Conference Paper

Antibacterial Activity of Nutmeg Oil

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Abstract

Indonesia is one of the largest producer of nutmeg oil (*Myristica fragrans*). This essential oil has a lot of usefulness for food and pharmaceutical industries, however antibacterial activity of Indonesian nutmeg oil has not been investigated yet. Antibacterial activity *Myristica fragrans oil* from two areas respectively (Sulawesi and Central Java) were investigated. The essential oils was extracted using water and steam distiller and then its antibacterial activity against pathogenic bacteria (gram-positive bacteria: *Staphylococcus aureus, Staphylococcus epidermis,* and gram-negative bacteria: *Shigella Dysenteriae, Salmonella Typhi)* was examined. Resistance pattern was studied by in vitro disc diffusion method using essential oil concentration 20%, 40%, 60%, 80% and 100%. The result showed that the two essential oils inhibited all bacteria. The highest inhibition zone on Central Java nutmeg oil was on 60% concentration of the oil (12.96 16.79, 13.46 and 16.50 mm for *S. aureus, S. epidermis, S. dysenteriae, S. typhi* respectively), while on Sulawesi nutmeg oil was on 100% concentration (18.84, 16.54, 17.84 and 12.54 mm for *S. aureus, S. epidermis, S. dysenteriae, S. typhi* respectively).

Keywords: Antibacterial activity; Nutmeg oil; Central Java; Sulawesi.

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1. Introduction

The Nutmeg tree (*Myristica fragrans*) was originated from the Banda Island [1], and is widely distributed all over in Indonesian and throughout the world (India, Grenada and Malaysia). Maluku (Moluccas), Sulawesi (Celebes), Aceh, Java and Papua are the major production areas of Indonesian nutmeg [2]. Indonesia produces three quarters of the total world output and export, while Grenada is in the second rank [3].

Nutmeg mainly used as a spice or extracted to nutmeg oil. Essential oils can be extracted from both the kernel and mace. Nutmeg refer to the dried kernel, while mace is a dried scarlet fibrous aril that covers the kernel. Steam and water distillation or steam distillation is generally used for the extraction oil. The main application used of nutmeg essential oils is in food flavoring. It has been used as the flavoring agent for beverage, biscuit, cake, pudding, candy and roasted food such as meat and sausages. In beverage industry the oils are used for soft drink of cola-type, beer, whisky and wine [4–6]. The adding aroma of nutmeg essential oils on these products linked to

spicy aroma. Nutmeg essential oils also used in pharmacy due to the antithrombotic, anti-dysentery, anti-inflammatory, rheumatism and narcotic activities [7].

Morover, nutmeg oil reveals their antibacterial activity. A number of report have been published about these activities. The oils can be effective against gram positive and gram negative bacteria: *Escherichia coli*, *Aeromonas hydrophila*, *Salmonella choleraesuis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Listeria innocua* [8] and others: *Acinebacter calcoacetica*, *Alcaligenes faecalis*, *Bacillus subtilis*, *Benecka natriegens*, *Brevibacterium linens*, *Brocothrix thermosphacta*, *Citrobacter fruendii*, *Enterobacter aerogenes*, *Erwinia carotovora*, *Flavobacterium suaveolens*, *Klebsiella pneumonia*, *Micrococcus luteus*, *Moraxella sp.*, *Proteus vulgaris*, *Serratia marcescens* and *Yersinia enterocolitica* [9].

However, research on antibacterial activity of nutmeg oil in Indonesia has not been reported widely. Therefore, this research aimed to examine the antibacterial activity of nutmeg oil from Sulawesi and Central Java against *Staphylococcus aureus, Staphylococcus epidermis, Shigella dysenteriae* and *Salmonella typhii.* Among the major production area in Indonesia, Sulawesi is the biggest. Moreover nutmeg oil from this area has been renowned since a long time ago as an export commodity. While Central Java is a new area for nutmeg oil production.

2. Materials and Method

2.1. Materials

Nutmeg oils from the kernel were obtained from distillers in Sulawesi and Central Java (2 samples for each), wich are distilled using water and steam distillation. *S. aureus, S. epidermis, S. dysenteria* and *S. typhii* were obtained from the Laboratory of Microbiology, Faculty of Pharmacy, Padjadjaran University.

