Vol. 12(1): 100-112, January 2024 DOI: https://doi.org/10.23960/jsl.v12i1.789

P-ISSN: 2339-0913
E-ISSN: 2549-5747

Jurnal Sylva Lestari

Journal homepage: https://sylvalestari.fp.unila.ac.id

Full Length Research Article

Characterization of Leaf Essential Oil from Nutmeg *(Myristica fragrans)* Cultivated on Agroforestry Land

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ARTICLE HISTORY:

Received: 22 August 2023
Peer review completed: 19 September 2023
Received in revised form: 9 December 2023
Accepted: 21 December 2023

KEYWORDS:

Agroforestry
Essential oil
Nutmeg leaves
Streptamine
Terpenoid compounds

ABSTRACT

Lampung is the third largest producer of nutmeg (*Myristica fragrans*) in Sumatra Island, Indonesia. Most nutmeg essential oil refining industries in Lampung process nutmeg seeds, but not many distill nutmeg leaves. Therefore, it is necessary to identify the chemical compounds of nutmeg essential oil to determine the potential for further processing products and the quality of nutmeg essential oil. The leaf essential oil samples were obtained from a refining industry in Pesawaran, Lampung, Indonesia. The chemical compounds of the essential oils were identified using gas chromatography-mass spectrometry. Nutmeg leaf essential oil quality was then evaluated according to SNI 06-2388-2006 as standard testing for nutmeg oil. The data were then analyzed descriptively. The results identified 28 active compounds in nutmeg leaf essential oil. Compounds with high relative abundance were streptamine (76.46%), aquinolizine (4.67%), boron (3.73%), kaurene (2.03%), pyrrole (1.95%), and naphthalene. There were also terpenoid compounds that produced aromas, such as guaiol, borneol, cedrene, cubenol, and spathulenol. The quality assessment of leaf nutmeg essential oils also fulfilled the requirement of SNI 06-2388-2006. However, the optical produced rotation towards the left, namely -9.48°, and a specific gravity of 0.87, less than the SNI 06-2388-2006 requirement of 0.88. Nutmeg leaf essential oil has the potential to become a raw material for health and beauty products.

1. Introduction

Indonesia is the largest nutmeg-producing country and the main producer of nutmeg essential oil globally, reaching the export of nutmeg oil of 200 tons/year (Suwarni et al. 2013). Nutmeg oil is processed from the seeds and mace of nutmeg and exported to the United States, England, and Germany (Woriwun et al. 2021). Grenada and Sri Lanka are the countries that produce nutmeg oil after Indonesia (Rodianawati et al. 2015). Lampung Province is the third largest producer of nutmeg plants (*Myristica fragrans*) in Sumatra Island, with an area of 2,444 ha and a production of 405 tons of nutmegs in 2022, after the provinces of Aceh and West Sumatra (Directorate General of Estates 2022). The largest nutmeg-producing in Lampung Province are Tanggamus Regency (Widayanti et al. 2020), Pesawaran Regency, and East Lampung Regency (BPS 2014).

The Indonesian government is implementing a nutmeg plant intensification program in the Lampung and Maluku regions in 2022 (Ministry of Agriculture 2022). Nutmeg in Lampung

Province is usually grown using an agroforestry system, which leads to a complex agroforestry system and has a high total density (Widayanti et al. 2020). It is applied to help farmers get income from other crops, apart from waiting for the nutmeg plants to produce, which takes 5 to 7 years from planting (Lestari et al. 2019).

Nutmeg is widely obtained in forest areas because the government is rehabilitating forests and land by planting nutmeg as a form of community welfare around forest areas and preventing forest damage (Senoaji et al. 2020). Nutmeg is also one of the superior products from non-timber forest products, which significantly impacts the economy and income of forest farmers (Ariandi et al. 2018). Nutmeg plants consist of trees, roots, stems, leaves, flowers, and fruit (Kapelle et al. 2022). The products that farmers sell are mainly in the form of dry nutmeg seeds and mace (Ministry of Agriculture 2022), while the fruit flesh and leaves are still under-utilized. Nutmeg leaves are a large part of the nutmeg tree. The nutmeg tree consists of female and male plants (Bermawie 2018). The population of female trees only reaches 45% of the plant population, and the rest is male nutmeg. Male nutmeg trees can not produce fruit (Kaihatu et al. 2021). Male nutmeg trees only produce nutmeg leaves and there is still a lack of farmers who can utilize the nutmeg leaves.

