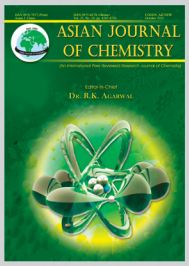
Asian Journal of Chemistry; Vol. 24, No. 2 (2012), 607-610

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Composition and Antimicrobial Activity of the Essential Oil of *Spilanthes paniculata* Growing Wild on the Gaoligong Mountains, China

S.M. ZHU, L. ZHU*, Y.J. TIAN and Y.C. YIN

College of Light Industry and Food Sciences, South China University of Technology, Guangzhou, Guangdong Province, P.R. China

*Corresponding author: Fax: +86 20 87113849; Tel: +86 20 87113849; E-mail: zhuliang@scut.edu.cn

(*Received*: 27 January 2011; *Accepted*: 10 October 2011) AJC-10490

The essential oil obtained by hydrodistillation of *Spilanthes paniculata* obtained from Gaoligong Mountains, China was analyzed by gas chromatography and gas chromatography/mass spectrometry (GC/MS), simultaneously. Main constituents of the oil was found as E-γ-cadinene (10.64 %), β-caryophyllene (6.31 %), thymol (5.55 %), β-pinene (5.42 %), 1,8-cineole (4.28 %), *p*-cymene (3.56 %) and bicyclogermacrene (3.17 %). The essential oil was also screened for its antimicrobial properties against various pathogens.

Key Words: *Spilanthes paniculata*, Essential oil, Chemical composition, Antimicrobial activity.

INTRODUCTION

The genus *Spilanthes*, classified in subtribe *Verbesininae Benth* of the *Anthemideae*, consists of about 60 species[1] of herbaceous annuals or perennials. The genus is widely distributed throughout the tropics and subtropics and can be found in damp pastures, at swamp margins, on rocks near the sea and as a weed of road sides and cultivations[2]. Two of them, *S. paniculata* and *S. callimorpha*, are found in China and used as folk medicines in the southern provinces of China. A series of studies has demonstrated the potential medicinal effect of the genus *Spilanthes*, for example, antifungal[3,4], antibacterial properties[5-7], diuretic[8], larvicidal[9-11], insecticidal properties[2,12,13], antifeedant[2], antiinflammatory[14] and antioxidant[15]. Several bioactive compounds, includes scopoletin[16], spilanthol[17] and a group of other isobutylamides[2], were obtained from this genus.

Spilanthes paniculata, mainly distributed in tropical and subtropical area in Asia, is an annual or short-lived perennial herb, 15-70 cm tall, with a prostrate or ascending branched cylindrical amaranth stem and simple needle opposite leaves without stipules. The flowers are yellow, with four or five petals on undercoat peduncles. The leaves and flowers have a pungent taste. In China, the plants have been growing in the southern hills and plateaus and have been used as folk medicine for dysentery, pyorrhea, toothache, stomatitis and throat complaints.

The aim of this study is to determine the chemical composition of the hydrodistilled essential oil of *Spilanthes paniculata* by GC-FID and GC-MS and to evaluate its antimicrobial activity properties.

EXPERIMENTAL

Spilanthes paniculata plants were collected in Gaoligong Mountains, Yunnan Province, China in June 2007 and identified by Dr. Gong Xun. Voucher specimen (No 721349) was deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Isolation of the essential oil: The dried powder (500 g) of *Spilanthes paniculata* was chopped and subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous Na_2SO_4 and preserved in a sealed vial at 4 ºC until further analysis.

GC-FID Analysis: The analytical GC was using an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with HP-5MS 5 % phenylmethylsiloxane capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) equipped with an FID detector. Helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. Injector and mass transfer line temperature were set at 250 and 280 ºC, respectively. Essential oil solution (1 μL) in hexane was injected and analyzed with the column held initially at 40 ºC for 1 min and then increased to 250 ºC with a 3 ºC/min heating ramp and subsequently kept at 250 ºC for 20 min.

