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Original Article

Chemical Composition and Antimicrobial Activity of the Essestial Oil from *Citrus medica* L. var. *sarcodactylis* (Sieber) Swingle Leaf

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Abstract The essential oil was extracted from the fresh leaf of *Citrus medica* L. var. *sarcodactylis* (Rutaceae) using steam distillation technique. Chemical composition of essential oil was identified by GC-MS technique. The major constituents were limonene (49.19%), geranial (25.93%) and neral (16.41%). Essential oil was tested for *in vitro* antimicrobial activity using agar diffusion method. It exhibited a significant antimicrobial activity against *S. aureus*, and *B. subtilis* with the same minimum inhibitory concentration of 2,500 ppm.

Keywords: antimicrobial activity, *Citrus medica* L. var. *sarcodactylis*, essential oil, Rutaceae

INTRODUCTION

Citrus medica L. var. *sarcodactylis* belonging to the family Rutaceae has originated in India and has been worldwide spread to other regions following the paths of civilization.[1] It is known as fingered citron or as fo-shou (Buddha's hand) in China and used in folk medicine as tonic, antispasmodic, antiemetic, expectorant and inhaler.[2,3]

Recent studies reported that *C. medica* L. var. *sarcodactylis* constituted coumarin compounds, *p*-coumaric acids, steroids, triterpenoids, limonin, nomilin, *etc*.[4,5] The major volatile components in the peel oil from the Japanese fingered citron were limonene and γ-terpinene.[6]

According to scientific literatures, no scientific information on the chemical composition and bioactivity for essential oil from *C. medica* L. var. *sarcodactylis* leaf has been reported. This study, thus, presents the identification of essential oil from the fresh leaf of this plant determined by GC-MS technique and its antimicrobial activity.

MATERIALS AND METHODS

Plant Material

The fresh leaf of *C. medica* L. var. *sarcodactylis* was collected from Pathumtani Province, Thailand, in June 2006. The specimen was identified by the expert botanist.

Essential Oil Analysis

The fresh leaf of *C. medica* L. var. *sarcodactylis* was ground into small pieces and was extracted using Clevenger apparatus by steam distillation technique. Essential oil was dissolved in HPLC grade methanol and analyzed using a Finnigan Trace GC ultra GC equipped with a Finnigan Trace DSC quadrupole mass spectrometric detector (MSD). The column used was a BPX 5 phenyl: dimethylpolysiloxane (5:95) capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness) and the gas carrier was helium at the flow rate of 1 ml/min. The temperature program was used for the analysis, *i.e.* the column temperature was held at 60°C for 1

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minute, raised to 240°C at the heating rate of 3.3°C/min and then held for 5 minutes. Temperatures for GC injector and GC-MSD interface were 180°C and 290°C, respectively.

Mass spectra were recorded in the electron ionization mode at 70 eV, scanning in the 40-500 m/z range. The tentative identification of isolated components was carried out by the comparison to the Adam terpene library. The relative yield of each individual component of essential oil was expressed as the percentage of the peak area relative to the total peak area.

Microorganisms

Three gram positive bacteria, *i.e*. *Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus* and one gram negative bacteria, *i.e. Escherichia coli* were used for *in vitro* antimicrobial activity test. These bacteria were supplied by Faculty of Medical Technology, Rangsit University, Pathumthani Province, Thailand.

Antimicrobial Assay

The antimicrobial assay of essential oil from *C. medica* L. var. *sarcodactylis* leaf was carried out using a modification of the agar diffusion method.[7]

