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Chemical composition and antimicrobial activity of essential oils of *Chaenomeles speciosa* from China

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Abstract

The chemical composition of essential oil obtained by hydrodistillation from the dried fruits of *Chaenomeles speciosa* was analyzed by GC–MS. Forty compounds, constituting about 85.13% of the total oil, were identified. The main constituents were β-caryophyllene (12.52%), α-terpineol (5.41%), terpinen-4-ol (4.56%) and 1,8-cineole (4.31%). The antimicrobial activity of the oil was evaluated against 10 microorganisms using disc diffusion and broth microdilution methods. The essential oil was found to show a broad spectrum of antimicrobial activity against all the tested bacterial strains. The essential oil had more sensitivity to gram-positive than gram-negative bacteria.

Keywords: *Chaenomeles speciosa*; Essential oil; GC–MS; Antimicrobial activity

1. Introduction

Chaenomeles speciosa (Sweet) Nakai (Rosaceae) is a well-known food spread in China and its dried fruit is commonly used for traditional medicine (Jiangsu New Medical College, 1977). An investigation into the effective constituents of *C. speciosa* suggests that carbohydrates, amino acids, proteins, tannins and organic acids are the main components of *C. speciosa* (Chen, Wu, & Dai, 2000).

Some reports have shown that *C. speciosa* possesses biological activities such as hepatoprotective effect (Zheng & Wang, 1985), antibacterial properties (Guo, Tian, & Tang, 1984), anti-inflammatory action (Dai, Wei, Shen, & Zheng, 2003), anti-tumor activity (Jing, 1975), and immunomodulatory effect (Gong, Wang, & Xu, 1995). However, there are no published reports on the chemical composition and anti-microbial activity of the essential oil of *C. speciosa* produced in China. Because many microorganisms have resistance to antibiotics, more and more researchers have been interested in extracting biologically active compounds from plant species in order to eliminate pathogenic microorganisms (Essawi & Srour, 2000). Therefore, we focused our study on the chemical composition and antimicrobial properties of the essential oil of *C. speciosa*.

2. Materials and methods

2.1. Plant material

Dried fruits of *C. speciosa* were collected from Changyang in Hubei Province (China) in September 2003. A voucher specimen was deposited at the herbarium of *College of Life Sciences, Wuhan University, China.*

2.2. Essential oil extraction and GC–MS analysis

Dried fruits of *C. speciosa* were distilled for 3 h using a Clevenger type apparatus. The essential oil obtained was dried using anhydrous sodium sulphate and then stored at −10 °C until tested.

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The oil was analyzed by GC–MS. The analyses were carried out 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 agilent company (Palo Alto, California, USA)]. The oven temperature was programmed to increase from 50 to 250 °C at a rate of 3 °C/min and finally held isothermal for 10 min. The carrier gas was helium introduced at a rate of 1.0 ml/min. Diluted samples (1/10 in ether) of 1.0 μl 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 NIST98 library data of the GC–MS system and Adams libraries spectra (Adams, 2001), as well as by comparison with the compounds' elution order with their retention indices reported in the literature (Adams, 2001). Retention indices of the components were determined relative to the retention times of a series of *n*-alkanes with linear interpolation.

2.3. Microbial strains

The essential oil was tested against 10 microorganisms. Reference strains were: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853; clinically isolated strains were: *Staphylococcus epidermidis*, *Staphylococcus simulans*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Proteus mirabilis* and *Klebsiella pneumoniae*.

2.4. Antimicrobial screening

The agar disc diffusion method was employed to determine the antimicrobial activity of the essential oil (NCCLS, 1997). Briefly, a suspension of the tested 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 (200.00 mg/ml) 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. The diameters of the inhibition zones were measured and expressed in millimetres. Each test was performed in three replicates and repeated twice. Levofloxacin served as positive control.

2.5. 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; Yu, Lei, Yu, Cai, & Zou, 2004). All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Serial doubling dilutions of the oil was prepared in a 96-well microtiter plate ranged from 0.05 to 200.00 mg/ml.

