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Full Length Research Paper

Composition and antimicrobial activity of essential oil from the aerial part of *Artemisia annua*

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Artemisia annua **is widely used as a key component of traditional Chinese medicine in China. The components of the essential oil extracted by hydro distillation from the stems and spikes of *A. annua* were identified using chromatography–mass spectrometry (GC/MS). The stem oil was composed of 50 components, while the spike oil contained 41 components. 30 compounds were found in both oils. 18 unreported compounds were Piperitone, Octanal and 1,4-Diphenyl-2- butanone etc. From the spike oil the relative content of terpenoids was higher than that from the stem oil. The antimicrobial activity of the spike oil was more active than that of the stem oil. Antimicrobial properties of the plant in Qingyang in China to *Escherichia coli and Staphylococcus aureus* may be stronger than those in Marseilles in France.**

Key words: *Artemisia annua*, essential oil, aerial part, chromatography–mass spectrometry, antimicrobial activity.

INTRODUCTION

Artemisia annua L. belongs to the family Asteraceae. It is an annual herb native of China and Eastern Europe. The dried aerial part is often widely used as a key component of traditional Chinese medicine. The plant has been used for many centuries to treat fevers and malaria, specifically cerebral malaria and has played an important role in discovering artemisinin that has antimalarial activity (Klayman et al., 1984). Since discovering artemisinin, people have mainly paid attention to this while ignoring the oil from this plant. The essential oil has many functions such as relieving heat exhaustion, fever and asthma as well as antibacterial, antifungal and antitumor properties (Bing et al., 2008). The useful kind of the plant is found not as frequently in nature (Yue et al., 2006).

The components of the essential oil extracted by hydro distillation from the stem and spike of *A. annua* were identified using chromatography–mass spectrometry (GC/MS). This is the first step aiming to identify the components of the stem and spike that have medicinal properties. The components and the behavior of the essential oil from *A. annua* L. are very different from other regions of the plant like the stems and the spikes. We want to obtain information about the antimicrobial activity of the essential oil.

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MATERIALS AND METHODS

Plant material

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

Extraction

280 g of each of the stems and spikes were subjected to hydro distillation in 6 L of saturated boiling salt solution with an essential oil collector. The stems and spikes of *A. annua* were distillated and dried over anhydrous sodium over night. A yellow and pale yellow essential oil with the weight of 7.84 and 27.16 g were extracted from the stems and spikes. The actual recovery was 2.8% of the stems and 9.7% of the spikes.

Analysis and identification

A Thermo Finnigan TRACE gas chromatography (GC) was used to analyze the essential oil extract. A polar column (30 m × 0.25 mm, 0.25 μm film thickness) was used. Oven temperature was

Table 1. Composition of essential oil from *A. annua*.

