

CHEMICAL COMPOSITION AND ANTIBACTERIAL
ACTIVITY OF ESSENTIAL OIL OF AERIAL PART OF
Artemisia absinthium L. (ASTERACEAE) FROM ALGERIA

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Abstract

Artemisia absinthium L. is a spontaneous medicinal plant grown in Algeria. This work aims to enhance the essential oil of *Artemisia absinthium* (EOAa) from the region of Sidi Bel Abbes by studying the chemical composition of the species and its antibacterial activity. The essential oil is analyzed by gas chromatography coupled with mass spectrometry. We identified 41 constituents, representing 98.43% of the oil total composition; the main compounds of the essential oil were Camphor (25.47%), followed respectively by β -Thujone (18.23%), Camphene (7.21%), and β -Caryophyllene (6.72%). The antibacterial activity of essential oils was evaluated against five microorganisms using the diffusion disc method.

The gram-negative micro-organisms (*Candida Albicans* ATCC 10231, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) had an inhibition zone with an important diameter, between 19 ± 09 mm and 12 ± 06 mm for concentration (100 mg/ml, 50 mg/ml and 25 mg/ml). Furthermore the gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus* ATCC 11778) were less sensitive since dilution (25 mg/ml).

Key words: *Artemisia absinthium* L., essential oil, chemical composition, GC-MS, antibacterial activity

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Introduction. Spontaneous flora is an important source of bioactive molecules [1]. In Algeria, medicinal plants are still a source of medical care. Several species of the genus *Artemisia* have great commercial and economic importance, used as traditional medicines and in the pharmaceutical industries [2]. More than 250 species of *Artemisia* are distributed worldwide [3]. Eleven species of *artemisia* have been identified in Algeria [4]. *Artemisia absinthium* L., a species of the family asteraceae, commonly called wormwood is a shrubby perennial aromatic and bitter plant with enormous ethno-pharmacological relevance. The native range of this herb are the warm Mediterranean countries restricting its growth to dry waste places. However, it has also been naturalized in certain parts of Asia, North America and Africa [5].

Numerous studies on chemical characterization and biological activities of this species have been carried out. The essential oil has been widely used mainly for its antifungal and insecticidal properties [4]. The study of BORA and SHARMA [6] on a methanol extract confirms that this plant has very interesting antidepressant and antioxidant biological activities. The extracts did not show any toxic effect up to a dose of 1.6 g/kg [6]. This plant is rich in phenolic acid and flavonoids. It is also recommended as a hepatoprotective, hypoglycemic, antiviral, and vermifuge. Our work is the first investigation that aims to determine the chemical composition of the essential oil of *Artemisia absinthium* in the northwest of Algeria and to assess their antimicrobial effects against some pathogenic bacteria.

Materials and methods. Plant material. The plant was harvested in March 2020. The collection site is located about 25 km east of the city of Sidi Bel Abbes (West of Algeria), at 35°10' N latitude and 0°22' W longitude. The leaves of the freshly harvested plants were dried in the shade in a dry and ventilated place, then weighed, coarsely crushed, and collected in paper bags.

Extraction of essential oil. The essential oils of the *Artemisia absinthium* leaves (EOAa) were obtained by hydrodistillation during 3 hours of extraction in a Clevenger type apparatus, according to European Pharmacopoeia [7]. It consists of directly immersing the plant material (200 g) in distilled water (1.5 L). The ascending vapours coming from the reactor progress then condense on cooling. The condensate is collected; then the oil is separated from the aqueous phase and stored at 4 °C.

Analysis of the essential oil. The evaluation of the chemical composition of (EOAa) and the identification of the main components were carried out by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm, 0.25 µm coating thickness) and a Varian Saturn 2000 ions. Analytical conditions were as follows: injector and transfer line temperatures 220 °C and 240 °C, respectively; programmed over temperature ranging from 60 °C to 240 °C at 3 °C/min; helium carrier gas at 1 ml/min; 0.2 µL injection (10% hexane solution); 1:30 division ratio. The compounds were iden-

tified by comparing their mass spectra with those of (Wiley and NIST libraries), and confirmed with data from mass spectrometry [8,9].

Antimicrobial study. Microorganisms. The antimicrobial activity of the EOaA was evaluated against five strains of bacteria, two of them with Gram-positive (*Staphylococcus aureus* **ATCC 25923**, *Bacillus* **ATCC 11778**) and three of them with Gram-negative (*Candida Albicans* **ATCC 10231**, *Escherichia coli* **ATCC 25922**, *Pseudomonas aeruginosa* **ATCC 27853**). The bacterial strains are prepared by transplanting on nutrient medium for 24 h at 37 °C before using them.