The final concentration of each strain was adjusted to 4×10^4 CFU/ml. Plates were incubated at 37 °C for 24 h. 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. The MBC was defined as the lowest concentration of the essential oil at which incubated microorganism was completely killed. Each test was performed in three replicates and repeated twice. Levofloxacin served as positive control.

3. Results and discussion

3.1. Chemical composition of the essential oil

By hydrodistillation the dried fruits of *C. speciosa* yielded 0.19% (v/w) of essential oil. By using two chromatographic

Table 1
Main chemical composition of *Chaenomeles speciosa* essential oil

Compounds	Percentage (value/weight)	RI[a]
Benzaldehyde	3.56	959
Linaloyl oxide	1.94	968
n-Octanal	1.59	1003
α-Terpinene	1.73	1016
ρ-Cymene	1.93	1023
Limonene	2.47	1028
1,8-Cineole	4.31	1031
(*Z*)-β-Ocimene	0.29	1035
(E)-*β*-ocimene	0.22	1045
γ-Terpinene	2.40	1057
n-Octanol	1.91	1069
(+)-4-Carene	1.71	1084
ρ-Cymenene	1.75	1089
trans-Limonene oxide	0.65	1097
Linalool	1.33	1100
n-Nonanal	3.80	1105
Iso-3-thujanol	0.09	1135
ρ-Menth-3-3-en-8-ol	0.29	1149
Menthol	0.77	1159
Borneol	0.97	1172
Terpinen-4-ol	4.56	1179
α-Terpineol	5.41	1194
n-Decanal	0.71	1204
trans-2-Decenal	1.79	1246
Carvenone	1.76	1257
Bornyl acetate	0.22	1282
ρ-Menth-3-3-en-8-ol, acetate	1.74	1317
α-Longipinene	2.76	1355
β-Elemene	0.32	1379
Longifolene	1.25	1405
(β)-Caryophyllene	12.52	1428
Neryl acetone	0.58	1446
E-Ethyl cinnamate	0.57	1464
(*E*,*E*)-α-Farnesene	1.35	1503
Germacrene A	1.51	1509
δ-Amorphene	2.61	1512
E-Nerolidol	0.93	1559
γ-Eudesmol	4.12	1628
Epi-α-Cadinol	2.48	1638
α-Cadinol	4.23	1651

[a] Retention indices on DB-5 capillary column. Identified 85.13%.

procedures forty compounds, representing 85.13% of the oil were identified. Quantitative and qualitative analytical results by GC–MS are shown in Table 1.

The essential oil consisted mainly of oxygenated monoterpenes and sesquiterpenes. α-Terpineol(5.41%), terpinen-4-ol (4.56%) and 1,8-cineole (4.31%) were the main oxygenated monoterpenes, while β-caryophyllene (12.52%) was the main sesquiterpene. Several minor compounds were also significant, i.e. ρ-cymene (1.93%), linalool (1.33%), borneol (0.97%) and menthol (0.77%). As far as our literature survey could ascertain, there was only one report on the chemical composition of the essential oil of *C. speciosa* (Horvat et al., 1994). Of the nineteen compounds identified previously, only two, α-terpineol and linalool, were found in this investigation. This may possibly be due to the fact that fruits were grown in different regions, which may have caused the differences in their chemical composition.

3.2. Antimicrobial activity

The disc diameters of zone of inhibition (DDs), minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of *C. speciosa* essential oil for the microorganisms tested are shown in Table 2.

The essential oil of *C. speciosa* showed inhibition zones against all microorganisms tested, which was similar to the results of MIC. Generally, larger DDs correlated with lower MICs. This was confirmed by both MICs and MBCs data, where the essential oil exhibited significant antimicrobial activity against the microorganisms tested, particularly against gram-positive bacteria.

The data obtained from disc diffusion method using *C. speciosa* essential oil, indicated that *S. aureus* was the most sensitive microorganism tested with the largest inhibition zone (23 mm) and *E. cloacae* exhibited the smallest inhibition zone (12 mm). The results of MIC indicated *S. aureus* and *S. epidermidis* had the lowest MIC (1.57 mg/ml), the highest MIC was 25.00 mg/ml for *K. pneumoniae*. The lowest MBC was 1.57 mg/ml for *S. aureus*; *K. pneumoniae* had the highest MBC of 100.00 mg/ml.