The nutmeg essential oil refining factory in the Padang Cermin of Pesawaran Regency focuses more on distilling nutmeg seeds, while refining nutmeg leaves is only a by-product because the essential oil of nutmeg leaves has a very low yield of 0.70%–1.70% (BSN 2006). Essential oils are concentrated liquids derived from aromatic plants that are hydrophobic, fat-soluble, alcohol-soluble, and most organic solvents (Naeem et al. 2018). Essential oils are volatile and contain active compounds widely used in the pharmacy, cosmetic, food, beverage, and textile industries (Awulachew 2023). The distillation capacity reaches approximately 1 ton/day using the steam method. The steam distillation process aims to break the oil glands in plants, and then the evaporated solid oil is separated from the water (Radwan et al. 2020). Steam refining is an effective method for increasing oil production in the field because supersaturated steam carries more latent heat than saturated steam under equivalent conditions (Liu et al. 2018).

Previous studies reported that nutmeg leaves could be extracted and showed promising results for utilization. According to Adibuduge (2023), local nutmeg leaves from Matale, Sri Lanka, were extracted using acetone to produce the highest compound, namely myristic acid, at 57.36%. Myristic acid compounds have biological activity as natural antimicrobials (Chen et al. 2019). The essential oil of nutmeg leaves from South Aceh also has physical properties in the form of a distinctive aroma of nutmeg oil and a pale-yellow color. Meanwhile, the density of the essential oil of nutmeg leaves is lower than that of nutmeg seeds, namely 0.58 g/cm^3 (Damayanti et al. 2015).

However, no one has identified the characteristics of nutmeg leaf essential oil in Lampung Province, let alone processed it directly from the essential oil refining industry in the Pesawaran Regency area. Therefore, this research aims to determine the characteristics of the chemical compounds contained in nutmeg leaf essential oil and compare the quality with the Indonesian National Standard (SNI) 06-2388-2006. The essential oil of nutmeg leaves is taken directly from the people's distillery in the Pesawaran Regency area, and the raw material is nutmeg leaves from the surrounding area. It is hoped that the distillation of nutmeg leaves can be improved and developed into a product that can be applied and is useful for society.

2. Materials and Methods

2.1. Materials

The material used in this study was nutmeg leaf essential oil from a nutmeg refining industry (DD) located in Pesawaran, Lampung, Indonesia (5.12°–5.84°S and 104.92°–105.34°E). The refining industry has a process capacity of 1 ton/day in one production with an oil yield of 0.70%, producing 2,555 kg of nutmeg leaf oil annually.

2.2. Methods

This research involved two stages. First, samples of nutmeg leaf essential oil from the nutmeg essential oil refining industry in Pesawaran Regency were collected, and the chemical components were identified. In the second stage, the quality of nutmeg essential oil was tested according to SNI 06-2388-2006.

2.2.1. Gas chromatography-mass spectrometry (GC-MS) analysis

Nutmeg leaf essential oil was injected into the GC-MS (CP-3800, Varian, California, USA). The following is method information: GC Varian CP-3800, Detector MS Saturn 2200, Column VF-5ms 30 m × 0.25 mm × 0.25 µm (CP 8944), manual injection method, 200°C temperature trap, 60°C temperature manifold, 250°C temperature transfer line, and front injector type 1177. The initial temperature was 70°C and held for 2 minutes, then increased to 300°C with an increase rate of 5°C. When the temperature reached 300°C, it was held for 8 minutes, totaling 58 minutes.

