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Chemical Composition and Antimicrobial Activity of Essential Oil from Aerial Parts of Pohpohan (*Pilea Trinervia* (Roxb.) Wight).

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ABSTRACT

In this research, chemical composition and antimicrobial activity of essential oil from aerial parts of Pohpohan (*Pilea trinervia* (Roxb.) Wight) was investigated. Methods :The chemical composition of the essential oil from aerial parts of Pohpohan was investigated by GC–MS. The antimicrobial activity of essential oil were evaluated against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Candida albicans, Aspergillus niger* and *Microsporum gypseum* by using micro broth dilution. Results: A total of 34 compounds were detected from essential oil and it inhibitted the growth of all the test microbe. The MIC values of essential oil against *S. aureus* (2.52 µg/mL, *B. subtilis* (5.04 µg/mL, *E. coli* (322.5 µg/mL, *C. albicans* (20.16 µg/mL, *A. niger* (1.26 µg/mL, and *M. gypseum* (5.04 µg/mL) respectively. Conclusion: A total of 34 compounds were identified from *Pilea trinervia* essential oil and it has antimicrobial activity.

Keywords: *Pilea trinervia*, essential oil, GC-MS, antimicrobial

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INTRODUCTION

Essential oils have been long recognized for their antibacterial, antifungal, antiviral, insecticidal and antioxidant properties. They are widely used in medicine and the food industry for these purposes i.e for preservative [1].

Many essential oils exert non-spesific antimicrobial effects due to the hydrophobic properties of the mixtures and components. For instance, the hydrophobic character of many essential oils facilitates entry into cell membranes leading to alteration in architecture, leakage of cell contents and eventually death. Changes in the hydrophobicity, surface charge, and membrane integrity with the subsequent K+ leakage from E. coli and S. aureus were observed after exposure to citronellol, citronellal, carveol, and carvone (some of essential oil components) [2,3].

Pilea trinervia (Roxb.) Wight belonging to the family Urticaceae is widely distributed in West Java. Traditionally, community of the national park of Halimun Salak mountain consume young leaves to cure cancer. *Pilea trinervia* has an unique odor, generally, it caused by essential oil content [4,5].

The studies of essential oil of *Pilea trinervia* have not been reported. Behalf of that we report here the isolation, analysis of chemical composition and antimicrobial activity of essential oil from aerial parts of *Pilea trinervia.*

MATERIALS AND METHODS

Plant materials:

The aerial parts of *Pilea trinervia* were collected from Manoko garden, Lembang in May 2011. The specimen was determined at the herbarium of Bandungense the School of Life Science and Technology, Institut Teknologi Bandung with 667/II.C02.2/PL/2012 as number of authentication.

Test microbes:

Test microbes were *Staphylococcus aureus* (American Type Culture Collection (ATCC) 6538), *Bacillus subtilis* (ATCC 6636), *Escherichia coli* ((ATCC) 8939), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 16404), and *Microsporum gypseum*. Those were obtained from Microbiology laboratory collection of Institut Teknologi Bandung.

Instruments:

A shimadzu QP 2010 ULTRA was used. The initial oven temperature was 40° C, this was held for 2 minutes then raised at the rate of 8°C /minutes to 150°C, then held for 2 minutes then raised again at the rate 10°C/minutes to 250°C. Injector temperature, 250°C. The constituents were identified by comparison of their mass spectra with those of NIST08 and Willey7 library data for the GC-MS system.

Methods:

Isolation of Essential Oil:

20 kg of fresh aerial parts were distilled in steam and water distillation apparatus. It was obtained 8 ml of essential oil (0.4 %).

Determination of the Minimum Inhibitory Concentration (MIC):

Antimicrobial activities test of essential oil were using by micro dilution broth method based on National Committee for Clinical Laboratory Standard (2012). The essential oil was emulsified with 2% (v/v) Tween 80 as surfactan. A series of dilutions of essential oil were prepared in Mueller Hinton Broth (MHB) or Sabouraud Dextrose Broth (SDB) at final concentrations ranging from 0.63 to 322.5 µg/mL. The inocula of microorganisms were prepared from 24 hours cultures and suspensions were adjusted to 0.5 McFarland

standard suspensions. The tubes were dispensed into 100 µL with different concentrations of essential oil and 10 µL inoculum. The control tubes contained only MHB or SDB and inoculums suspension. The positive or reference controls were prepared using tetracycline HCl, and ketoconazol. The inoculated tubes of bacteria were incubated at 37° C for 24 hours, yeasts at 28° C for 48 hours and fungi at 28° C for 120 hours. The MIC was calculated as no visible growth of tested microorganism appeared, which were expressed in µg/ml. The tests were conducted in triplicate. The least concentration of each essential oil showing a clear of inhibition was taken as the MIC [6].

