



GC-MS Analysis and Antibacterial Activity of the Essential Oil Isolated from Wild *Artemisia herba-alba* Grown in South Jordan

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Authors' contributions

This work was carried out in collaboration between all authors. Author JAS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SAS, YAS and AAQ helped in data collection and managed the analyses of the study. Author MA and IA performed the statistical analysis, literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: There is a high variability in chemical composition of essential oil from *Artemisia herba-alba* grown in different countries and different localities in the same country. This has led to the characterization of many oil-dependent chemotypes assigned to the plant. Only one report was published on the essential oil composition of *Artemisia herba-alba* grown in Jordan.

Aim: The current study aims to determine the essential oil composition of *Artemisia herba-alba* grown wild in south Jordan and test their activity against clinical isolate antibiotics resistant bacteria.

Methodology: The essential oils were isolated by hydrodistillation and analysed by Gas Chromatography-Mass Spectrometry (GC-MS). The screening for essential oil activity was carried out using disc diffusion method on methicillin-resistant *Staphylococcus aureus*, methicillin-sensitive

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Staphylococcus aureus, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.

Results: Fifty-eight components accounting for 98.8% of the oil were identified, with oxygenated monoterpenes accounting for about 75% of the total oil content. Major identified compounds were cis-chrysanthenol (13.83%), 1,8-cineole (12.84%), cis-limonene (12.57%), α -terpinenol (6.97%), and γ -muurolene (4.50%). The volatile fractions exhibited potent activity against all resistant strains except *Pseudomonas aeruginosa*.

Conclusion: We report here a new chemotype of *Artemisia herba-alba* grown in Jordan characterized by the presence of chrysanthenol, 1, 8-cineole, cis-limonene, and α -terpinenol.

Keywords: Chemotypes; oxygenated monoterpenes; chrysanthenol; 1, 8-cineole.

1. INTRODUCTION

Essential oils are volatile, natural compounds characterized by strong odor that are usually obtained by steam or hydro-distillation. Essential oils are very complex natural mixtures which can contain between 20 to 60 components although 2 to 3 components are found at higher concentrations (20-70%). These major components that determine the biological properties of the essential oil fall into two groups having different biosynthetic origins. The main group is composed of terpenes and the other is composed of aromatic and aliphatic compounds, all characterized by low molecular weight [1,2].

The genus *Artemisia* comprises more than 300 species. *Artemisia herba-alba* Asso (syn. *A. inculta*), known as desert wormwood, is a dwarf shrub growing wild throughout the Mediterranean basin and extending into the North-western Himalayas [3].

Artemisia herba-alba has been used in traditional medicine in Jordan for treatment against constipation, hypercholesterolemia, jaundice, abdominal pains, diabetes, parasitic worms, flatulence, inflammations, common cold, kidney sand and stones [4]. The strong and aromatic smell of some species of *Artemisia* genus is mainly due to high concentrations of volatile terpenes. Many studies have shown that *Artemisia* species display significant variations in terpene constituents of their essential oils. The quality and yield of essential oils from *Artemisia* species is influenced by the harvesting season, fertilizer and pH of soils, choice and stage of drying conditions, geographic location, chemotype, choice of plant part or genotype and extraction method.

Large variability in essential oil composition of the aerial parts of *Artemisia herba-alba* has been reported [5,2]. Diversity in oil composition from

plants grown in different countries and from different localities in the same country has led to the assignment of many oil-dependent chemotypes to the plant [6]. For example, in Morocco seven chemotypes have been reported with monoterpenes as being the major components followed by sesquiterpene. The chemotypes are characterized by the presence of one major component such as camphor (40-70%), α - or β -thujone (32-82%), chrysanthenone (51-70%), chrysanthenyl acetate (32-71%), or davanone (20-70%). One chemotype is characterized by the presence of camphor (34-55%) and α -thujone (26-37%) [7]. Data from Spain showed that monoterpene hydrocarbons and oxygenated monoterpene were the most abundant components in *Artemisia herba-alba* oil. Large amounts of sesquiterpenes were found for some populations with camphor, 1,8-cineole, *p*-cymene and davanone being the major components found [8,9].