GC-MS analysis: Quantitative and qualitative analysis of the essential oil was performed using a GC-MS 6890-5975 system (Agilent Technologies, Palo Alto, CA, USA) equipped

with a HP-5 MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. Injector and mass transfer line temperature were set at 250 and 280 ºC, respectively. Essential oil solution (1 μL) in hexane was injected and analyzed with the column held initially at 40 ºC for 1 min and then increased to 250 ºC with a 3 ºC/min heating ramp and subsequently kept at 250 ºC for 20 min. The Kovats indices were calculated for all volatile constituents using a homologous series of *n*-alkanes C_8-C_{25} on HP-5 MS column.The major components of oils were identified by co-injection with standards (wherever possible), confirmed with Kovats indices using the Wiley (V.7.0) and National Institute of Standards and Technology (NIST) V.2.0 GC-MS library. The relative concentration of each compound in essential oil was quantified based on the peak area integrated by the analysis program.

Antibacterial activity

Test bacteria: The *in vitro* antibacterial activity of the essential oil was evaluated against five pathogenic microorganisms *viz*., *Pseudomonas aeruginosa* CCTCC AB93066, *Escherichia coli* CCTCC AB91112, *Bacillus subtilis* CCTCC AB92068, *Staphylococcus aureus* CCTCC AB91053, *Hansenula anomala* CCTCC AY92046 procured from China Center for Type Culture Collection (CCTCC), Wuhan, China. All the strains were stored in the appropriate medium before use.

Inhibitory effect by disc diffusion method: Petri plates were prepared by pouring 20 mL of LB medium and allowed to solidify. Plates were dried and 0.1 mL of standardized inoculum containing $10^{6.7}$ CFU/mL of bacterial suspension was poured and uniformly spread and the inoculum was allowed to dry for 5 min. A Whatman No. 1 sterile filter paper disc (6 mm diameter) was impregnated with 1000 μg/disc of essential oil. Negative controls were prepared using the same solvent employed to dissolve the samples. Standard reference antibiotics, streptomycin and tetracycline (10 μg/disc) were used as positive controls for the tested bacteria. The plates were incubated at 37 ºC for 24 h. Antibacterial activity was evaluated by measuring the diameter of the zones of inhibition against the tested organisms. The experiments were repeated in triplicate and the results were expressed as average values.

Determination of minimum inhibitory concentration (MIC): Dilutions of the essential oil were prepared in Mueller-Hinton broth (Hi Media, Mumbai) ranging from 0.06-125 μL/mL. To each tube 0.5 mL of the inoculum containing approximately 10^8 CFU/mL microorganisms was added. A control test was also performed containing inoculated broth supplemented with only DMSO under identical conditions with gentamicin as reference. All the tubes were then incubated at 37 ºC for 24 h and examined for evidence of the growth.

Statistical analysis: Tests were carried out in triplicate and the results were calculated as mean ± SD.

RESULTS AND DISCUSSION

Chemical composition of the essential oil: The essential oil compositions of the *Spilanthes paniculata* has not been reported before and these results were first evidenced on the composition of this unique and endemic species.

The steam distillation of 500 g of dried plant material yielded 2.9 mL (0.58 % v/w) greenish oil with a distinct smell. The oil sample was analyzed by GC-FID and GC-MS and the components were identified on the basis of their RI values and by comparison of their mass spectra with those reported in the literatures. The GC-MS analysis of the essential oil of *Spilanthes paniculata* was resulted in detection of 67 components representing 98.21 % of the oil (Table-1). Among these, the amount of the monoterpene hydrocarbon fraction was 15.86 % of the oil while the sesquiterpene hydrocarbon fraction was 41.16 %. The oxygenated monoterpene fraction was 14.62 % and the oxygenated sesquiterpenoid fraction was 8.46 % in the oil. The main constituents in the oil were: E-γ-cadinene (10.64 %), β-caryophyllene (6.31 %), thymol (5.55 %), β-pinene (5.42 %), 1,8-cineole (4.28 %), *p*-cymene (3.56 %) and bicyclogermacrene (3.17 %).

