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The Influence of Myristicin Lost in Myristica Fragrans Volatile Oils to Antimicrobial Activity Against *B. Subtilis*, *E. Coli* and *S. Aureus*

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***Abstract*—Objectives: Myristica fragrans volatile oil has presented strong antimicrobial activity against a wide variety of bacteria. The antimicrobial activity would be expected to relate to the respective composition and their relative percentage, and possibly synergistic interaction between its components. M.fragrans volatile oil without myristicin have different relative percentage composition, so it is expected to have different antimicrobial activities. This study aimed to evaluate the correlation between different compound and percentage of M.fragrans volatile oil and myristicin to antimicrobial activities against different bacterial strains. Myristicin was isolated by fractional distillation method at 473.5 K, 25 mmHg. The M. fragrant volatile oil and myristicin screened for antimicrobial activity against B. Subtilis, E.Coli and S.Aureus using an agar diffusion technique. The concentration of each component range between 2,5-20%. The antimicrobial activity determined by measured zones of growth inhibition with 50 µl tetracycline disc as a positive control. The result showed that m. Fragrant volatile oils containing 31 compounds with myristicin content of 12.94%, while the essential oils of nutmeg without myristicin contain 16 compounds, both of them has same 5 major components i.e., α-pinene, sabinene, β-pinene, 4-terpineol and limonene, The volatile oil of M.fragrans exerting greatest inhibitory activity against S.Aureus followed by B.Subtilis and E.Coli with the zones of growth inhibition 18,3; 9,3; and 7,6mm respectively. The myristicin lost in M.fragrans volatile oil had lower antimicrobial activity against all bacterial strains. The zone of growth inhibition of fraction against S.Aureus, B.Subtilis, and E.Coli were 13,6; 8,6; and 7,3mm respectively.**

Keywords: myristica fragrans volatile oil, myristicin lost, antimicrobial activity

I. INTRODUCTION

Indonesia has reported as a world-leading producer of Myristica fragrans. M.fragrans have been acclaimed to have several health benefits, probably due to the presence of essential oil. Essential oils are secondary metabolite that is made up of volatile compounds such as pinene, camphene, dipentene, terpenes, etc. M.fragrans have full medicinal potentials, and its chemical constituents have been scientifically validated to have hypolipidemic and hypocholesterolemic activities. Antimicrobial, antidepressant, aphrodisiac, memory-boosting, antioxidant, hepatoprotective properties, and anti-parasitic potentials.

Myristica fragrans volatile oil has presented strong antimicrobial activity against a wide variety of bacteria. The antimicrobial activity would be expected to relate to the respective composition and their relative percentage, and possibly synergistic interaction between its components. M.fragrans volatile oil has a main myristicin compound, 4-terpineol, safrole, sabinene, α-pinene, and δ-limonene [4]. Its volatile oil without myristicin have different relative percentage composition, so it is expected to have different antimicrobial activities. This study aimed to evaluate the correlation between different compound and percentage of M.fragrans volatile oil and myristicin lost in m.fragrans volatile oil to antimicrobial activities against three different bacterial strains i.e., Bacillus Subtilis, Escherichia Coli and Staphylococcus Aureus.

II. MATERIAL AND METHOD

A. Plant materials

Myristica fragrans volatile oils obtained from Caringin, Bogor, West Java. The Separation of myristicin from m.fragrans volatile oils submitted with fractional distillation at 423 K, 25 mmHg.

B. Analysis of chemical constituents

M.fragrans volatile oils and volatile oils of m.fragrans without myristicin is analyzed by GC-Ms, and its structure was analyzed using IR and ^{1}H-NMR.

C. Bacterial strains

Three bacterial strains were used to assess the antibacterial properties of the test samples, 2 Gram-positive (Bacillus subtilis and Staphylococcus aureus), and 1 Gram-negative bacteria (Escherichia coli). All strains were subcultured every two weeks.

D. Dilution of the sample volatile oils

The volatile oils were considered as 100% concentration and the further diluted into 1,25%, 2,5%, 5%, 10% and 20% of the original volume with aquadest and 100µl tween 80 added.

E. Antimicrobial activity test

Antimicrobial activity test was carried out using the agar well diffusion technique. Nutrient agar was prepared according to the manufacturer's instruction. About 20 ml of the autoclaved nutrient agar was poured on a sterile petri dish and allowed to solidify. 0,3 ml of the isolates were placed in in the

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solidified agar plates, then spread over the surface using a sterile spreader. About 50µl sample solution was dropped using micropipette into disc paper for about 10 seconds and then placed at solidified agar plates. The plates were labeled according and incubated for 24 hours. The result of the diameter of the inhibition zone was measured using meter rule.

III. RESULT

A. Analysis of compound

The M.fragrans volatile oils containing 31 compounds with 6 major component were sabinene (26,62%), α-pinene (20,96%), β-pinene(15:10%), myristicin (12.94%), Limonene (5:24) and γ-terpinene (3:24%) while the volatile oils of m.fragrans without myristicin contain 16 compounds with same 5 latest major compounds without myristicin . All the same present components on myristicin lost m.fragrans volatile oil has higher concentration compared to its volatile origin oils. The analysis results are present in Table I.