The results of essential oil injection were detected in the form of peaks with different retention times. After obtaining the possible compounds (based on the NIST Library 11), each compound was grouped based on its similarity of each compound. The compounds used in this study had a similarity above or equal to 70%. If there were compounds with a similarity value below 70%, but these compounds often appear in other types of nutmeg, these compounds were then used. The concentration of compounds is presented in percentage (%) of area (Sipahelut 2019).

2.2.2. Quality testing of nutmeg leaves essential oil

Subsequent tests were carried out according to SNI 06-2388-2006 standards, including organoleptic testing, refractive index testing, solubility testing in alcohol, density testing, evaporation residue, and optical rotation testing. In this study, the analysis of nutmeg essential oil samples was carried out three times. The data is taken from the average value and expressed descriptively. The detailed method is described in the following sections.

2.2.2.1. Organoleptic testing

Nutmeg essential oil was tested to determine its color and smell with 20 respondents, according to SNI 06-2388-2006. The organoleptic test was carried out by 20 homogeneous respondents aged 25 years during the day. The color assessment scores were as follows: very brown (score 1), brown (score 2), pale yellow (score 3), clear yellow (score 4), and clear colorless (score 5). Moreover, the odor assessment was as follows: very not typical for nutmeg oil (score 1),

not typical for nutmeg oil (score 2), not very specific for nutmeg oil (score 3), typical for nutmeg oil (score 4), very typical for nutmeg oil (score 5). The data was then analyzed descriptively.

2.2.2.2. Refractive index testing

Refractive index testing was carried out according to SNI 06-2388-2006. This method is based on the direct measurement of the refractive angle of the oil maintained at proper temperature conditions using the chemical acetone. Water flowed through the refractometer so that this tool was at the temperature at which the reading was carried out. The reference temperature was maintained with a tolerance at the same temperature as the temperature at which the measurement was performed, and the reading was taken when the temperature was stable. The refractive index testing was calculated using Equation 1.

$$Bias\ Index = ntl\ D = nt\ D + 0.0004\ (tl - t) \quad (1)$$

where *ntl D* is the working temperature reading, *nt D* is the refractive index at 20°C, *tl* is the temperature at the time of work, and *t* is the reference temperature of 20°C.

2.2.2.3. Solubility testing in alcohol

Solubility testing in ethanol is carried out to determine the quality of essential oils. Essential oils that dissolve entirely in ethanol indicate that the constituent compounds are more polar (Loppies et al. 2021). The test was carried out according to SNI 06-2388-2006. An essential oil sample of 1 ml was measured in a 10 ml measuring cup. Then, 90% ethanol was added drop by drop. Each addition was shaken at 20°C until a clear solution was obtained. If the solution was unclear, ethanol was added again until the solution was clear and dissolved. Solubility was expressed in the ratio of 1 ml of oil to 1 ml of alcohol that dissolves it.

2.2.2.4. Density testing

The specific gravity of nutmeg oil was determined according to SNI 06-2388-2006. First, the hydrometer and measuring cup were cleaned and rinsed with ethanol, and then dried. Nutmeg leaf essential oil was then added to the measuring cup to approximately 80 ml. The hydrometer was then dipped into the nutmeg essential oil and readings were taken on the hydrometer.

2.2.2.5. Evaporation residue testing

The analysis of the evaporation residue was based on non-volatile compounds obtained by evaporating nutmeg oil over a water bath following SNI 06-2388-2006. The following test procedure was used: the beaker was heated in a water bath at 104°C for 60 minutes, cooled for 20 minutes, and then weighed (W_0). Nutmeg essential oil of 1 g was put into a beaker (W_1) and then heated in a water bath at 104°C for 4 hours, followed by cooling for 20 minutes, and then weighed (W_2). The evaporation residue (%) was calculated using Equation 2.

$$Evaporation\ residue = \frac{W_2 - W_0}{W_1 - W_0} \times 100\% \quad (2)$$

where W_0 is the empty evaporating cup weight (g), W_1 is the weight of the evaporating cup containing the essential oil (g), and W_2 is the weight of the evaporating cup containing essential oil after heating (g).

