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Original article:

CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OIL OF *BLUMEA MEGACEPHALA*

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ABSTRACT

Blumea megacephala essential oil, obtained through steam distillation of samples collected from the Shiwang Mountains in Guangxi Province, China, was analyzed using GC-FID and GC-MS. Among the 65 compounds identified in the oil, the main compounds were borneol (13.6 %), β-caryophyllene (9.56 %), germacrene D (9.09 %), sabinene (6.37 %), and α-humulene (4.78 %). Antimicrobial activity revealed that the essential oil (1000 µg/disc) has promising antimicrobial effects against several pathogens, giving satisfactory inhibition zone diameter values (21.5, 21.6, 23.4, 23.8, 21.9) and MIC values (125, 125, 62.5, 125, 125 µg/ml) against Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*), Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), and yeast (*Hansenula anomala*). Antioxidant and antimicrobial activities were correlated with chemical composition.

Keywords: *Blumea megacephala,* essential oil, GC/MS, antimicrobial activity

INTRODUCTION

Blumea is a genus of shrubs and small trees characterised by disciform capitula with outer filiform female florets and inner tubular bisexual florets, tailed anthers, and cypsela wall epidermis with one large oxalate crystal in each cell (Pornpongrungrueng et al., 2007). The genus, classified in subtribe *Matricariinae* of *Anthemideae*, comprises of about 80 species distributed in tropical and subtropical Asia, Africa, and Oceania; thirty of which are distributed in the southern provinces of China (Chen et al., 2009). This genus includes some important medicinal plants largely used in traditional medicine. Pharmacological studies of the essential oils of several of its species have been performed. The essential oil from the leaves of *B. mollis* has a significant toxic effect against early fourth-instar larvae of *C. quinquefasciatus* (LC_{50}=71.71 LC_{90}=143.41 ppm) (Senthilkumar et al., 2008) and promising antibacterial activity against 14 clinically isolated bacterial strains (Senthilkumar et al., 2009). The essential oil from the aerial parts of *Blumea perrottetiana* demonstrates notable insecticidal activity against the red flour beetle, *Tribolium castaneum* (Owolabi et al., 2010).

The essential oil of *B. membranacea* produces a marked and long-lasting fall in the blood pressure of anaesthetized dogs, exerts a direct depressant action on frog hearts, and a spasmolytic effect on rabbit ilea (Mehta et al., 1986). The essential oil from *B. membranacea* shows significant antifungal activity through the filter-paper-disk method (Geda and Bokadia, 1979). The essential oils of *B. lacera* and *B. malcomii* increase the insecticidal activity of

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pyrethrum, as illustrated by the knockdown activity and toxicity against houseflies (Musca domestica) (Gupta et al., 1977).

B. megacephala is chiefly distributed in the south of China and has long been collected both as an edible and medicinal plant for malaria, bronchitis, puerperal metrorrhagia, puerperal edema, and barrenness. This study aims to determine the chemical composition of the hydrodistilled essential oil of *B. megacephala* grown in the Shiwang Mountains in Guangxi Province, China by GC-MS and to evaluate its antimicrobial activity against microorganisms.

MATERIALS AND METHODS

Plant material

Fresh aerial parts of *B. megacephala* were collected in the Shiwang Mountains in Guangxi Province, China in June 2008, and were identified by Dr. Gong Xun. A voucher specimen (No. 50360) 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 *B. megacephala* was chopped and subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The oil was dried over anhydrous Na_2SO_4 and was preserved in a sealed vial at 4 °C until further analysis.

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 (i.d: 30 m×0.25 mm, film thickness: 0.25 μm). For GC-MS detection, an electron ionization system (ionization energy: 70 eV). Helium gas was used as the carrier gas at a constant flow rate of 1 ml/min. Injector and mass transfer line temperature were set at 250 and 280 °C, respectively. Essential oil solution (1 μL) in hexane was injected and analyzed with the column held initially at 40 °C for 1 min, increased to 250 °C with a 3 °C/min heating ramp, and subsequently kept at 250 °C for 20 min. The Kovats indices were calculated for all volatile constituents using a homologous series of n-alkane C_8–C_{25} on the HP-5 MS column. The major components of the oils were identified by co-injection with standards (wherever possible) and 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 the essential oil was expressed as percentage by peak area normalization.

