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Chemical Compositions and Antimicrobial Activities of Essential Oil from *Melaleuca leucadendron* var. *Minor*(องค์ประกอบทางเคมีและฤทธิ์ต้านจุลินทรีย์ของน้ำมันห...

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นิพนธ์ปฐมภูมิ

องค์ประกอบทางเคมีและฤทธิ์ต้านจุลินทรีย์ของน้ำมันหอมระเหยจากเสม็ดขาว

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บทคัดย่อ

องค์ประกอบทางเคมีของน้ำมันระเหยที่กลั่นได้โดยวิธีต้มกับน้ำจากใบสดของต้นเสม็ดขาว (*Melaleuca leucadendron* Linn. var. *minor* Duthie) วงศ์ Myrtaceae เมื่อวิเคราะห์ด้วยเครื่องก๊าซโครมาโตกราฟี-แมสสเปคโตรเมตรี พบว่า มีองค์ประกอบ 45 ชนิด โดยมี terpinolene (ร้อยละ 29.21), α -terpinene (ร้อยละ 22.55), 2- δ -carene (ร้อยละ 8.53) และ α -phellandrene (ร้อยละ 7.61) เป็นองค์ประกอบหลัก การทดสอบฤทธิ์ต้านจุลินทรีย์พบว่า น้ำมันระเหยจากต้นเสม็ดขาวแสดงฤทธิ์ต้านเชื้อ *Staphylococcus aureus* (MIC ร้อยละ 2.50 โดยปริมาตร) *Bacillus subtilis* (MIC ร้อยละ 1.25 โดยปริมาตร) *Candida albicans* (MIC ร้อยละ 0.63 โดยปริมาตร) และ *Trichophyton mentagrophytes* (MIC ร้อยละ 0.16 โดยปริมาตร)

กุญแจคำ

น้ำมันระเหย, องค์ประกอบทางเคมี, ฤทธิ์ต้านจุลินทรีย์, ต้นเสม็ดขาว

*Original Article***Chemical Compositions and Antimicrobial Activities of Essential Oil from *Melaleuca leucadendron* var. *minor***Puangnoi Lohakachornpan^{1,*} and Watcharin Rangspanuratn²¹Faculty of Pharmaceutical Sciences, Huachiew Chalermprakiet University, Samutprakarn 10540, Thailand²Faculty of Medical Technology, Huachiew Chalermprakiet University, Samutprakarn 10540, Thailand

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Abstract

The fresh leaves of *Melaleuca leucadendron* Linn. var. *minor* Duthie (Myrtaceae) was hydrodistilled by Clevenger apparatus. Gas chromatography–mass spectrometry analyses revealed the presence of 45 components. Terpinolene (29.21 %), α -terpinene (22.55%), 2- δ -carene (8.53 %) and α -phellandrene (7.61 %) were found to be the major components. The essential oil exhibited antimicrobial activities against *Staphylococcus aureus* (MIC 2.50 % v/v), *Bacillus subtilis* (MIC 1.25 % v/v), *Candida albicans* (MIC 0.63 % v/v) and *Trichophyton mentagrophytes* (MIC 0.16 % v/v).

Key wordsEssential oils, Chemical compositions, Antimicrobial activities, *Melaleuca leucadendron* var. *minor*.

Introduction

The family Myrtaceae comprises of about 140 genera and 3,000 species, mostly shrubs or trees with schizogenous secretory cavities containing aromatic volatile oils, some of which are used for their therapeutic action such as oil of eucalyptus. *Melaleuca leucadendron* Linn. var. *minor* Duthie, locally named Samed-khao, also belongs to the family Myrtaceae.

In Thai folk medicine, the oil obtained from Samed-khao was used for toothache, bronchitis, expectorant, carminative and vermifuge. The oil was also applied topically as an insect repellent, scabicide and for traumatic pain (1). Various types of oils from *Melaleuca* species are available for use as sources of therapeutic agent (2). The variety *minor* of *M. leucadendron*, widely found in southern Thailand, still has never been reported on its chemical compositions and antimicrobial activities. Herein, we reported the terpenoid constituents of the oil obtained from this particular species and its activities against some tested microorganisms.

Materials and Methods

Plant material and the separation of essential oil.

Fresh leaves of *M. leucadendron* were collected and chopped into small pieces. The prepared plant material was immediately hydrodistilled by Clevenger apparatus for 3 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 (3).

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°C–210°C rate 3.1 °C /min
Injector temperature	200°C

Detector temperature	230°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 (4) for its activities against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, as well as a clinical isolate fungus, *Trichophyton mentagrophytes*.

