องค์ประกอบทางเคมีและฤทธิ์ต้านเชื้อจุลชีพของน้ำมันระเหยจากหัสคุณ

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บทคัดย่อ

องค์ประกอบทางเคมีของน้ำมันระเหยที่กลั่นได้ โดยวิธีต้มกับน้ำจากใบสด ของต้นหัสคุณ (*Micromelum minutum* Wight & Arn. วงศ์ Rutaceae) เมื่อวิเคราะห์องค์ประกอบทางเคมี ด้วยเครื่องก๊าซโครมาโตรกราฟี-แมสสเปคโตรเมตรี พบว่า มีองค์ประกอบ 37 ชนิด โดยมีสารประกอบจำพวก bicyclogermacrene (ร้อยละ 19.79), 9-epi-*(E)*-caryophyllene (ร้อยละ 15.65) และ tricyclene (ร้อยละ 8.74) เป็นองค์ประกอบหลัก การทดสอบฤทธิ์ต้านจุลชีพ พบว่า น้ำมันระเหยจากต้นหัสคุณ แสดงฤทธิ์ต้านเชื้อ *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* และ*Pseudomonas aeruginosa* (ความเข้มข้นร้อยละ 0.25-1 โดยปริมาตร)

คำสำคัญ: น้ำมันระเหย, องค์ประกอบทางเคมี, ฤทธิ์ต้านจุลชีพ, ต้นหัสคุณ

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Chemical Compositions and Antimicrobial Activities of Essential Oil from *Micromelum minutum*

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Abstract

The essential oil from fresh leaves of *Micromelum minutum* Wight & Arn. (Rutaceae) was hydrodistilled by Clevenger apparatus. Gas chromatography-mass spectrometry analyses revealed the presence of 37 components. Bicyclogermacrene (19.79%), 9-epi-*(E)*-caryophyllene (15.65%) and tricyclene (8.74%) were found to be the major components. The essential oil exhibited antimicrobial activities against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* (concentration 0.25-1%v/v).

Key words: Essential oil, Chemical compositions, Antimicrobial activities, *Micromelum minutum*

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Introduction

The family Rutaceae consists of *ca* 150 genera and 900 species; mainly shrubs and trees; distributed in both temperate and tropical countries, but particularly abundant in South Africa and Australia. Oil glands are present in the leaves and other parts. The flowers are usually in cymes with 4-5 sepals, 4-5 petals, 8 or 10 stamens and a superior ovary. The fruits are of various types. Constituents of the Rutaceae include a wide variety of alkaloids, volatile oils, coumarins and terpenoids[1]. *Micromelum minutum* Wight & Arn. (*M. pubescens* Blume.), locally named as Hatsakhun (Saraburi), also belongs to the family Rutaceae[2].

In Thai folk medicine, the oil obtained from Hatsakhun was used for carminative, antitussive, anthelmintic, and expectorant[3]. The variety of *M. minutum*, widely found in Thailand, still has never been reported on its chemical composition and antimicrobial activities. Herein, we reported the terpenoid constituents of the oil obtained from this particular species and its activities against some tested microorganisms.

Material and Method

Plant material and the separation of essential oil.

Fresh leaves of *M. minutum* were collected from Sakaeraj Environmental Research Station, Nakorn-Ratchasima province and chopped into small pieces. The prepared plant material was immediately hydrodistilled by Clevenger apparatus for 4 hours. After cooling, the oil yield was recorded and the essential oil was collected.

Analysis of the compositions of essential oil.

The essential oil was diluted to 1:100 in methanol and injected into gas chromatography-mass spectrometry apparatus at the condition described below. The spectra were recorded and compared with the terpene library program[4].

GC-MS condition.

Instrument model	Varian Saturn III
Column	fused silica capillary column (30 m x 0.25 mm i.d.) coated with DB-5 (J&W), film thickness 0.25 μm
Column programming	60°C 1 minute, 60°-180°c rate 3.0°C/min
Injector temperature	220°C
Carrier gas	helium: flow rate 1.0 ml/min
Split ratio	100:1
Accelerating voltage	1700 volts
Sample size	0.5 μL
Solvent	methanol (HPLC grade)

Antimicrobial activity testing.