Microbial cultures for antimicrobial assay were freshly cultured on tryptic soy broth (TSB) medium and incubated at 37°C for 24 hours. After that, the TSB medium was approximately adjusted to solution concentrations of 0.5 McFarland with 0.9% sterile normal saline solution. The mixtures were spread on tryptic soy agar (TSA) plates with sterile cotton swab and allowed to dry. Sterilized paper filter discs with a diameter of 6 mm were impregnated with 20 µl of an essential oil solution in 5% v/v dimethylsulphoxide (DMSO) in TSB and placed on the TSA plate. The plates were left 30 minutes at room temperature to allow the diffusion of the oil, and then they were incubated at 37°C for 24 hours. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. In this experiment, two controls were used, *i.e.* a control having microorganism but no test material and a control having 5,000 ppm standard tetracycline solution in 5% v/v DMSO in TSB. The tetracycline solution was used as a positive control. Experimental tests were performed in triplicate and the developing inhibition zones were compared with those of reference discs.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by the broth dilution method.[8,9] The inocula of microorganisms were prepared from 24 hours broth cultures and suspensions were adjusted to 1 Mc-Farland standard turbidity. Essential oil dissolved in 5% v/v DMSO in TSB at the highest concentration (20,000 ppm) was first tested, followed by serial two-fold dilutions at concentrations ranged from 20,000 to 156.25 ppm in 10 ml sterile test tubes containing nutrient broth. Solvent, antibiotic and microorganism controls were also analyzed. The mixture in each tube was incubated at 37°C for 24 hours. The MIC value of essential oil was determined as the lowest concentration of essential oil that completely prevented any turbidity or growth of the test organisms. All samples were tested in triplicate.

RESULTS AND DISCUSSION

Essential oil hydrodistilled from the fresh leaf of *C. medica* L. var. *sarcodactylis* was found to be 0.75% w/w of the fresh weight. The GC-MS chromatogram of essential oil from the fresh leaf of *C. medica* L. var. *sarcodactylis* was shown in Figure 1.

The GC-MS analysis showed that limonene (49.19%), geranial (25.93%) and neral (16.41%) were the three major components of essential oil from the fresh leaf of *C. medica* L. var. *sarcodactylis*. The yields of limonene in both leaf and peel essential oils of this plant are insignificantly different whereas the yields of neral and geranial in leaf are higher than those in peel. In addition, the GC-MS analysis indicated that γ-terpinene in peel is higher than that in leaf (Table 1). The structures of three major components of essential oil from the fresh leaf of *C. medica* L. var. *sarcodactylis* were shown in Figure 2.

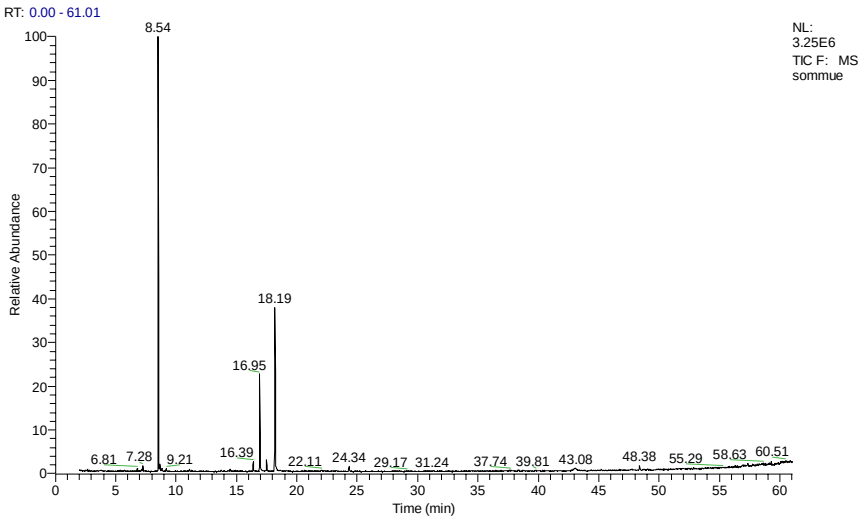


Figure 1. The GC-MS chromatogram of essential oil from the fresh leaf of *C. medica* L. var. *sarcodactylis.*

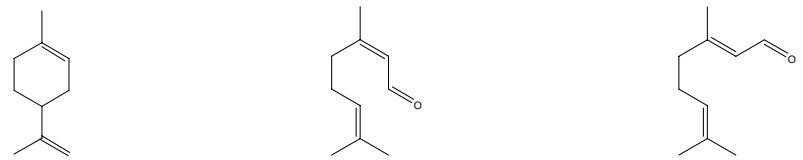
Table 1. Chemical composition of essential oil from the fresh leaf of *C. medica* L. var. *sarcodactylis*