2.2.2.6. Optical rotation testing using a polarimeter

The optical rotation test was performed to determine the amount of light polarization. The analysis used a polarimeter (LWZZ-2A, AMTAST, USA) following SNI 06-2388-2006. The test was carried out by switching on the light source and waiting until a full glow was obtained. The polarimeter tube was filled with a sample of essential oil and set to a temperature of 20°C. During this period, the tube was maintained to avoid the bubbles. The tube was then placed in the polarimeter, and the dextro optical rotation (+) or levo (-) of the oil was checked on the scale contained in the tool. The oil temperature in the tube was maintained at 20°C ± 1°C. The optical rotation was also expressed in degrees of a circle until it closed to 0.01°.

3. Results and Discussion

3.1. Identification of Essential Oil Compounds of Nutmeg Leaves

Essential oils are volatile oils from medicinal plants. Essential oils consist of various molecules, namely monoterpenes, terpenes, and phenyl propanoids (Khayyat and Roselin 2018). Nutmeg leaf essential oil is taken from the nutmeg essential oil refining industry in Padang Cermin District, Pesawaran Regency (**Fig. 1a**). The raw material for nutmeg leaves comes from the Pesawaran area. Essential oil distillation equipment has a capacity of 1 ton, and the distillation method uses the steam method, as shown in **Fig. 1b**.

Fig. 1. The appearance of (a) nutmeg leaves and (b) distillation of nutmeg essential oil.

The distillation of nutmeg leaves and small twigs in Pesawaran takes 4–6 hours. According to Rangkuti (2018), the yield of nutmeg essential oil was obtained from the 3 hours of distillation, namely 5.58%. The lowest yield of nutmeg essential oil was obtained at 30 hours, namely 0.12%. Steam distillation of essential oils is the official method for isolating essential oils from plants. The principle of this technique is that when the vapor pressure equals the ambient pressure of about 100°C, volatile components with boiling points between 150°C and 300°C can be vaporized. This method can be improved by performing it under pressure, depending on the chemical properties of the essential oil. The advantage of the steam method is that the amount of steam is easy to control, and there is no thermal decomposition of the oil constituents (Smith et al. 2001).

The disadvantage of using steam distillation is that the capital spent is more expensive than the method in water or the water and steam method (Rassem et al. 2016). Nutmeg leaf essential oil had a low yield, from distilling 1 ton of raw material to only 7 kg, yielding 0.7%. The yield of nutmeg leaf essential oil was lower than that of nutmeg essential oil, reaching 2–15%, and that of nutmeg mace, reaching 7–18% (Robert et al. 2015). Factors that affect the yield of essential oils are genetic factors, climate, extraction method (Juliarti et al. 2020), soil altitude (Sulaswatty 2019), and location (Dacosta et al. 2017). The best yield of nutmeg essential oil is 198.2 ml/kg, affected by moderate sunlight intensity of 25–75% (Ariandi et al. 2018). Therefore, its production is only as a by-product of nutmeg refining, so it is necessary to carry out further tests to increase the interest of the distilling industry in distilling nutmeg leaves and the potential for further processing.

The chromatogram of nutmeg leaf essential oil is presented in **Fig. 2**. The identified chemical compounds obtained are presented in **Table 1**. In total, there are 28 compounds identified by GC-MS. In nutmeg leaf extract using acetone, 18 compounds were identified (Adibuduge 2023). The O-2-amino-2-deoxy-.deoxy-.alpha.-D-glucopyranosyl-(14)-O-[O-2,6-diamino-2,6-dideoxy-.beta.-L-idopyranosyl-(13)-.beta.-D-ribofur-anosyl-(15)]-2-deoxy-D-Streptamine had the highest relative abunce, reached 76.46%. This compound was a very complex amine sugar with groups attached. The structure is the basis of many aminoglycoside antibiotics used in the pharmaceutical world as ingredients for manufacturing antibiotic drugs such as streptomycin. Adibuduge (2023) stated that 57.36% of the compounds in nutmeg leaves are myristic acid, which has natural antibacterial properties. The second highest compound, with a relative abundance of 14.14%, is hexanoic acid, myristic acid, and sucrose, which are antimicrobial (Chen et al. 2019).

