Chemical Constituents in Essential Oils from *Elsholtzia ciliata* and Their Antimicrobial Activities

TIAN Guang-hui*

School of Chemical and Environmental Sciences, Shaanxi University of Technology, Hanzhong 723000, China

Abstract: **Objective** To compare the chemical constituents in the essential oils from the leaves, flowers, and seeds of *Elsholtzia ciliata* and their antimicrobial activities. **Methods** The chemical constituents in essential oils were extracted by the hydro-distillation method and analyzed by GC-MS. The chemical constituents in essential oils were identified on the basis of comparison on their retention indices and MS spectrum with published data. Moreover, the antimicrobial activities of the chemical constituents in the oils against the growth of six bacteria strains and one pathogenic yeast strain were evaluated by using minimum inhibitory concentration and minimum bactericidal concentration methods. **Results** A total of 58 compounds were identified, while compounds **31**, **35**, and **36** were identified in the essential oils from the leaves, flowers, and seeds, respectively. Fifteen compounds were identified as shared constituents in the leaves, flowers, and seeds. The chemical constituents in the essential oils showed the inhibitory activities against the six bacteria strains and the yeast strain. **Conclusion** The major constituents are different in the essential oils of the leaves, flowers, and seeds. The major chemical constituents in the essential oils are monoterpenoids and sesquiterpenoids. And the chemical constituents in the essential oils obtained from the leaves show higher inhibitory activities especially against *Bacillus subtillis* CMCC63501 and *Escherichia coli* ATCC25922.

Key words: antimicrobial activity; *Bacillus subtillis*; *Elsholtzia ciliata*; essential oils; monoterpenoids
DOI: 10.3969/j.issn.1674-6348.2013.02.004

Introduction

The genus *Elsholtzia* Willd. of Lamiaceae comprises about 40 species which occur in the tropics, Southern Africa, and subtropics of Asia (Xu *et al*, 1987; Dongsa, Ryongung, and Shen, 1992). The most of them are widely used in folk medicine by local practitioner for their anti-inflammation, anticancer, antibacteria, and antitumor activities (Fujita and Node, 1984; Yin and Guo, 1994). *Elsholtzia ciliata* (Thunb.) Hyland. is a herbaceous plant up to 30—50 cm height and widely grows wild in Qinba Mountain of China. Its seed is a brownish oblong berry, its leaf is thick, oval leaflet growing villus, and its pink flower is alike lip in which down lobe is deeper than up lobe containing 3—4 pistils (Agendac Acade Mica Sinicas Edita, 1977). As the constituents of herbal medicines from its branch and leaf, it is widely used for the treatment of blood clotting, gastralgia, dysphonia, jaundice, diarrheic, throat infections, and as an astringent, antipyretic, and antiviral medicine for the treatment of cough and fever (Janssen, Scheffer, and Baerheim, 1987). Therefore, the aims of the present paper were: (1) to characterize the chemical constituents in the essential oils obtained from leaves, flowers, and seeds of wild *E. ciliata* collected from Qinba Mountain of China; (2) to assess the antimicrobial activities of the obtained essential oils against the growth of six bacteria strains and one pathogenic yeast strain; and (3) to seek for the part with high biological activities of *E. ciliata*. This research will provide scientific proof to seek for bioactive constituents from the essential oils of *E. ciliata*.

Materials and methods

Plant material

The leaves, flowers, and seeds of *Elsholtzia ciliata* (Thunb.) Hyland. were collected in the morning of July

* Corresponding author: Tian GH Address: School of Chemical and Environmental Sciences, Shaanxi University of Technology, 505 Chaoyang Road, Hanzhong 723000, China Tel: +86-138 9263 1822 Fax: +86-916-2641 574 E-mail: tianguanghui123@yahoo.com.cn
Received: July 12, 2012; Revised: August 13, 2012; Accepted: October 18, 2012
Fund: The Society Progress Project Supported by Shaanxi Province of China (2012K19-03-03); Shaanxi Provincial Education Department of China (2010JK471)
Online time: April 20, 2013 Online website: http://www.cnki.net/kcms/detail/12.11410.R.20130420.1113.006.html

10, 2010 (temperature range, 25—28 ℃) from the same fresh plant growing on the Qinba Mountain of Shaanxi province in China, and were identified by Prof. ZHAO Hua (Shaanxi University of Technology) by comparing with herbarium specimens of the voucher (No SNU 97-08-09). The samples were air-dried for 5 d and weighed for extracting the essential oils. These researching culture media contained Tryptic soy agar, Mueller-Hinton Broth, and broth culture. The seven bacterium strains are from National Institute for Food and Drug Control.