The essential oil was screened by agar diffusion method[5] for its activities against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa.*

Test samples. The essential oil was diluted to 8% concentration with 0.5% Tween 80. The oil solutions were sterilized by Millipore filter paper (pore size 0.45 μm).

Preparation of test microorganisms. Each bacterial strain was cultured overnight on Mueller-Hinton agar plate at 37°C. The isolated colonies were inoculated into a 5 ml Mueller-Hinton broth and incubated at 37°C for 2-3 hours. The turbidity of these inocula was adjusted to match that of a 0.5 McFarland standard (approximately 10^8 CFU/ml for bacteria).

Preparation of test plates. Thirty ml of melted Mueller Hinton agar (MHA) were dispensed into sterile glass petri dishes with internal diameter of 9 cm. The harden plates were dried for 1 hour at 37°C. A sterile cotton swab was dipped in each inoculum and inoculated the entire surface of the MHA plate for bacteria.

Test procedure. A quantity (100 μl) of each test sample was pipetted into each well (6 mm diameter) in test plate. This was done in triplicate. Bacterial plates were incubate at 37°C overnight. The oil samples showing inhibition zone were further examined for their minimize concentration.

Result

Chemical compositions of essential oil.

Hydrodistillation of the fresh leaves yielded 0.2% of an essential oil. By means of gas chromatography-mass spectrometry, it was found that the main groups of chemical compositions were monoterpene hydrocarbon, oxygenated monoterpene, sesquiterpene hydrocarbon and oxygenated sesquiterpene. The percentages of these terpene groups are shown in Figure 1. Comparison of mass spectra with terpene library program revealed the presence of 37 chemical components. These chemical components are shown in Table 1. The main constituents were bicyclogermacrene (19.79%), 9-epi-*(E)*-caryophyllene (15.65%) and tricyclene (8.74%) (Figure 2).

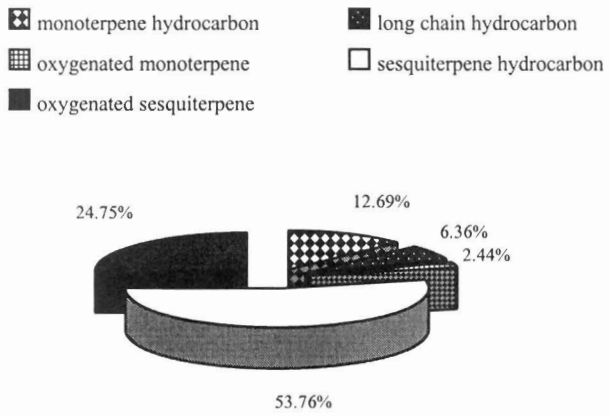
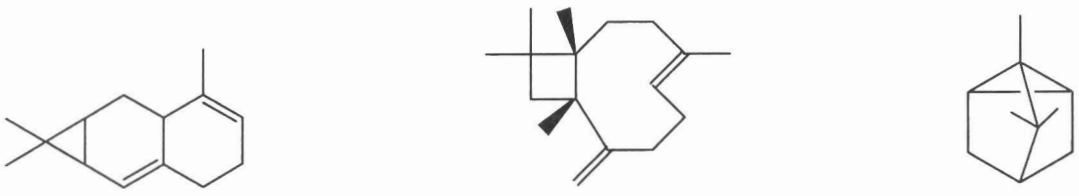


Figure 1. The percentage of various terpenoid groups found in the essential oil from *Micromelum minutum* Wight & Arn.

Table 1. The chemical compositions of essential oil from *M. minutum* Wight & Arn.

