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Chemical composition and antimicrobial activity of the essential oil of *Scutellaria barbata*

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Abstract

The essential oil of *Scutellaria barbata* was obtained by hydrodistillation with a 0.3% (v/w) yield and analysed by GC and GC-MS. The main compounds in the oil were hexahydrofarnesylacetone (11.0%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (7.8%), menthol (7.7%) and 1-octen-3-ol (7.1%). The antimicrobial activity of the oil was evaluated against 17 microorganisms using disc diffusion and broth microdilution methods. The gram-positive bacteria, including methicillin-resistant *Staphlococcus aureus*, were more sensitive to the oil than gram-negative bacteria and yeasts.

Keywords: *Scutellaria barbata*; Essential oil; Antimicrobial; Lamiaceae

1. Introduction

Scutellaria barbata D.Don (*Lamiaceae*) is a plant native to southern China. This herb is known in traditional Chinese Medicine as Ban-Zhi-Lian and has been used as an anti-inflammatory and antitumor agent and as a diuretic (Jiangsu New Medical College, 1977). Extracts of *S. barbata* have shown in vivo growth inhibitory effects in a number of cancers (Qian, 1987). In the clinic, the herb has been used in the treatment of digestive system cancers, hepatoma, lung cancer, breast cancer and chorioepithelioma.

The herb is known to contain alkaloids and flavones (Jiangsu New Medical College, 1977). Flavonoids (apigenin and luteolin) were isolated from a 50% ethanolic extract of the plant as active constituents against methicillin-resistant *Staphylococcus aureus* (Sato et al., 2000). *E*-1-(4′-Hydroxyphenyl)-but-1-en-3-one was isolated from methanol extract of the plant and displayed strong cytotoxicity in K562 human leukaemia cell lines (Ducki et al., 1996). To our knowledge, there are no published reports on the chemical composition and antimicrobial activity of the essential oil of *S. barbata*. Therefore, we focused our study on the chemical composition and antimicrobial property of the essential oil of *S. barbata*.

2. Results and discussion

Hydrodistillation of dried aerial parts of *S. barbata* yielded 0.3% (v/w) of a yellowish essential oil. Forty-one compounds, representing 85.6% of the oil, were identified using two chromatographic procedures (see Experimental). Quantitative and qualitative analytical results are shown in Table 1.

The essential oil consisted mainly of oxygenated monoterpenes and sesquiterpenes. Menthol (7.7%), linalool (6.7%), α-terpieol (1.5%) and thymol (1.4%) were the main oxygenated monoterpenes, whereas (*z*)-α-*trans*-bergamotol (5.1%) and globulol (4.2%) were the main oxygenated sesquiterpenes. The levels of several sesquiterpene hydrocarbons, such as β-bourbonene (2.8%) and β-himachalene (1.5%), were also significant. Among other compounds, considerable amounts of hexahydrofarnesylacetone (11.0%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (7.8%), 1-octen-3-ol (7.1%) and methyleugenol (5.6%) were detected.

The disc diameters of zone of inhibition (DDs), minimum inhibitory concentrations (MICs) and

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Table 1
Main components (%) of the essential oil of *Scutellaria barbata*

Compounds[a]	Percentage
Benzaldehyde	0.2
1-Octen-3-ol	7.1
3-Octanol	0.2
Benzeneacetaldehyde	0.2
Linalool	6.7
Camphor	0.1
Menthone	0.3
Phenetol	0.3
Menthol	7.7
α-Terpineol	1.5
trans-2-Hydroxycinnamic acid	0.7
Perillol	0.1
Nerol	0.2
Anethole	0.3
Thymol	1.4
Eugenol	1.6
α-Copaene	0.2
β-Bourbonene	2.8
Methyleugenol	5.6
α-Cedrene	0.2
Isoledene	0.2
α-*trans* Bergamotene	0.2
Geranyl acetone	0.6
β-Ionone	3.1
β-Guaiene	0.3
β-Himachalene	1.5
α-Terpinyl butyrate	2.8
δ-Cadinene	1.2
Cis-Asarone	0.2
Isoaromadendrene epoxide	0.4
Globulol	4.2
(*Z*)-α-*trans*-Bergamotol	5.1
Torreyol	0.4
α-Cadinol	0.5
3,7,11-Trimethyl-1-dodecanol	0.5
Hexahydrofarnesylacetone	11.0
Farnesylacetone	0.5
Octadecanal	2.5
n-Heptadecanol	5.0
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	7.8
Heptacosane	0.2

[a] The compounds are listed in order of their elution on DB-1.

minimum bactericidal concentrations (MBCs) of *Scutellaria barbata* essential oil for the microoganisms tested are shown in Table 2. Although the MICs and MBCs results varied between organisms tested, in the most cases the MIC was equivalent to the MBC, indicating a bactericidal activity of the oil.

