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Phytochemical analysis and biological activity of the essential oil of field mugwort: *Artemisia campestris* L. subsp. glutinosa from the Algerian Sahara

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Abstract

This work focuses on the phytochemical study and biological activity (antimicrobial) of the aerial parts of the *Artemisia campestris* collected from the Ahaggar National Park in southern Algeria. After extraction of the essential oil, chromatographic and biological analyzes were carried out on this species.

The results show an average essential oil yield of 0.6±0.02 ml per 100 g of dry plant. Chromatographic analysis of the essential oil of *Artemisia campestris subsp. glutinosa* is rich in monoterpene compounds (50.47%), particularly in hydrogenated forms (40.24% of α and β-pinene) and sesquiterpenes (35.75% of Spathulenol (8.47%), β-Eudesmol (4.67%), and Carvomenthene (3.37%)...

The study of antimicrobial activity revealed a remarkable antifungal activity for *Candida albicans* that could be exploited therapeutically.

Keywords: Essential oil; *Artemesia campestris*; GC/MS; Antimicrobial activity; Candida albicans

1. Introduction

Artemisia campestris is a Mediterranean species that originates from the high plains of the Maghreb. It grows widely in the high plateaus, but more rarely in the pre-Saharan region, and is absent from the northern Sahara, reappearing in the central Sahara mountains up to an altitude of 2000 m. It is quite common in rocky and sandy riverbeds, where it descends quite low in the tropical zone of hot areas of the Hoggar [1-3], but less so in the Tassili des Ajjer [4].

These highly fragrant aerial parts [5] are widely used in traditional medicine as an anti-venom and anti-inflammatory. The leaves are prepared as an infusion or decoction, ground into a powder, and sometimes added to food sauces or porridges as a purifier and regulator of the blood circulation of pregnant or postpartum women, hence the Arabic name "mother of breath", "um nefsa" [1,2].

2. Material and methods

2.1. Plant Material

The aerial parts of *Artemisia campestris L. subsp. Glutinosa* were collected during the month of September for several years from flowering branches in the Ahaggar region at Oued Tizouyeg (65 km north of Tamanrasset and at an altitude of 2095 meters). A voucher specimen was deposited, and after botanical identification, it was placed in the herbarium

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of the National Higher School of Agronomy of Algiers. To prevent putrefaction, the leaves and fruiting branches were carefully separated and dried on paper in a well-ventilated place, protected from light and humidity for ten days at room temperature in the pharmacognosy laboratory. They were then stored in dry containers until use.

Figure 1 *Artemisia campestris* L. subsp. glutinosa : general aspect (D. Boukhalfa)

2.2. Microbiological material

To determine the antimicrobial activity of the essential oil of this plant, five bacterial strains *(Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, and Enterococcus faecium*) and one mold (*Candida albicans*) were selected (Table 1).

The strains used are reference strains (American Type Culture Collection : ATCC) that are sensitive. All strains are pure and derived from the collection of the microbiology laboratory at the Research and Development Center (CRD), SAIDAL. The bacterial strains were maintained by subculturing on nutrient agar suitable for their growth for 24 hours in the dark at 37° C, while the only fungal strain tested was grown on Sabouraud nutrient medium for 24 hours at 37°C.

Table 1 Characteristics of the tested microbial (bacterial and fungal) strains

Names of strains	**N° ATCC**	**Forms**	**Taxon**
Staphylococcus aureus	6538	CG+ Aerobic / Facultative anaerobic	*Micrococcaceae*
Bacillus subtilis	9372	BG+ Strict aerobe	*Bacillaceae*
Pseudomonas aerugenosa	9027	BG- Strict aerobe	*Pseudomonadaceae*
Escherichia coli	4157	BG- Aerobic / Facultative anaerobic	*Enterobacteriaceae*
Enterococcus faecium	6569	BG+ Facultative anaerobic	*Enterococcaceae*
Candida	24433	yeast	*Cryptococcaceae*

2.3. Physicochemical Equipment

2.3.1. Refractometer

The refractometer used to determine the refractive index of the essential oil is a CARL ZEISS instrument, reference 89717.

2.3.2. Hydrodistillation Apparatus:

Standardized apparatus for essential oil extraction in compliance with the European Pharmacopoeia 8th edition.

2.3.3. Physicochemical Analysis Equipment:

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

- Injector: Split-splitless set at 250 °C, injection mode: Split 50:1. Sample is introduced with a micro- syringe.
- Column:
  - Type: Hewlett Packard-5MS, non polar.
  - Dimensions: Length: 30 m, internal diameter (I.D.): 0.25 mm, film thickness: 0.25 µm
  - Stationary phase: 5% phenyl 95% dimethylpolysiloxane.
- • Detector: The detector used is a mass spectrometer (MS) or triple quadrupole mass filter (QQQ) of the HP (Agilent Technologies) MSD 5973 type.

