CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL OF THE *Toona sur*eni (Blume) Merr

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ABSTRACT

The essential oil composition of the *Toona sureni* (Blume) Merr leaf was analyzed by GC-MS. More than 68 peaks, representing 99.99% of total oil, forty three components were identified, this represents 80.65% of the total oil component. The major components were *α*-terpinene (9.58%), *α*-copaene (8.39%), bicyclogermacrene (7.61%), *δ*-cadinene (6.65%), *β*-elemene (4.88%), germacrene-D (4.65%), *δ*-selinene (3.58%), caralene (3.10%), *β*-caryophyllene (2.88%), *α*-cubebene (2.82%), *δ*-gurjunene (2.20%), and (-)-isoledene (2.05%). The antibacterial activity of the essential oil of *Toona sureni* (Blume) Merr leaf was evaluated using disk diffusion method. The oil was effective on the inactivation of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis.*

Keywords: *Toona sureni (Blume) Merr, antibacterial activity, GC-MS, essential oil*

INTRODUCTION

World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants which are widely used as medicine and constitute a major source of natural organic compounds[1].

The essential oils are complex mixers comprising many single compounds. Chemically they are derived from terpenes and their oxygenated compounds. The essential oils have been shown to possess antibacterial. They demonstrated an interesting selectivity as its have a modes antimicrobial activity against some the tested gram-positive and gram-negative strains[2-10].

Toona sureni (Blume) Merr is one of among five or six species of trees in the mahogany family Meliaceae[11]. Indonesia it is found in Sumatra, Java and Sulawesi. Various parts of the tree, especially the bark and root, are used for medicinal purposes, e.g. to treat diarrhoea. Leaf extracts have antibiotic effect. The bark and fruits can be used for production of essential oils[12].

The literature search revealed that a number different compounds have previously been isolated from the leaves of the plant, including tetranortriterpenoid (surenin, surenone and surenolactone)[13,14], and carotenoids[15-16]. Another species of Toona genus, *Toona ciliate* contains the essential oil from the leaves (0.05%, V/W) and the stems (0.05%, V/W). The oil contains *β*-caryophyllene, germacrene-D and bicyclogermacrene as the major compounds[17].

Toona sinensis contains the essential oil from the leaves (0.02%, V/W). The oil contains germacrene-D, germacrene-B, *α*-terpinene, *α*-humulene, *β*-caryophyllene, *α*-elemene, bicyclogermacrene and *α*-copaene as the major compounds[18].

Herein we report the chemical composition of and antibacterial activity of the essential oil from the leaves of the *Toona sur*eni (Blume) Merr.

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MATERIALS AND METHODS

Plant Material

Plant materials were collected in Padang, West Sumatera, Indonesia in Juli 2007, and identified in the Herbarium of the Andalas University (ANDA), Padang, with specimen M.Taufik Ekaprasada, 0107 (ANDA.Fr).

Isolation of Volatile Oil

The fresh leaves of *Toona sureni* (Blume) Merr (6500 g) were sliced and subjected to steam distillation (4 h) to yield (0.04%) oil. The work was repeated to produce the oil that enough for analysis. The oil was dried over anhydrous sodium sulfate and stored at low temperature prior to analysis. The oil obtained was greenish yellow with a strong odor. The physicochemical characteristics of the oil were determined according to AFNOR[19] standards at 20°C: d(20;20) = 0.9197, n(D;30) = 1.5085.

Identification Components

Identification of volatile compounds was performed using gas chromatography-mass spectrometry (GC-MS). The component relative concentrations in each essential oil were calculated based on GC peak areas. Each oil was analyzed by GC-MS using a Agilent Technologies 6890 Gas Chromatograph with Auto Sampler and 5973 Mass Selective Detector and Chemstation data system with a Innowax Capilarry Coloumn (30 m×0.25 mm I.D×0.25 μm film thickness). GC oven initial temperature was 65°C hold for a minute, rising at 3°C/min to 150°C for 2 minutes, rising at 15°C/min to 240°C for 20 minutes. Injection port temperature at 250°C; ion source temperature at 230°C, interface temperature at 280°C, quadrupole temperature at 140°C, carrier gas was helium, flow column: 0.6 μL/min, injected volume: 1 μL, split: 250:1, method file was AKRWAX.

The mass spectra were performed at 70 eV with ionization mode: electron impact. Identification of the constituents was based on comparison of the retention times with those of authentic samples on computer matching against commercial libraries (Wiley 275 L).

Test Organism

Microorganisms were obtained from the Department of Biology Faculty of Mathematic and Natural Science, University of Andalas, Padang, Indonesia. A strain of gram-negative bacteria (*Escherichia coli*) and two strains of gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) were used. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures.

Antibacterial Assay

The disk diffusion susceptibility method was used in order to examine the sensitivity of the bacteria of interest toward the essential oil of the *Toona sureni* (Blume) Merr. Antimicrobial assay was measured using the methods of Rojas, J. *et. al*[20] but with slight modifications. One loopful of the given test strain was inoculated into 2.5 mL of N-broth (Nutrient Broth) and incubated for 24 h in an incubator at 37°C in order to activate the bacterial strain.