Determination of the Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The minimum bactericidal/fungicidal concentration of the essential oil on the clinical bacterial isolates was done according to the method highlighted in National Committee for Clinical Laboratory Standard (2000). Briefly 5µL that was pipetted from the microbe mixture obtained in the determination of MIC stage was streaked out on the nutrient agar/ Sabouraud dextrose agar at 37°C for 24 hours, yeasts at 28 °C for 48 hours and fungi at 28° C for 120 hours. The least concentration of the essential oil with no visible growth was taken as the minimum bactericidal/fungicidal concentration [7].

RESULTS AND DISCUSSION

Results and Discussion

The fresh of aerial parts yielded 0.4 % of volatile oil and it was colorless. The chromatogram of the essential oil is displayed in Fig. 1. The amounts of the components from the volatile oil were determined by the peak area normalization method. This presence of several overlapping peaks shows the complexity of the mixture. The chemical constituents of the essential oil are listed in Table 1. A total of 34 compounds were detected. The major components were: 2 (10) pinene (14.85%); 1R-alpha-pinene (12.95%) and sabinene (12.66%); o-menth-8-ene (8.33%) and germacrene-D (5%). The essential oil consisted of monoterpenes and sesquiterpenes. Ones of monoterpenes were 2(10)-pinene, 1R-alpha-pinene, camphene and sabinene. Ones of sesquiterpenes were alpha-humulene, germacrene-D, alpha-gurjunene and delta-cadinene. The essential oil also consisted of sulfur containing sesquiterpenes. It was mintsulfide (2.95%). This result is different with those reported by other author for *Pilea* spesies that growing in China, Zhi-Yuan *et al.*(2009) reported for *P. aquarum*, a major contents (48,92 %) of 9 vinyl compound [8].

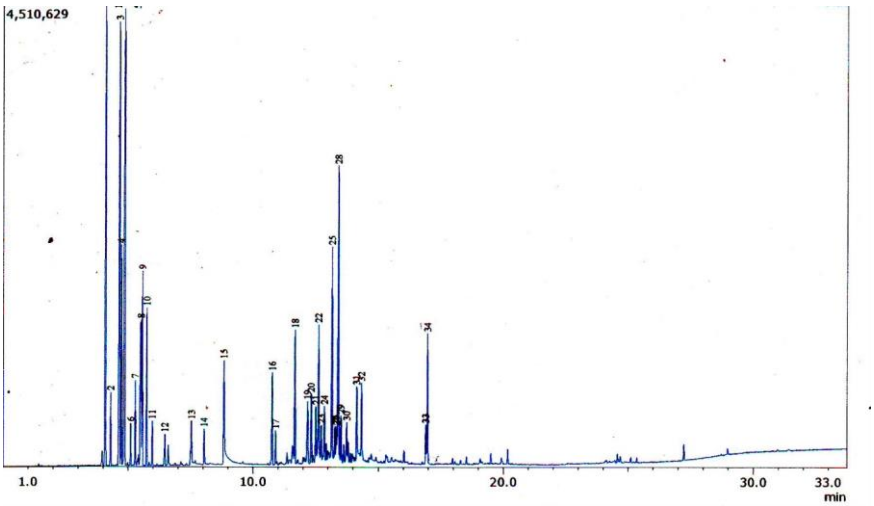

Figure 1. Chromatogram of essential oil of *Pilea trinervia*

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Table 1. Chemical constituents of essential oil of the aerial parts *Pilea trinervia*