In Jordan oxygenated monoterpenes were found to be the major oil components (39.3% of the oil), with α - and β -thujones as being most predominant (24.7%). The other major identified components were: santolina alcohol (13.0%), artemisia ketone (12.4%), and *trans*-sabinyl acetate (5.4%) [6]. In Tunisian oil oxygenated monoterpenes were found to be the major components of *Artemisia herba-alba* oil extracted from aerial parts. The main compounds were β -thujone and α -thujone, 1,8-cineole, camphor, chrysanthenone, *trans*-sabinyl acetate, *trans*-pinocarveol and borneol [5,10]. In the essential oil of *Artemisia herba-alba* growing in Pakistan, chrysanthenyl propionate and elixene were identified for the first time for any *Artemisia* species [11]. Two oil types were found for plants grown in Sinai Peninsula namely cineol-thujanebornane type and the pinane type. The essential oil of all studied populations were found to contain 1,8-cineole although in varying concentrations [12]. More recently, it has been

reported that a further five chemotypes could be distinguished in plants growing in Sinai based on variation in composition of the oil, suggesting the existence of a greater number of chemotypes in the region. For Algerian oil, monoterpenes were the major components with the main compounds being camphor, α - and β -thujones, 1,8-cineole and chrysanthenyl derivatives [13,2].

The chemical composition of the oil of *Artemisia herba-alba* grown in south Jordan has never been reported. The aim of the present study was to report on the chemical composition of the essential oil from the aerial parts of *Artemisia herba-alba* grown in southern Jordan and its biological activity.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plants

Fresh amount of the *Artemisia herba-alba* was collected from Mutah, Alkarak, south Jordan, during the flowering period and the vegetative phase. The plant materials were taxonomically identified and authenticated by the Botanical Survey of Yarmouk University.

2.2 Isolation of Essential Oil

A fresh aerial parts (200 g) of *Artemisia herba-alba* was finely chopped and subjected to hydrodistillation for 4 h using a Clevenger-type apparatus, yielding 0.24% (v/wt), pale yellowish oil. Subsequently, oil was dried over anhydrous sodium sulfate and immediately stored in GC-grade hexane at 4°C until the analysis by gas chromatography/mass spectrometry (GC/MS) was done.

2.3 Essential Oil Composition

2.3.1 GC-FID analysis

The oils were analyzed in an Agilent (Palo Alto, USA) 6890N gas chromatograph fitted with a 5% phenyl-95% methylsilicone (HP5, 30 m \times 0.25 mm \times 0.25 μ m) fused silica capillary column. The oven temperature was programmed to run from 60°C to 240°C at 3°C/min with hydrogen being used as the carrier gas (1.4 mL/min). 1.0 μ L of a 1% solution of the oils in hexane was injected in split mode (1:50). The injector was kept at 250°C and the flame ionization detector (FID) was kept at 280°C. Concentrations (% contents) of oil

ingredient for *Artemisia herba-alba* were determined using their relative area percentages obtained from GC chromatogram, assuming a unity response by all components.

2.3.2 GC-MS analysis

Chemical analysis of the essential oils was carried out using gas chromatography-mass spectrometry (Agilent (Palo Alto, USA) 6890N gas chromatograph). The chromatographic conditions were as follows: column oven program, 60°C (1 min, isothermal) to 246°C (3 min, isothermal) at 3°C/min, the injector and detector temperatures were 250°C and 300°C, respectively. Helium was the carrier gas (flow rate 0.90 ml/min) and the ionization voltage was maintained at 70eV. A HP-5 MS capillary column (30 m \times 0.25 mm i.d., 0.25 μ m film thicknesses) was used. A hydrocarbon mixture of *n*-alkanes (C₈-C₂₀) was analyzed separately by GC-MS under same chromatographic conditions using the same HP-5 column. Kovats Retention Indexes (KRIs) were calculated by injection of a series of *n*-alkanes (C₈-C₂₀) in the same column and conditions as above for gas chromatography analyses.

Identification of the oil components were based on computer search using the library of mass spectral data and comparison of calculated Kovats retention index (KRI) with those of available authentic standards and literature data.