Compounds	Formula	Similarity (%)		MW	Content (%)	
		Stem	Spike		Stem	Spike
Camphene	$C_{10}H_{16}$	94	93	136	1.32	0.70
β-Phellandrene	$C_{10}H_{16}$	92	92	136	1.19	1.69
β-Myrcene	$C_{10}H_{16}$	91	92	136	0.70	1.17
β-Pinene	$C_{10}H_{16}$	94	95	136	1.22	2.90
p-Methylisopropylbenzene	$C_{10}H_{14}$	95	95	134	1.52	1.12
Limonene	$C_{10}H_{16}$	94	95	136	0.81	2.81
1,8-Cineole	$C_{10}H_{18}O$	93	92	154	1.68	1.30
2-Methoxy-3-allylphenol	$C_{10}H_{12}O_2$	92	93	164	1.10	1.43
Linalool	$C_{10}H_{18}O$	90	91	154	1.05	1.83
Borneol	$C_{10}H_{18}O$	91	95	154	1.39	3.16
Terpinene-4-ol	$C_{10}H_{18}O$	93	94	154	2.82	3.39
Cuminic alcohol	$C_{10}H_{14}O$	91	94	150	1.20	1.51
Piperitone	$C_{10}H_{16}O$	94	92	152	3.03	1.42
Phenylacetic acid	$C_8H_8O_2$	92	94	136	1.28	4.88
γ-Humulene	$C_{15}H_{24}$	90	92	204	0.70	1.86
Methyl cinnamate	$C_{10}H_{10}O_2$	95	96	162	4.53	9.70
Isobornyl acetate	$C_{12}H_{22}O_2$	91	93	196	1.12	3.85
β-Copaene	$C_{15}H_{24}$	93	93	204	2.36	2.49
Alloaromadendrene	$C_{15}H_{24}$	92	92	204	1.50	1.64
β-Guaiene	$C_{15}H_{24}$	94	94	204	3.52	3.51
γ-Elemene	$C_{15}H_{24}$	92	92	204	1.31	2.50
α-Caryophyllene	$C_{15}H_{24}$	92	93	204	1.12	1.75
Hexadecane	$C_{16}H_{34}$	90	93	226	0.85	2.81
2-Benzyl octanal	$C_{15}H_{22}O$	92	93	218	1.08	3.31
Ethyl laurate	$C_{14}H_{32}O_2$	91	92	232	1.62	1.59
Benzyl cinnamate	$C_{16}H_{14}O_2$	92	94	238	1.70	3.17
Methyl 9-octadecenoate	$C_{19}H_{36}O_2$	90	92	296	0.79	1.67
Ethyl palmitate	$C_{18}H_{36}O_2$	92	92	284	2.29	2.68
Phytol	$C_{20}H_{40}O$	92	93	296	1.42	2.20
Palmitic acid	$C_{16}H_{32}O_2$	93	92	256	1.59	1.50
Isovaleraldehyde	$C_5H_{10}O$	90		86	0.40	-
2-Hexenal	$C_6H_{10}O$	91		98	0.40	-
Octanal	$C_8H_{16}O$	92		128	0.62	-
1,4-Cineole	$C_{10}H_{18}O$	93		154	2.89	-
Artemisia ketone	$C_{10}H_{16}O$	93		152	2.21	-
Citronellol	$C_{10}H_{20}O$	92		156	1.23	-
Carvone	$C_{10}H_{14}O$	92		150	1.08	-
Camphor	$C_{10}H_{16}O$	93		152	2.20	-
Lauric acid	$C_{12}H_{24}O_2$	92		200	1.18	-
3-Allyl-6-methoxyphenol	$C_{10}H_{12}O_2$	94		164	3.92	-
Caryophyllene oxide	$C_{15}H_{24}O$	96		220	5.13	-
Germacrene D	$C_{15}H_{24}$	94		204	2.09	-
Pentadecane	$C_{15}H_{32}$	93		212	0.69	-
1,4-Diphenyl-2-butanone	$C_{16}H_{160}$	93		224	1.20	-
α-Cubebene	$C_{15}H_{24}$	93		204	1.30	-
Nerolidol	$C_{15}H_{26}O$	93		222	1.70	-
(-)-Dihydro new caryophyllene	$C_{15}H_{26}$	92		206	1.08	-
Docosane	$C_{22}H_{46}$	92		310	1.50	-
Pentacosane	$C_{25}H_{52}$	92		352	2.05	-
Di-(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	91		390	0.60	-

Table 1. Contd.

2-Cymene-5-isopropyl bicyclo[3,1,0]hexa-2-ene	$C_{10}H_{16}$	92	136	-	0.50
trans-Ocimene	$C_{10}H_{16}$	93	136	-	3.50
1,5,5-Trimethyl-6-methene-cyclohexene	$C_{10}H_{16}$	90	136	-	0.83
1-Thujanol	$C_{10}H_{18}O$	91	154	-	1.43
α-Terpinolene	$C_{10}H_{16}$	91	136	-	1.57
(+)-4-Carene	$C_{10}H_{18}$	92	138	-	1.79
α-Copaene	$C_{15}H_{24}$	92	204	-	2.78
δ-Cadinene	$C_{15}H_{24}$	93	204	-	2.34
Cadin-5,8-diene	$C_{15}H_{24}$	93	204	-	2.34
β-Farnesene	$C_{15}H_{24}$	92	204	-	1.90
Ethyl laurate	$C_{14}H_{32}O_2$	91	232	-	1.59

programmed for 80 to 290°C with a final hold time of 30 min. The detector temperature was at 250°C. Helium was used as the carrier gas. Mass spectra were acquired at the electron ionization (EI) mode with a temperature 230°C, an electron multiplier voltage of 2200 V and an ionization voltage of 70 eV. The mass scanning ranged between *m/z* 45 and *m/z* 550 under scan mode. Identification of components was achieved by matching their mass spectra with those in the National Institute of Standards in Technology (NIST) libraries and with those reported in literature. Quantitative data was obtained from the total ion current chromatogram peak area percentages without the use of correction factors (Table 1).

Antimicrobial activity

Measuring the inhibition zone

A paper disc (10 mm) diffusion method was used to determine the growth inhibition caused by the 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 area of the inhibition zone. The experiment was repeated three times (Table 2).

Measuring MICs

MIC of the essential oil of *A. 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°C (Table 3).