Test samples. The essential oil was diluted to 10% concentration with 0.1% 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 trypticase soy agar (TSA) plate at 37°C. Four well isolated colonies were inoculated into a 5 ml trypticase soy broth (TSB) and incubated at 37°C for 2-3 hours. The inoculum of *Candida albicans* was prepared by suspending the culture which was grown on Sabouraud dextrose agar (SDA) slope at 30°C for 24 hours in sterile normal saline solution. Spores of *Trichophyton mentagrophytes* were washed from the SDA slope after 4 days of incubation at 30°C with sterile 0.05 % Tween 80. The turbidity of these inocula was adjusted to match that of a 0.5 McFarland standard (approximately 108 CFU/ml for bacteria and fungi).

Preparation of test plates. Twenty-five ml of melted Mueller Hinton agar (MHA) or SDA 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 and the SDA plate for fungi.

Test procedure. A quantity (50 µl) of each test sample was pipetted into each well (6 mm diameter) in the test plate. This was done in triplicate. Bacterial plates were incubated at 37°C overnight and fungal

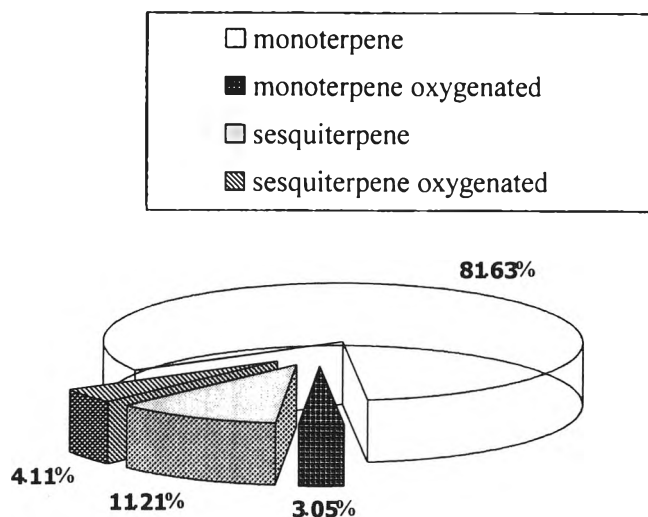


Figure 1. The percentage of various terpenoid groups found in the essential oil from *Melaleuca leucadendron* Linn. var. *minor*.

plates were incubated at 30°C for 48-72 hours. The oil samples showing inhibition zone were further examined for their minimum inhibitory concentrations (MIC).

Determination of minimum inhibitory concentration (MIC). MIC of essential oil was determined by the broth microdilution method (5).

Preparation of test microorganisms. The bacterial and fungal inocula prepared as described for agar diffusion test were further diluted to 1: 100 in MHB and SDB, respectively (approximately 10⁶ CFU/ml of microorganism).

Assay samples. The sterile solution of 10% essential oil in 0.5% Tween 80 was prepared and two-fold dilution was performed to give the concentrations of oil sample ranging from 5.00 to 0.04% v/v. This was done with sterile 0.15% w/v agar solution in MHB for bacteria and SDB for fungi.

Assay procedure. The assay was performed in duplicate in 96-well sterile U-bottom microtiter plates. A 50 µl volume of each concentration of the test sample was dispensed to the corresponding well. A 50-µl volume of diluted inoculum was added into each well. The 100 µl volume of assay organisms was used as growth control and 100 µl volume of sterile

0.15% w/v agar solution in MHB was used as sterility control.

The incubating conditions were 37°C, 24 hours for bacteria and 30°C, 48-72 hours for fungi. The lowest concentration of oil sample that showed growth inhibition was considered as the MIC.

Results and Discussion

Chemical compositions of essential oil. Hydrodistillation of the fresh leaves yielded 1.0% of an essential oil. By means of gas chromatography-mass spectrometry, it was found that the main groups of chemical compositions were monoterpene, oxygenated monoterpene, sesquiterpene and oxygenated sesquiterpene. The percentages of these terpene groups are shown in Figure 1. Comparison of spectra with terpene library program revealed the presence of 45 chemical components, 8 of which constituted 78.71% of the oil. These chemical components are shown in Table 1. The main constituents were terpinolene (29.21%) and α-terpinene (22.55%), while cineole which was reported in previous studies on *M. leucadendron* (6-7) was not found in this particular chemovar.

Antimicrobial activities and minimum inhibitory concentration. The screening test showed that the oil exhibited antimicrobial activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans* and *Trichophyton mentagrophytes* (Table 2).

active against *C. albicans* (MIC 0.63% v/v) and *T. mentagrophytes* (MIC 0.16% v/v).

Antimicrobial activities and minimum inhibitory concentration. The screening test showed that the oil exhibited antimicrobial activities against *Staphylococcus aureus*, *Bacillus subtilis*,

Candida albicans and *Trichophyton mentagrophytes* (Table 2). The MIC (Table 3) showed that the oil was quite active against *C. albicans* (MIC 0.63% v/v) and *T. mentagrophytes* (MIC 0.16% v/v).