The bacterial inoculum was diluted in the sterile saline solution (0.9% NaCl) to obtain turbidity visually comparable to a McFarland No. 0.5 Standard (10^{6-8} CFU/mL). Mueller Hinton Agar (MHA), sterilized in a flash and cooled to 40-50°C, was poured (15 mL) into sterilized Petri dishes (9 mm diameter) and allowed to harden under room temperature. This is followed homogenous distribution of 0.1 mL bacteria culture (10^{6-8} cfu/mL) onto medium in Petri dishes. The essential oils were dissolved in dimethylsulfoxide (DMSO).

The sterile paper disk (5 mm diameter) was impregnated with 20 μL of the different dilutions, 10, 5, 3, 1, 0.75, 0.625, 0.5, 0.375, 0.25, and 0.125 (% v/v), of the previously prepared solution. The reading of the plates, incubated at 37°C, showed after 48 h inhibitory zones around the paper disks. When the inhibitory zone diameter is lower or equal to 5 mm, the sample tested was considered as not active. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the test compound that demonstrated no visible growth. Chloramphenicol; 3% (b/v) as the standard antibiotic used in order to provide a control for

the sensitivity of the test organisms in the experiments. Two replicates were performed for each analysis.

RESULTS AND DISCUSSION

Essential Oils

After four hours of steam distillation, the essential oil yield was 0.04% (v/b). The chromatogram of the essential oil is given in Figure 1 and the composition of the essential oil is given in Table 1. Component concentrations were calculated from GC peak areas. Out 68 peaks, representing 99.99% of total oil, forty three components were identified, this represents 80.65% of the total oil component.

Inspection of Table 1 clearly shows that the oil consists largely of sesquiterpene hydrocarbons which constitute about 66.50% of the total oil with *α*-copaene (8.39%), bicyclogermacrene (7.61%), *δ*-cadinene (6.65%), *β*-elemene (4.88%), germacrene-D (4.65%), *δ*-selinene (3.58%), (+)-*β*-gurjunene (3.10%), *β*-caryophyllene (2.88%), *α*-cubebene (2.82%), *δ*-gurjunene (2.20%), and (-)-isoledene (2.05%) as the major sesquiterpene. Furthermore, 4.57% was found to be present as sesquiterpenoid i.e, sphatulenol, torreyol, isosphatulenol, t-cadinol, t-muurolol, and d-nerolidol.

There were only one monoterpene which accounted for 9.58% of the oil total i.e, *α*-terpinene. No diterpene was found so far from the essential oil composition of this plant. Some major components of the oil (*β*-caryophyllene bi-cyclogermacrene, germacrene-D) also were found on the leaves of *Toona ciliata* and *Toona sinensis* as major components[17,18].

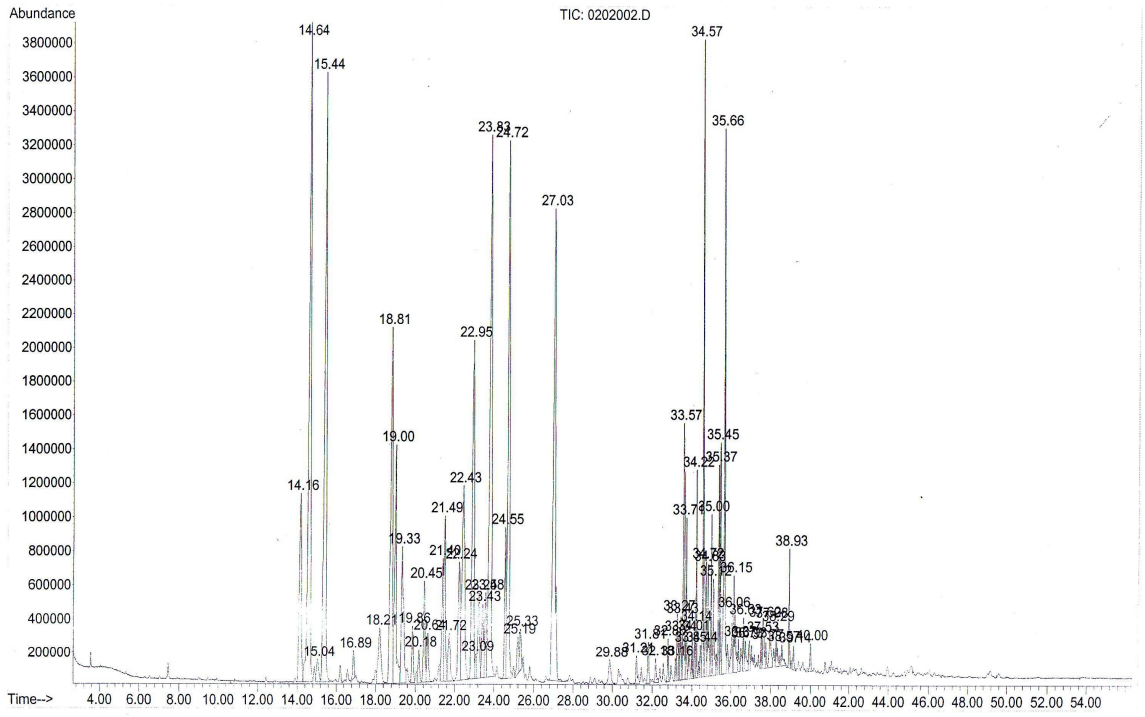

Figure 1. Chromatogram of the essential oil of the leaves of *Toona sureni*

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Table 1. Identified Chemical Constituents in the Essential Oil of the Leaves of *Toona sureni*