TABLE-1
CHEMICAL COMPOSITION OF ESSENTIAL OIL OF *S. paniculata*

Peak No.	RI[a]	Components	RA (%)[b]	Identification methods[c]
1	852	(Z)-3-Hexenol	2.06	MS, RI
2	856	(E)-2-Hexenal	0.97	MS, RI
3	862	(E)-2-Hexen-1-ol	2.62	MS, RI, Co
4	871	Hexanol	1.28	MS, RI
5	902	Heptanal	0.99	MS, RI
6	928	α-Thujene	0.54	MS, RI
7	936	α-Pinene	1.24	MS, RI, Co
8	973	Sabinene	0.23	MS, RI
9	978	β-Pinene	5.42	MS, RI
10	980	1-Octen-3-ol	0.59	MS, RI
11	986	α-Myrcene	0.23	MS, RI
12	998	α-Phellandrene	0.20	MS, RI, Co
13	1017	*p*-Cymene	3.56	MS, RI
14	1023	Benzyl alcohol	0.90	MS, RI
15	1028	Limonene	2.82	MS, RI, Co
16	1030	1,8-Cineole	4.28	MS, RI
17	1032	β-Phellandrene	1.39	MS, RI
18	1051	(E)-β-Ocimene	0.23	MS, RI, Co
19	1093	1-Undecene	0.44	MS, RI
20	1099	Linalool	0.94	MS, RI
21	1117	Benzeneethanol	0.40	MS, RI
22	1153	Z-3-Nonen-1-ol	0.52	MS, RI
23	1158	(E,Z)-2,6-Nonadienal	0.59	MS, RI, Co
24	1168	Borneol	0.72	MS, RI
25	1170	Nonanol	0.64	MS, RI
26	1178	Terpinene-4-ol	0.72	MS, RI
27	1196	Estragole	0.79	MS, RI
28	1205	Verbenone	0.45	MS, RI
29	1258	Piperitone	0.54	MS, RI, Co
30	1265	(E)-2-Decenol	0.44	MS, RI
31	1292	Thymol	5.55	MS, RI
32	1294	2-Undecanone	0.25	MS, RI
33	1305	Carvacrol	0.26	MS, RI
34	1357	Eugenol	1.16	MS, RI
35	1375	α-Copaene	0.40	MS, RI
36	1388	β-Elemene	2.10	MS, RI, Co
37	1390	β-Cubebene	0.32	MS, RI
38	1410	α-Cedrene	0.20	MS, RI

39	1418	β-Caryophyllene	6.31	MS, RI, Co
40	1437	γ-Elemene	1.91	MS, RI
41	1440	α-Guaiene	0.22	MS, RI
42	1454	α-Humulene	0.79	MS, RI
43	1479	α-Amorphene	0.22	MS, RI, Co
44	1486	Germacrene D	3.12	MS, RI
45	1489	β-Selinene	0.52	MS, RI
46	1492	Valencene	0.40	MS, RI, Co
47	1496	2-Tridecanone	2.26	MS, RI
48	1498	Bicyclogermacrene	3.17	MS, RI
49	1504	(E)-α-Farnesene	0.25	MS, RI
50	1506	β-Bisabolene	7.15	MS, RI
51	1514	E-α-Cadinene	10.64	MS, RI
52	1522	δ-Cadinene	1.26	MS, RI, Co
53	1525	β-Sesquiphellandrene	0.25	MS, RI, Co
54	1537	α-Bisabolene	0.62	MS, RI
55	1548	β-Elemol	0.91	MS, RI
56	1556	Germacrene B	1.31	MS, RI, Co
57	1562	E-Nerolidol	1.09	MS, RI
58	1565	Geranyl N-butyrate	0.60	MS, RI
59	1575	Spathulenol	1.93	MS, RI
60	1578	Caryophyllene oxide	0.44	MS, RI
61	1608	β-Oplopenone	0.97	MS, RI
62	1646	α-Muurolol	1.28	MS, RI
63	1652	α-Cadinol	0.45	MS, RI
64	1752	α-Sinensal	1.39	MS, RI
65	1762	Benzyl benzoate	0.40	MS, RI
66	1830	Neophytadiene	0.76	MS, RI
67	1971	*n*-Hexadecanoic acid	0.61	MS, RI
		Total identified (%)	98.21	
		Monoterpene hydrocarbons	15.86	
		Monoterpenoids	14.62	
		Sesquiterpene hydrocarbons	41.16	
		Sesquiterpenoids	8.46	
		Others	18.11	

[a]Retention index relative to *n*-alkanes on HP-5 MS capillary column. [b]Relative area (peak area relative to the total peak area). [c]RI is the retention index, MS = mass spectrum, Co = co-injection with authentic compound.

