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RESEARCH ARTICLE

Chemical composition and antimicrobial activity of the essential oil of *Cacalia tangutica* (Maxim.) Hand.-Mazz

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Abstract Essential oil of the subterranean part of *Cacalia tangutica* (Maxim.) Hand.-Mazz was analyzed by gas chromatography (GC)-mass spectrum (MS) technique in two different capillary columns of different polarities. Thirty-one components were identified in the oil and the main compounds were α-zingiberene (13.49%), germacrene D (10.76%), α-pinene (8.54%), caryophyllene(Z-) (6.36%), linalool (6.16%), β-myrcene (4.89%), β-ocimene (Z-) (4.40%)and ocimenone(Z-) (3.58%). The antimicrobial activity of the oil was evaluated against 2 fungi and 12 bacteria including 6 clinically isolated strains using the agar disc diffusion and broth microdilution methods. The results show that the oil presented a broad antimicrobial spectrum and had better antimicrobial activity against yeast and gram-positive bacteria. The minimum inhibitory concentration values were 0.16–5.00 g/L and minimum bactericidal concentration values were 0.16–5.00 g/L.

Keywords *Cacalia tangutica*, antimicrobial activity, essential oil, gas chromatography (GC)-mass spectrum (MS)

1 Introduction

Cacalia tangutica (Maxim.) Hand.-Mazz, also called "Zhuduzi", "Shuihuluqi", "Dengyuxie", belongs to the tribe *Senecioneae* with about 50 species of genus *Cacalia* distributed in China, wherein approximately 26 species are used as traditional folk herbs. According to traditional medicine, they could guide qi downward, dissipate phlegm, relieve cough and act as a cathartic. Several species of the genus *Cacalia* have been investigated with respect to their anti-oxidation, anti-radiation and anti-histamine activities (Northwest Plateau Institute of Biology, 1987; Hou, 1998; Yue et al., 2005). The presence of pyrrolizidine alkaloids, sesquiterpenes, fatty acid compounds, diterpenes, monoterpenes and sterides in many species of the tribe *Senecioneae* is well documented (Zhang et al., 1998; Wang et al., 2003; Zhou et al., 2006).

In recent years, studies have focused on the separation and identification of the chemical components of *Cacalia tangutica* such as coumarin (Yue et al., 2005) and sesquiterpenes (Liu and Tian, 2004). Although research of the chemical composition of the essential oil have been conducted from this herb (Zhang et al., 2005), studies of its biological functions, especially the antimicrobial activity have rarely been reported. We isolated the essential oil from the herb of *Cacalia tangutica* using the hydrodistillation method, identified the chemical components of *Cacalia tangutica* by gas chromatography (GC)-mass spectrum (MS) technique and evaluated the antimicrobial property of the oil using agar disc diffusion and broth microdilution methods (Yu et al., 2004; Zhu et al., 2005). As far as we know, this is the first report on the antimicrobial effect of the oil on bacteria, fungi, especially, clinically isolated pathogens.

2 Materials and methods

2.1 Plant material

The herb of *Cacalia tangutica* (Maxim.) Hand.-Mazz was collected from the Shennongjia area in Hubei Province, China in October, 2004. A voucher specimen was deposited at the herbarium of the College of Life Sciences, Wuhan University, China.

2.2 Extraction of the essential oil

The herb of *Cacalia tangutica* (Maxim.) Hand.-Mazz was ground and distilled for 3 h using a Clevenger type apparatus. The essential oil obtained was dried using

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anhydrous sodium sulphate and then stored at 4°C until tested.

2.3 GC/MS analysis

The oil was analyzed by GC/MS technology using two different fused-silica capillary columns (30 m × 0.25 mm i.d., film thickness 0.25 μm) of different polarities [DB-5 and HP-Innowax from the Agilent Company (Palo Alto, California, USA)]. The oven temperature was programmed to increase from 50°C to 250°C at a rate of 3°C per min and finally held isothermally for 10 min. The carrier gas was helium introduced at a rate of 1.0 mL/min. Diluted samples (1.0 μL, 1/100 in ether) were injected manually and the split ratio was adjusted to 40:1. GC/MS analyses were performed using a Thermo Finnigan-TRACE GC (Waltham, Massachusetts, USA) coupled with a TRACE MS plus (Waltham, Massachusetts, USA) (EI 70 eV) of the same company. The components were identified by comparison of their mass spectra with those of NIST2002 library data of the GC/MS system and the Adams libraries spectra (Adams, 2001). The results were further confirmed by comparison of the compounds' elution order with their retention indices reported in the literature. The retention indices of the components were determined relative to the retention times of a series of *n*-alkanes with linear interpolation. The relative percentages of the separated compounds were calculated from total ion chromatograms by computerized integrator.