2.4 Maintenance and Preparation of Cultures

Eight clinical isolates antibiotics resistant bacteria were used in this study. Four strains of Gram positive bacteria: Methicillin-resistant *Staphylococcus aureus* (MRSA), Methicillin-sensitive *Staphylococcus aureus* (MSSA), *Staphylococcus epidermidis*, *Streptococcus pyogenes*, and four strains of Gram negative bacteria: *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*, were studied. Isolates were purified on specific nutrient agar plates and characterized by standard microbiological and biochemical methods like Gram stain, catalase test, coagulase test and an API system (bioMerieux, France).

The bacteria were incubated at 37°C for 24 h by inoculation into broth. Inoculums (1 mL) per plate containing 10⁶ bacterial cell/ ml were spread on

Mueller Hinton agar (Oxoid, Hampshire, England).

2.5 Disc Diffusion Assay

The antibacterial activity of the *Artemisia herba-alba* essential oil was determined by the disc diffusion method according to the National Committee for Clinical Laboratory Standards. Sterile paper discs of 6 mm in diameter were impregnated with 5 μ L essential oil and deposited on the agar surface. Petri dishes were placed at 4°C for 2 h to facilitate the dissemination of extract on the culture medium followed by incubation at 37°C for 24 h. For each sample, negative water control and positive antibiotic disc control were used. At the end of the period, inhibition zones formed on the medium were evaluated in mm. Studies were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of the Essential Oil

Hydrodistillation of the aerial parts of the *Artemisia herba-alba* sample gave a pale yellowish oil with a yield of 0.24%. The chemical composition of the oil was investigated using GC-MS techniques. The identified components of the essential oils, their percentages and retention indices are given in Table 1. Fifty-eight components accounting for 98.8% of the oil were identified with oxygenated monoterpenes as being the major constituent (accounting for about 75% of the total oil content). The major identified compounds were *cis*-chrysanthenol (13.83%), 1,8-cineole (12.84%), *cis*-limonene (12.57%), α -terpinenol (6.97%) and γ -muurolene (4.50%). Other oil component included: α -terpinyl acetate (3.16%), isobornyl acetate (3.15%), germacrene D (2.94%), iso-menthyl acetate (2.23%), γ -terpinene (2.18%), *trans*-thujone (1.98%), neoiso-isopulegyl acetate (1.98%), camphene (1.90%), neryl acetate (1.79%), terpinen-4-ol acetate (1.67%), carvenone (1.58%), Z-jasmone (1.54%), α -terpinene (1.38%), neo-verbanol (1.30%), α -thujplicin (1.15%), and perilla aldehyde (1.04%).

Table 1. Constituents (%) of the essential oil of *Artemisia herba-alba* grown in south Jordan

K.I	Compound	%A
935	α -Pinene	0.43
956	Camphene	1.90
976	Sabinene	0.58
1021	α -Terpinene	1.38
1031	o-Cymene	0.82
1040	1,8-Cineole	12.84
1063	γ -Terpinene	2.18
1092	Para-Mentha-2,4(8)-diene	0.39
1118	<i>trans</i> -Thujone	1.98
1131	<i>cis</i> -Limonene	12.57
1135	<i>cis</i> -para-Mentha-2,8-dien-1-ol	0.52
1153	<i>cis</i> -Sabinol	0.93
1163	<i>cis</i> -Chrysanthenol	13.83
1176	Umbellulone	0.45
1186	neo-Verbanol	1.30
1194	α -Terpinenol	6.97
1208	γ -Terpinenol	0.94
1251	Carvenone	1.58
1254	<i>cis</i> -Chrysanthenyl acetate	0.71
1259	<i>trans</i> -Ascaridol glycol	0.78
1272	Perilla aldehyde	1.04
1276	<i>trans</i> -Carvone oxide	0.36
1283	Isobornyl acetate	3.15
1287	<i>trans</i> -Sabinyl acetate	0.39
1296	Terpinen-4-ol acetate	1.67
1302	Iso-menthyl acetate	2.23
1313	neoiso-Isopulegyl acetate	1.98
1323	Z-Patchenol	0.67
1329	neo-Verbanol acetate	0.53
1339	Myrtenyl acetate	0.32
1351	alpha-Terpinyl acetate	3.16
1358	neoiso-Dihydro carveol acetate	0.16
1363	Neryl acetate	1.79
1374	α -Copaene	0.24
1380	<i>trans</i> -Myrtenol acetate	0.31
1396	E-Jasmone	0.10
1398	Methyl cinnamate	0.18
1407	Z-Jasmone	1.54
1415	alpha-Thujplicin	1.15
1423	E-Caryophyllene	0.46
1433	<i>cis</i> -Thujopsene	0.26
1457	E-beta-Farnesene	0.54
1460	Sesquisabinene	0.46
1466	7-epi-1,2-Dehydro Sesquicineole	0.25
1473	Isobornyl n-butanoate	0.23
1475	Gernayl propanoate	0.12
1476	<i>trans</i> -4,10-epoxy-amorphane	0.51
1480	γ -Muurolene	4.50
1488	Germacrene D	2.94
1505	Bicyclogermacrene	0.65
1510	α -Chamigrene	0.41
1562	E-Nerolidol	0.63
1567	Geranyl butanoate	0.35
1585	Caryophyllene oxide	0.24
1610	Khusimone	0.18
1624	Z-Bisabolol	0.73
1639	E-Sesquilandulol	0.77
1725	Farnesol	0.49
	Total	98.80
	Monoterpene hydrocarbons	7.68
	Oxygen-containing monoterpenes	75.04
	Sesquiterpene hydrocarbons	10.46
	Oxygen-containing sesquiterpenes	3.80
	Others	1.82
	Total	98.80

