarbons (benzene, 1, 2,4-trimethyl-, cyclohexane, (1-methylpropyl)-) as well as other trace compounds.

The chemical analysis of the essential oil of this species has been the subject of several studies, including the work of Dob T. and Chelghoum C., 2006 [18], in which the essential oil of *Artemisia judaïca* harvested in In Amenas in southern Algeria yielded 62 compounds by GC-MS, with a predominance of piperitone (61.9%), which is a significant percentage compared to the yield of our plant, followed by terpinen-4-ol (4.6%) and bornyl acetate (3.0%).

In the work of Gherib M., [23], the essential oils of this species harvested in Djebel Antar (Mechria W. Naama), analyzed by gas chromatography (GC) and by nuclear magnetic resonance (NMR) carbon 13 (13C NMR) , showed a predominance of camphor (20.2%) followed by borneol (8.8%) and davanone (7.6%), which is another chemotype for this plant compared to those of Ahaggar and In Amenas since our sample is low in camphor (0.10%) .

4.3. Biological activity: Microbiological determination

The study of the antibacterial activity showed that the essential oil of *Artemisia judaïca* is active against all Gram-positive cocci bacteria (*Staphylococcus aureus*, MIC: 0.06 mg/ml) and Gram-negative bacilli with significant sensitivity to *Escherichia coli* (MIC: 0.125 mg /ml), including *Bacillus subtilis* (MIC: 0.5 mg/ml) and *Pseudomonas aeruginosa* (MIC: 0.5 mg/ml).

From these results, it appears that *Artemisia judaïca* has bactericidal properties against the microorganisms studied. The highest antifungal activity was observed on *Candida albicans* (MFC: 0.25mg/ml), making it a fungicide against this microorganism, which is consistent with the results reported in the literature, particularly those of Cherchari [24] on *Staphylococcus aureus*, but especially on *Candida albicans*.

Other fungi are also sensitive to the essential oil of *Artemisia judaïca*, such as *Enterococcus faecium*. This microbiological activity could be explained by the richness of this oil in oxygenated derivatives (monoterpenes and sesquiterpenes), mainly piperitone. The inhibitory action of the oil is weak for enterobacteria.

4.4. Antioxidant activity

This activity was demonstrated by the DPPH free radical scavenging test, which showed that the essential oil of *Artemisia judaïca* has an antioxidant power that increases with the concentration of the extract, with an efficiency of 2.77±0.05 mg/ml. The β-carotene bleaching test with linoleic acid confirmed the scavenging power of the essential oil; according to this test, the antioxidant activity is significant but lower than that of the control, butylated hydroxytoluene (BHT).

Studies, including those by Liu C.Z., et al. [25], have demonstrated that the crude extract of *Artemisia judaïca* from Egypt has significant antioxidant potential. In general, the species *judaïca*, regardless of its habitat, has antioxidant activity, but this activity varies from one geographical area to another. This antioxidant activity could be exploited for food preservation during storage. However, due to its high content of ketone compounds, *Artemisia judaïca* could be toxic.

5. Conclusion

The Ahaggar, a mountain range in central Sahara, is subject to a dual Mediterranean temperature and Saharan-Arabic tropical climate influence. This environment provides the flora of Ahaggar with certain characteristics, such as higher essential oil content, which is confirmed with *Artemisia judaïca*, whose essential oil content in this work is 3.5%.

The chemotype of this essential oil could be confirmed (essential oil with piperitone 73.65%).

The study of antimicrobial activity showed that this essential oil has an interesting antimicrobial power.

At the end of our study, we can say that the traditional uses of aromatic and medicinal plants in Ahaggar by the local population are justified.Compliance with ethical standards

Compliance with ethical standards

Acknowledgments

We would like to thank all the members of the Pharmacognosy laboratory, the CRD-SAIDAL research and development center as well as the National Office of the Ahaggar Cultural Park (ONPCA), Tamanrasset-Algeria) for their help ; they were invaluable to us in carrying out this work.

Disclosure of conflict of interest

The authors and all co-authors declare that they have no conflicts of interest in relation to this document, and the material described is not in publication or intended for publication elsewhere.

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