2.4 Antimicrobial activity

2.4.1 Microbial strains

The activities of the essential oil were tested against 14 microorganisms. The reference strains were: *Bacillus subtilis* CCTCC AB92068, *Staphylococcus aureus* CCTCC AB91053, *Staphylococcus aureus* CCTCC AB91118, *Escherichia coli* CCTCC AB91107, *Proteus vulgaris* CCTCC AB91103, *Salmonella* Typhimurium CCTCC AB94010, *Candida* sp. CCTCC AY91001 and *Hansenula anomala* CCTCC AY92046. Clinically isolated strains were: *Enterococcus faecalis*, *Staphylococcus saprophyticus*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. The microorganisms were provided and identified by the Chinese Center for Type Culture Collection (CCTCC) and Renmin Hospital of Wuhan University. They were preserved at −80°C.

2.4.2 Antimicrobial screening

The agar disc diffusion method was employed to determine the antimicrobial activity of the essential oil (Yu et al., 2004; Zhu et al., 2005). Briefly, a suspension of the tested microorganism (2×10^8 CFU/mL) was spread on the solid media plates [Mueller Hinton and Sabouraud Dextrose agars (Merck, Darmstadt, Germany)]. Filter paper discs (6 mm in diameter) were individually impregnated with 15 μL of the diluted oil aliquots (200.00 g/L) and placed on the incubated plates. The plates were placed at 4°C for 2 h, followed by incubation at 37°C for 24 h for bacteria and at 30°C for 48 h for fungi. Then, the disc diameters of inhibition zones (DDs) were measured and expressed in millimeters. Each test was performed using three replicates and repeated twice. Modal values were selected. Levofloxacin served as a positive control and Tween 80 at a final concentration of 0.5% (v/v) was used as a negative control.

2.4.3 Determinations of minimum inhibitory concentration and minimum bactericidal concentration

The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (Yu et al., 2004; Zhu et al., 2005). All tests were performed in Mueller Hinton broth and Sabouraud Dextrose broth (Merck, Darmstadt, Germany) both supplemented with Tween 80 at a final concentration of 0.5% (v/v) for both bacteria and fungi, respectively. Serial doubling dilutions of the oil were prepared in a 96-well microtiter plate ranging from 0.05 to 200.00 g/L.

Overnight broth cultures of each strain (37°C for 12 h for bacteria and 30°C for 24 h for fungi) were prepared and the final concentration of each strain in each well was adjusted to 5×10^4 CFU/mL. Plates were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for fungi. The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. The microorganism growth was indicated by the turbidity.

To determine MBC, broth was taken from each well and incubated in Mueller Hinton agar at 37°C for 24 h for bacteria or in Sabouraud Dextrose agar at 30°C for 48 h for fungi. The MBC was defined as the lowest concentration of the essential oil at which the incubated microorganism was completely killed. Each test was performed using three replicates and repeated twice. Levofloxacin served as a positive control in parallel experiments and Tween 80 at a final concentration of 0.5% (v/v) was used as a negative control.

3 Results and discussion

3.1 Chemical composition of the essential oil

The essential oil was obtained by hydrodistillation from *Cacalia tangutica* with a yield of 0.32% (v/w). By using two chromatographic procedures 73 compounds, representing 74.55% of the oil were identified. Qualitative and

quantitative analytical results by GC-MS are shown in Figures 1, 2 and Table 1.