A study conducted by Hudaib and Aburjai [6] on the chemical composition of *Artemisia herba-alba* growing in south Amman Jordan, it was found that oxygenated monoterpenes were the major oil components (39.3% of the oil), with α - and β -thujones as being the principal components (24.7%). The other identified components as shown in Table 2 were: santolina alcohol (13.0%), artemisia ketone (12.4%), and α -thujone (16.2%) [6]. To the best of our knowledge, this study was the only one to report on the chemical composition of *Artemisia herba-alba* essential oil grown in Jordan. We report here the existence of new chemotype of *Artemisia herba-alba* grown in Jordan. This new chemotype is characterized by the presence of chrysanthenol, 1,8-cineole, *cis*-limonene, and α -terpinenol. This combination of components may also represent a new chemotype of *Artemisia herba-alba*.

3.2 Antimicrobial Activity

The results in Table 3 show the activity of *Artemisia herba-alba* essential oil against some

clinical antibiotic resistant bacteria. The results show a good activity of the oil against *E. coli* a moderate activity against Methicillin-resistant *Staphylococcus aureus*, Methicillin-sensitive *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Proteus mirabilis* while *Pseudomonas aeruginosa*, remained resistant. The essential oil of *Artemisia herba-alba* showed no specificity to Gram-positive or Gram-negative bacteria.

4. CONCLUSION

We report here a new chemotype of *Artemisia herba-alba* growing in Jordan characterized by the presence of chrysanthenol, 1,8-cineole, *cis*-limonene, and α -terpinenol as a major ingredients. The results show a good activity of the oil against all tested pathogens except for *Pseudomonas aeruginosa* that remained resistant.

Table 2. Percentage composition of the major oil obtained from *Artemisia herba-alba* from Jordan obtained by Hudaib and Aburjai

Compound	Percentage
santolina alcohol	13.0
artemisia ketone	12.4
α -thujone	16.2
β -thujone	8.5
trans-sabinyol acetate	5.4
caryophyllene acetate	5.7

Table 3. Antibacterial activity of *Artemisia herba-alba* essential oil grown in south Jordan

Name of Bacteria used	Zone of inhibition of essential oil in mm	Antibiotic used	Zone of inhibition by antibiotic in mm
MRSA	7 \pm 0.1	Vancomycin	19 \pm 0.8
MSSA	8 \pm 0.3	Vancomycin	18 \pm 0.3
S.epid	9 \pm 0.2	Vancomycin	21 \pm 1.1
Strep. Pyog	12 \pm 1.0	Clindamycin	26 \pm 0.9
<i>E. coli</i>	18 \pm 1.0	Chloramphenicol	28 \pm 1.2
Klebsla. pneu	11 \pm 0.3	Chloramphenicol	23 \pm 0.8
Pseudo. aeru	9 \pm 0.2	Ceftazidime	25 \pm 1.1
Prot. <i>mirabilis</i>	2 \pm 0.1	Ampicillin	14 \pm 0.8

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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