Antimicrobial activity

The *in vitro* antimicrobial 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, and *Hansenula anomala* CCTCC AY92046; all of which were procured from the China Center for Type Culture Collection (CCTCC), Wuhan, China. All strains were stored in appropriate medium before use.

A standard agar diffusion method was used for antibacterial assay (Murray et al., 1999). Petri plates were prepared by pouring 20 ml of the LB medium which was then allowed to solidify. Plates were dried and 0.1 ml of standardized inoculum containing $10^{6–7}$ CFU/ml bacterial suspension was poured and uniformly spread; the inoculum was allowed to dry for 5 min. A Whatman No. 1 sterile filter paper disc (diameter: 6 mm) was impregnated with 1000 μg/disc of essential oil. Negative controls were prepared using the same solvent employed to dissolve the samples, whereas standard reference antibiotics, streptomycin, and tetracycline (10 μg/disc) were used as positive controls for the tested bacteria. The plates were incubated at 37 °C for 24 h. Antimicrobial activity was evaluated by measuring the inhibition zone diameter against the tested organisms. The experi-

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ments were performed in triplicate and results are expressed as average values.

The minimum inhibitory concentration (MIC) of the essential oils was determined by the two-fold serial dilution technique (Baker et al., 1980). Dilutions of the essential oil were prepared in a Mueller-Hinton broth (Hi Media, Mumbai) ranging from 0.06 to 125 µl/ml. To each tube, 0.5 ml of the inoculum containing approximately 10^8 CFU/ml microorganisms was added. A control test, containing inoculated broth supplemented with only DMSO under identical conditions with gentamicin, was also performed as reference. All the tubes were then incubated at 37 °C for 24 h and examined for any evidence of 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

Shiwang Mountains is one of the richest areas of the world owing to its substantial number of medicinal plant species which grow in tropic and subtropic ecological conditions. Investigation of the antimicrobial properties of these plants has brought attention to the opportunity of producing a natural and environment-friendly source that could replace the synthetic antimicrobial compounds. Based on the current literature survey, no phytochemical and pharmacological study has been reported on *B. megacephala*. The essential oil composition of *B. megacephala* has no previous records; the results presented here are the first evidence regarding the composition of this unique and endemic species.

The steam distillation of 500 g dried plant material yielded 3.6 ml (0.72 % v/w) of a greenish oil with a distinct smell. The oil sample was analyzed by 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 literature. The GC-MS analysis of the *B. megacephala* essential oil resulted in the detection of 65 components comprising 94.86 % of the oil (Table 1). Among these, the monoterpene hydrocarbon fraction was 15.18 %, whereas the sesquiterpene hydrocarbon fraction was 36.44 %. The oxygenated monoterpene fraction was 23.87 % and the oxygenated sesquiterpenoid fraction was 11.83 %. The main constituents of the oil were borneol (13.6 %), β-caryophyllene (9.56 %), germacrene D (9.09 %), sabinene (6.37 %), and α-humulene (4.78 %).

Table 1: Chemical composition of *B. Megacephala* essential oil

Peak no.	RI[a]	Components	%RA[b]	Identification methods[c]
1	854	3-Hexen-1-ol	0.42	MS, RI
2	928	α-Thujene	0.32	MS, RI
3	936	α-Pinene	0.57	MS, RI
4	949	Camphene	0.25	MS, RI
5	973	Sabinene	6.07	MS, RI
6	978	β-Pinene	0.23	MS, RI, Co
7	982	1-Octen-3-ol	0.91	MS, RI
8	986	β-Myrcene	0.31	MS, RI
9	1017	p-Cymene	3.06	MS, RI
10	1030	d-Limonene	1.10	MS, RI, Co
11	1032	β-Phellandrene	0.50	MS, RI, Co
12	1040	(Z)-β-Ocimene	1.19	MS, RI
13	1051	(E)-β-Ocimene	0.92	MS, RI
14	1059	γ-Terpinene	3.72	MS, RI
15	1078	cis-Linalool oxide	0.60	MS, RI
16	1099	Linalool	1.94	MS, RI
17	1116	Phenylethyl alcohol	0.31	MS, RI
18	1143	Camphor	1.70	MS, RI
19	1160	Isoborneol	1.46	MS, RI
20	1168	Borneol	13.60	MS, RI, Co
21	1178	Terpinene-4-ol	1.45	MS, RI
22	1191	α-Terpineol	0.31	MS, RI
23	1229	Nerol	0.57	MS, RI
24	1235	Thymol methyl ether	0.40	MS, RI
25	1255	Geraniol	0.25	MS, RI
26	1270	Perillaldehyde	0.31	MS, RI
27	1286	Bornyl acetate	0.57	MS, RI
28	1300	Perillaalcohol	0.19	MS, RI

[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 = retention index, MS = mass spectrum, Co = co-injection with authentic compound.