The components of the essential oil were separated into five classes, including monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and others (Table 1). The oil consisted mainly of sesquiterpene hydrocarbons (32.10%), monoterpene hydrocarbons (21.76%), and

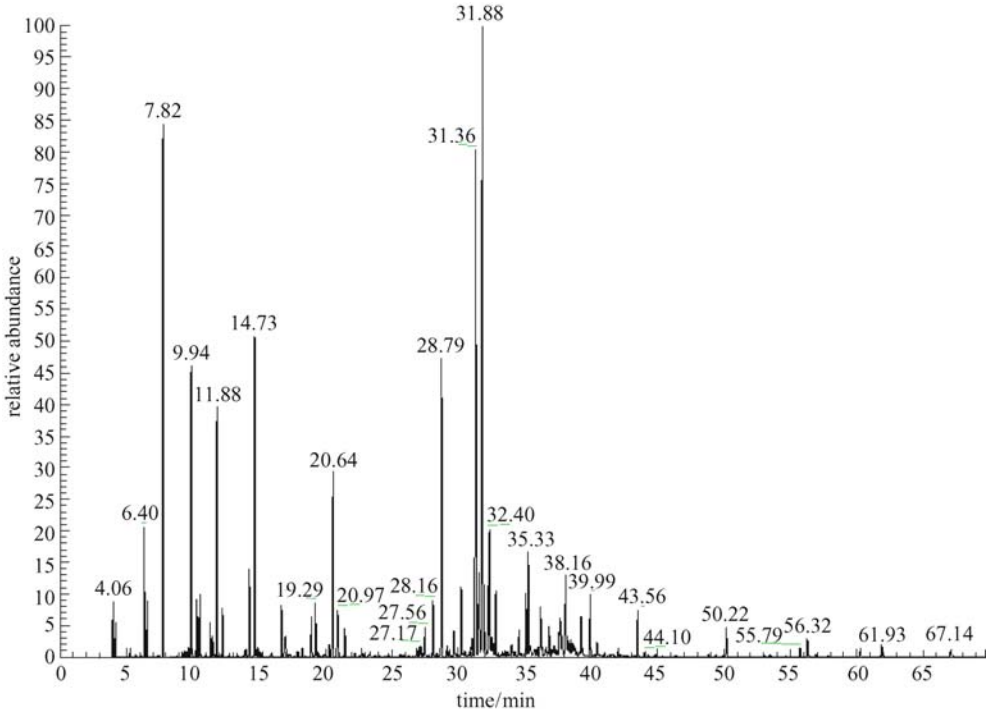

Fig. 1 GC-MS total ion count chromatograms for the essential oil of *Cacalia tangutica* with *n*-alkanes in DB-5.

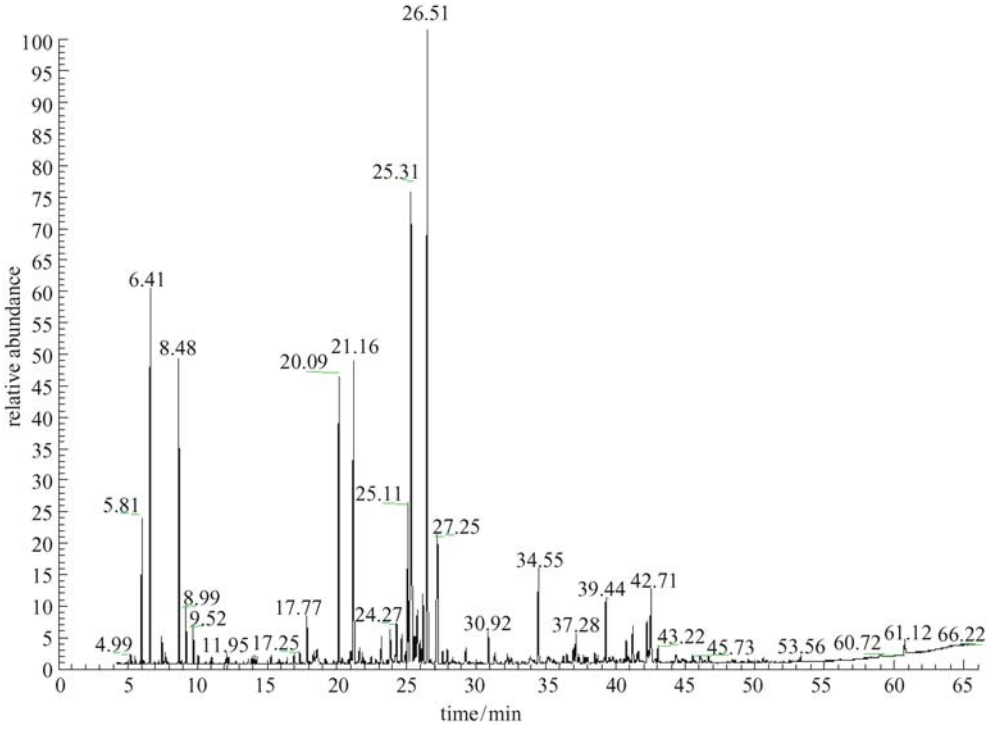

Fig. 2 GC-MS total ion count chromatograms for the essential oil of *Cacalia tangutica* without *n*-alkanes in HP-Innowax.