RT[a]	Compound	Molecular formula	Percentage	
			Leaf oil	Peel oil[b]
6.81	Sabinene	$C_{10}H_{16}$	0.28	-
7.28	Myrcene	$C_{10}H_{16}$	0.63	1.7
8.54	Limonene	$C_{10}H_{16}$	49.19	47.8
8.67	1,8-Cineole	$C_{10}H_{18}O$	1.00	-
8.83	(Z)-β-Ocimene	$C_{10}H_{16}$	0.27	-
9.21	γ-Terpinene	$C_{10}H_{16}$	0.47	32.1
14.49	α-Terpineol	$C_{10}H_{18}O$	0.24	-
16.39	Nerol	$C_{10}H_{18}O$	1.48	-
16.95	Neral	$C_{10}H_{16}O$	16.41	1.6
17.51	Geraniol	$C_{10}H_{18}O$	2.27	-
18.19	Geranial	$C_{10}H_{16}O$	25.93	2.5
24.34	γ-Caryophyllene	$C_{15}H_{24}$	0.63	-
-	α-Pinene	$C_{10}H_{16}$	-	2.9
-	Terpinolene	$C_{10}H_{16}$	-	1.4
-	γ-Thujene	$C_{10}H_{18}$	-	1.3

- Not present
[a] Retention time
[b] Data obtained from reference 6.

Table 2 shows *in vitro* antimicrobial activity of essential oil from the fresh leaf of *C. medica* L. var. *sarcodactylis* and the inhibition zone observed on standard antibiotic disc. It was found that essential oil tested had strong inhibitory activity against gram positive bacteria (*S. aureus*, *B. subtilis* and *M. luteus*) but it showed weak activity against gram negative bacteria (*E. coli*). The antimicrobial activity was significantly increased as the concentration of essential oil increased from 5,000 to 20,000 ppm.

Furthermore, essential oil from the fresh leaf of *C. medica* L. var. *sarcodactylis* in this study showed the strongest antimicrobial activity against *S. aureus* and *B. subtilis* (MIC 2,500 ppm). However, the antimicrobial efficiency of essential oil from this plant was much lower (about 40%) than that of



Limonene Neral Geranial

Figure 2. Chemical structures of three major components of essential oil from the fresh leaf of *C. medica* L. var. *sarcodactylis*.

Table 2. Antimicrobial activity of essential oil from the fresh leaf of *C. medica* L. var. *sarcodactylis* and the standard antibiotic

Concentration (ppm)	Inhibition zone, mm (mean ± S.E.M.)[a]			
	Gram -positive			Gram-negative
	S. aureus	*B. subtillis*	*M. luteus*	*E. coli*
20,000	11 ± 0.3	15 ± 0.8	11 ± 0.3	8 ± 0.7
5,000	9 ± 0.3	9 ± 0.6	7 ± 0.3	No inhibition
Tetracycline[b]	13 ± 2	16 ± 2	35 ± 3	26 ± 0.7

[a] The diameter of inhibition zone included diameter of paper filter disc (6 mm).
[b] Tetracycline (5,000 ppm) was used as a standard antibiotic.

Table 3. Minimum inhibitory concentrations (MIC) of essential oil from the fresh leaf of *C. medica* L. var. *sarcodactylis*

Compound	MIC (ppm)			
	Gram-positive			Gram-negative
	S. aureus	*B. subtilis*	*M. luteus*	*E. coli*
Essential oil	2,500	2,500	5,000	10,000
Tetracycline	156	156	78	625

tetracycline solution at the same concentration (Table 3).

The antimicrobial activity of essential oil from the fresh leaf of *C. medica* L. var. *sarcodactylis* is suspected to be associated with the high limonene content, which has been tested previously.[10,11]

CONCLUSION

This is the first report for the studies of chemical composition and antibacterial activity of essential oil from the fresh leaf of *C. medica* L. var. *sarcodactylis* and the experimental data obtained might provide useful information on chemotaxonomic, phytochemical and biological activity studies of this plant genus. In addition, these results suggest that essential oil from the fresh leaf of this plant would probably be a good therapeutic agent against *B. subtilis* and *S. aureus*.

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