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Table 1 (cont.): Chemical composition of *B. Megacephala* essential oil

Peak no.	RI[a]	Components	%RA[b]	Identification methods[c]
29	1351	α-Cubebene	0.33	MS, RI
30	1357	Eugenol	1.49	MS, RI
31	1375	α-Copaene	0.59	MS, RI
32	1384	β-Bourbonene	0.48	MS, RI
33	1390	β-Elemene	0.21	MS, RI
34	1409	α-Gurjunene	2.22	MS, RI
35	1418	β-Caryophyllene	9.56	MS, RI, Co
36	1436	β-Gurjunene	0.57	MS, RI
37	1437	γ-Elemene	0.48	MS, RI
38	1441	Aromadendrene	0.64	MS, RI
39	1484	β-Selinene	0.28	MS, RI
40	1454	α-Humulene	4.78	MS, RI, Co
41	1458	(E)-β-Farnesene	0.22	MS, RI
42	1460	Allo aromaden-drene	1.62	MS, RI
43	1486	Germacrene D	9.09	MS, RI, Co
44	1502	α-Muurolene	0.57	MS, RI
45	1506	β-Bisabolene	3.85	MS, RI
46	1524	δ-Cadinene	0.42	MS, RI
47	1531	Cadina-1,4-diene	0.32	MS, RI
48	1549	Elemol	0.21	MS, RI, Co
49	1562	E-Nerolidol	0.61	MS, RI
50	1567	Palustrol	0.69	MS, RI
51	1578	Spathulenol	0.42	MS, RI
52	1578	Caryophyllene oxide	1.11	MS, RI
53	1598	Guaiol	1.48	MS, RI
54	1619	10-epi-γ-Eudesmol	1.28	MS, RI
55	1621	Fonenol	0.45	MS, RI
56	1624	7-epi-γ-Eudesmol	1.56	MS, RI
57	1639	Isospathulenol	0.58	MS, RI
58	1645	δ-Cadinol	0.33	MS, RI
59	1646	Torreyol	0.57	MS, RI
60	1643	Cubenol	0.42	MS, RI
61	1648	β-Eudesmol	1.38	MS, RI
62	1652	Pogostol	0.18	MS, RI
63	1654	α-Eudesmol	0.77	MS, RI
64	1948	Isophytol	0.42	MS, RI
65	1971	n-Hexadecanoic acid	1.45	MS, RI
		Total identified (%)	94.86	
		Monoterpene hydrocarbons	15.18	
		Monoterpenoids	23.87	
		Sesquiterpene hydrocarbons	36.44	
		Sesquiterpenoids	11.83	
		Others	7.54	

[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 = retention index, MS = mass spectrum, Co = co-injection with authentic compound.

The essential oils of several species of the genus *Blumea* were examined. Essential oil from the aerial parts of *B. perrottetiana* was dominated by 2,5-dimethoxy-p-cymene (30.0 %) and 1,8-cineole (11.0 %) with lesser amounts of sabinene (8.1 %), delta-cadinene (5.3 %), and (E)-caryophyllene (3.9 %) (Owolabi et al., 2010). The dominant components in the essential oil of *B. balsamifera* leaves were borneol (33.22 %), caryophyllene (8.24 %), ledol (7.12 %), tetracyclo[6,3,2,0,(2.5).0(1,8)tridecan-9-ol, 4,4-dimethyl] (5.18 %), phytol (4.63 %), caryophyllene oxide (4.07 %), guaiol (3.44 %), thujopsene-13 (4.42 %), dimethoxy-durene (3.59 %), and γ-eudesmol (3.18 %) (Bhuiyan et al., 2009). The major chemical compounds of the essential oil of *B. mollis* leaves were identified as linalool (19.43 %), γ-elemene (12.19 %), copaene (10.93 %), estragole (10.81 %), allo-ocimene (10.03 %), γ-terpinene (8.28 %), and allo-aromadendrene (7.44 %) (Senthilkumar et al., 2008). The main components of the *B. brevipes* essential oil were terpinen-4-ol (27.6 %), germacrene-D (15.4 %), sabinene (8.0 %), and **γ**-terpinene (5.5 %) (Mwangi et al.,1994), whereas that of the *B. lanceolaria* essential oil was methyl thymol (Dung et al., 1991). The main constituents of the essential oil of *B. lacera* leaves were thymoquinol di-mether, β-caryophyllene, α-humulene, and E-β-farnesene (Laakso et al., 1989).

