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Composition and Antimicrobial Activity of Essential Oil from the Aerial Part of *Artemisia annua*

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Artemisia annua is widely used as key components of traditional Chinese medicine in China. The compositions of essential oil extracted by hydro distillation from the stem and spike of *Artemisia annua* were identified using GC/MS. The stem oil comprized of 50 components, while the spike oil contained 41 components. There were 30 same compounds. Twenty seven unreported compounds were β-pinene, β-phellandrene, citronellol, borneol, linalool and cuminic alcohol *etc*. The relative content of terpenoids from the spike oil was more than it from the stem oil, so the antimicrobial activity of the spike oil was more active than it of the stem oil. MICs of the essential oil of them were different from microorganisms.

Key Words: *Artemisia annua*, Essential oil, Aerial part, GC/MS, Antimicrobial activity.

INTRODUCTION

Artemisia annua L. is an annual herb native of China and Eastern Europe. The plant has been used for many centuries to treat fevers and malaria, specifically cerebral malaria. It is the dried aerial part is often widely used as key components of traditional Chinese medicine. The essential oil has the function of cleaning summer heat, cleaning hectic heat, relieving asthma, antibacterial, antifungal and antitumor, *etc*. They are very different from these of other regions. The compositions of essential oil extracted by hydro distillation from the stem and spike of *Artemisia annua* were identified using GC/MS.

EXPERIMENTAL

The aerial part were collected from mature plants in September growing near the Longdong University in Qingyang City, Gansu Province, China. Plant material was dried at room temperature for 3 weeks. Stems were shut in 5 mm and spikes were ground into fine powders until extraction.

Extraction: 280 g each of stems and spikes were subjected to hydro distillation in 6 L boiling saturation salt with an essential oil collector. Distillated and dried over anhydrous sodium all night long, yellow and pale yellow essential oil with the weight of 7.84 g and 27.16 g were extracted from the stems and spikes of *Artemisia annua*. The actual recovery were 2.8 and 9.7 % from stems and spikes, respectively.

Analysis and identification: Analysis of essential oil extract employed a thermo finnigan trace gas chromatography (GC). Mass spectra were acquired at the electron ionization (EI) mode (Figs. 1 and 2)[1]. Identifications of components were achieved by matching their mass spectra with those in the NIST02L libraries or with published MS data bank. Quantitative data were obtained from the total ion current chromatogram peak area percentages without the use of correction factors (Table-1).