RESULTS AND DISCUSSION

The components of the stem oil and the spike oil of *A. annua* were given in Table 1. In both oils there were 30 compounds that were the same. 18 compounds were firstly reported such as Piperitone, Octanal and 1,4-Diphenyl-2-butanone etc (Afaq et al., 2009; Chenfei et al., 2007; Perazzo et al., 2003; Qin et al., 2001; Yaohong et al., 1999; Yinta et al., 2007). Piperitone can be applied to relieve asthma, cough and antibiosis. The stem oil was comprised of 50 identifiable components, making up 81.28% of the total oil composition. Found in the stem oil terpene compounds made up 25.3%. Terpene alcohol made up 15.4%. Terpene ketone made up 8.5%. 5 components each made up more than 3% of the total content. The spike oil was 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. Found in the spike oil terpene compounds made up 41.7%. Terpene alcohol compounds made up 11.7%. Esters compounds made up 22.7%. 9 components each made up more than 3% of the total content. The percentages of *β*-guaiene, ethyl laurate and palmitic acid from the stem oil was similar to that from the spike oil. The percentages of methyl cinnamate, phenylacetic acid, isobornyl acetate and 2-benzyl octanal were different in the two kinds of the essential oil.

Both of the essential oils remarkably inhibited the growth of all tested bacteria and fungi. Terpenoids were useful for their antibacterial and antioxidant properties. The stem oil only had 49.2% of terpenoids whereas the spike oil had 53.4% of terpenoids, therefore the relative content of terpenoids from the spike oil was more than that from the stem oil. The antimicrobial activity of the spike oil was more active than that of the stem oil (Figures 1 and 2). It shows that the spike oil has a better potential for use in aromatherapy. Both stem and spike oils had the same antimicrobial activity for *E. coli* and *Bacillus subtilis* (MICs 31.3 and 7.81 µg/ml respectively). The stem oil had stronger antimicrobial properties than the spike oil for *S. aureus* ($MICs_{stem}$=15.6 µg/ml and $MICs_{spike}$=31.3 µg/ml), but the spike oil had stronger antimicrobial properties than the stem oil for *B. thuringiensis* ($MICs_{stem}$=31.3 µg/ml and $MICs_{spike}$=15.6 µg/ml). For *E. coli* and *S. aureus* the diameters of inhibition zone of the stem oil at 30% concentration in China are 5.1±0.2 and 2.5±0.2 mm,

Table 2. Antimicrobial activity of *Artemisia annua* essential oil.

Microorganisms	Aerial part	Diameters of inhibition zone(mm)						Acetone	*Penicillin*
		5.00%	10.00%	15.00%	20.00%	25.00%	30.00%		
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 *A. annua*.

Microorganisms		*S. aureus*	*E. coli*	*B. subtilis*	*B. thuringiensis*	*Penicillin*
MICs	Stem	15.6	31.3	7.81	31.3	-
(µg/ml)	Spike	31.3	31.3	7.81	15.6	7.81

Mass spectra were acquired at the electron ionization (EI) mode with a temperature 230°C, an electron multiplier voltage of 2200 V and an ionization voltage of 70 eV. The mass scanning ranged between *m/z* 45 and *m/z* 550 under scan mode.