Disc diffusion method. Susceptibility to a panel of antimicrobial agents was determined by the standardized disc diffusion assay [10]. Microbial inoculum with a standard 0.5 McFarland turbidity is prepared with a few colonies in a NaCl solution (0.9%) to seed the petri dishes containing the Muller-Hinton agar with a sterile swab.

Then four sterile paper discs (6 mm diameter) impregnated with 20 µl of the different AEOa concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml) are uniformly distributed on the surface of the petri dishes. AEOa dilutions have been prepared with DMSO and another disc soaked with this solvent is deposited in the middle of each petri dish as a negative control. The antibiogram (positive control) was performed with the same technique and with the following marketed antibiotic discs: Trimethoprim 1.25 µg + Sulfamethoxazole (SXT) 23.75 µg, Chloramphenicol (C 30) 30 µg, Clindamycin (CMN2) 2 µg, Penicillin (PEN) 6 µg. After incubating 37 °C petri dishes for 24 h, the diameters of the inhibition zones are measured in mm.

Statistical analysis. Descriptive analysis was performed to describe the entire results of anti-bacterial activity. Then, results were expressed as mean ± SE. ANOVA one-way was used to compare results. Statistical analyses were performed using SPSS v.18 software.

Results and discussion. The extraction yield of EOaA flowering aerial parts was (0.43 ± 0.08)% as v/w. The oil showed an intense dark blue colour. The yield of essential oil for this species varies between 0.3% and 0.57% [4].

Chemical composition. The chemical composition of the EOaA is presented in Table 1. EO analyses by GC-MS shown in Table 1 identify 41 compounds, representing 98.43% of the total oil, with Camphor at 25.47% followed by: β-Thujone 18.23%, Camphene 7.21%, β-Caryophyllene 6.72%, cis-sabinene hydrate 4.58%, Chrysanthenyl acetate 4.36%, Terpinene-4-ol 3.81%, Chamazulene 3.78%, Germacrene D 3.74%, Eucalyptol 2.23%, Thymol 2.04%, Bornyle acetate 1.87%, α-Pinene 1.73%, γ-Terpinene 1.28%, and traces of other compounds. According to our results, the species *A. absinthium* in Algeria has a specific chemotype that is Camphor. The study done by BENKHALED et al. [11] from the region of M'sila (East of Algeria) confirms our results. Using the same analysis method, we found that the oxygenated monoterpenes are the main con-

T a b l e 1
Chemical composition of the EOAs

No	Chemical compounds	RI calculated (DB5)	RI Liturature (Adams 2017)	%
1	α -Pinene	930	932	1.73
2	α -Thujene	923	924	0.39
3	Sabinene	967	946	7.21
4	Camphene	945	954	7.21
5	β -Pinene	975	974	0.11
6	6-Methyl-5-hepten-2-one	972	969	0.27
7	α -Phellandrene	1000	1002	0.18
8	Myrcene	990	988	0.83
9	p-Cymene	1022	1020	1.17
10	α -Terpinene	1013	1014	0.28
11	Eucalyptol	1028	1026	2.23
12	Limonene	1026	1024	0.4
13	Linalool	1093	1086	0.17
14	Terpinolene	1085	1065	0.35
15	cis-Sabinene hydrate	1100	1095	4.58
16	Linalool	1093	1086	0.17
17	β -Thujone	1114	1112	18.23
18	α -Thujone	1101	1101	0.13
19	Camphor	1143	1141	25.47
20	P-menth-2-en-1-Ol	1120	1118	0.12
21	Terpinen-4-ol	1172	1174	3.81
22	Borneol	1167	1165	0.87
23	Nerol	1228	1227	0.12
24	α -Terpineol	1185	1186	0.65
25	Perillaldehyde	1270	1269	0.88
26	Chrysanthenyl Acetate	1262	1261	4.36
27	Thymol	1288	1289	2.04
28	Bornyle acetate	1286	1284	1.87
29	β -Bourbonene	1385	1387	0.12
30	α -Copaene	1372	1374	0.27
31	β -Caryophyllene	1419	1419	6.72
32	Methyl eugenol	1401	1403	0.27
33	Germacrene D	1483	1484	3.74
34	α -Caryophyllene	1419	1419	6.72
35	γ -Cadinene	1513	1513	0.53
36	α -Farnesene	1507	1505	0.16
37	Elemol	1545	1548	1.33
38	δ -Cadinene	1522	1522	0.11
39	Chamazulene	1728	1730	3.78
40	β -Eudesmol	1650	1649	0.19
41	Caryophyllene oxide	1584	1582	0.11
	Total			98.43

T a b l e 2
Sensitivity of the strains tested to EOAA and antibiotics as mean value $\pm$ standard deviation