2.4. Extraction and determination of essential oil

The essential oil is stored in tinted glass bottles to protect it from heat and light, which are necessary for its preservation.

Table 2 Operating conditions

Dry weight (gr)	Solvent (ml)	Extraction time
50	Water + glycerine: (250+150)	3 hours

2.5. Physical indices

- Refractive index

The refractive index at 20°C and density are determined by methods conforming to AFNOR standards, 2011 [6].

- Relative density:

This is the ratio of the mass of a certain volume of essential oil at 20°C to the mass of an equal volume of distilled water at 20°C [6].

2.6. Chemical analysis of essential oils :

This involves separation by gas chromatography/mass spectrometry (GC/MS).

- Injector: 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
- Ionization type : Electron impact
- Filament intensity: 70 eV
- Mass analyzer type : Quadrupole
- Quadrupole temperature: 150°C, Source temperature: 230°C
- Oven temperature: programmed to 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.

Each essential oil sample is injected three times, as well as the internal calibration solution containing a mixture of hexanes.

2.7. Biological methods: Method for evaluating antimicrobial activity:

We adopted the disk diffusion method on agar medium using sterile cellulose disks impregnated with essential oil called aromatograms [7].

Determination of minimum fungicidal concentration (MFC):

MFC is the lowest concentration of the essential oil capable of killing microorganisms after 48 hours of incubation to achieve 99.99% destruction of the initial inoculum.

Strains are considered resistant, slightly sensitive, sensitive, very sensitive, and extremely sensitive when the diameters of the inhibition zones are, respectively:

- Resistant strain (- or D ≤ 6mm)
- Sensitive strain (+ or 9mm ≤ D ≤ 14mm)
- Very sensitive strain (++ or 15mm ≤ D ≤19mm)
- Extremely sensitive strain (+++ or D ≥ 20mm), according to the criteria defined in the literature.

It should be noted that all fungal and bacterial tests were repeated three times. Positive (Gentamicin 20mg/ml) and negative (physiological water) controls were also prepared.

3. Results

The essential oil obtained after extraction is a clear yellow liquid with a strong characteristic odor.

3.1. Physical clues

Table 3 Yield, refractive index, and relative density

Plant	Average yield (ml /100 gdm)	IR_n^{x20}	Density (g/ml)
Artemisia campestris subsp. glutinosa	0.60±0.2	1.4768 ±0.0005	0.9344

3.2. Analysis of the chemical composition of the essential oil:

Gas chromatography coupled with mass spectrometry analysis of the essential oil of the Wild Wormwood was performed under the analytical conditions mentioned above..