No	Compound	Retention time (Min)	Concentration (%)
A	**Monoterpene hydrocarbon**	-	**9.58**
1	*α*-terpinene	14.64	9.58
B	**Sesquiterpene hydrocarbons**	-	**66.50**
1	*α*-cubebene	14.16	2.82
2	*α*-copaene	15.45	8.39
3	*β*-cubenene	16.90	0.26
4	*β*-elemene	18.81	4.88
5	*β*-caryophillene	19.01	2.88
6	(-)-isoledene	19.34	2.05
7	(+)-*δ*-selinene	19.87	0.55
8	*γ*-elemene	20.46	1.01
9	allo-aromadendrene	20.64	0.56
10	valencene	21.40	1.27
11	*α*-humulene	21.49	1.78
12	(+)-*β*-guaiene	21.72	0.76
13	*α*-amorphene	22.24	1.62
14	*δ*-selinene	22.44	3.58
15	germacrene-D	22.95	4.65
16	(+)-aromadendrene	23.24	1.08
17	*α*-selinene	23.44	0.88
18	*α*-muurolene	23.58	0.86
19	bicyclogermacrene	23.83	7.61
20	E,E- *α*-farnesene	24.56	1.18
21	*δ*-cadinene	24.72	6.65
22	ar-curcumene	25.19	0.45
23	epizonaren	25.33	0.43
24	*α*-calacorene	29.88	0.38
25	calarene	31.81	0.35
26	*γ*-gurjunene	32.18	0.20
27	*α*-guaiene	32.80	0.30
28	*δ*-gurjunene	33.57	2.20
29	*β* –selinene	33.71	0.85
30	*α*-elemene	33.84	0.28
31	*β*-maaliene	34.43	0.22
32	(+)-*β*-gurjunene	34.58	3.10
33	*α*-gurjunene	34.72	0.91
34	*δ*-cadinene	34.83	0.79
35	selin-4,7(11)-diene	36.07	0.56
36	patchoulane	37.76	0.16
C	**sesquiterpenoid**	-	**4.57**
1	sphatulenol	34.22	0.99
2	torreyol	35.12	0.70
3	isosphatulenol	35.38	0.92
4	t-cadinol	35.45	1.11
5	t-muurolol	34.99	0.73
6	d-nerolidol	33.15	0.12

Table 2. Antibacterial Activities of of *Toona sureni* (Bl) Merr Essential Oil from the Leaves[a]

Bacterial species	Concentrations of the oil (% v/v)						Chloramphenicol (3 %, b/v)	MIC (% v/v)
	10	5	3	1	0.75	0.675		
B. subtilis	14	12	11	8	6	b	31	0.75
S. aureus	15	9	8	6	6	b	31	0.75
E. coli	8	8	7	b			29	3

[a] Values represent diameters of inhibitory zone (mm) at indicated dilutions (% v/v)
[b] Not active (the inhibitory zone diameter is lower or equal to 5 mm)

Antibacterial Activity of the Essential Oil

Table 2 shows the antibacterial activity the oil against Gram-positive and Gram-negative bacteria. It was showed an antibacterial activity against all bacterial strains used in this study. Diameter values of the inhibitory zones of the oil are lower for *Escherichia coli* than *Bacillus subtilis* and *Staphylococcus aureus.* It showed that *Escherichia coli* is the most resistant. It was observed that *Bacillus subtilus* was the most sensitive to the oil. As seen in Table 2, all three bacteria show an antibacterial activity to the oil which cantains *α*-terpinene (9.58%), and *β*-caryophillene (2.88%), and this is in agreement with literature reference[3].

CONCLUSION

In conclusion, our GC and GC-MS study of *Toona Sureni* (Blume) Merr essential oil led to the identification of out 68 peaks, representing 99.99% of total oil, forty three components were identified, this represents 80.65% of the total oil component. The major components were monoterpene i.e *α*-terpinene (9.58%) and *α*-copaene (8.39%), bicyclogermacrene (7.61%), *δ*-cadinene (6.65%), *β*-elemene (4.88%), germacrene-D (4.65%), *δ*-selinene (3.58%), (+)-*β*-gurjunene (3.10%), *β*-caryophyllene (2.88%), *α*-cubebene (2.82%), *δ*-gurjunene (2.20%), and (-)-isoledene (2.05%) as the major sesquiterpene. Furthermore, 4.57% was found to be present as sesquiterpenoid.

The results presented here for the antibacterial activity study demonstrate the activity of *Toona Sureni* (Blume) Merr essential oil and support the use of parts of this plant in used for medicinal purposes.

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