Reagents

Ether (analytically pure) was produced by Tianjin No. 6 Chemical Reagent Factory; Anhydrous sodium sulfate and sodium chloride (analytically pure) were all bought from Xi'an Chemical Reagent Factory. Water used in this experiment was distilled water.

Instruments

Finnigan-Trace DSQ Mass Spectrometer with a Heweltt-Packard GC-5890 Series II Instrument equipped with HP-WAX and HP—5 capillary column (30 m × 0.25 mm, 0.25 μm); GC—6890N America Agilent Series GC with DB—5 fused silica capillary column (30 m × 0.25 mm, 0.25 μm); ZNCI-T Intelligent Constant Temperature Magnetic Stirrer was produced by Gongyi City Yuhua Instrument Co., Ltd.; HH-Constant Temperature Water Bath was produced by Bohai Electric Appliance Plant of Huanghua City; BP1 Balance was bought from Shanghai Hardware & Electrical Co., Ltd.; 96-well microtiter plate was used.

Extraction of essential oils

An aliquot of 100 g samples from the leaves, flowers, and seeds of *E. ciliata* was steam-distilled in triplicate with a Clevenger type apparatus for 6 h (Liu, 2011; Tian, Liu, and Wang, 2007), then each essential oil was weighed 0.26, 0.43, and 1.1 g, respectively. The rates of essential oils were 0.26%, 0.43%, and 1.1%, then each essential oil was collected in a small breaker to which a small amount of anhydrous sodium sulfate was added to absorb water and sample was kept in a stopper vial at −4 ℃ for further analysis. The oils were dissolved 1:10 in capillary GC grade *n*-hexane before GC-MS analysis.

GC-MS analysis

Essential oil analysis was carried out with the following temperature program: 40 ℃ for 5 min, then increasing to 250 ℃ at the speed of 4 ℃/min; injector and detector temperature of 250 ℃; The flow rate of carrier gas Helium is 1 mL/min; injection of 1 μL. The MS conditions were as follows: ionization mode EI from 40 to 500 amu with split ratio of 1:50; The relative retention time (t_R) obtained in GC for the constituents in essential oils was compared with that obtained at the same conditions for commercial compounds, and the MS was compared with that of pure samples and with the MS from standards NIST Library.

Gas chromatography analysis

Qualifications of their oils were conducted by GC and detector dual flame ionization detection (GC-FID) on GC—6890N America Agilent series GC with DB—5 fused silica capillary column (30 m × 0.25 mm, 0.25 μm). All constituents in essential oils were calibrated with a standard mixture of homologous *n*-alkane series as standard and were determined by comparison of their t_R, and calculated as Kovats retention indices (RI). A aliquot of the sample oils (0.5 μL) was injected into DB—5 fused silica capillary column with the same GC conditions of HP—5 capillary columns.

Antibacterial assays

The antibacterial activities of the essential oils obtained from the leaves, flowers, and seeds of *E. ciliata* were studied. The experiments were carried out by using a set of bacterium in infection intestines and stomach involving seven strains included six bacteria strains and one yeast strain: *Bacillus subtillis* CMCC63501; *Escherichia coli* ATCC 25922; *Salmonella enteritidis* 50040; *Shigella flexneri* 51065; *Salmonella typhi* 50127; *Staphylococcus aureus* ATCC 25925; and *Candida albicans* 85021. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the three oils were determined using a broth microdilution method (Liu and Tian, 2007; Liu, 2009). All tests were performed in NB for six bacteria strains and one yeast strain. Each experiment was repeated at least three times for each oil and under each test concentration.

Results

Chemical constituents

The contents of constituents in essential oil were reported in Table 1. As it is shown, total 58 compounds were characterized in the essential oils of *E. ciliata*,