The data obtained from disc diffusion method indicated that *Staphylococcus epidermidis* was the most sensitive microorganism tested with the strongest inhibition zone (29 mm), followed by methicillin-resistant and methicillin-senstive *Staphylococcus aureus* and *Staphylococcus heamolyticus* with stronger inhibition zones (21–26 mm). *Salmonella paratyphi*-A, *Enterococcus faecalis* (Group D) and *Candida albicans* exhibited weak inhibition zones (7–9 mm).

The results of MIC indicated the oil inhibited all microorganisms tested, with the exception of *S. paratyphi*-A. The gram-positive bacteria were more sensitive to the oil than gram-negative bacteria and yeasts. *S. epidermidis* had the lowest MIC (0.77 mg/ml).

The oil was demonstrated to have a strong bactericidal effect. The lowest MBC was 0.77 mg/ml for *S. epidermidis*. Methicillin-resistant and methicillin-sensitive *S. aureus and S. heamolyticus* had lower MBC of 1.53 mg/ml. *C. albicans* had the highest MBC of 24.50 mg/ml.

Overall, the essential oil displayed a broad antimicrobial spectrum and exerted a much stronger bactericidal effect against gram-positive bacteria, including methicillin-resistant *S. aureus* (MRSA). Among the microorganisms tested, only *S. paratyphi*-A was resistant to the essential oil. The antimicrobial activity of the oil could be due to menthol and long chain alcohols such as linalool and 1-octen-3-ol. Menthol has been reported to have significant antimicrobial activity (Pattnaik et al., 1997; Osawa et al., 1999). Linalool has been demonstrated to have strong inhibitory effect against 17 bacteria and 10 fungi (Pattnaik et al., 1997). In fact, long chain (C_6–C_{10}) alcohols were particularly active against gram-positive bacteria (Delaquis et al., 2002), the antimicrobial properties of alcohols were known to increase with molecular weight (Morton, 1983). In addition, minor components such as thymol and α-terpineol could also contribute to antimicrobial activity of the oil. Thymol and α-terpineol have been reported to be antibacterial (Cosentino et al., 1999; Carson and Riley, 1995). In fact, it is also possible that the minor components may be involved in some type of synergism with the other active compounds (Lattaoui and Tantaoui-Elaraki, 1994; Marino et al., 2001).

The oil is a potential source of novel antimicrobial essential oils because of stronger bactericidal effect on clinically isolated microorganisms, particularly on methicillin-resistant *Staphylococcus aureus* (MRSA). According to recent studies, community-acquired or outpatient MRSA infections are increasing in both children and adults (Herold et al., 1998), MRSA is responsible for worldwide outbreaks of nosocomial infections (Voss and Doebbeling, 1995). At present, however, the pharmaceutical arsenal available to contral MRSA is limited (Sato et al., 2000). Our study suggest that essential oil could be one of new medicinal resources for antibacterial agents against MRSA.

3. Experimental

3.1. Plant material

Dried plant material of *Scutellaria barbata* was collected in the Hubei (China) in August 2001. A voucher specimen has been deposited at the herbarium of College of Life Science, Wuhan University, China.

Table 2
Antimicrobial activity of the essential oil of *Scutellaria barbata*

Microorganisms	Essential oil			Levofloxacin		
	DD[a]	MIC[b]	MBC[b]	DD[c]	MIC[d]	MBC[d]
Reference strains						
Staphylococcus aureus ATCC 25923	25	1.53	1.53	34	0.30	0.30
Escherichia coli ATCC 25922	15	3.06	6.12	34	0.30	0.61
Pseudomonas aeruginosa ATCC 27853	13	6.12	6.12	20	0.15	0.30
Clinically isolated strains						
Methicillin-sensitive *Staphylococcus aureus*	24	1.53	1.53	32	0.61	1.22
Methicillin-resistant *Staphylococcus aureus*	26	1.53	1.53	11	9.75	9.75
Staphylococcus epidermidis	29	0.77	0.77	28	1.22	1.22
Staphylococcus heamolyticus	21	1.53	1.53	13	4.87	9.75
Staphylococcus simulans	15	3.06	3.06	30	0.61	0.61
Enterococcus faecalis (Group D)	8	12.25	12.25	11	9.75	9.75
Citrobacter freundii	19	3.06	3.06	15	2.44	4.87
Klebsiella pneumoniae	14	3.06	3.06	15	4.87	4.87
Shigella flexneri	14	6.12	6.12	17	2.44	4.87
Salmonel paratyphi-A	7	>49.00	–	14	1.22	2.44
Serratia liquefaciens	12	6.12	6.12	22	1.22	1.22
Serratia marcescens	16	3.06	6.12	26	0.61	1.22
Stenotrophomonas maltophilia	19	3.06	3.06	20	0.61	0.61
Candida albicans	9	24.50	24.50	NT	NT	NT
Candida tropicalis	10	12.25	12.25	NT	NT	NT

