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Essential Oil Composition of Sinai *Artemisia monosperma* and the Biological Activity

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Abstract *Artemisia* spp. exhibit several biomedical and pharmaceutical applications as the anti-inflammatory in malaria treatment, in reducing the withdrawal syndrome of morphine, anthelminthic drug and as natural antimicrobial agents that could be used in the preservation of foods as natural additives. Artemisia spp (Asteraceae) contains valuable secondary metabolites and could be used in many targets. Oil of the fresh aerial parts (include stems and leaves) of *Artemisia monosperma*, grown in El Arish city (the capital of the Egyptian governorate of North Sinai, as well as the largest city on the entire Sinai Peninsula), was obtained by hydrodistillation. The chemical composition of the isolated oil was characterized by gas chromatography/mass spectrometer (GC/MS). Forty-five compounds were identified. *A. monosperma* oil was rich in biphenyl (51.5%), a substance prevents the growth of molds and fungi, and is therefore used as a preservative with maximum peak area of (51.5%), besides hydrocarbon curcumin (9.45 %) peak area, (7.35%) of 2-á-pinene, (2.55%) of (-)-Spathulenol, and, (3.45 %) of 1,**3,**-dimethyl-2-(1-methylethylidene)imidazolidinewere identified. Other minor components were, γ-terpinene, methyl eugenol, limonene, α-cymene, myrcene, α-pinene, Sabinenebicyclic monoterpene, and ocimene monoterpenes. The presence of significant amounts of terpenes greatly suggests its preferable use as flavoring agents in foods.

Keywords *A. monosperma*, GC/ MS, biphenyl, curcumin, sesquiterpene

Introduction

Medicinal plants have been used for a long time to treat human diseases because of their essential oils chemical components and the antioxidant activities [1]. There was a great interest in exploring antimicrobial properties of extracts from natural flora essential oils [2-3]. A World Health Organization in 2008 declared that above 80% of the world's population depend on herbal medicine [1]. *Artemisia L.* is a genus of small shrubs, mostly perennial herbs, in family Compositae (Asteraceae), which includes about 1,000 genera and over 20,000 species. Within this family, *Artemisia* is included in the species Anthemideae, which are generally found in North America, Asia, and Europe.

Artemisia Essential oils possessed antibacterial, antifungal, antiviral, insecticidal and antioxidant properties [4-5]. Some oils used in cancer [6] and El Zheimer treatment, others used in food preservation [7], and aromatherapy [8] as main sources of biologically active compounds. Therefore, their antimicrobial and antibiotic potential are expected [9]. However, *Artemisia monosperma* caused a direct harm to the pregnant rats, therefore, taking the leaves of the plant by pregnant women must be avoided [10].

Artemisia spp. In particular are used for the treatment of many diseases such as malaria, hepatitis, inflammation, and infections by fungi, bacteria, and viruses [11], Some *Artemisia* components used in manufacturing insecticides and allelopathic chemicals. The relationship between the antimicrobial activity and the chemical composition of the isolated oils undergo several investigations. Recently, 3,000 essential oils are recorded, only about 300 of which are significant in the fields of pharmaceutical, agricultural, food [12] sanitary, cosmetics, and perfume industries. Essential oils or more precisely, their certain components are used in perfumes and cosmetics products, in sanitary products, in dentistry, in agriculture, as food preservatives and additives and as natural flavors. The essential oil consists of mixture of terpene (mono-, sesqui- and diterpenes), esters, epoxides, aldehydes, ketones, amines, and sulfides. Their antimicrobial or biological activities are strictly related to the presence of these bioactive volatile components.

When plant volatile oils were taken out either by steam or hydrodistillation, this mixture of low molecular weight aliphatic hydrocarbons (linear, branched, saturated and unsaturated), homologues of phenylpropanoids, acids, alcohols, aldehydes, acyclic esters or lactones , coumarin and homologues of phenylpropanoids, were detected [13]. Monoterpenes [C10] and sesquiterpenes [C15], although diterpenes [C20] may also be present. Terpenes cause the medicinal, and fragrant effect of medicinal plantsMost terpenes obtained from the combination of branched five-carbon isoprene units and are systemized according to the number of these units incorporated within the carbon structure [13].

