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Analysis Of Essential Oils From Mace And Banda Nutmeg (Myristica Fragrans Houtt) From Romang Island, Maluku-Indonesia

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Abstract:
Background*: Maluku has essential oil-producing plants, including Banda Nutmeg (Myristica fragrans Houtt). This plant's spread is widespread; some are scattered on Romang Island, Southwest Maluku Regency, Indonesia, especially in Hila Village and Jerusu Village. Banda nutmeg and mace seed oil were isolated using the steam distillation method. The results of oil isolation showed that the highest percentage of Banda nutmeg oil yield was from Hila Village, 3.36% for mace, and 1.36% for seeds from Jerusu Village. Analysis of the composition of Banda nutmeg oil using GC-MS showed that there were six major components, namely Alpha-Pinen (21.24–24.51% in seeds and 15.80–15.81% in mace), Sabinen (34.87–36.03% in seeds and 25.73–27.43% in mace), Beta-Myrcene (13.41–18.27% in seeds and 13.26–14.8 7% in mace), Limonen (3.04–3.25% in seeds and 2.84–3.03% in mace), Linalool (2.47–4.40% in seeds and 3.02–4.39% in mace), Myristicin (3.78–7.80% in seeds and 15.32–19.40% in mace).*
Key Word*: Essential Oils, chemical composition, Banda Nutmeg, Romang Maluku-Indonesia*

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I. Introduction

Nutmeg is a spice plant native to Maluku and has been cultivated for generations in the form of community plantations in most of the Maluku Islands[1]. Nutmeg has high economic value[2] and is essential to people's economy in various regions, especially in Eastern Indonesia. Nutmeg plants are also one of the plants that are in great demand in the development of social forestry[3]. Apart from being the largest producer of nutmeg in the world, Indonesia is also the largest supplier of nutmeg globally, with a share reaching 60-75% of world demand[4,5].

Nutmeg, with its main products in the form of seeds and mace, is an indigenous spice plant from the Maluku Islands (Moluccas), Banda Island, to be precise[6]. Most of the 99% of nutmeg plants are cultivated as community plantations[7]. For a long time, nutmeg has been used to remedy diarrhea, mouth sores, and insomnia[8]. Nutmeg seeds are used naturally as insect repellents, toothache remedies, digestive disorders, and herbal ingredients to reduce muscle pain, joints, gout, and headaches[9]. In the Middle Ages, nutmeg was used as an upset stomach, stimulant, carminative, catarrh, colic colitis, stimulates appetite, controls flatulence, and has a reputation as an emmenagogue and abortifacient[10].

Several studies have been carried out on the isolation of Myristica fragrans Houtt nutmeg oil and the identification of nutmeg oil components. Isolated and identified essential oils from fruit flesh and mace based on the age of the nutmeg and showed that the dominant compound components in the fruit flesh and mace were myristicin, sabine, α-pinene, β-pinene which contributed to the characteristic aroma of nutmeg[11]. The same thing was also reported by Ismiyarto[12], which obtained the results of oil isolation received clear yellow essential oil with a distinctive nutmeg odor with a yield of 3.22%, a specific gravity of 0.9397 g/mL at 25°C and a refractive index of 1.493 (25°C). Identification of chemical components using the GC-MS method showed that nutmeg mace essential oil isolated contained components a-pinene (13.08%), b-pinene (15.14%), sabinene (22.93%), limonene (5.60%), and myristicin (26.46%). Differences in oil yields in each region vary depending on environmental conditions. The samples selected in this study were the seeds and flowers (mace) of Banda nutmeg (Myristica et al.) taken from the Romang Archipelago, Southwest Maluku in particular, namely Hila village and Jerusu village, which represent Romang Island as a whole. This study aimed to analyze the chemical components of essential oils from the seeds and mace of banda nutmeg (Myristica fragrans Houtt) from Hila and Jerusu villages, Romang Islands, Southwest Maluku, Maluku, Indonesia.

II. Material and Methods

Material and Methods Sample preparation: Banda nutmeg (Myristica fragrans Houtt) seeds and mace were obtained from Hila village and Jerusu village, Romang Island (Southwest Maluku). Mace samples were dried by storing in a room at 16ºC for 4 days. Nutmeg seed samples were dried in an oven at 68ºC for seven days.

Distillation and sample identification: Isolating nutmeg oil uses the distillation method, separating compounds based on vapor pressure. In the distillation process, the dried Banda nutmeg flower (mace) sample is put into a distillation apparatus and distilled for 6 hours, while the dried Banda nutmeg seed sample is mashed first by cutting or grinding to increase the surface area of the model. The distillation results are collected, and there are two layers of the distillation results: the top layer is nutmeg oil, and the bottom layer is water. The oil and water were then separated using a separatory funnel, and the distillate nutmeg oil was added with anhydrous sodium sulfate, filtered, and analyzed using GC-MS.

