SUPPLEMENTARY MATERIAL

Chemical composition and biological activities of essential oil from *Filifolium sibiricum* (L.) Kitam

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Abstract

The essential oil from *Filifolium sibiricum* (L.) Kitam were extracted using hydro-distillation and GC-MS was used to analyse the essential oil. The main components were Espatulenol (8.55%), Geranyl acetate (8.03%), Caryophyllene oxide (5.47%), Calamenene (4.79%), Geraniol (4.28%), calamenene (4.53%), Geraniol (4.06%), Cedrene epoxide (3.23%), Myrtenol (3.18%), trans-Geranylgeranio (3.13%), etc. The essential oil showed intensive inhibitory effects against MCF-7 with IC₅₀ levels of 0.78 mg/mL, HepG-2 with IC₅₀ levels of 0.44 mg/mL, SKOV-3 with IC₅₀ levels of 0.27 mg/mL, BGC-823 with IC₅₀ levels of 0.34 mg/mL. In antibacterial test, the essential oil showed significantly antibacterial activity. The lowest MIC and MBC values were 5.20 mg/ml and 5.20 mg/mL against *Staphylococcus aureus*

Keywords: *Filifolium sibiricum* (L.) Kitam, essential oil, GC-MS, cytotoxicity, antibacterial activity.

1. Experimental

1.1. Plant material and essential oil isolation

The fresh *Filifolium sibiricum* (L.) Kitam was collected at Lindian County, Heilongjiang Province, People's Republic of China, in September 2015 and identified by Dr. Lianjie Su, a specialist in department of Chinese Medicine Resources, Heilongjiang University of Chinese Medicine, where the voucher specimen (20151062) has been deposited.

1.2 Essential oil isolation

The dried plant (250 g) was crushed into powder, then hydrodistillation for 4 hours by a circulatory Clevenger-type apparatus. The sample yielded about 0.3% (on a dry mass) of yellowish oil (0.74 g) with a pleasant smell. The obtained essential oil was dried over anhydrous sodium sulfate and stored 4°C before analysis and bioassay.

1.3 Essential oil analysis

Gas-chromatography-flame ionization detector (GC-FID) analyses of essential oil were conducted using a Agilent GC (Model 7890A) instrument equipped with

Agilent HP-5 capillary column (5% phenyl-methylpolysiloxane, 60 m×0.25 mm i.d. 0.25 µm). Helium was the carrier gas at the flow of 1.2 mL/min as well as the split ratio was 1/10. The oven temperature was raised from 120 °C to 280 °C (hold on 2 minutes) at a rate of 3 °C/min. The injector and detector (FID) temperatures were kept at 250 °C and 300 °C, respectively.

GC-MS was carried out in Agilent 6890 gas chromatograph equipped with a mass spectrometer (Agilent, Model 5975C) and parameters as mentioned for GC. The interface and quadrupole temperatures were 250 °C and 150 °C, respectively. Ion source temperature was 230 °C with EI mode and the mass ranges m/z 20-550 (Xue et al, 2015).

1.4 Cytotoxicity activity

1.4.1 Cell lines

The cytotoxicity activity of the *Filifolium sibiricum* (L.) Kitam essential oil was tested against four cell lines. MCF-7 (ATCC HTB-22), HepG-2 (ATCC HB-8065), SKOV-3 (ATCC CRL-1740) and BGC-823(ATCC CL-0034).

1.4.2 Cytotoxicity assay

The cytotoxicity activity of the *Filifolium sibiricum* (L.) Kitam essential oil was tested against four human cancer cell lines MCF-7, HepG-2, SKOV-3, BGC-823. 5-Fluorouracil (Sigma Chemical Co; purity≥98%) was used as reference compound to assess the cytotoxicity of tested essential oils. Cell numbers were counted with a hemocytometer by trypan blue dye exclusion technique to make sure that cell viability exceeded 95%. Briefly, cells were plated in 96-well plates 24 h before treatment and continuously exposed to different concentrations . Then cells were cultured in triplicate for 48 h with the test compounds. Cells cultured with medium alone were used as controls. MTT (5 mg/mL) reagent was added 4 h before the end of culture, the plates were centrifuged (1400 rpm, 5 min), and the untransformed MTT was removed carefully by pipetting. DMSO working solution (150 µL) was added to each well, and the absorbance was evaluated using an ELISA reader at 490 nm. The percentage of cell death was calculated using the following formula: Cytotoxicity % = [Compounds (OD490)-background (OD490)] / [control (OD490)-background (OD490)] ×100% (Wang et al.)

1.5 Antibacterial activity

1.5.1 Bacterial strains

The antibacterial activity of the *Filifolium sibiricum* (L.) Kitam essential oil was tested against four bacterial strains: two Gram-positive strains (*Staphylococcus aureus* CMCC 26003 and *Staphylococcus albus* SCTCC 100266) and two Gram-negative strains (*Pseudomonas aeruginosa* CMCC 10104 and *Escherichia coli* CMCC 44825).