Artemisia monosperma contains volatile oil, flavonoids alkaloids, and coumarins [14]. Several studies using *Artemisia* spp. oil showed their noticeable effect on antimicrobial and antioxidant activities. So, these plants secondary metabolites are applied in the cosmetic, food [15-16] pharmaceutical industries, and in medical stuff, Cai and Wu 1996.

The essential oil of *A. monosperma* was tested for antimicrobial activity against *Agrobacterium* tumefaciens and *Erwiniacarotovora* var. *carotovora* plant pathogenic bacteria, and was the most powerful inhibitor against *Alternaria alternata*, *Botrytis cinerea*, *Fusarium oxysporum*and *Fusarium solanI* fungi, and was found to decline spore germination percentage of these fungi [17].

Materials and Methods

Plants Material

Aerial parts of *Artemisia monosperm* were collected from El Arish, Sinai collected in February 2017 allowed to dry under shadow away from direct sunlight at room temperature. Then the dried parts were ground into fine powder with a mechanical grinder and collected in airtight containers individually .The plant was identified and confirmed taxonomically by Dr. Eweis, Department of botany, Cairo university, Egypt. Samples were prepared under laboratory conditions before hydrodistillation.

Extraction of Essential Oils from Artemisia Cultivars

The aerial parts of *Artemisia monosperma* were hydrodistilled for 3 h, using a Clevenger-type apparatus, according to the European Pharmacopoeia method (Council of Europe, 2007). The essential oils were stored at -20oC in the dark until gas chromatography-mass spectrometry (GC/MS) analysis.

Analysis of Essential Oil

Gas Chromatography/ Mass Spectrometery

The GC/MS analysis of the essential oil samples was carried out using gas chromatography-mass spectrometry instrument stands at the Laboratory of Medicinal and Aromatic Plants Research, National Research Centre with the following specifications. Instrument: a trace GC ultra gas chromatographs (THERMO Scientific Corp., USA), coupled with a thermos mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC/MS system was equipped with a TR5 MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min at a split ratio of 1:10 and the following temperature program: 60°C for 1 min; rising at 4.0 C/min to 240°C and held for 1 min. The injector and detector were held at 200 and 240°C, respectively. Diluted samples (1:10 hexane, v/v) of 1 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450.Most of the compounds were identified using two different analytical methods: mass spectra (authentic chemicals, Wiley spectral library collection, and NSIT library The identification of the compounds was achieved on the basis of retention time, Kovats Index, by comparing with the mass spectral literature data retention index reported in the literature (based on the same stationary phase)

Results and Discussion

Essential oil composition of *A. monosperma*

The GC/MS analyses of the *A. mmonosperma* essential oil identified 46 compounds, (Table 1). From the data obtained, the essential oil detected is complex mixture of several components, predominating biphenyl. Retention time (Rt) and absorbance allowed the identification of almost all individual compounds detected, (Fig. 1).

GC/ MS analyses of *A. monosperma* essential oil allowed chemical characterization of forty-five compounds,