III. Result and Discussion

The process of isolating Banda mace oil and nutmeg seeds used the steam distillation method, which is based on differences in the boiling points of the essential oil constituents. The sample is mashed first (especially for the nutmeg seed sample) to increase the surface area to maximize the contact between the model and water vapor, thereby accelerating the diffusion process. Reducing the sample size will open up the tissue in the stem bark, which causes the amount of extracted oil to be higher, and the small size of the material causes the diffusion process to speed up. In extracting essential oils using the steam distillation method, water is heated to a temperature close to the boiling point of water, namely 95-105 ºC. In this condition, the water will evaporate. The water vapor will extract the essential oil in the sample (nutmeg seeds and mace), then go to the condenser, and condensation occurs (condensation). The condensed steam is collected in a separatory funnel, where there are two layers: the upper layer is oil, and the lower layer is water. The distilled oil is separated from water using a separatory funnel. The remaining water in the distilled oil is removed by adding anhydrous sodium sulfate (Na_2SO_4). The result of the distillation is a pale yellow oil rendition with a weight percentage that can be seen in Table 1.

Table 1. Percentage of mace and nutmeg oil yields

Village of origin sample	Sample weight (g)		Oil weight (g)		Yield %	
	Mace	Seed	Mace	Seed	Mace	Seed
Hila	376,77	1431,70	12,66	11,60	3,36	0,81
Jerusu	338,08	1776,11	9,20	24,20	2,72	1,36

The percentage of Banda nutmeg mace oil and Banda nutmeg seed oil yields in Table 1 shows that the Banda nutmeg mace oil yield is greater than the Banda nutmeg seed oil yield. The highest result of Banda nutmeg oil (3.36%) was for nutmeg mace in Hila Village and (1.36%) for nutmeg seeds in Jerusu Village (% w/w). Differences in oil content in mace and banda nutmeg originating from Romang Island and Banda Island are caused by differences in natural conditions, namely the position, and altitude where the nutmeg plant grows to sea level. Also, the natural conditions of Romang Island are unsuitable for Banda nutmeg plants. Based on field data, Banda nutmeg does not grow well on the mainland of Romang Island, and the number of Banda Nutmeg trees that grow is small.

The secondary metabolites' constituent components determine the aroma and quality of natural ingredients that produce essential oils. The essential oil of nutmeg seeds and mace is the most valuable component in nutmeg. Therefore, Banda nutmeg is a species of nutmeg that is considered in trade and has high economic value because it is widely used in various food and pharmaceutical industries. The results of chemical composition analysis using a Gas Chromatography-Mass Spectroscopy (GC-MS) instrument on Banda nutmeg and mace oil from Hila Village, Romang island, showed 17 components for nutmeg seed oil (Figure 1) and 19 components for nutmeg mace oil (Figure 2). The differences and similarities of the components for the samples of nutmeg seed oil and nutmeg mace are presented in Table 2.

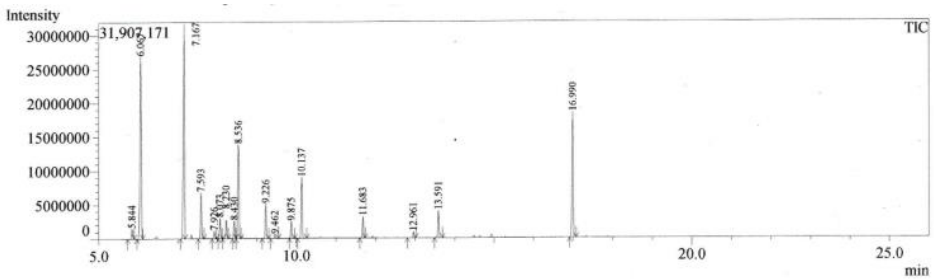

Figure 1. Chromatogram of nutmeg seed oil in Hila-Romang village

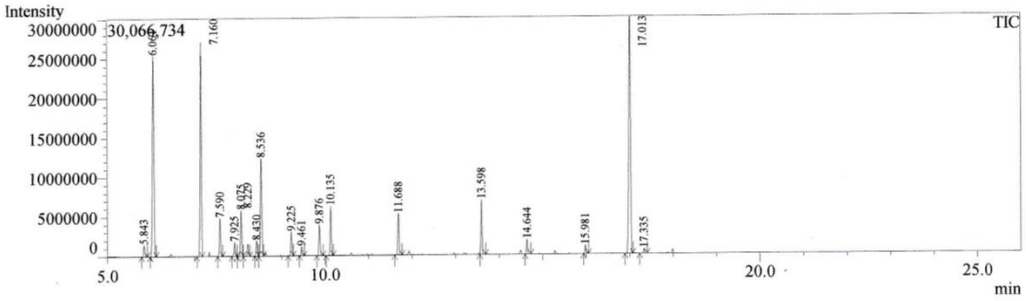

Figure 2. Chromatogram of mace oil in banda nutmeg in Hila-Romang village

Table 2. Chemical composition of nutmeg seed oil and fuli oil from nutmeg in Hila-Romang village