In previous studies, the essential oils of several species of the genus *Spilanthes* have been examined and sesquiterpene hydrocarbons were found to be the major compounds. *Spilanthes acmella*, traditionally used in treatment of toothache, flu, cough and tuberculosis[18] and found in India, Sri Lanka and other tropical countries[19], has been well documented for its uses as antibacterial, antifungal and antimalarial activity[20]. The main constituents of the oil include the germacrene-D, β-caryophyllene, γ-cadinene, (Z)-β-ocimene, limonene, myrcene, thymol, (E)-2-hexenol, 2-tridecanone and hexanol[15,21-23]. The constituents of the major volatile secondary metabolites from *S. americana*, used in Colombian cuisine, were β-bisabolene, N-isobutyl-2(E),6(Z),8(E)-decatrienamide, 5-phenyl-2,4-pentadienyl acetate, caryophyllene, β-pinene, 1,8-cineole, *p*-cymene[24]. The results indicate that the essential oil of *S. paniculata* share some relatively similar components with other species of *Spilanthes* and serve as chemosystematic markers of *S. paniculata*.

Antibacterial activity: The antimicrobial activity of essential oils was evaluated by the standard agar diffusion method and minimum inhibitory concentrations (MICs) method against 2 gram-negative bacteria, 2 gram-positive bacteria and 1 yeast.

The *in vitro* antibacterial activities of the essential oil against the bacteria were qualitatively and quantitatively assessed by the presence or absence of inhibition zones. As shown in Table-2, in every cases, the essential oil exhibited higher antibacterial activity than that of standard streptomycin, while tetracycline showed highest antibacterial effect in every cases (Table-3). The essential oil exhibited remarkable antibacterial effect as minimum inhibitory concentration against all bacteria, *P. aeruginosa* CCTCC AB93066, *E. coli* CCTCC AB91112, *B. subtilis* CCTCC AB92068, *S. aureus* CCTCC AB91053, *H. anomala* CCTCC AY92046, with MIC values of 125, 62.5, 62.5, 62.5 and 125 μg/mL, respectively.

TABLE-2
ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF *S. paniculata* AGAINST THE GROWTH OF PATHOGENS

Microorganism	Diameter of zones of inhibition		
	Essential oil[A]	Standard[B]	
		SM	TC
Pseudomonas aeruginosa	21.3 ± 0.8	20.8 ± 0.7	21.5 ± 0.4
Escherichia coli	21.7 ± 0.7	21.2 ± 0.6	22.1 ± 0.7
Bacillus subtilis	23.2 ± 0.6	20.5 ± 0.6	23.4 ± 0.6
Staphylococcus aureus	23.3 ± 0.8	20.7 ± 0.7	23.5 ± 0.6
Hansenula anomala	21.9 ±0.9	20.8± 0.8	23.1 ± 0.6

Diameter of inhibition zones (mm) including the diameter of disc (6 mm), values are given as mean ± SD of triplicate experiment. [A]Diameter of inhibition zones of essential oil (tested volume 1000 μg/disc). [B]Standard antibiotics: SM, streptomycin; TC, tetracycline (tested volume 10 μg/disc).

TABLE-3
MINIMUM INHIBITORY CONCENTRATIONS OF ESSENTIAL OIL OF *S. paniculata* AGAINST THE GROWTH OF PATHOGENS

Microorganism	MICs of essential oil*
Pseudomonas aeruginosa	125
Escherichia coli	62.5
Bacillus subtilis	62.5
Staphylococcus aureus	62.5
Hansenula anomala	125

*MIC, Minimum inhibitory concentration (values in lg/mL).

It has frequently been reported that gram-positive bacteria were more susceptible to essential oils than gram-negative bacteria[25,26], which supports the present finding. Resistance of gram-negative bacteria against essential oils has been attributed the presence of a hydrophilic outer membrane which possessed hydrophilic polysaccharide chain as a barrier hydrophobic essential oil[27].

Conclusion

The results obtained in this study showed that the essential oil of *Spilanthes paniculata* may be suggested as a new potential source of natural antibacterial. The essential oil of *Spilanthes paniculata* presented inhibited the growth of different pathogens that can causes health problems. However, further studies are needed to understand the origin of the activity. Particularly, major constituents of the essential oil need to be tested for their antibacterial and antioxidant activities.