Table 1 Chemical constituents of essential oils from *E. ciliata*

No.	RI	Compounds	Molecular formula	Contents / %		
				Leaves	Flowers	Seeds
1	419	ethyl acetate	$C_4H_8O_2$	1.55	1.26	2.28
2	424	1,1-diethoxy-ethane	$C_6H_{14}O_2$		2.37	
3	488	acetic acid, bcptycester	$C_6H_{12}O_2$	0.18	1.56	3.41
4	519	1-ethoxy-pentane	$C_7H_{16}O$	2.05		
5	523	1,3-dioxolane	$C_3H_6O_2$			1.38
6	558	toluene	C_7H_8	0.75	0.82	0.64
7	569	ethylcyclohexane	C_8H_{16}		2.35	1.12
8	592	2,4-pentanedione	$C_5H_8O_2$	1.04	2.62	1.27
9	628	1-octen-3-ol	$C_8H_{16}O$	8.89	3.25	6.08
10	665	1,3-dimethylbenzene	C_8H_{10}	0.28		
11	693	3-hexen-1-ol	$C_6H_{12}O$	1.16	1.89	1.74
12	718	1,2-pentanediol	$C_5H_{12}O_2$		1.66	
13	752	6-methyl-3-heptanol	$C_8H_{18}O$	2.02		
14	763	3-octanol	$C_8H_{18}O$			0.85
15	797	5-methyl-3-heptyne	C_8H_{14}		0.52	
16	821	2-pinen-4-one	$C_{10}H_{14}O$	6.87	1.34	2.09
17	853	formic acid, 1-methyle thylester	$C_4H_8O_2$			0.21
18	886	5-benzoylpentanoic acid	$C_{12}H_{14}O_3$		0.64	
19	901	2-methoxy-2-methyl-propane	$C_5H_{12}O$			1.07
20	935	(*R*)-(−)-*p*-menth-1-en-4-ol	$C_{10}H_{18}O$	3.34	1.03	1.12
21	974	(*Z*)-cinerone	$C_{10}H_{14}O$	2.15	1.28	2.06
22	991	*cis*-geraniol	$C_{10}H_{18}O$		3.61	4.52
23	1023	β-linalool	$C_{10}H_{18}O$	12.06	11.52	10.86
24	1041	copaene	$C_{15}H_{24}$	1.31		
25	1066	2,2,5-trimethyl-3,4-hexanedione	$C_9H_{16}O_2$		0.56	
26	1082	3,3,6,8-tetramethyl-1-tetralone	$C_{14}H_{18}$			0.75
27	1097	coumaran	C_8H_8O	2.37	1.43	3.16
28	1113	*cis*-dihydro-2-copaene-8-ol	$C_{15}H_{26}O$		0.83	
29	1127	3-methyl-tetradecane	$C_{15}H_{32}$	0.79		
30	1146	caryophyllene	$C_{15}H_{24}$	11.02	9.57	11.13
31	1161	2-[1-phenylethyl]-phenol	$C_{14}H_{14}O$	0.30		
32	1178	launi acid, vinylester	$C_{14}H_{22}O_2$			1.94
33	1195	nerolidyl acetate	$C_{17}H_{28}O_2$		1.12	
34	1216	eugenol	$C_{10}H_{12}O_2$	9.67	10.56	9.32
35	1229	humulane-1,6-dien-3-ol	$C_{15}H_{26}O$	2.31		
36	1241	cadina-1(10),4-diene	$C_{15}H_{21}$			1.37
37	1258	(−)-spathulenol	$C_{15}H_{24}O$	7.16	2.35	1.97
38	1272	epiglobulol	$C_{15}H_{26}O$	2.13		2.85
39	1286	palmitic acid, ethylester	$C_{18}H_{36}O_2$			0.62
40	1298	isocaryophillene	$C_{15}H_{24}$	0.81	4.18	1.81
41	1315	β-lonone	$C_{13}H_{20}O$		11.08	
42	1336	caryophyllene oxide	$C_{14}H_{21}O$	9.61		8.83
43	1357	3-buthyl-cyclobexanone	$C_{10}H_{18}O$	1.29		
44	1373	*n*-eicosane	$C_{20}H_{42}$		1.96	
45	1391	linolenic acid, ethyl ester	$C_{20}H_{34}O_2$			3.05
46	1416	*n*-heneicosane	$C_{21}H_{44}$	0.25		
47	1438	4-methyl-dodec-3-en-1-ol	$C_{13}H_{26}O$		1.68	
48	1466	retinal	$C_{20}H_{28}O$		3.81	1.42

(To be continued)

(Continued Table 1)

No.	RI	Compounds	Molecular formula	Contents / %		
				Leaves	Flowers	Seeds
49	1482	agaruspirol	$C_{15}H_{26}O$	2.32	0.59	
50	1501	gual-1(5),7(11)-diene	$C_{15}H_{26}O$		1.62	1.95
51	1533	(13*R*)-8,13-epoxy-labda-14-ene	$C_{20}H_{34}O$		0.28	0.34
52	1564	dibutyl phthalate	$C_{16}H_{22}O_4$	0.38		
53	1583	(13*R*)-8,13-diol-labd-14-ene	$C_{20}H_{36}O_2$		1.85	1.09
54	1616	pimara-7,15-dien-3-ol	$C_{20}H_{32}O$		0.39	0.24
55	1638	8,13-epoxy-androst-14-en-3-one	$C_{20}H_{34}O$	1.65	1.78	1.59
56	1652	linolenic acid	$C_{18}H_{30}O_2$		1.45	1.16
57	1683	7-hexyl-eicosane	$C_{26}H_{54}$	0.63		
58	1703	*n*-triacontane	$C_{30}H_{62}$	0.21		0.21
	Total			96.55	94.81	95.55