These results indicate that the essential oil of *B. megacephala* shares some relatively similar components with other the species of *Blumea* and serve as chemosystematic markers of *B. megacephala*.

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Antimicrobial activity

Antimicrobial activity of the essential oils against 2 Gram-negative bacteria, 2 Gram-positive bacteria, and yeast was evaluated using the Standard agar diffusion method and the MIC method.

The *in vitro* antimicrobial activity of the essential oil against these bacteria was qualitatively and quantitatively assessed by the presence or absence of inhibition zones. As shown in Table 2, the essential oil exhibits higher antimicrobial activity than standard streptomycin; in some cases, the essential oil exhibits higher antimicrobial activity than standard tetracycline against Gram-positive bacteria (*B subtilis* CCTCC AB92068, *S. aureus* CCTCC AB91053).

Table 2: Antibacterial activity of *B. megacephala* essential oil against the growth of pathogens

Microorganism	Inhibition Zone Diameter		
	[A]Essential oil	[B]Standard	
		SM	TC
Pseudomonas aeruginosa	21.5 ± 0.8	20.5 ± 0.4	21.5 ± 0.5
Escherichia coli	21.6 ± 1.1	21.0 ± 0.6	22.2 ± 0.9
Bacillus subtilis	23.4 ± 0.8	20.4 ± 0.7	23.2 ± 0.5
Staphylococcus aureus	23.8 ± 1.1	20.3 ± 0.7	23.6 ± 0.8
Hansenula anomala	21.9 ±0..7	20.5± 0.8	23.2 ± 0.6

Inhibition zone diameter (mm) including disc diameter (6 mm), values are given as mean ± SD of triplicate measurements.

[A] Inhibition zone diameter of the essential oil (tested volume 1000 µg/disc)

[B] Standard antibiotics: SM, streptomycin; TC, tetracycline (tested volume 10 µg/disc).

As shown in Table 3, the essential oil has remarkable antimicrobial effect with the minimum inhibitory concentration against bacteria *P. aeruginosa* CCTCC AB93066, *E. coli* CCTCC AB91112, *B. subtilis* CCTCC AB92068, *S. aureus* CCTCC AB91053, and *H. anomala* CCTCC AY92046; MIC values are 125, 125, 62.5, 125 and 125 µg/ml, respectively.

Table 3: Minimum inhibitory concentrations of *B. megacephala* essential oil against the growth of pathogens

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

[a] MIC, Minimum inhibitory concentration (values in µg/ml)

Various publications have documented the antimicrobial activity of essential oils and plant extracts (Morris et al., 1979). The antibacterial activity of the *B. megacephala* essential oil could, in part, be associated with major constituents such as terpene hydrocarbon and oxygenated terpene. Several researchers also report mono- and sesquiterpenoids as the major components of essential oils which are phenolic in nature (Oyedeji and Afolayan, 2005; Cakir et al., 2004). It is therefore reasonable to assume that their antimicrobial activity might be related to the phenolic compounds presented.

It has been reported that Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria (Burt 2004); this is supported by the current results. Resistance of Gram-negative bacteria against essential oils has been attributed the presence of a hydrophilic outer membrane containing a hydrophilic polysaccharide chain which acts as a barrier hydrophobic essential oil (Kalemba and Kunicka 2003; Mann et al., 2000).

CONCLUSION

The essential oil of *B. megacephala* is a newly discovered potential source of natural antimicrobial compounds. This report demonstrates for the first time that *B. megacephala* essential oil inhibits the growth of different pathogens that can cause health problems. However, further studies are needed to understand the origin of this activity. Particularly, major constituents of the essential oil need to be tested for

their antimicrobial and antioxidant activities.

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