2.2. Methods

Antibacterial activity of nutmeg oils (zone of inhibition). The nutmeg oils was prepared into solutions with concentration of 20%, 40%, 60%, 80% and 100% (v/v) by diluting with ethanol. *S. aureus, S. epidermis, S. dysenteria* and *S. typhii* suspension were prepared with cell content of 3×10^8 CFU/ml (absorption in spectrometry was compared with the Mc Farland scale 1 for each microorganism). *S. aureus, S. epidermis, S. dysenteria* and *S. typhii* were cultured on Beef Extract Peptone Agar (NA) medium. With the aid of moist sterile swab the suspensions were spread on plates of Mueller Hilton Agar (MHA). Paper disc of 0.5 cm was soaked in oil solution and left to dry for about 7 minute. Paper disc was placed on MHA and incubated at 37°C for 48 h.

After incubation, the inhibition zones were measured to determine the antibacterial activity. The experiment was conducted at three replications. Diameter of inhibition was calculated by subtracting the diameter of bacterial activity with the diameter of paper disc. The resulting diameters of the inhibition zones were compared with control disc without nutmeg oils.

GC-flame ionization detection analysis. The nutmeg oil was analyzed using GC 2010 Shimadzu machine with a ZB-1MS fused mμsilica capillary column (30m × 0.25mm × 0.25µm film thickness). The injector and detector temperature was 280 and 230°C, respectively. The oven temperature was programmed to rise from 60°C to 290°C in 29 min at rate of 8°C/min. The carrier gas was hydrogen with a flowrate of 1.31 mL/min. The percentage of constituents were based on electronic integration of peak area without the use of response factor correction.

GC-MS Analysis. The analysis of nutmeg oil was performed on Shimadzu QP 2010 Ultra with a ZB-5MS fused silica capillary column (30m × 0.25mm × 0.25µm film thickness). The GC operating conditions were the same as described above. The detector was operated in EI mode with a mass scan range from m/z 35 to 500 at 0.7 kV. The components were identified by using The National Institute of Standards and Technology (NIST 3.0) and WILEY 275 libraries provided with controlling the GC –MS system and mass spectra with published data.

3. Result and Discussion

3.1. Antibacterial activity

The antibacterial activity of Sulawesi nutmeg oil on both gram positive (*S. aureus* and *S. epidermis*) and gram negative (*S. dysenteriae* and *S. typhii*) were represented in Table 1, while antibacterial activity of Central Java nutmeg oil were represented in Table 2. The results from the disc diffusion method indicated that Sulawesi and Central Java nutmeg oils had inhibitory effect against all bacteria tested in middle to strong area. Sulawesi *Myristica fragrans* oil had smaller antibacterial activity average better than Central Java *Myristica fragrans* oil. However, the increasing concentration of oils did not reveal increasing of inhibition diameter. It is more influenced by the cell wall of each type of bacteria, gram-positive bacteria have a thicker peptidoglycan than gram-negative bacteria. In addition, the gram-negative bacteria had proteins and lipoproteins layer that serves as a protective. The mechanism of inhibition against the bacteria include damage cell membranes, inhibits protein synthesis, and specific enzymes disrupt cell membranes and cell biological functions [10].

Phenolic compound in essential oil appears to contribute on most antibacterial activity, while other constituens contributes little to this activity [11]. However, the

Table 1: Antibacterial activity units of Sulawesi *Myristica fragrans* oil.

Bacteria	0% (control)	20%	40%	60%	80%	100%
Stapphylococcus aureus	0	11.59	14.29	12.96	12.75	18.84
Stapphylococcus epidermi.	0	9.63	10.79	11.46	11.88	16.54
Shigella dysenteriae	0	10.04	12.17	13.04	15.59	17.84
Salmonella typhi	0	9.54	10.75	10.38	10.79	12.54

Table 2: Antibacterial activity units of Central Java *Myristica fragrans* oil.

Bacteria	0% (control)	20%	40%	60%	80%	100%
Stapphylococcus aureus	0	12.16	12.54	12.96	9.37	8.35
Stapphylococcus epidermis	0	9.54	13.62	16.79	9.91	9.29
Shigella dysenteriae	0	13.46	14.62	13.46	12.71	14.21
Salmonella typhi	0	12.46	12.75	16.50	10.04	8.71

antibacterial activity of nutmeg oils was primarily caused by pinene-β component [12]. This constituent presents in both nutmeg oils (Sulawesi dan Central Java). Although -β pinene component is higher in Central Java nutmeg oils (Table 3), it did not show higher antibacterial activity. The differences of antibacterial activity in the same component allegedly influenced by many other active components found in the nutmeg oils. It has been identified 45 components were identified in Sulawesi nutmeg oil and 39 components were identified in Central Java nutmeg oil. Each component can synergize to produce antibacterial activity. However, further research needs to be done on the interaction between the active component in nutmeg oils.