1.5.2 Antibacterial activity

Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined by microdilution method in 96 well microtitre plates, then dimethyl sulfoxide (DMSO) at a final concentration of 2% in each well was used to dissolve the essential oil. Briefly, fresh over night cultures of bacteria were adjusted with sterile saline to a concentration of 1.0×10^5 CFU per well. The essential oil were added in TSB medium for bacteria. And a negative control containing DMSO (2%) without essential oil was analysed as well. The microplates were incubated for 24 h at 37 °C for bacteria. The MIC was defined as the lowest concentration of the essential oil at which the bacteria did not exhibit visible growth after 24 h of incubation at 37 °C. The minimal bactericidal concentration (MBC) was determined by subculture on LB agar at 37 °C for 24 h. Each test was carried out in triplicates and repeated three times (Kazemi et al, 2015).

1.6 Statistical analysis

The quantitative data of major components of oil were statistically examined by one-way analysis of variance (ANOVA). Correlation and regression coefficients were performed using Statistical Package for the Social Sciences (SPSS).

References

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- Xue J, Sun YC, Wei Q, Wang CF, Yang BY, Kuang HX, Wang QH. 2015. Chemical composition and cytotoxicity of the essential oil from different parts of *Datura metel* L. Nat Prod Res. doi:10.1080/14786419.2015.1088541.

122 **Table S1** Chemical composition and relative contents of the essential oil from
 123 *Filifolium sibiricum* (L.) Kitam

No.	RI	Compound	%Area
1	804	1,3-Cycloheptadiene	1.02
2	940	α -Pinene	3.21
3	980	β -Pinene	2.31
4	982	cis-Meta-mentha-2,8-diene	0.27
5	1035	Limonene	0.45
6	1038	Eucalyptol	1.32
7	1106	Linalool	1.06
8	1125	Campholenic aldehyde	0.26
9	1142	trans-Pinocarveol	2.06
10	1164	3-Nopinenone	1.06
11	1179	Terpinen-4-ol	0.26
12	1200	α -Terpineol	1.83
13	1202	Myrtenol	3.18
14	1231	Nerol	0.34
15	1232	cis-Carveol	0.44
16	1267	Geraniol	4.28
17	1276	Citral	0.28
18	1288	Bornyl acetate	1.95
19	1349	Dehydro-ar-ionene	0.46
20	1350	α -Cubebene	0.47
21	1351	α -Longipinene	0.53
22	1354	Eugenol	0.37
23	1386	Geranyl acetate	8.03
24	1391	β -Elemen	2.29
25	1441	Aromadendrene	0.97
26	1444	Caryophyllene	0.33
27	1458	Thymylisobutyrate	1.40
28	1472	Chamigrene	1.43
29	1481	α -Curcumene	2.45
30	1483	α -Selinene	1.02
31	1488	β -Ionone	2.96
32	1525	delta-Cadinene	0.47
33	1531	Calamenene	4.79
34	1544	Isolongifolene, 4,5,9,10-dehydro-	1.77
35	1565	Nerolidol	0.58
36	1570	Espatulenol	8.55
37	1582	Cedrene epoxide	3.23
38	1583	Caryophyllene oxide	5.47
39	1617	Santalol	1.57
40	1619	Humulene epoxide II	1.92
41	1629	(Z)-4-Hexadecen-6-yne	0.69
42	1680	8-Cedren-13-ol	1.00
43	1844	Perhydrofarnesyl acetone	1.79
44	1919	Farnesylacetone	0.24
45	1990	Ethyl palmitate	0.59
46	2000	Eicosane	1.54
47	2110	Phytol	0.98

48	2152	Linoleic acid ethyl ester	0.26
49	2201	trans-Geranylgeraniol	3.13
50	2400	Tetracosane	0.32
51	2500	Pentacosane	0.41
	Monoterpene hydrocarbons	5.79%	
	Oxygenated monoterpenes	17.19%	
	Sesquiterpene hydrocarbons	16.52%	
	Oxygenated sesquiterpenes	22.32%	
	Diterpenoids	1.54%	
	Oxygenated diterpenoids	4.37%	
	Aliphatic compounds	2.27%	
	Aromatic compounds	3.2%	
	others	14.39%	
	Total identified	87.59%	

Table S2 The IC₅₀ values of MCF-7, HepG-2, SKOV-3, BGC-823 treated with essential oils from *Filifolium sibiricum* (L.) Kitam.

	IC ₅₀ (mg/mL)
MCF-7	0.78
HepG-2	0.44
SKOV-3	0.27
BGC-823	0.34

Table S3 Antibacterial activity of the essential oil of *Filifolium sibiricum* (L.) Kitam. (n=3)

	MIC(mg/mL)	MBC(mg/mL)
<i>Staphylococcus aureus</i>	5.20	5.20
<i>Staphylococcus albus</i>	41.63	83.25
<i>Pseudomonas aeruginosa</i>	10.41	10.41
<i>Escherichia coli</i>	10.41	20.81

Fig S1 Total ion chromatogram of *Filifolium sibiricum* (L.) Kitam

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