Table 1 Chemical composition of the essential oil of *Cacalia tangutica*

compounds	percentage/%	R.I.[a]	R.I.[b]
monoterpene hydrocarbons	**21.76**		
α-pinene	8.54	931	939
β-myrcene	4.89	988	991
α-phellandrene	1.10	1005	1003
ρ-cymene	0.58	1023	1025
limonene	0.48	1027	1029
β-ocimene(Z-)	4.40	1034	1037
β-ocimene(E-)	0.87	1044	1050
γ-terpinene	0.90	1055	1060
oxygenated monoterpenes	**13.63**		
linalool	6.16	1099	1097
tagetone(E-)	0.99	1144	1144
α-terpineol	0.82	1194	1189
ocimenone(Z-)	3.58	1232	1229
ocimenone(E-)	1.29	1238	1240
geraniol(Z-)	0.57	1253	1253
geranyl acetate	0.22	1379	1381
sesquiterpene hydrocarbons	**32.10**		
β-elemene	0.67	1397	1391
caryophyllene(Z-)	6.36	1415	1409
(Z)- β-farnesene	0.60	1438	1443
β-farnesene	1.43	1451	1457
γ-muurolene	0.40	1471	1480
germacrene D	10.76	1477	1485
β-selinene	1.95	1484	1490
α-zingiberene	13.49	1490	1494
α-farnesene	2.47	1503	1506
γ-cadinene	0.33	1509	1514
oxygenated sesquiterpenes	**6.84**		
nerolidol(E-)	0.52	1559	1563
spathulenol	1.39	1572	1578
caryophyllene oxide	2.28	1577	1583
α-cadinol	1.87	1651	1654
α-bisabolol	0.78	1682	1686
others	**0.22**		
nonanal	0.22	1104	1101
total	**74.55**		

[a]: Retention indices calculated against *n*-alkanes on DB-5 MS column; [b]: Retention indices reported in the literature.

oxygenated monoterpenes (13.63%). α-zingiberene (13.49%), germacrene D (10.76%), caryophyllene(Z-) (6.36%) and α-farnesene (2.47%) were the main sesquiterpene hydrocarbons. Monoterpene hydrocarbons accounted for 21.76% of the oil with α-pinene (8.54%), β-myrcene (4.89%) and β-ocimene (Z-) (4.40%) as the main compounds. Oxygenated sesquiterpenes were rather weakly represented (13.63%) with linalool 6.16%, ocimenone (Z-) (3.58%) and ocimenone (E-) (1.29%) as the main components. Oxygenated sesquiterpenes constituted the lowest level (6.84%) of the oil. Among them, considerable amounts of caryophyllene oxide (2.28%), α-cadinol (1.87%) and spathulenol (1.39%) were detected.

It is noteworthy that the composition of the essential oil is only in partial agreement with the previous report. For example, germacrene D (10.76%), caryophyllene (Z-) (6.36%) and α-phellandrene (1.10%) were present at a lower concentration in our sample than that reported before, and α-pinene (8.54%) was in a higher percentage. Some components such as α-zingiberene, linalool, β-myrcene, β-ocimene(Z-), which were not detected in previous reports, were found in our sample. Likewise, other constituents such as 1H-benzocycloheptene, (E)-1.2-(methylenedioxy)-4-propenyl-benzene, 4-carene, and (+)-(Z)-longipinane, which were not detected in our sample, were found before. The differences might be resulted from the variability associated with origins of the herb, collection seasons or environmental growing conditions.

3.2 Antimicrobial activity

The disc diameters of inhibition zones (DDs), MICs, and MBCs of *Cacalia tangutica* essential oil for the microorganisms tested are shown in Table 2.