3.2. Composition of nutmeg oils

Chemical composition of Sulawesi and Central Java nutmeg oils with GC-MS analysis was presented in Table 3. In total, 45 components were identified in Sulawesi nutmeg oil and 39 components were identified in Central Java.

Table 3: Composition of Sulawesi and Central Java Nutmeg Oils.

No	Retention Time	Chemical component	Sulawesi (%)	Central Java (%)
1	3.844	alpha.-Thujene	0.81	2.1
2	3.976	1R-.alpha.-Pinene	4.24	16.54
3	4.155	Beta-Fenchene		0.03
4	4.180	Camphene	0.5	0.87
5	4.532	Sabinene	9	18.82
6	4.603	Beta- Pinene	5.31	13.82
7	4.702	Beta -Pinene	3.75	3.52
8	4.959	Phellandrene	1.38	1.49
9	5.055	Carene	1.16	2.96

Table 3: Continued.

No	Retention Time	Chemical component	Sulawesi (%)	Central Java (%)
10	5.146	alpha-Terpinene	4.23	2.59
11	5.272	Cymene	2.14	1.63
12	5.352	Limonene	10.31	8.41
13	5.592	beta-Ocimene	0.1	0.09
14	5.822	gamma-Terpinene	5.4	3.18
15	5.962	Dimethylsiloxane pentamer	0.14	
16	6.240	trans Sabinene hydrate	0.41	0.43
17	6.317	alpha-Terpinolene	2.78	2.43
18	6.409	Adamantanol	0.14	
19	6.485	Linalool		0.82
20	6.949	Terpeniol		0.13
21	7.145	Mentha-tiene	0.05	
22	7.910	Therpinen-ol	7.99	2.8
23	8.286	alpha-Terpineol	0.88	0.8
24	9.099	Linalyl acetate	0.06	0.08
25	9.707	Borneol acetate	0.46	0.25
26	9.786	Isosafrole	3.68	2.17
27	9.919	Terpinene acetate	0.23	
28	9.980	Anisole	0.82	0.39
29	10.733	alpha Terpinenyl acetate	0.81	0.41
30	10.770	alpha Cubebene	0.42	0.17
31	10.984	Eugenol	0.42	0.34
32	11.235	Copaene	1.95	1.16
33	11.457	Germacrene	0.14	
34	11.648	Methyl isoeugenol	8.5	0.19
35	11.902	Cyclohexene	0.11	
36	11.983	Caryophyllen	0.12	0.23
37	12.150	Norpinene	0.3	0.21
38	12.418	Isoeugenol		0.37
39	12.403	Farnesol	0.12	
40	12.872	Alpha Curcumene	0.27	0.17
41	12.943	Germacrene	0.22	0.09
42	13.060	Zingibere	0.25	0.11
43	13.106	Cis-methyl isoeugenol	2.04	
44	13.200	alpha-Farnesene	0.46	0.11
45	13.267	beta-Bisabolene	0.32	0.13
46	13.586	Myristicine	13.73	9.32
47	13.720	Cadina diene	0.03	
48	13.780	Alpha humulene	0.02	
49	13.971	Elemicin	3.81	0.39

The differences of component caused by differences in species of nutmeg. The main components for both nutmeg oils were alpha pinene, beta pinene, sabinene, limonene and myristicin. This findings were likely to be similar with other previous studies [4, 13–16]. The highest component of Sulawesi nutmeg oil was myristicine (13.73%), while in Central Java nutmeg oil, the highest component was sabinene (18.82%).

4. Conclusion

The study showed that Sulawesi and Central Java nutmeg oils were effective against bacteria (*S. aureus, S epidermis, S. dysenteriae* and *S. typhi*). The highest inhibition zone on Central Java nutmeg oil was on 60% concentration of the oil (12.96 16.79, 13.46 and 16.50 mm for *S. aureus, S. epidermis, S. dysenteriae, S. typhi* respectively), while on Sulawesi nutmeg oil was on 100% concentration (18.84, 16.54, 17.84 and 12.54 mm for *S. aureus, S. epidermis, S. dysenteriae, S. typhi* respectively). Therefore nutmeg oils can be considered as a potential natural antibacterial product. The main components of the nutmeg oils were sabinene, myristicin, pinene and limonene.

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