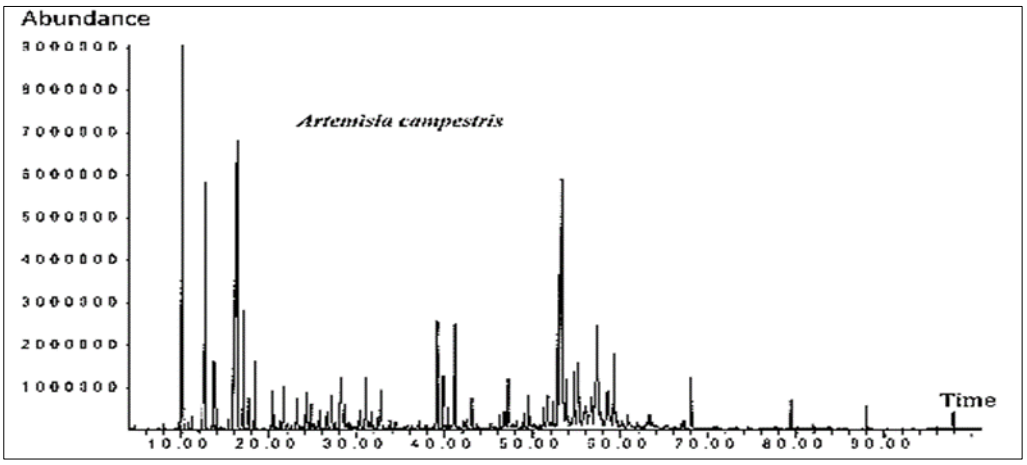

Figure 2 Chromatographic profile of the essential oil *of Artemisia campestri*

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

N°	Identified compounds	TR	IR	IR réf.	%
01	ethanone,1-(2-méthyl-2-cyclopentèn-1-yl)-	07.40	881	-	0.04
02	4- cis-heptanal	07.97	897	893	0.08
03	α-thuyene	09.66	926	924	0.14
04	α-pinene	10.20	935	932	9.96
05	camphene	10.89	946	946	0.13
06	verbenene	11.25	952	967	0.18
07	β-pinene	12.74	977	974	6.06
08	β-myrcene	13.69	993	988	1.14
09	1,9-decadiene	14.04	998	1003	0.34
10	1(7),5,8-o-menthatriene	14.52	1005	1003	0.08
11	α-terpinene	15.34	1017	1014	0.22
12	p- cymene	16.21	1024	1024	9.42
13	α-limonene	16.42	1032	1029	5.51
14	trans-β-ocimene	17.04	1032	-	2.08
15	cis- β-ocimene	17.68	1050	1044	0.48
16	γ-terpinene	18.37	1059	1054	1.10
17	α-terpinolene	20.40	1087	1086	0.70
18	benzene, 1-methyl-2-(1methylethenyl)-	20.65	1088	1086	0.27
19	rose furane	21.17	1098	1095	0.15
20	Perillene	21.41	1102	1102	0.12
21	Linalool	21.69	1106	1107	0.76
22	cis-rose oxyd	22.10	1111	1111	0.21
23	cyclopropane methanol,2-methyl-2-(4-methyl-3-pentenyl)	22.53	1117	1111	0.10
24	α-campholenal	23.21	1126	1122	0.67
25	neo-allo-ocimene	23.50	1130	1128	0.09
26	β-pinone	23.91	1136	1137	0.11
27	trans-pinocarveol	24.26	1141	1141	0.87
28	di hydro linalol	24.78	1148	1134	0.49
29	(+)-pinocarvone	25.72	1161	1160	0.33
30	α-phellandren-8-ol	26.58	1173	1172	0.39
31	terpinen-4 ol	27.07	1180	1179	0.80
32	Cryptone	27.58	1187	1189	0.20
33	p-cymen-8-ol	28.17	1195	1196	1.91
34	Myrtenol	28.56	1201	1202	0.47
35	l-verbenone	29.13	1209	1204	0.19

36	(4R,8R)-8,9-epoxy-p--menth-1-ene	29.77	1218	-	0.12
37	trans-(+)-carveol	30.29	1225	1223	0.47
38	β-citronellol	31.00	1236	1333	1.45
39	cuminaldehyde	31.43	1242	1238	0.16
40	carvone	31.70	1246	1242	0.36
41	piperitone	32.36	1255	1251	0.32
42	p-menth-1-en-7-al	32.74	1261	1276	0.93
43	p-cymen-7-ol	35.43	1299	1287	0.12
44	perilla alcohol	35.74	1294	1295	0.19
45	carvacrol	36.27	1312	1314	0,23
46	cis-3-hexenyl tiglate	37.13	1325	1322	0.18
47	p-mentha-1,4-dien-7-ol	37.84	1336	1333	0.17
48	α- cubebene	38.52	1346	1345	0.06
49	acetate de citronellyl	39.13	1355	1354	2.18
50	acetate de neryle	39.81	1365	1365	1.04
51	α-copaene	40.26	1372	1374	0.40
52	acetate de geranyle	41.14	1385	1383	2.18
53	methyl Eugenol	42.58	1407	1410	0.17
54	β-caryophyllene	43.06	1416	1415	0.60
55	β- cubebene	43.65	1425	1461	0.11
56	α-caryophyllene	45.19	1450	1454	0.14
57	α-amorphene	46.70	1475	1478	0.31
58	germacrene-D	46.92	1478	1484	0.36
59	α-curcumene	47.21	1483	1487	1.02
60	α-muurolene	48.14	1498	1502	0.20
61	γ-cadinene	49.00	1513	1511	0.41
62	δ-Cadinene	49.56	1522	1522	0.74
63	cyclooctene, 4-ethenyl-	50.09	1531	1536	0.14
64	α-calacorene	50.75	1542	1544	0.10
65	nerolidol *(E)*	52.36	1570	1565	0.60
66	(+)-carvomenthene	52.91	1579	-	3.37
67	(+) spathulenol	53.28	1585	1577	8.47
68	Salvialenone	53.82	1595	-	1.37
69	Globulol	54.39	1604	-	0.20
70	10-epi-γ-eudesmol	54.68	1610	-	1.52
71	caryophylene oxide	55.13	1618	1606	2.32
72	γ-eudesmol	56.08	1635	1629	1.43
73	(+)-epi-bicyclosesquiphellandrene	56.70	1646	-	1.43