TABLE 1: THE COMPOUNDS OF M. FRAGRANS VOLATILE OILS AND M.FRAGRANS OIL WITHOUT MYRISTICIN

No.	*Retention time (minute)*	*Compounds*	*Compositions (%)*	
			M.fragrans volatile oil	*Nutmeg essential oil without myristicin*
1	9661	α-pinene	1:41	1.66
2	10 008	α-pinene	20.96	24.66
3	10 557	Camphene	0:29	0:34
4	11 548	Sabinene	26.62	31.32
5	11 720	β-pinene	15:10	17.76
6	12 079	Myrcene	2:08	2:45
7	12 583	4- (Bromomethyl) Cyclohexene	0:04	-
8	12 698	α-phellandrene	0:50	0:59
9	12 773	β-ocimene	0:52	0.61
10	13,099	alpha-terpinene	2:22	2.61
11	13 407	1-methyl-2- (1-methylethyl) benzene	0:31	0:36
12	13 582	Limonene	5:24	6:16
13	14 630	γ-terpinene	3:27	3.85
14	15,100	Sabinenehydrate	0:17	-
15	15 580	α-terpinolene	1:00	1:25
16	16,200	Linalool	0:15	-
17	17 010	1-terpineol	0:12	-
18	18 913	4-terpineol	3.71	4:36
19	19 431	α-terpineol	0:46	0:58
20	22 080	bornyl acetate	0:09	-
21	22 383	Safrole	1:15	1:44
22	22 649	Amylanisole	0:43	-
23	23 923	Myrtanylacetate	0:31	-
24	24 241	Eugenol	0:09	-
25	24 771	α-Copaene	0:46	-
26	25 496	Methyleugenol	0:09	-
27	26 038	β-caryophyllene	0:07	-
28	26 316	α-bergamotene	0:10	-
29	28 320	β-bisabolene	0:04	-
30	28 632	β-cadinene	0:06	-
31	28 858	Myristicin	12.94	-

IV. DISCUSSION

The result of the antibacterial activity test is shown in Table 2. From the test results can be seen that all the concentration demonstrated some degree of antimicrobial activity in all strains bacteria. M.fragrans volatile oils showed higher activity compared to m.fragrans volatile oils without myristicin at all level concentration and all starin bacteria. The activity of the sample would be expected to relate to the respective composition, the structural configuration of the constituent component and their functional group, and possible synergistic interaction between components. The stereochemistry had an influence on bioactivity. M.fragrans volatile oils without myristicin had relative higher α-isomer rather than its common oils. It was observed that α-isomers are inactive relative β-isomers.

The tested samples were exerting greater inhibitory activity against Gram-positive bacteria, and it appeared preferentially more active to Gram reaction. Table 2 summarizes the antibacterial activity at different concentration of the sample and form this table the highest activity was found to be m.fragrans volatile oils in S.aureus bacteria followed by B.subtilis and E. coli. Myristicin lost in m.fragrans has lower antimicrobial activity in all strain bacteria compared to its origin volatile oil. The previous study showed that m.fragrans volatile oils had higher activity compared to single components. Complex compound predicted give multi-action and synergistic effect as antibacterial.

TABLE 2: ANTIMICROBIAL ACTIVITY OF TEST SAMPLE WHEN TESTED AGAR-DIFFUSION TECHNIQUE

Samples	The diameter of zone inhibition (mm)		
	B. subtilis	**S. aureus**	**E. Coli**
***M.Fragrans Volatile Oils**			
1.25%	6.1	7.2	5.5
2.5%			
5%	7.2	12.4	6.1
10%			
20%	9.3	18.3	7.6
***Myristicin lost in M.Fragrans Volatile oils**			
1.25%	5.1	6.4	6.4
2.5%			
5%	5.5	10.5	6.6
10%			
20%	8.6	13.6	7.3

An alkenyl substituent resulted in increased antibacterial activity as seen in limonene. Terpenoid action appeared to be at the phospholipid bilayer, caused by biochemical mechanism catalyzed by the phospholipid bilayers of the cell. These processes include the inhibition of electron transport, protein translocation, phosphorylation steps, and other enzyme-dependent reactions. Lost of myristicin had a significant influence on the antibacterial activity of its volatile oils. M.fragrans without myristicin lost not only myristicin but also some components, so it is predicted lowering the antimicrobial activity.

V. CONCLUSION

The test results obtained that the component and concentration of compounds at m.fragrans have a correlation with antimicrobial activity. M.fragrans volatile oils had higher activity against all strain bacteria with the potential activity against S.aureus, B.subtilis and E.coli 18.3, 9.3, and 7.6 mm respectively while m.fragrance without myristicin had 13.6, 8.6 and 7.3 mm. Based on the result, both m.fragrans volatile oils m.fragrans volatile oils without myristicin is categorized as a strong antibacterial to S.aureus and weak antibacterials to B.subtilis and E.coli.

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