No	Component Name	m/z	Presentation	
			Nutmeg Seed Oil	Fuli oil from nutmeg
1	Alpha-Thujene	136	1,16	0,99
2	Alpha-Pinen	136	22,06	19,35
3	Sabinen	136	24,30	17,31
4	Beta-Myrcene	136	4,70	3,0
5	Beta-Ocimene	136	2,17	3,59
6	Alpha-Terpinen	136	2,00	0,97
7	Limonen	136	10,71	8,62
8	Gama-Terpinen	136	3,39	1,78
9	Alpha-Terpinolen	136	1,7	2,44
10	Linalool	154	6,05	3,56
11	Terpinen-4-ol	154	1,96	3,08
12	Safrole	162	2,54	3,97
13	Eugenol	164	-	1,04
14	Myristicin	192	13,41	26,29

Based on the compositional data shown in Table 2, it can be seen that there are six major components in Banda mace and nutmeg oil from Hila Island Romang, with different compositions and concentrations, namely sabinene in mace (17.31%), while seeds (24.30%). α-pinene is concentrated in mace (19.35%) while in seeds (22.06%). The concentration of myristicin compounds in mace (26.29%) while in seeds (13.41%). The β-Myrcene combination has a concentration in mace (of 3.0%) in seeds (4.7%), while the concentration of linalool compounds in mace (3.56%) in seeds (6.05%). Limonene compounds have concentrations in mace (8.62%) while in seeds (10.71%). In addition, there are differences in the composition and concentration of minor compounds, as seen in Table 2. The eugenol compound is only present in nutmeg mace oil, with a presentation of 1.04%. Safrole compounds it is found in both types of oil, but the largest production is in mace oil (3.97%) while in seeds (2.54%). The results of chemical composition analysis using a Gas Chromatography-Mass Spectroscopy (GC-MS) instrument on nutmeg seed oil and Banda nutmeg mace from Jerusu Village, Romang Island, showed 17 components for nutmeg seed oil (Figure 3) and 23 components for nutmeg mace oil (Figure 4).

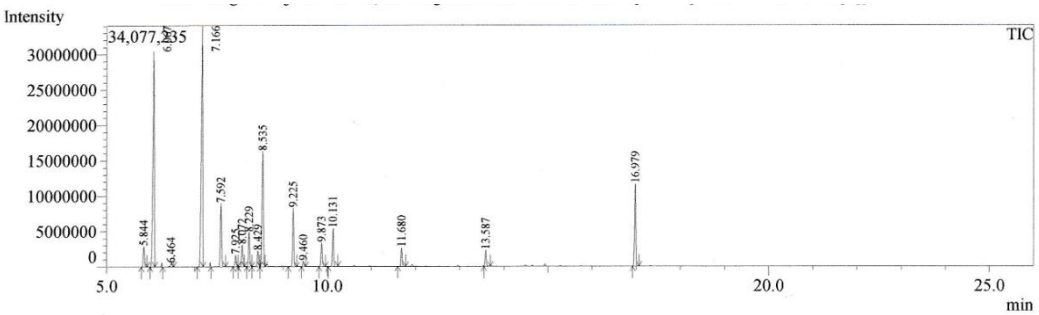

Figure 3. Chromatogram of nutmeg seed oil in Jerusu-Romang village

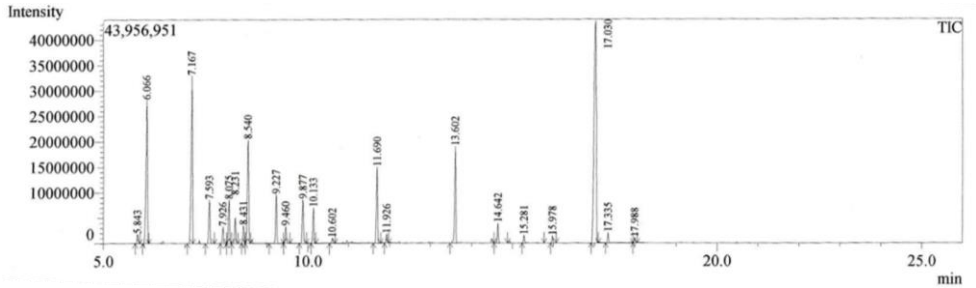

Figure 4. Chromatogram of mace oil in banda nutmeg, Jerusu-Romang village

Table 3. Chemical composition of nutmeg seed oil and fuli oil from nutmeg in the village of Jerusu-Romang