74	β-eudesmol	57.31	1657	1654	4.67
75	α-bisabolol oxide	57.70	1664	1666	0.78
76	trans-Z-.alpha.-bisabolene epoxide	58.51	1678	-	4.06
77	Valerenol	60.08	1707	-	0.21
78	Farnesol	60.27	1711	1727	0.16
79	2,6-diisopropylnaphthalene	60.80	1721	1728	0.50
80	1H-3a,7-methanoazulène, octahydro- 1,4,9,9-tetra-methyl-	61.90	1742	-	0.21
81	vulgarol A	62.93	1761	-	0.19
82	bicyclo[5.2.0]nonane,4-methylene-2,8,8-trimethyl-2-vinyl-	63.26	1767	-	0.41
83	(3E)-5-isopropylidene-2,7-dimethyl -6-oxa- 1,3,7,10 -undécatetraene	63.58	1774	-	0.24
84	1H-cycloprop[e]azulene, decahydro-1,1,7- trimethyl-4-methylene-, [1aR-(1a.alpha.,4 a. alpha., 7.alpha. 7a. beta. , 7b.alph a.)]-	64.01	1782	-	0.15
85	8 methyltricyclo [5.3.1.0(3,8)]undecane-2,6-dione	66.96	1839	-	0.17
86	6-methyl-2-tridecanone	67.25	1845	-	0.16
87	cis,cis-5,9-tetradécadiene	68.02	1860	-	1.07
88	ethyl ester de l'acide hexadecanoique	74.54	1947	-	0.07
89	heneicosane	79.36	1947	2020	0.44
90	Tricosane	88.02	2297	2300	0.37
91	heptacosane	103.42	2645	2647	0.07
92	tricosane, 2-méthyl-	110.36	-	-	0.11
93	9-hexacosene	116.84	-	-	0.09
94	(s)(+)-Z-13-methyl-11-pentadecen-1-ol acetate	120.97	-	-	0.58
Total (%)					96,07

3.3. Testing for antimicrobial activity

Table 5 Antimicrobial activity of essential oils of *Artemisia campestris*

Micro-organisms	**Qualitative test**			**Quantitative test**
	Diameter of inhibition in mm			
	Essential oils	**Negative Controls (Physiological water)**	**Gentamycin**	**Essential oils**
				MFC
P. aeruginosa ATCC 9027	14	-	37	-
E. coli ATCC 4157	10	-	25	-
S. aureus ATCC 6538	15	-	36	-
E. feacium ATCC 6569	15	-	31	-
C. albicans ATCC 24433	23	-	25	> 2%

4. Discussion

4.1. To. Yield and Physical Indices

The essential oil yield of *Artemisia campestris* from Hoggar is slightly higher than that of the Naama region (0.3% MS) and Djelfa (0.29%) (8). It is almost identical to those of the northern Algerian Sahara (In Amenas (0.70% MS) (9), the Boussaada region (0.66%) (10), and Tunisia (Matmata region (Tunisia, 0.41-0.65%) (11) However, it is low (0.6 ml % DM); when compared to those from Morocco (Ouarzazate region (Morocco) (1.2%) (12).

The refractive index of this oil is low (1.4768 ±0.0005) and complies with AFNOR purity standards [6], which could promote its use in cosmetic products.

4.2. Gas Chromatography-Mass Spectrometry

From a phytochemical point of view, ninety-four compounds were identified, representing 96.07% of the essential oil of *Artemisia campestris*, which is rich in:

- Monoterpenes (50.47%): of which 40.24% are monoterpene hydrocarbons (MH)(α-pinene (9.96%), p-cymene (9.42%), β-pinene (6.06%), α-limonene (5.51%), and β-ocimene (2.56%) and 10.23% are monoterpene oxygenated compounds(MO) (p-cymen-8-ol (1.91%), β-citronellol (1.45%), citronellyl acetate (2.1:8%)...).
- Sesquiterpenes represent 35.75% : of which 18.18% are sesquiterpene hydrocarbons (SQH) (+)-carvomenthene (3.37%), (+)-epi-bicyclosesquiphellandrene (1.43%), α-curcumene (1.02%), ...) and 17.46% are oxygenated sesquiterpenes (SQO) (+) spathulenol (8.47%), β-eudesmol (4.67%), trans-Z-.α-bisabolene epoxide (4.06%)...
- Other compounds include alkanes (heneicosane, tricosane, heptacosane, etc.) and alkenes (1,9-decadiene, 9-hexacosene), etc.