Interestingly, other *Melaleuca leucadendron* chemovars contain 1,8-cineol as the main component,

Table 1. The chemical compositions of essential oil from *Melaleuca leucadendron* Linn. var. *minor* Duthie

Peak No.	Retention time	Components [†]	% Area
1	4.73	α -thujene	2.19
2	4.93	3- δ -carene	2.59
3	5.96	sabinene	0.45
4	6.11	β -phellandrene	0.40
5	6.41	myrcene	0.65
6	6.98	α -phellandrene	7.61
7	7.33	2- δ -carene	8.53
8	7.61	<i>o</i> -cymene	1.46
9	7.74	limonene	2.12
10	7.96	(<i>E</i>)- β -ocimene	0.10
11	8.34	(<i>Z</i>)- β -ocimene	0.10
12	8.79	α -terpinene	22.55
13	9.84	terpinolene	29.21
14	10.53	linalool	0.72
15	11.56	<i>trans</i> - <i>para</i> -menth-2-en-1-ol	0.25
16	12.34	<i>trans</i> -sabinene hydrate	0.19
17	14.00	terpin-4-ol	0.10
18	14.46	<i>p</i> -cymene-9-ol	1.57
19	14.73	α -terpineol	0.17
20	15.34	<i>cis</i> -piperitol	0.05
21	20.99	δ -elemene	0.21
22	22.09	(<i>E</i>)-isoeugenol	0.16
23	22.83	α -copaene	0.05
24	23.18	(<i>Z</i>)-caryophyllene	1.44
25	23.53	β -elemene	0.05
26	24.29	methyl eugenol	3.51
27	24.81	(<i>E</i>)-caryophyllene	1.62
28	26.46	α -humulene	0.06
29	27.26	β -patchoulene	2.52
30	27.61	β -cubebene	0.24
31	27.98	viridiflorene	1.88
32	28.28	bicyclogermacrene	0.14
33	28.63	γ -cadinene	0.27
34	28.79	α -bulnesene	1.96
35	29.08	δ -cadinene	0.45
36	29.31	elemol	0.20
37	30.79	germacrene B	0.32
38	30.99	spathulenol	0.18
39	31.94	globulol	0.51
40	32.30	β -eudesmol acetate	^a tr
41	32.66	guaiol	0.18
42	32.83	α -cadinol	^a tr
43	33.08	isomenthone [2-(3-oxobutyl)-]	0.90
44	35.39	selin-11-en-4 α -ol	0.95
45	35.66	bulnesol	1.19

[†]All components identified by Retention Indices and Mass spectra: terpene library program (3)

^atr = trace < 0.05 %

whereas in this study it was found that terpinolene and α -terpinene were the major constituents. It is known that 1,8-cineol displays broad spectrum antimicrobial activities (6). However, in spite of the lack of 1,8-cineol in this particular oil compared to the other chemovars, it still showed antibacterial activity. According to Cuong et al (6), terpinolene was revealed as active against *S. aureus*. Therefore, terpinolene which was the major component in this report might play this important role. Moreover, the oil was also found to exhibit antifungal activities against *C. albicans* (ATCC 10231) and *T. mentagrophytes* (clinical isolate).

Conclusion

The variety *minor* of *Melaleuca leucadendron* produced a fair yield of essential oil of approximately 1% of its fresh weight [0.5-2.5% is preferable for *M. leucadendron* (8)] so it might be a suitable source in

large scale commercial growth.

In this particular study, this oil was characterized as comprising two major components (terpinolene 29.21% and α -terpinene 22.55%) but had no 1,8-cineole content. This suggested the fingerprint characterization of Thai *M. leuca-dendron* chemovar. The oil also displayed promising antifungal activities against *Candida albicans* and *Trichophyton mentagrophytes*.

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Table 2. Antimicrobial activities of essential oil from *Melaleuca leucadendron* Linn. var. *minor* Duthie.

Organism	Result
<i>Staphylococcus aureus</i> ATCC 29213	++++
<i>Escherichia coli</i> ATCC 25922	-
<i>Pseudomonas aeruginosa</i> ATCC 27853	-
<i>Bacillus subtilis</i> ATCC 6633	++++
<i>Candida albicans</i> ATCC 10231	+++
<i>Trichophyton mentagrophytes</i> (clinical isolate)	+++

Remark : - No activity
 + Diameter of clear zone = 10-19 mm. (less activity)
 ++ Diameter of clear zone = 20-25 mm.
 +++ Diameter of clear zone = 26-30 mm.
 ++++ Diameter of clear zone = >30 mm.

Table 3. Minimum inhibitory concentration (% v/v) of essential oil from *Melaleuca leucadendron* Linn. var. *minor* Duthie.

Organism	MIC (%v/v)
<i>Staphylococcus aureus</i> ATCC 29213	2.50
<i>Bacillus subtilis</i> ATCC 6633	1.25
<i>Candida albicans</i> ATCC 10231	0.63
<i>Trichophyton mentagrophytes</i> (clinical isolate)	0.16

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