No	Component Name	m/z	Presentation	
			Nutmeg Seed Oil	Fuli oil from nutmeg
1	Alpha-Thujene	136	2,14	0,68
2	Alpha-Pinen	136	22,5	11,59
3	Sabinen	136	26,4	13,07
4	Beta-Myrcene	136	6,05	3,01
5	Alpha-Phelandrene	136	1,21	1,21
6	Beta-Ocimene	136	2,14	2,96
7	Alpha-Terpinen	136	3,34	1,76
8	Limonen	136	12,17	8,20
9	Gama-Terpinen	136	5,27	3,43
10	Alpha-Terpinolen	136	2,14	3,09
11	Linalool	154	3,37	2,33
12	Terpinen-4-ol	154	1,59	5,11
13	Safrole	162	1,42	6,95
14	Eugenol	164	-	1,24
15	Myristicin	192	7,59	29,48

Based on the analysis data shown in Table 3 above, it can be seen that there are six major components in mace oil and Banda nutmeg from Jerusu Village, Romang Island, with different compositions and concentrations, namely the concentration of sabinene compounds in mace is (13.07%), while in seeds (26.4%). α-pinene is concentrated in mace (11.59%) and seeds (22.5%). The highest concentration of myristicin compounds was in mace (29.48%), while in seeds (7.59%). The concentration of β-Myrcene is in mace (3.01%) while in seeds (6.05%)—the concentration of linalool compounds in mace (2.33%) while in seeds (3.37%). Limonene compounds have concentrations in mace (8.2%) while in seeds (12.17%). In addition, there are differences in the composition and concentration of minor compounds in Table 3. The eugenol compound is only found in nutmeg mace oil with a presentation of 1.24%, which aligns with the results in Hila village. The safrole compound in nutmeg mace oil has a relatively large percentage of 6.95% and 1.42% for nutmeg seed oil. Safrol produced from nutmeg mace oil in Jerusu village was more than that from Hila village, which was only 3.97%. In the species Myristica fragrans out originating from Banda Island, there are four major components, namely α-pinene (10.80-18.34% for mace and (22.62-25.23% for seeds), sabinene (15.60-16.28% for mace and 24.81-25.61% for nutmeg), β-pinene 8.43-17.18% for mace and 6.24 -6.44% in seeds), myristicin (18.80-21.56% in mace and 6.52-10.23% in seeds). Based on the results of these studies, it can be seen that the place of growth also influences the chemical composition of the nutmeg oil obtained.

Table 4. Significant components of nutmeg seed oil and mace in the villages of Jerusu and Hila

No	Component Name	Presentation in Hila village		Presentation in Jerusu village	
		Nutmeg Seed Oil	Fuli oil from nutmeg	Nutmeg Seed Oil	Fuli oil from nutmeg
1	Alpha-Pinen	22,06	19,35	22,5	11,59
2	Sabinen	24,30	17,31	26,4	13,07
3	Beta-Myrcene	4,70	3,0	6,05	3,01
4	Limonen	10,71	8,62	12,17	8,20
5	Linalool	6,05	3,56	3,37	2,33
6	Myristicin	13,41	26,29	7,59	29,48