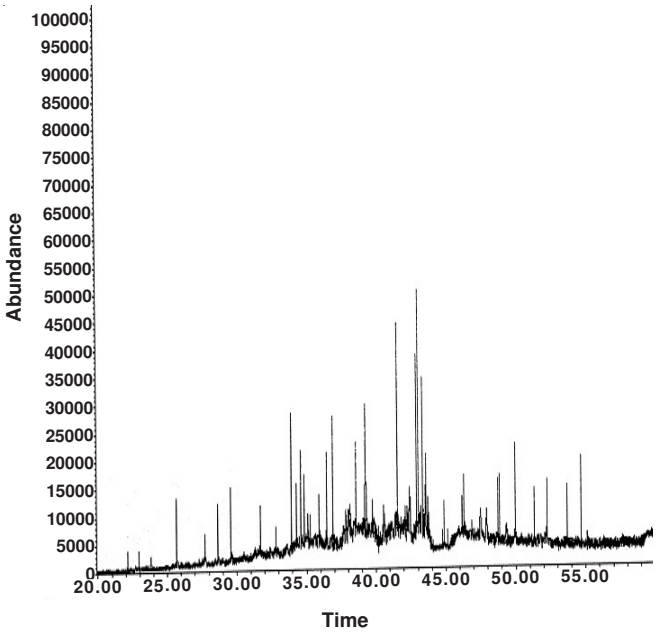

Fig. 1. Total ion current for the stem oil

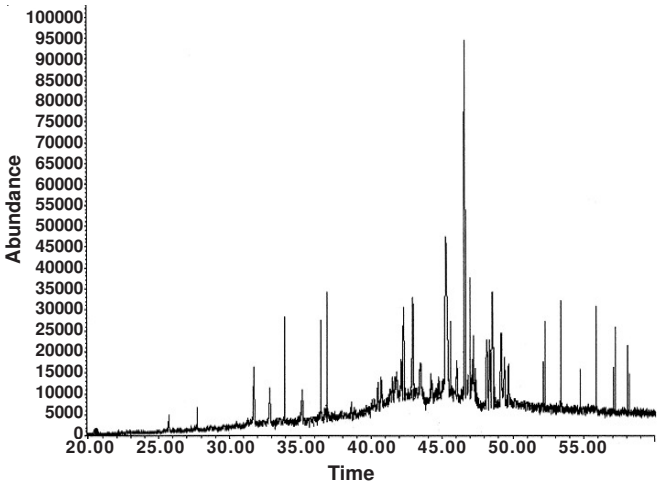

Fig. 2. Total ion current for the spike oil

TABLE-1
COMPOSITION OF ESSENTIAL OIL FROM *ARTEMISIA ANNUA*

Compounds	m.f.	m.w.	Content (%)	
			Stem	Spike
Camphene	$C_{10}H_{16}$	136	1.32	0.70
β-Phellandrene	$C_{10}H_{16}$	136	1.19	1.69
β -Myrcene	$C_{10}H_{16}$	136	0.70	1.17
β -Pinene	$C_{10}H_{16}$	136	1.22	2.90
p-Methylisopropylbenzene	$C_{10}H_{14}$	134	1.52	1.12
Limonene	$C_{10}H_{16}$	136	0.81	2.81
1,8-Cineole	$C_{10}H_{18}O$	154	1.68	1.30
2-Methoxy-3-allylphenol	$C_{10}H_{12}O_2$	164	1.10	1.43
Linalool	$C_{10}H_{18}O$	154	1.05	1.83
Borneol	$C_{10}H_{18}O$	154	1.39	3.16
Terpinene-4-ol	$C_{10}H_{18}O$	154	2.82	3.39
Cuminic alcohol	$C_{10}H_{14}O$	150	1.20	1.51
Piperitone	$C_{10}H_{16}O$	152	3.03	1.42
Phenylacetic acid	$C_8H_8O_2$	136	1.28	4.88
γ-Humulene	$C_{15}H_{24}$	204	0.70	1.86
Methyl cinnamate	$C_{10}H_{10}O_2$	162	4.53	9.70
Isobornyl acetate	$C_{12}H_{22}O_2$	196	1.12	3.85
β-Copaene	$C_{15}H_{24}$	204	2.36	2.49
Alloaromadendrene	$C_{15}H_{24}$	204	1.50	1.64
β-Guaiene	$C_{15}H_{24}$	204	3.52	3.51
γ-Elemene	$C_{15}H_{24}$	204	1.31	2.50
α-Caryophyllene	$C_{15}H_{24}$	204	1.12	1.75
Hexadecane	$C_{16}H_{34}$	226	0.85	2.81
2-Benzyl octanal	$C_{15}H_{22}O$	218	1.08	3.31
Ethyl laurate	$C_{14}H_{32}O_2$	232	1.62	1.59
Benzyl cinnamate	$C_{16}H_{14}O_2$	238	1.70	3.17
Methyl 9-octadecenoate	$C_{19}H_{36}O_2$	296	0.79	1.67
Ethyl palmitate	$C_{18}H_{36}O_2$	284	2.29	2.68
Phytol	$C_{20}H_{40}O$	296	1.42	2.20
Palmitic acid	$C_{16}H_{32}O_2$	256	1.59	1.50
Isovaleraldehyde	$C_5H_{10}O$	86	0.40	-
2-Hexenal	$C_6H_{10}O$	98	0.40	-
Octanal	$C_8H_{16}O$	128	0.62	-
1,4-Cineole	$C_{10}H_{18}O$	154	2.89	-
Artemisia ketone	$C_{10}H_{16}O$	152	2.21	-
Citronellol	$C_{10}H_{20}O$	156	1.23	-
Carvone	$C_{10}H_{14}O$	150	1.08	-
Camphor	$C_{10}H_{16}O$	152	2.20	-
Lauric acid	$C_{12}H_{24}O_2$	200	1.18	-
3-Allyl-6-methoxyphenol	$C_{10}H_{12}O_2$	164	3.92	-
Caryophyllene oxide	$C_{15}H_{24}O$	220	5.13	-
Germacrene D	$C_{15}H_{24}$	204	2.09	-
Pentadecane	$C_{15}H_{32}$	212	0.69	-
1,4-Diphenyl-2-butanone	$C_{16}H_{160}$	224	1.20	-
α-Cubebene	$C_{15}H_{24}$	204	1.30	-
Nerolidol	$C_{15}H_{26}O$	222	1.70	-
(-)-Dihydro new caryophyllene	$C_{15}H_{26}$	206	1.08	-
Docosane	$C_{22}H_{46}$	310	1.50	-
Pentacosane	$C_{25}H_{52}$	352	2.05	-
Di-(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	390	0.60	-
2-Cymene-5-isopropyl bicyclo [3,1,0]hexa-2-ene	$C_{10}H_{16}$	136	-	0.50
trans-Ocimene	$C_{10}H_{16}$	136	-	3.50
1,5,5-Trimethyl-6-methene-cyclohexene	$C_{10}H_{16}$	136	-	0.83
1-Thujanol	$C_{10}H_{18}O$	154	-	1.43
α-Terpinolene	$C_{10}H_{16}$	136	-	1.57
(+)-4-Carene	$C_{10}H_{18}$	138	-	1.79
α-Copaene	$C_{15}H_{24}$	204	-	2.78
δ-Cadinene	$C_{15}H_{24}$	204	-	2.34
Cadin-5,8-diene	$C_{15}H_{24}$	204	-	2.34
β-Farnesene	$C_{15}H_{24}$	204	-	1.90
Ethyl laurate	$C_{14}H_{32}O_2$	232	-	1.59

Antimicrobial activity

Measuring the inhibition zone: Paper disc (10 mm) diffusion method was used to determine the growth inhibition caused by essential oil against the following bacterial strains: *Staphyloccocus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus thuringiensis* and *Penicillin*. The volume concentrations of each tested compound were 5, 10, 15, 20, 25 and 30 %. The activity was determined by measuring the probable area of the inhibition zone. Three replicas were prepared in each case (Table-2).