The essential oil of *Cacalia tangutica* showed different inhibitory activities against the 14 microorganisms and it demonstrated bactericidal activity to a certain extent. Inhibition zones were similar to the results of MIC and MBC and larger DDs correlated with lower MICs and MBCs. Although the MIC and MBC results varied between the organisms tested, in most cases the MIC was equivalent to the MBC, indicating bactericidal activity of the oil. Generally, the Gram-positive bacteria and yeasts were more sensitive to the oil than Gram-negative bacteria. The oil showed no activity against *B. subtilis* CCTCC AB92068 or *E. coli* CCTCC AB91107 at the tested concentrations. The MIC and MBC data obtained with the broth microdilution method indicated that *Candida* sp., *S. aureus* CCTCC AB91053 and *E. faecalis* were the most sensitive microorganism to the oil with the largest inhibition zone and lowest MIC and MBC, followed by *S. aureus* CCTCC AB91118, *S. saprophyticus* and *P. mirabilis*, which exhibited stronger antimicrobial and bactericidal capability. Meanwhile, a relatively weaker antimicrobial activity was observed against *P. vulgaris*, *S.* Typhimurium, *H. anomala*, *K. pneumoniae*, *C. freundill* and *E. coli*.

The antimicrobial activity of the *Cacalia tangutica* oil could be attributed to the high content compounds. α-zingiberene has shown very strong antimicrobial activity (Tan et al., 2003) and germacrene D has been frequently reported to have pronounced antimicrobial effects (Juteau et al., 2002). α-pinene is a well-known chemical possessing an antimicrobial potential against Candida albicans (Xia and Yu, 2000). Linalool has antimicrobial, antiviral and ataractic capacity (Liao et al., 2004). Caryophyllene oxide has been found to inhibit growth of *S. aureus*, *E. coli* and *C. albicans* strains (Sibanda et al., 2004).

In addition, minor components may also contribute to the antimicrobial activity of the oil. γ-terpinene, ρ-cymene and α-terpineol have been demonstrated to have significant antimicrobial activity (Carson and Riley, 1995; Cosentino et al., 1999). Limonene has strong inhibitory

Table 2 Antimicrobial activity of the essential oil of *Cacalia tangutica*

microorganisms	*essential oil*			levofloxacin		
	DD[a]	MIC[b]	MBC[b]	DD[c]	MIC[d]	MBC[d]
reference strains						
B. subtilis CCTCC AB92068	8	NA	NA	39	0.49	0.49
S. aureus CCTCC AB91053	20	0.31	0.31	41	0.24	0.24
S. aureus CCTCC AB91118	20	0.63	0.63	40	0.24	0.24
E. coli CCTCC AB91107	7	NA	NA	28	3.91	3.91
P. vulgaris CCTCC AB91103	8	5.00	5.00	40	0.24	0.24
S. Typhimurium CCTCC AB94010	10	1.25	5.00	30	1.95	1.95
Candida sp. CCTCC AY91001	18	0.16	0.16	NT	NT	NT
H. anomala CCTCC AY92046	9	5.00	5.00	NT	NT	NT
clinically isolated strains						
E. faecalis	18	0.31	0.31	30	3.91	3.91
S. saprophyticus	13	0.63	1.25	25	7.81	62.50
C. freundill	9	5.00	5.00	17	31.25	31.25
E. coli	9	2.50	2.50	33	1.95	1.95
K. pneumoniae	8	5.00	5.00	40	0.12	0.98
P. mirabilis	15	0.63	0.63	20	31.25	62.50

DD: diameter of zone of inhibition (mm) including disc diameter of 6 mm; NT: not tested; NA: not active. [a] : Tested at a concentration of 3 mg/disc; [b] : Values given as g L^{-1}; [c] : Tested at a concentration of 5 μg/disc; [d] : Values given as mg L^{-1}.

effect against several bacteria and fungi (Wang, 2005). Geraniol has shown effective inhibitory effect against bacteria, fungi and insects, and is an antiseptic agent (Chen et al., 2000). Elemene has an anticonvulsant activity which could relieve asthma and also contribute to the antimicrobial and antiviral activity of the oil (Wei et al., 2005). It is also possible that the minor components may be synergistic with other constituents of the essential oil in their antimicrobial activity (Marino et al., 2001).

4 Conclusions

Our study suggests that the *Cacalia tangutica* essential oil has good antimicrobial activity against microorganisms, especially some clinically isolated pathogens attributable to the many antimicrobial components. With the increase of antibiotic resistance, development of new natural plant species with antibacterial activity has been in urgent need. The antimicrobial effect demonstrated by the *Cacalia tangutica* essential oil suggests that it may be one of new medicinal resources for antibacterial and antifungal agents.

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