Number of peak	Retention time	Components†	%Area	Number of peak	Retentio n time	Components†	%Area
1	5.3	Tricyclene	8.74	20	28.11	*(E)*-Caryophyllene	0.92
2	6.51	*β*-Pinene	0.42	21	28.28	Germacrene A	5.68
3	6.78	Myrcene	0.95	22	28.74	*δ*-Cadinene	0.64
4	8.13	Limonene	2.58	23	30.6	*(Z)*-Nerolidol	0.64
5	9.79	*n*-Nonanal	0.67	24	31.11	Spathulenol	7.68
6	10.74	Linalool	1.9	25	31.31	Caryophyllene oxide	1.56
7	13.69	*n*-Decanal	0.66	26	31.43	Unknown	4.86
8	14.76	*α*-Terpineol	0.26	27	31.81	Globulol	0.8
9	16.33	Citronellyl formate	0.28	28	32.21	*β*-Oplopenone	1.74
10	18.46	Undecanal	4.14	29	32.43	Unknown	0.72
11	18.56	*n*-Undecanal	0.89	30	33.18	Unknown	0.2
12	23.3	*β*-Elemene	3.63	31	33.34	*Bicyclo*-Vertivenol	0.96
13	24.56	*9-epi-(E)*-Caryophyllene	15.65	32	33.53	*14-hydroxy-9-Epi-(E)*-	0.64
14	25.81	Viridiflorene	0.71			caryophyllene	
15	26.09	*α*-Humulene	4.23	33	33.73	*Epi-α*-Cadinol	1.15
16	26.84	*β*-Selinene	0.27	34	34.03	*β*-Bisabolenol	0.3
17	27.18	Germacrene D	1.46	35	34.24	*α*-Cadinol	1.62
18	27.51	*α*-Selinene	0.78	36	34.34	*Epi-α*-Muurolol	1.12
19	27.79	Bicyclogermacrene	19.79	37	34.76	Longiborneol acetate	0.76

†All components identified by Retention Indices and Mass spectra: terpene library



Bicyclogermacrene 9-epi-*(E)*-Caryophyllene Tricyclene

Figure 2. Structure of the major components of essential oil from *M. minutum* Wight & Arn.

Antimicrobial activities.

The screening test showed that the oil exhibited antimicrobial activities against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa* (Table 2).

Table 2. *In vitro* antimicrobial activities of essential oil from *M. minutum* Wight & Arn. (determined by diameter of inhibition zones)

Organism	Concentrations (%v/v)					
	8	6	4	3	2	1.5
S. aureus	1.95±0.05	1.65±0.05	1.55±0.05	1.45±0.05	1.30±0.10	1.05±0.05
B. subtilis	3.20±0.10	2.10±0.30	2.13±0.08	1.56±0.44	1.78±0.13	1.25±0.45
E. coli	2.35±0.05	2.15±0.15	1.50±0.10	1.30±0.10	1.50±0.05	1.45±0.05
Ps.aeruginosa	2.23±0.08	2:10±0.10	1.73±0.08	1.60±0.10	1.28±0.03	-
Organism	Concentrations (%v/v)					
	1	0.75	0.5	0.375	0.25	0.125
S. aureus	0.95±0.05	-	-	-	-	-
B. subtilis	1.64±0.12	1.18±0.13	1.40±0.10	1.03±0.08	1.08±0.08	-
E. coli	1.30±0.05	-	-	-	-	-
Ps.aeruginosa	-	-	-	-	-	-

Values are mean±S.D. (cm) for four separate experiments. -, No inhibition zone.

Discussion

The leaves of *M. minutum* Wight & Arn. produced a fair yield of essential oil of approximately 0.2% of its fresh weight so it might be a suitable source in large scale commercial growth.

In this particular study, this oil was characterized as comprising three major components: bicyclogermacrene (19.79%), 9-epi-*(E)*-caryophyllene (15.65%) and tricyclene (8.74%). This suggested the fingerprint characterization of *M. minutum*. Moreover, the essential oil displayed broad spectrum antimicrobial activities in both gram-positive and gram-negative bacteria.

It can be concluded that *M. minutum* has the potential to be an antibacterial agent against *S. aureus, B. subtilis* and *E. coli*. In the present study, concentration 0.25-1%v/v of the essential oil has the minimum antibacterial activities against *S. aureus* (1%), *B. subtilis* (0.25%) and *E. coli* (1%). Moreover, 2%v/v of the oil was active against *Ps. aeruginosa*. Further investigations should be performed to examine the active principles of the oil for the antifungal and antibacterial properties. Toxicological studies on the essential oil should also be performed to ensure the safety of the oil.

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