Concentration of HE	Diameter of inhibition in (mm) <i>Artemisia absinthium</i>							
	100 mg/ml	50 mg/ml	25 Mg/ml	12.5 mg/ml	CMN	SXT 25	C 30	PEN
<i>Candida albicans</i>	17 $\pm$ 0.9*	17 $\pm$ 1.2*	12 $\pm$ 0.6*	–	29 $\pm$ 5.10*	30 $\pm$ 1.26*	22 $\pm$ 0.50*	35 $\pm$ 1.03*
<i>Staphylococcus aureus</i>	14 $\pm$ 0.6*	7 $\pm$ 1.5*	7 $\pm$ 0.6*	–	29.3 $\pm$ 1.2**	21 $\pm$ 0.29**	22 $\pm$ 0.90**	34 $\pm$ 2.25**
<i>Escherichia coli</i>	17 $\pm$ 0.6*	17 $\pm$ 0.9*	12 $\pm$ 1*	–	30 $\pm$ 0.85**	27 $\pm$ 1.26**	25 $\pm$ 0.71**	35 $\pm$ 0.50**
<i>Bacillus</i>	18 $\pm$ 1**	12 $\pm$ 0.6**	8 $\pm$ 1.4**	7 $\pm$ 0.6**	19 $\pm$ 0.52**	30 $\pm$ 1.32**	20 $\pm$ 0.45**	23 $\pm$ 1.31**
<i>Pseudomonas aeruginosa</i>	19 $\pm$ 0.9*	19 $\pm$ 1.5*	12 $\pm$ 1.5*	9 $\pm$ 0.8*	25 $\pm$ 0.52**	33 $\pm$ 1.32**	28 $\pm$ 0.45**	44 $\pm$ 1.31**

*: Significant effects

**: Very significant effects

stituents representing 59.64% of the total oil with 19 components. Camphor with 47.59% was quantitatively the main element of this chemical class and total EO. We conclude that the same chemotype exists in northern Algeria, from East to West.

Each geographic region has its chemotype, eucalyptol 25.59%, linalool 11.99%, and β -myrcene 10.05% were identified as major constituents of EOAA growing in Xinjiang province of China [12]. EOAE from France mainly contains Z-epoxyocimene and chrysanthenyl acetate, while Croatian oils from *A. absinthium* are known by the Z-epoxyocimene/ β -thujone chemotype [13]. MORTEZA-SEMNANI and AKBARZADEH [14] reported that β -thujone (35.1%), p-cymene (16.5%), were abundant in the EOAA growing in Iran.

Antibacterial activity. It reveals the sensitivity of the bacteria tested to different antibiotics and indicates that the EOAA of the plant studied has a remarkable action after 24 h, compared to the action of antibiotics as indicated in Table 2. The results obtained show that the EOAA exhibits an antibacterial effect against the strains tested. The Gram-positive bacteria are sensitive to the EOAA; where the diameter of the zone of inhibition for *Staphylococcus aureus* ATCC 25923 is 7 to 14 mm, and for *Bacillus* ATCC 11778 is 8 to 18 mm. As well as to Gram-negative bacteria, where the diameter of the zone of inhibition for *Escherichia coli* ATCC 25922 is 12 to 17 mm, and for *Pseudomonas aeruginosa* ATCC 27853 is 12 to 19 mm. The antibacterial activity varies according to the components constituting the essential oil. Many researchers have reported that Camphor, as a considerable constituent of EOAA, has a strong antibacterial activity against Gram-positive bacteria [15]. Subsequently, the richness present in Camphor in the essential oil may explain the good antibacterial effect that *Artemisia absinthium* had in this study. This compound is also an excellent treatment for candidiasis caused by fungi and against contamination of medical devices [16]. It can be used to prepare powerful fungistats [17] which was confirmed by our results on *Candida albicans* where the diameter of the zone of inhibition was 12 to 17 mm. Other works on the antimicrobial activity of OAEa in different study areas have recorded remarkable results similar to ours; such as those of MSAADA et al. [18] and EREL et al. [19] where the following inhibition zone diameters were obtained: *Staphylococcus aureus* (18 to 25 mm), *Listeria monocytogenes* (17 to 20 mm), *Fusarium culmorum* (23 to 46 mm), *Fusarium oxysporum* (17 to 27 mm) and *Staphylococcus aureus* (18 mm), *Candida albicans* (23 mm), respectively.

Conclusion. The present study revealed the composition of the hydro-distilled EOAA cultivated in the region of Sidi Bel Abbes and determined by GC and GC/MS. Forty-one components representing 98.43% of the essential oil have been identified. The main components are Camphor, Camphene, and β -Thujone which is well confirmed by the results obtained in other regions. The different chemical composition contents of essential oils of the same species could be due to several factors, such as climate, soil, seasonal variations, and the method used

for essential oil extraction [20]. Due to its main components, the essential oil has shown good antibacterial activity. This biological effect is largely due to the high Camphor content of this wormwood.

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