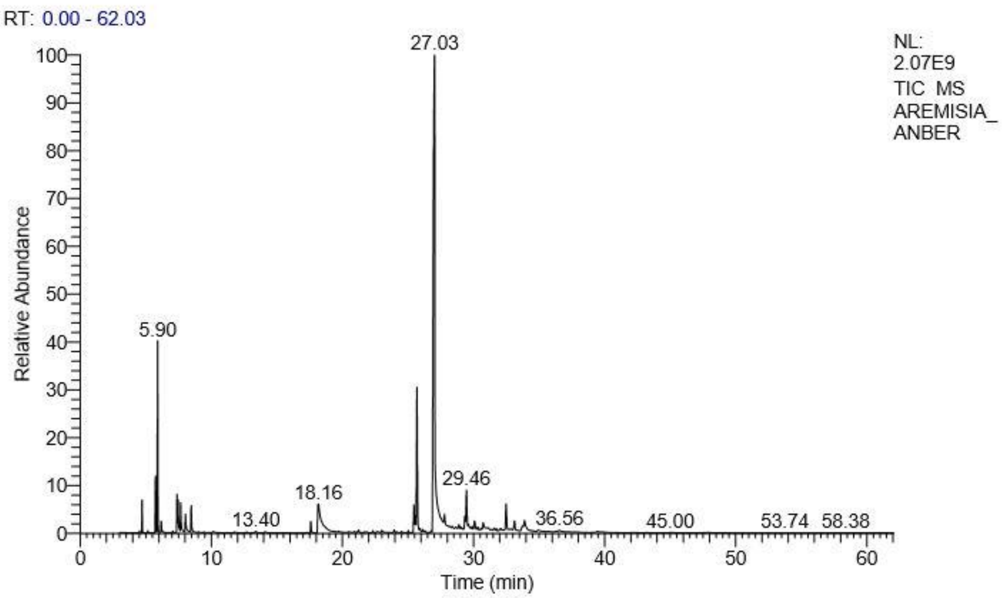

Figure 1: GC/MS Chromatogram of Sinai Artemisia monosperma essential oil

From the data obtained in table 1, the essential oil is a complex mixture of many components, with the highest percentage of, biphenyl, monoterpene and sesquiterpenes. The identified compounds abundance and their Retention time values are presented in table 1. The essential oils from *A. monosperma* composed of the monoterpene with peak area of pinene (1.24%), for b-myrcene (0.65%), sesquiterpene hydrocarbon sesquphelandrene (0.22 %). However, 30 trace components were detected. The role of the trace components could not be ignored in the biological activity. This data showed that sesquiterpene hydrocarbons and oxygenated monoterpenes are present in the essential oil of *A. monosperma*. General speaking, the antimicrobial activities are due to C10 and C15 terpenes with aromatic rings and phenolic hydroxyl groups that are able to form hydrogen bonds with active sites of specific enzymes, although alcohols, aldehydes, and esters are causative for overall antimicrobial effect of essential oils [18].

On the other hand, optical isomers of à-pinene, à-pinene, limonene, and linalool have a marked antibacterial activity [19-21]. Similarly, Pinene-type monhydrocarbons (à-pinene and à-pinene) are well-known chemicals having antimicrobial potentials [13].

Data presented in table 1 showed plant volatiles component, The interaction between components of plant volatiles in structural form, and functional groups of the constituent resulted in oil activity, comes in agreement with findings of [13, 22]. Terpenes have known as remarkable antimicrobial agents against, both Gram-positive, Gram-negative bacteria and fungi [23]. Several monoterpenes have antibacterial properties in many reports. For example, eugenol inhibits about thirty *Helicobacter pylori*strains [24]. Chemical structures of monoterpenes have the effect on various pharmacological properties [25]. Studies literature expresses that volatiles in total represents better antibacterial activity than the specific components. It also shows that minor components have a possible effect on the activity [26-27].