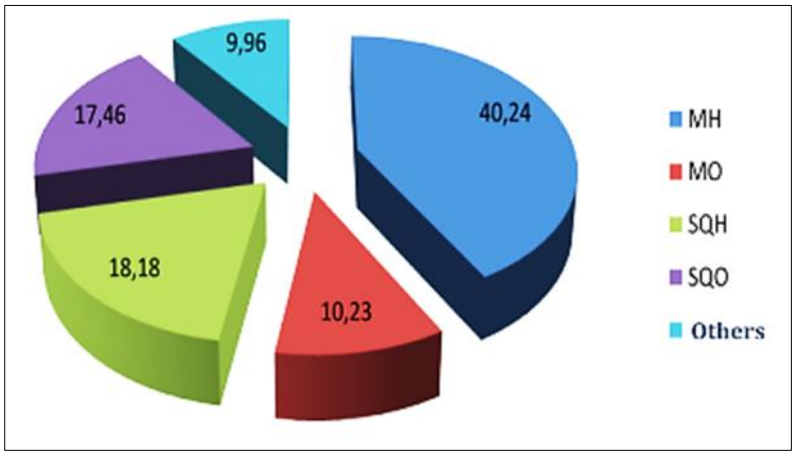

Figure 3 Distribution by chemical class of the essential oil of *Artemisia campestris*

The essential oil of this species showed a richness in hydrogenated monoterpenes with a predominance of the α and β-pinene couple. The other most important compounds are α-limonene, β-eudesmol, and β-ocimene. Several alkanes were also counted, although at relatively low levels (the most important being cis,cis-5,9 tetradecadiene), as well as acids and ester acids (ethyl hexadecanoic acid ester).

Compared to literature data, the essential oil of *Artemisia campestris* from the Ahaggar region is compositionally very different from those of other regions of Algeria, notably Djelfa (8), the latter being particularly rich in spathulenol (58.2%). Other compounds are present in variable amounts, such as caryophyllene oxide, β-caryophyllene, β-guaiene, p-menth-1-en-8-ol, and α-copaene.

It is also different from that of In Amenas (9), whose major compound is (Z, E) farnesol (10.3%), followed by cedrol (5.4%) and verbenone (3.8%).

However, this species is compositionally very close to those of certain regions in Tunisia, Italy (13), and Turkey, with a predominance of the α and β-pinene couple (11,14), such as the essential oil of *Artemisia campestris* from Matmata

(Tunisia). In fact, the α, β-pinene couple predominates, although the proportions are significantly higher compared to our sample, limonene and p-cymene are also present in both species with almost similar amounts.

In general, the compounds in common with all these species are the α and β-pinene couple, spathulenol, α-limonene, β-eudesmol, and β-ocimene.

These variations in yield and chemical composition seem to depend closely on the harvesting, extraction, and separation conditions as well as geographic variations.

4.3. Antimicrobial activity

The essential oil of *Artemisia campestris* is less active against all the studied bacteria and fungi, except for *Candida albicans*.

We observe that the essential oil of *Artemisia campestris* has weak activity against both Gram-positive and Gram-negative bacteria.

Our results seem to be consistent with those found with the essential oil of the same species from southern Tunisia (Matmata) (11) and those of Naili MB and al. (2010) (15), who tested the antibacterial activity of the methanolic extract of *Artemisia campestris* leaves. They found that the extract was more effective against Gram-positive bacteria (*Staphylococcus aureus*) than against Gram-negative bacteria (*Escherichia coli*).

These results tend to justify its use by the local population as an anti-flu and hemostatic agent, but above all as an antiseptic and healing agent during circumcision of children.

Like most Saharan aromatic species, the essential oil of *Artemisia campestris* shows a very pronounced activity against *Candida albicans*.

5. Conclusion

The essential oil of *Artemisia campestris* from Hoggar is mainly composed of hydrogenated monoterpenic compounds (40.24%), with the main ones being α-Pinene (9.96%), p-Cymene (9.42%) and β-Pinene (6.06%). The study of antimicrobial activity revealed strong antifungal activity against *Candida albicans*, which is often involved in digestive and gynecological fungal infections. This oil could indeed find its usefulness in various areas of health applications, particularly in infectiology. These results should be supplemented by more extensive studies to determine the toxicity and possibly the galenic forms of administration of this oil.

Compliance with ethical standards

Acknowledgments

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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.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

Statement of informed consent:

The studies presented in this manuscript do not involve any information on individuals.

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