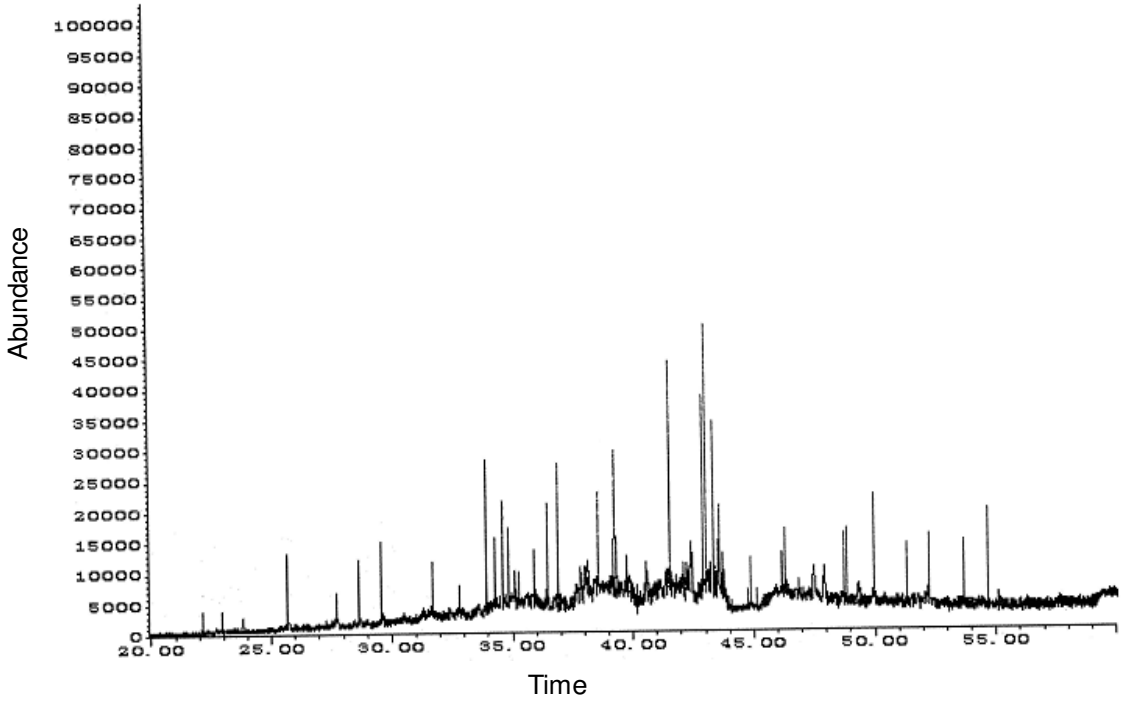

Figure 1. The total ion current for the stem oil.

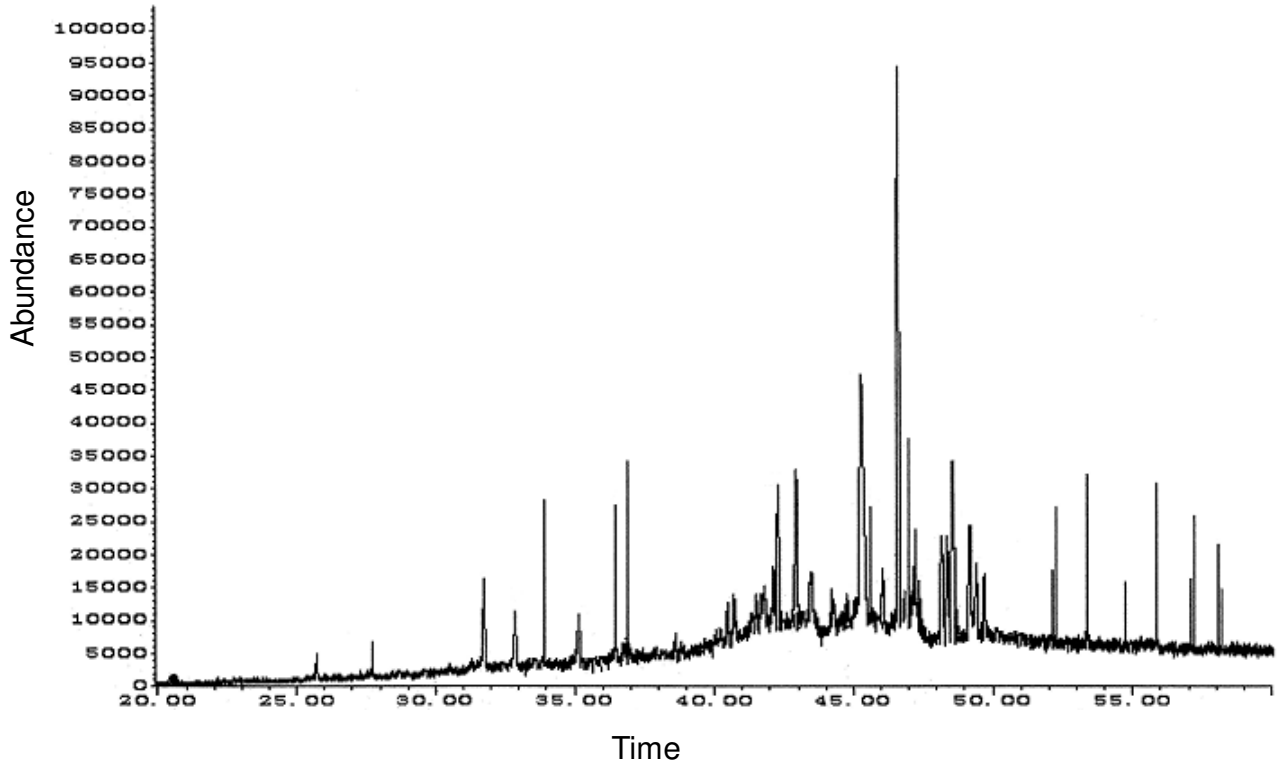

Figure 2. The total ion current for the spike oil.

respectively, similarly those of the spike oil are 10.0 and 5.1±0.3 mm, but the oil in France had no effect at 50% concentration (Fabien et al., 2002). Antimicrobial properties of the plant in Qingyang in China to *E. coli* and *S. aureus* may be stronger than those in Marseilles in France.

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