No	Retention time	Compound	Relative content (%)
1	4.099	1R-alpha-pinene	12.95
2	4.307	Camphene	1.48
3	4.655	Sabinene	12.66
4	4.724	2-beta-pinene	3.91
5	4.863	2(10)pinene	14.85
6	5.102	1-phellandrene	0.68
7	5.288	alpha-terpinene	1.46
8	5.513	beta-phellandrene	4.86
9	5.569	cis-ocimene	3.54
10	5.745	1,3,6-octarienene,3,7 dimethyl	2.78
11	5.967	terpinen-4-ol	1.52
12	6.473	alpha-terpinolene	1.24
13	7.528	6-octenal-3,7dimetil	1.68
14	8.039	3-cyclohexen-1-ol, 4-methyl- 1-methylethyl	1.63
15	8.838	6-octen-1-ol,3,7-dimethyl	2.14
16	10.755	delta-elemene	1.75
17	10.892	6-octen-1-ol,3,7-dimethyl acetate	0.61
18	11.666	beta-elemene	2.81
19	12.175	Isocaryophyllene	0.79
20	12.322	elixene	1.51
21	12.505	aristolen	1.11
22	12.619	cyclosativene	3.40
23	12.720	alpha-humulene	0.76
24	12.851	epi-bicyclosesquiphelandrene	0.56
25	13.153	germacrene-D	5.00
26	13.270	naphtalene,1,24a,5,8,8ahexahydro4,7-dimethyl-1-(1-methylethyl)	0.51
27	13.328	(+)-epi-bicyclosesquiphellandrene	0.56
28	13.410	o-menth-8-ene	8.33
29	13.513	alpha-gurjunene	0.85
30	13.739	delta-cadinene	1.53
31	14.142	o-menth-8-ene-4-methanol	1.44
32	14.331	germacrene B	1.44
33	16.902	zerumbone	2.95
34	16.971	mintsulfide	2.04

Table 2. Minimum inhibitory concentration of the essential oil

No	Sample	Minimum inhibitory concentration (µg/ml) against						
		Sa	Bs	Ec	Pa	Ca	An	Mg
1	Essential oil	0.63	5.04	322.5	20.16	20.16	1.26	0.63
2	Tetracycline HCl	0.39	0.098	1.563	12.5			
3	Ketoconazole					12.5	0.781	1.563

Sa: *Staphylococcus aureus*; Bs: *Bacillus subtilis*; Ec : *Escherichia coli*; Pa: *Pseudomonas aeruginosa*; Ca: *Candida albicans*; An: *Aspergillus niger*; Mg: *Microsporum gypseum*

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Table 3. Minimum bactericidal/fungicidal concentration of the essential oil

No	Sample	Minimum bactericidal/fungicidal concentration (µg/ml) against						
		Sa	Bs	Ec	Pa		An	Mg
1	Essential oil	2.52	5.04	322.5	20.16	20.16	1.26	5.04
2	Tetracycline HCl	0.39	0.098	1.563	12.5			
3	Ketoconazole					12.5	0.781	1.563

Sa: *Staphylococcus aureus*; Bs: *Bacillus subtilis*; Ec : *Escherichia coli*; Pa: *Pseudomonas aeruginosa*; Ca: *Candida albicans*; An: *Aspergillus niger*; Mg: *Microsporum gypseum*

The essential oil of *Pilea trinervia* inhibitted the growth of all the test microbes. It showed the lowest MIC value of 0.63 µg/ml againts *S. aureus* and the lowest MBC value of 1.26 µg/ml against *A. niger*.

It confirmed that the antimicrobial properties of *Pilea trinervia* essential oils are mainly related to their contents. This study has identified two components, beta-pinene and naphtalene analog, which appear to contribute significantly to the antimicrobial activity of the essential oil examined. Beta-pinene and naphtalene analog have been reported by Da silva and Rokade *et al*. with have antimicrobial properties [9,10]. The possibility that other components possess some antimicrobial power still remains. In addition, synergistic or antagonistic effects between some components may also affect the observed antimicrobial activity of the essential oil.

CONCLUSIONS

In this study, 34 constituents of the essential oil of *Pilea trinervia* was sucessfully identified. 2 (10) pinene was one of the major constituents. It inhibitted the growth of *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *C. albicans*, *A. niger* and *M. gypseum*. . It showed the lowest MIC value of 0.63 µg/ml againts S. aureus and the lowest MBC value of 1.26 µg/ml against A. niger.

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