Measuring MICs: MICs of the essential oil of *Artemisia annua* was carried out. The stem oil and the spike oil were dissolved in distilled water with a concentration of 2000 μg/mL. The serial two fold dilutions of the polysaccharide (1000, 500, 250, 125, 62.5, 31.3, 15.6 and 7.81 μg/mL) were prepared for MIC tests, respectively. A series of dilutions were incubated on a rotary shaker at 250 rpm for 48 h at 37 ºC (Table-3).

RESULTS AND DISCUSSION

The constituents of the stem oil and the spike oil of *Artemisia annua* are given in Table-1. There are 30 same compounds. Twenty seven unreported compounds[2-5] are β-phellandrene, cuminic alcohol, piperito, *etc*. The stem oil comprised of 50 components, making up 81.28 % of the total oil composition. Terpene compounds identified from the stem oil only 25.3 % of the oil, terpene alcohol did 15.4 % and terpene ketone did 8.5 %. Major compounds over 3 % were 5 ones.

The spike oil comprised of 41 components, making up 96.34 % of the total oil composition. Major compounds were methyl cinnamate (9.70 %), phenylacetic acid (4.88 %), isobornyl acetate (3.85 %), β-guaiene (3.51 %) and *trans*-ocimene (3.50 %) *etc*. Terpene compounds identified from the spike oil 41.7 % of the oil, terpene alcohol compounds did 11.7 % and esters compounds did 22.7 %. Major compounds over 3 % were 9 ones.

TABLE-2
ANTIMICROBIAL ACTIVITY OF *ARTEMISIA ANNUA* ESSENTIAL OIL

Microorganisms	Aerial part	Diameters of inhibition zone (mm)						*Penicillin*
		5	10	15	20	25	30	
Staphyloccocus aureus	Stem	7.4 ± 0.2	-	2.5 ± 0.2	2.4 ± 0.3	5.2 ± 0.3	2.5 ± 0.2	5.1 ± 0.2
	Spike	5.1 ± 0.3	5.1 ± 0.3	5.2 ± 0.3	5.0 ± 0.3	4.9 ± 0.3	5.1 ± 0.3	5.0 ± 0.2
Escherichia coli	Stem	5.1 ± 0.1	2.5 ± 0.3	10.0	7.6 ± 0.1	10.0	5.1 ± 0.2	5.1 ± 0.2
	Spike	5.0 ± 0.2	4.9 ± 0.2	10.0	10.0	10.0	10.0	7.6 ± 0.2
Bacillus subtilis	Stem	10.0	5.1 ± 0.3	7.6 ± 0.2	7.5 ± 0.3	10.0	10.0	-
	Spike	9.9 ± 0.1	10.0	10.0	9.9 ± 0.1	10.0	10.0	-
Bacillus thuringiensis	Stem	5.0 ± 0.2	5.1 ± 0.3	2.4 ± 0.4	2.4 ± 0.2	9.9 ± 0.2	4.9 ± 0.2	5.1 ± 0.2
	Spike	7.5 ± 0.2	7.4 ± 0.3	10.0	10.0	10.0	9.9 ± 0.1	5.1 ± 0.2
Penicillin	Stem	-	5.0 ± 0.3	7.6 ± 0.2	7.5 ± 0.2	7.5 ± 0.2	7.6 ± 0.2	-
	Spike	10.0	10.0	9.9 ± 0.1	9.9 ± 0.1	10.0	10.0	-

TABLE-3
MICs OF THE ESSENTIAL OIL OF *ARTEMISIA ANNUA*

Microorganisms		*Staphyloccocus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Bacillus thuringiensis*	*Penicillin*
MICs (μg/mL)	stem	15.6	31.3	7.81	31.3	-
	spike	31.3	31.3	7.81	15.6	7.81

The percentage composition of β-guaiene, ethyl laurate and palmitic acid from the stem oil was similar to that from the spike oil. But the percentage composition of methyl cinnamate, phenylacetic acid, isobornyl acetate and 2-benzyl octanal was more different from the two kinds of the essential oil. Both of the essential oils remarkably inhibited the growth of all tested bacteria and fungi. And the antimicrobial activity was made a distinction between the stem and spike oil. Terpenoids were useful for their broad bioactivities, notably antibacterial and antioxidant properties. Then the relative content of terpenoids from the spike oil was more than it from the stem oil, so the antimicrobial activity of the spike oil was more active than it of the stem oil. It shows that the spike oil has a better potential for use in aromatherapy. MICs of the essential oil of them were different from microorganisms. MICs of the stem was less than the other against *Staphyloccocus aureus,* but more against *Bacillus thuringiensis*. Antimicrobial properties of the plant in Qingyang in China to *Escherichia coli* and *Staphylococcus aureus* may be different from those in Marseilles in France[4,6].

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