Table 1: Chemical components of Sinai *Artemisia monosperma* essential oil

S. No.	RT	% Area	Peak area	Compound name
1	4.71	1.04	310669681.05	à-Pinene
2	5.47	2.04	613078080.27	Sabinene
3	5.90	7.37	2208973057.06	2-á-Pinene
4	6.17	0.65	194795817.92	á-Myrcene
5	7.38	1.58	474291414.23	Benzene, 1-methyl-4-(1-methyl ethyl)(CAS)
6	7.46	1.43	428269544.10	l-Limonene
7	7.55	0.24	73300758.27	ç-Terpinene
8	7.66	1.45.	435739760.87	cis-Ocimene
9	8.03	1.15	344322235.61	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-
10	8.47	1.40	418892014.90	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)
11	10.12	0.16	49464007.95	LINALOOLL
12	13.40	0.20	59346703.84	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)
13	17.59	0.67	201132773.58	Bornyl acetate
14	18.14	3.45	1034109597.51	1,**3,**-dimethyl-2-(1-methylethylidene)imidazolidine
15	21.23	0.22	66731771.32	Camphene (CAS)
16	21.71	0.15	44402996.02	à-Copaene
17	22.32	0.16	47952961.42	à-Gurjunene (CAS)
18	22.63	0.16	49287384.85	Benzene, 2,5-cyclohexadien-1-yl-
19	23.00	0.19	57555160.76	Methyleugenol
20	23.94	0.22	64587345.53	á-Sesquiphellandrene (CAS)
21	25.02	0.15	45816304.95	Caryophyllene oxide
22	25.45	1.56	466545416.41	á-Himachalene (CAS)
23	25.55	0.86	258647878.71	Germacrene-D
24	25.68	9.45	2833879656.51	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl (CAS)
25	27.02	51.50	15445851341.34	Biphenyl
26	27.78	0.54	163067530.62	Dehydroxy-isocalamendiol
27	28.65	0.19	57712655.81	1,1 Biphenyl(CAS
28	28.89	0.41	122841515.03.	Diepicedrene-1-oxide
29	29.33	0.73	218384960.04	Butanoic acid, 2-methyl-, 3,7-dimethyl-6-octenyl ester
30	29.46	2.55	764342844.76	(+) spathulenol
31	29.61	0. 16	47010727.85	(-)-Caryophyllene oxide
32	29.87	0.16	46764938.68	Limonene-6-ol, pivalate
33	30.07	0.75	224424515.23	Diepicedrene-1-oxide
34	30.32	0.24	73405417.28	6,9-Octadecadiynoic acid, methyl ester
35	30.73	0.82	245873285.79	Veridiflorol
36	31.03	0.16	49112356.60	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetr
37	31.15	0.17	50673217.24	Cedrenol (CAS)
38	31.60	0.22	65743582.24	á-Guaiene
39	32.08	0.22	65250144.27	.tau.-Muurolol
40	32.48	2.02	606879334.91	2-Naphthalenemethanol, decahydro-à,à,4a-trimethyl-8-methylene -, [2R-(2à,4aà,8aá
41	33.13	0.86	257609591.40	Patchouli alcohol
42	33.74	0.50	150830277.67	trans-Sesquisabinene hydrate
43	33.88	1.26	378378595.59	2,3,4,5,8,8a-hexahydro-6,8a-dimethyl3-(1-methylethyl)-, [3R-(3à,3aà,8aà)](CAS)
44	34.92	0.20	60785136.59	(1,5,5,8-Tetramethyl-bicyclo[4.2.1]non9-yl)-acetic acid
45	36.55	0.38	114542194.38	2-Methyl-4-(2,6,6-trimethylcyclohex-1 -enyl)but-2-en-1-ol

The components *of A. monosperma* oil were mixture of monoterpenoid, γ-terpinene, methyl eugenol, limonene, p-cymene, myrcene, α-pinene and α- cymene, and sabinene. Cyclic monoterpenes were prominent in the oil obtained from *A. monosperma* plants collected from Sinai. Terpinene is a perfume and flavoring chemical used in the cosmetics and food industries.

The maximum peak areas were found in six major components: Biphenyl(has the highest peak area equivalent to (51.50 %) and is used as a food preservative and flavoring agent, this suggest the use of *Artemisia monosperma* as flavoring agent; 2-á-Pinene monoterpene which is known to exhibit microbicidal activity against all fungi and bacteria with peak area of (7.37%); oxygenated sesquiterpene spathulenol (2.55 %) with known antimicrobial effect; (9.45%), benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl (CAS) or curcumine which supplement, cosmetics ingredient, food flavoring, and food coloring; (2.02 %) 2-Naphthalenemethanol, decahydrate-à,à,4a-trimethyl-8-methylene-, 2R-, and (3.45%) 1,3-dimethyl-2-(1-methylethylidene)imidazolidine. Imidazolidines (saturated imidazoles), also known as tetrahydroimidazoles are biologically active nitrogen containing heterocyclic moiety which have been reported to show wide bioactivities such as anti-inflammatory, analgesic, α-adrenergic receptor agonist, antimicrobial, antiparasitic, oral hypoglycemic and anticonvulsant activities.