DD, diameter of zone of inhibition (mm) including disc diameter of 6 mm. NT, not tested.
[a] Tested at a concentration of 2.7 mg/disc.
[b] Values given as mg/ml.
[c] Tested at a concentration of 5 μg/disc.
[d] Values given as μg/ml.

3.2. Essential oil extraction and analysis

Aerial parts (1.5 kg) of *S. barbata* were air dried and then distilled for 3 h using a Clevenger type apparatus. Anhydrous sodium sulphate was used to absorb the little water that the essential oil contained. The oil was then stored at −10 °C until tested.

The oil was analyzed by GC and GC–MS. GC analyses were performed using a Shimadzu GC-14C instrument with a data handing system and FID. The analyses were carried out by using two different fused-silica capillary columns: DB-1 (30 m×0.25 mm i.d.; film thickness 0.25 μm) and HP-Innowax (30 m×0.25 mm i.d.; film thickness 0.25 μm). The oven temperature was programmed from 50 to 250 °C at 3 °C/min and held at this temperature for 10 min. Injector and FID detector temperatures were 220 and 280 °C, respectively. Carrier gas was helium at a flow rate of 1 ml/min. Diluted samples (1/10 in ether) of 1.0 μl were injected manually and split ratio was adjusted to 20:1. GC–MS analyses were performed with a Thermo-Finnigan TRACE GC coupled with a TRACE MS^{plus} (EI 70 eV) from the same company. The same two columns as described for GC analyses were used, with operating conditions as above. Components were identified by comparison of their mass spectra with those in the NIST98 GC–MS library and those in the literature (Adams, 1995), as well as by comparison of their retention indices with literature data (Davies, 1990; Pedro et al., 2001). Retention indices of the components were determined relative to the retention times of a serie of *n*-alkanes with linear interpolation.

3.3. Microbial strains

The essential oil was tested against 17 microorganisms. Reference strains were: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853; clinically isolated strains were as follows: methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus heamolyticus*, *Staphylococcus simulans*, *Enterococcus faecalis* (Group D), *Citrobacter freundii*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Salmonella typhi*, *Salmonella paratyphi*-A, *Serratia liquefaciens*, *Serratia marcescens*, *Stenotrophomonas maltophila*, *Candida albican* and *Candida tropicalis*.

3.4. Antimicrobial screening

The agar disc diffusion method was employed for the determination of antimicrobial activity of the essential oil (NCCLS, 1997). Briefly, a suspension of the test microorganism (2×10^8 cfu/ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were individually impregnated with 15 μl of the diluted oil aliquots (180 mg/ml), then placed on the inoculated plates, for 2 h at 4 °C following which they were

incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for yeast. The diameters of the inhibition zones were measured in millimeters.

3.5. Determinations of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

A broth microdilution method was used to determine the MIC and MBC (NCCLS, 1999; Bassole et al., 2003). All tests were performed in Mueller Hinton broth supplemented with Tween 80 [final concentration of 0.5% (v/v)], with the exception of the yeasts (Sabouraud dextrose broth + Tween 80). A serial doubling dilution of the oil was prepared in a 96-well microtiter plate over the range 0.02–49.00 mg/ml.

Overnight broth cultures of each strain were prepared and the final concentration in each well was adjusted to 2×10^4 cfu/ml. Plates were incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for the yeasts. 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 inoculated in Mueller Hinton Agar for 24 h at 37 °C for bacteria or in Sabouraud dextrose agar for 48 h at 30 °C for the yeasts. The MBC is defined as the lowest concentration of the essential oil at which inoculated microorganism was completely killed. Each test was performed in duplicate and repeated twice. Levofloxacin was served as positive control.

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