ACKNOWLEDGEMENTS

The authors are grateful for financially supported by the National Natural Science Foundation of China (20676041) and by the China Postdoctoral Science Foudation (2004036506).

REFERENCES

1. J.C. Willis, A Dictionary of the Flowering Plants and Ferns, Cambridge University Press, Cambridge (1977).
2. R.S. Ramsewak, A.J. Erickson and M.G. Nair, *Phytochemistry*, **9**, 732 (1999).
3. W. Fabry, P.O. Okemo and R. Ansong, *Mycoses*, **39**, 67 (1996).
4. W. Fabry, P.O. Okemo and R. Ansong, *J. Ethnopharmacol*., **60**, 79 (1998).
5. M. Rai, A. Varma and K. Pandey, *Mycoses*, **47**, 479 (2004).
6. F.B. Holetz, G.L. Pessini, N.R. Sanche, D.A.G. Cortez, C.V. Nakamura and B.P.D. Filho, *Mem. Inst. Oswaldo. Cruz*., **97**, 1027 (2002).
7. J.H. Leal-Cardoso and M.C. Fonteles, *An. Acad. Bras. Cienc*., **71**, 207 (1999).
8. W.D. Ratnasooriya, K.P.P. Pieris, U. Samaratunga and J. Jayakody, *J. Ethnopharmacol*., **91**, 317 (2004).
9. G.S. Pendse, N.L. Phalnikar and B.V. Bhide, *Curr. Sci*., **2**, 37 (1945).
10. K.V. Saritha, E. Prakash, N. Ramamurthy and C.V. Naidu, *Biol. Plant*., **45**, 581 (2002).
11. V. Pandey, V. Agrawal, K. Raghavendra and A.P. Dash, *Parasitol. Res*., **102**, 171 (2007)
12. I.J.O. Jondiko, *Phytochemistry*, **25**, 2289 (1986).
13. H.A. Khadir, M.B. Zakaria, A.A. Ketchil and M.S. Azirum, *Pestic. Sci*., **25**, 329 (1989).
14. L.C. Wu, N.C. Fan, M.H. Lin, I.R. Chu, S.J. Huang, C.Y. Hu and S.Y. Han, *J. Agric. Food Chem*., **56**, 2341 (2008).
15. R. Kawaree, S. Okonogi, S. Chowwanapoonpohn and W. Phutdhawong, *Acta Hort*., **786**, 209 (2008).
16. S. Prachayasittikul, S. Suphapong, A. Worachartcheewan, R. Lawung, S. Ruchirawat and V. Prachayasittikul, *Molecules*, **14**, 850 (2009).
17. V.G. Gokhale and B.V. Blude, *J. Indian Chem. Soc*., **22**, 250 (1945).
18. A.B. Haw and C.L. Keng, *J. Appl. Hortic*., **5**, 65 (2003).
19. W.D. Ratnasooriya, K.P.P. Pieris, U. Samaratunga and J.R.A.C. Jayakody, *J. Ethnopharmacol*., **91**, 317 (2004).
20. S.K. Singh, *Acta. Physiol. Plant*, **31**, 649 (2009).
21. L. Jirovetz, G. Buchbauer, A. Wobus, M.P. Shafi and G.T. Abraham, *J. Essent. Oil Res*., **17**, 429 (2005).
22. R.N. Baruah and P.A. Leclercq, *J. Essent. Oil Res*., **5**, 693 (1993).
23. T.L.G. Lemos, O.D.L. Pessoa, F.J.A. Matos, J.W. Alencar and A.A. Craveiro, *J. Essent. Oil Res*., **3**, 369 (1991).
24. E.E. Stashenko, M.A. Puertas and M.Y. Combariza, *J. Chromatogr. A*, **752**, 223 (1996).
25. S. Burt, *Int. J. Food Microbiol*., **94**, 223 (2004).
26. D. Kalemba and A. Kunicka, *Curr. Med. Chem*., **10**, 813 (2003).
27. C.M. Mann, S.D. Cox and J.L. Markham, *Lett. Appl. Microbiol.*, **30**, 294 (2000).