Overall, the essential oil displayed a broad antimicrobial spectrum and exerted a much stronger antimicrobial effect against gram-positive bacteria than gram-negative bacteria. The antimicrobial activity of the oil could be due to α-terpineol, terpinen-4-ol, 1,8-cineole. α-Terpineol has been reported to have significant antimicrobial activity (Carson & Riley, 1995; Cosentino et al., 1999). Terpinen-4-ol has been demonstrated to have bacteriostatic activity against several microorganisms (Barel, Segal, & Yashphe, 1991). Antimicrobial activity of 1,8-cineole has been reported (Mourey & Canillac, 2002; Tzakou, Pitarokili, Chinou, & Harvala, 2001; Viljoen et al., 2003). In addition, the components in lower amount such as, ρ-cymene, linalool, borneol and menthol could also contribute to the antimicrobial activity of the oils (Carson & Riley, 1995; Cosentino et al., 1999; Knobloch, Pauli, Iberi, Wegand, & Weis, 1989; Osawa et al., 1999; Pattnaik, Subramanyam, Bapaji, & Kole, 1997; Tabanca, Kirimer, Demirci, Demirci, & Baser, 2001). It is also possible that the components present at lower concentrations might be involved in some type of synergism with the other active compounds (Marino, Bersani, & Comi, 2001).

In general, the active antimicrobial compounds of essential oils, namely terpenoids such as eugenol, thymol, and carvacrol, which are phenolic in nature, it would seem reasonable that their antimicrobial mode of action might be related to phenolic compounds present. Most of the studies on the mechanism of phenolic compounds has focused on their effects on cellular membranes. Actually, phenolics not only attack cell wall and cell membranes, thereby affecting their permeability and release of intracellular constituents (ribose, Na glutamate, etc.) but also interfere with

Table 2
Antimicrobial activity of *Chaenomeles speciosa* essential oil

Microorganisms	Essential oil			Levofloxacin		
	DD[a]	MIC[b]	MBC[b]	DD[c]	MIC[d]	MBC[d]
Reference strains						
Staphylococcus aureus ATCC 25923	23	1.57	1.57	29	0.30	0.30
Escherichia coli ATCC 25922	21	3.13	3.13	34	0.61	0.61
Pseudomonas aeruginosa ATCC 27853	15	6.25	12.50	28	0.30	0.61
Clinically isolated strains						
Staphylococcus epidermidis	18	1.57	3.13	25	0.61	1.22
Staphylococcus simulans	20	3.13	6.25	30	1.22	1.22
Staphylococcus saprophyticus	20	3.13	12.50	27	2.44	9.77
Enterococcus faecalis	16	12.50	12.50	18	4.88	9.77
Enterobacter cloacae	12	12.50	25.00	13	9.77	19.53
Proteus mirabilis	14	12.50	50.00	12	39.06	NA
Klebsiella pneumoniae	16	25.00	100.00	24	4.88	4.88

DD: diameter of zone of inhibition (mm) including disc diameter of 6 mm. NA, not active.
[a] Tested at a concentration of 3 mg/disc.
[b] Values given as mg/ml.
[c] Tested at a concentration of 5 μg/disc.
[d] Values given as μg/ml.

membrane function such as electron transport, nutrient uptake, protein and nucleic acid synthesis and enzyme activity. Thus, active phenolic compounds might have several invasive targets which could lead to their inhibition of bacteria. Tassou, Koutsoumanis, and Nychas (2000) observed that the leakage of intracellular material from *E. coli* and *S. aureus* correlated with the concentration of phenolics present.

In conclusion, the essential oil of *C. speciosa* presented a wide spectrum of antimicrobial activity. It constitutes a potential source of novel antimicrobial essential oils because of resurgence of interest in aromatherapy (Lis-Balchin, 1997), particularly for the local population which need cheap drug.

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