Beta-Phellandrene and delta 3 carene are flavor ingredients. Germacrenes are typically produced in a variety of plant species for their antimicrobial and insecticidal properties that play a role as insect pheromones. In *Artemisia* species, monoterpene and sesquiterpene compounds are produced in the glandular trichomes present on plant leaf surfaces(Ruhland*et al.,* 2013). Table 1 demonstrated that the copaene (tricyclic sesquiterpene) had the least peak area (0.15%.), caryophyllene oxide, is a natural minor bicyclic sesquiterpene (0.15%), guaienes with peak area(0.22%)are sesquiterpenes that are used in the fragrance and flavoring industries to impart, spicy tastes and aromas The antimicrobial activity of *Artemisia monosperma* against human pathogenic bacteria and fungi (unpublished work) suggested *Artemisia monosperma* application in traditional medicine.

This study showed that *Artemisia monosperma* extract possesses different antimicrobial activities against tested organism (unpublished work). As there is a requirement for novel antimicrobial compounds produced from the plant oils, that will be applied medicinally [28-29]. Essential oils have been used as antibiotics alternatives for curing of various infectious diseases. Many researchers reported that different plant extracts and essential oils have been used as antiseptics that possess antimicrobial properties.

Table 2 shows the composition of the essential oil of the different *Artemisia species* from different areas. Components different percentages of *Artemisia* species may also be attributed to the climatic and geographic conditions such as temperature, altitude, wind direction, rainfall, and soil type [30] and hence the chemical variability for each species is prominent. Asia has the greatest number of species, with 150 spp for China, 174 in the ex-Union of Soviet Socialist Republics, about 50 present in Japan, and 35 species of the genus in Iran. Table 2 proves variation in the components concentration of essential oil with the different geiographical area, Italy, Libya, India, Algeria and Jordan within different Artemisia spp.

Table 2: Variation in chemical position of *Artemisia spp*. in different areas

***Artemisia* species**	**Major Chemical**		**Origin**	**Reference**
	Chemical Composition	**% Content**		
A. arborescens	Camphor	4.9	Libya	[31]
	Chamazulene	24.7		
	Linalool	20.9		
	Bornyl acetate	6.0		
	Germacrene			
A. arborescens	Camphor	35.7	IItaly	[32]
	β-thujone	23.9		
	Chamazulene	7.6		
A. arborescens	Chamazulene β-	30.2	Algeria	[33]
	thujone	27.8		
A.anna	camphor	44	China	[34]
	germacrene D,	16		
	trans-pinocarveol	11		
	beta-selinene beta-	9		

	caryophyllene	9		
A. maderaspatana	a-humulene), b-Caryophyllene a-Copaene Z(E)- a-FarneseneCalarene	46.3 9.3 8.2 3.7 3.5	Lucknow, UP, India.	[35]
A. judaica	Camphor Ethyl cinnamate Pipertone	16.1 11 30.4	Aboriginal Bedouins in the North Badia region of Jordan	[36]

The difference in essential oil constituents depends on some factors; the stage of the plant sample collection, time, soil nature, storage conditions, the part of a plant used, the method of extraction, and the concentration of the extract.

Many reports indicated that essential oil high antimicrobial potential may be due to oxygenated terpenes, especially phenolic compounds [37-41]. *A. monosperma* essential oil contains oxygenated compounds, it has antimicrobial effect. Oxygenated monoterpenes represent the highest antimicrobial activity in whole cells. On the contrary, hydrocarbon derivatives have the least antimicrobial activity due to their less solubility and spreading through the medium [42]. According to their configuration, hydrocarbons are almost inactive in relation to their hydrogen bonds capacity, and solubility in water [43]. Ketones, aldehydes, and alcohols are more active, but with a variant level of specificity and activity, which is related to the functional group, which is also regarded as hydrogen-bonding in all cases.

It can be concluded that phytochemicals content in *A. monosperma* plant may be a promising alternatives to synthetic drugs that could be used under certain conditions.

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