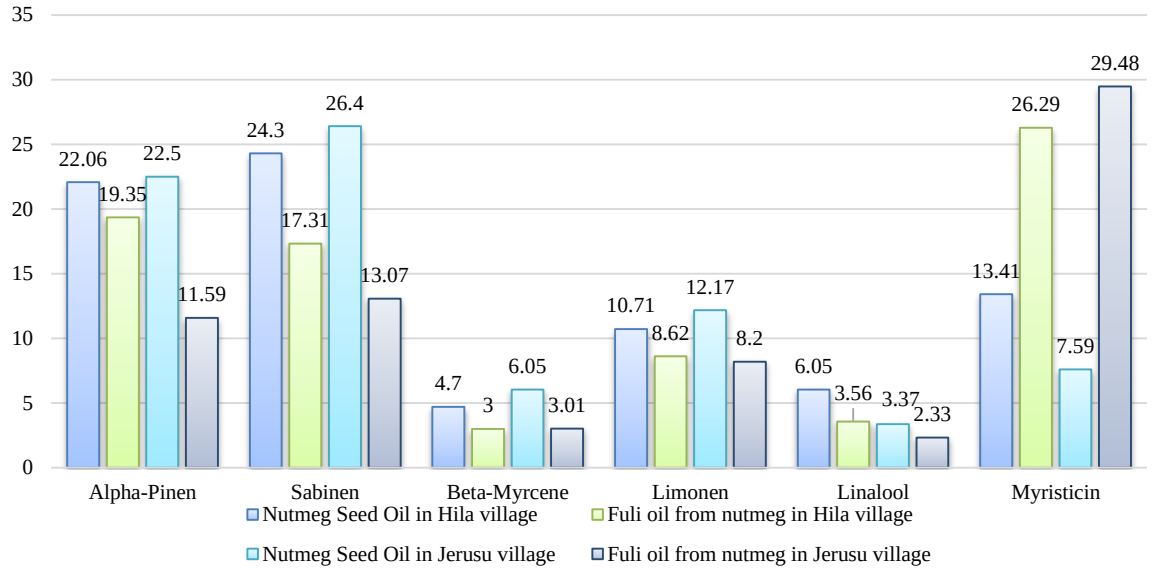

Figure 5. Major component histogram of Romang Island nutmeg oil

In Figure 5, it can be seen that the presentation of the six main components in mace and banda nutmeg varies. Mace contains the most myristicin compounds compared to seeds, while seeds contain the most sabinene compared to mace. Sabinene is the dominant compound in nutmeg essential oil. Sabinene is a compound that can rotate the plane of polarized light to the right (dextrorotatory). This compound is not very broad, but it is often used as a component of synthetic pepper oil. Sabinene is a compound that belongs to the third monoterpenes (bicyclic) group, which has two circles accompanied by one double bond. The aroma of an essential oil is formed by all the components that make up the essential oil, both major and minor components. The distinctive aroma differences between essential oil-producing plants indicate differences in chemical composition.

The analysis results show a similarity in the composition of the chemical components, both in the mace and the seeds. The major components of nutmeg oil are Alpha-Pinene 22.06–22.5% in seeds and 11.59–19.35% in mace), Sabinen (24.3–26.4% in seeds and 13.07–26.4% in mace), Beta-Myrcene (4.7–6.05% in seeds and 3.0–3.01% in mace), Limonen (10.71–12.17% on seeds and 8.2–8.62% on mace), Linalool (3.37–6.05% on seeds and 2.33–3.56% on mace), and Myristicin (7.59–13.41% on seeds and 26.29–29.48% on mace). When viewed from the myristicin content as the most important component of nutmeg oil, it turns out that the myristicin component in the nutmeg mace is more than in the seeds. The results are the same as those reported by Dorman[13]: Mace oil contains more myristicin than nutmeg oil.

Jukic[14] stated that the main components of nutmeg oil are terpenes, terpene alcohols, and phenolic ethers. The monoterpene hydrocarbon component, which is the main component of nutmeg oil, consists of β-pinene (23.9%), α-pinene (17.2%), and limonene (7.5%). Meanwhile, the phenolic ether components were mainly myristicin (16.2%), followed by safrole (3.9%) and methyl eugenol (1.8%). Furthermore, Dorman13 stated that 25 identified components in nutmeg oil (92.1% of the total oil) were obtained by hydrodistillation using an oil distiller, according to the British Pharmacopeia. In principle, these oil components were identified as α-pinene (22.0%) and β-pinene (21.5%), sabinene (15.4), myristicin (9.4), and terpinene–4-ol (5.7).

IV. Conclusion

Based on the results of the research that has been done, it can be concluded that the yield of Banda nutmeg mace oil from Hila Village is 3.36%, and the yield of Banda nutmeg mace oil from Jerusu Village is 2.72%. Meanwhile, the yield of Banda nutmeg seed oil from Hila Village was 0.81%, and the Banda nutmeg seed oil yield from Jerusu Village was 1.36% (% w/w). The results of the analysis of the chemical composition of nutmeg oil using GC-MS showed six major components, namely alpha-pinene (21.24–24.51% in seeds and 15.80–15.81% in mace), Sabinen (34.87–36.03% in seeds and 25.73–27.43% in mace), Beta-Myrcene (13.41–18.27% in seeds and 13.26–14.8 7% in mace), Limonen (3.04–3.25% in seeds and 2.84–3.03% in mace), Linalool (2.47–4.40% in seeds and 3.02–4.39% in mace), and Myristicin (3.78-7.80% in seeds and 15.32-19.40% in mace).

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