then compounds **31**, **35**, and **36** were from the leaves, flowers, and seeds, accounting for 96.55%, 94.81%, and 95.55%, respectively. In the 58 compounds, 2 aromatics, 8 alkenes, 12 enols, 3 alcohols, 2 phenols, 5 ethers, 9 aldehydes and ketones, 8 esters, 2 carboxylic acids, 1 alkyne, and 6 alkylations were included. Among them, the terpenoids formed the major portion in all studied samples. The compounds with higher contents in the essential oils from the leaves were β-linalool (12.06%), caryophyllene (11.02%), eugenol (9.67%), caryophyllene oxide (9.61%), and 1-octen-3-ol (8.89%). But β-linalool (11.52%), β-lonone (11.08%), eugenol (10.56%), caryophyllene (9.57%), and retinal (3.81%) were the major compounds from the flowers, and the major constituents from the seeds were caryophyllene (11.13%), β-linalool (10.86%), eugenol (9.32%), caryophyllene oxide (8.83%), and 1-octen-3-ol (6.08%). About 16 compounds were identified as shared constituents in the three parts. The result revealed that their major constituents were different. The property of many herbs has been associated with their antibacterial activities, which is mainly attributed to their compounds whose structures contained unsaturated bond.

Microbiological analysis

Table 2 showed the MIC and MBC of the essential oils tested against *B. subtillis* CMCC63501, *E. coli* ATCC 25922, *S. enteritidis* 50040, *S. flexneri* 51065, *S. typhi* 50127, and *S. aureus* ATCC 25925 (six bacteria strains), and *C. albicans* 85021 (one yeast strain). The oils from the leaves, flowers, and seeds showed higher activities to inhibit those microorganisms at the highest concentration tested (MIC and MBC < 12.56 μL/mL), and especially the essential oil obtained from the leaves showed the best antimicrobial activities against *B. subtillis* CMCC63501 with MIC = 0.02 μL/mL and MBC = 0.08 μL/mL, against *E. coli* ATCC 25922, *S. enteritidis* 50040, *S. flexneri* 51065, *S. typhi* 50127, *S. aureus* ATCC 25925, and *C. albicans* 85021 with MIC and MBC < 9.52 μL/mL. The oils from the flowers and the seeds exhibited relatively low activity against *S. aureus* ATCC25925, and *C. albicans* 85021 (MIC and MBC > 10.02 μL/mL), comparing with that from the leaves. The results suggested that the oils could be used to treat the disease of digest system in accordance with the literature (Janssen *et al*, 1987). The oils showed potential to be exploited as effective medicines of antimicrobials for curing the disease of digest system.

Table 2 MIC and MBC of essential oils obtained from leaves, flowers, and seeds

Strains	MIC / (μL·mL^{-1})			MBC / (μL·mL^{-1})		
	Leaves	Flowers	Seeds	Leaves	Flowers	Seeds
B. subtillis CMCC63501	0.02	0.14	0.16	0.08	0.22	0.28
E. coli ATCC25922	1.08	2.32	4.18	1.16	3.60	5.50
S. enteritidis 50040	2.56	5.96	6.06	3.72	7.84	9.18
S. flexneri 51065	5.14	7.08	8.28	7.36	9.30	10.02
S. typhi 50127	6.12	8.54	7.52	8.36	10.22	9.06
S. aureus ATCC25925	6.88	10.16	11.32	9.52	11.06	13.56
C. albicans 85021	7.64	10.02	10.80	8.28	12.38	12.90

Discussion

In this paper, the essential oils from the different parts of wild *E. ciliata* growing on Qinba Mountain were analyzed by GC-MS. The constituents in the essential oils obtained from the leaves, flowers, and seeds are different, only 15 of 58 compounds identified are shared one. The constituents in the essential oils obtained from the leaves, flowers, and seeds containing terpenoids compounds have 14 hemiterpenoids (major semiterpenoids: caryophyllene), five diterpenoids (major diterpenoids: 8,13-epoxy-androst-14-en-3-one), and 11 monoterpenoids (major monoterpenoids: *p*-menth-1-en-4-ol), respectively. Terpenoids have high bioactive properties on many facets (Dongsa, Ryongung, and Shen, 1992; Janssen, Scheffer, and Baerheim, 1987). Here, we only tested the antimicrobial activities and found that the essential oils exhibited relatively good activities against the six bacterial strains and the yeast strain correlating with the diseases of infectious intestines and stomach. Among them, the oils obtained from the leaves showed the best inhibitory activities against the six bacteria strains and the yeast strain, and the fresh leaves may have the potential to be developed as a new herbal medicine.

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