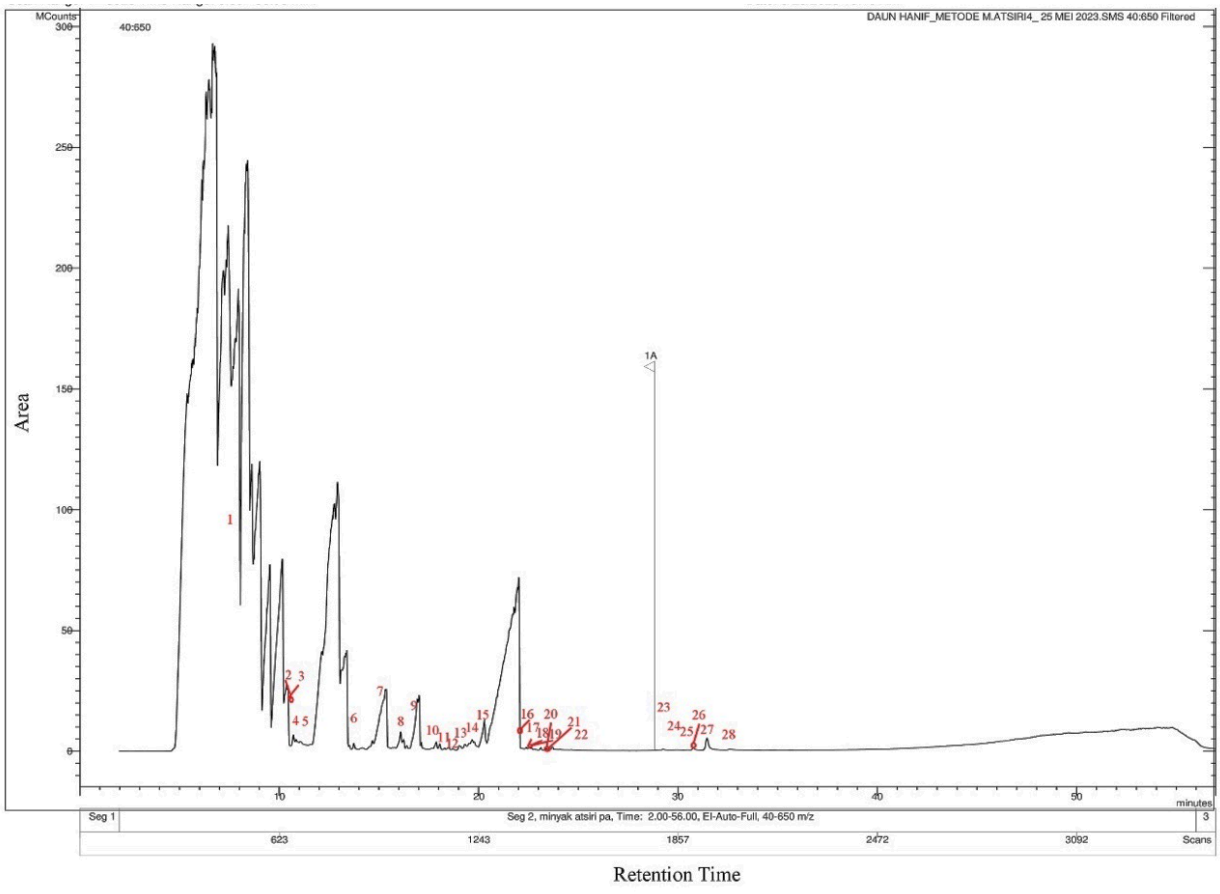

Fig. 2. Chromatogram of nutmeg leaf essential oil.

Table 1. Chemical components in nutmeg leaves essential oil

No.	RT	m/z	Compound name	Area (%)
1	7.95	42	D-Streptamine, O-2-amino-2-deoxy-.deoxy-.alpha.-D-glucopyranosyl-(14)-O-[O-2,6-diamino-2,6-dideoxy-.beta.-L-idopyranosyl-(13)-.beta.-D-ribofuranosyl-(15)]-2-deoxy-	76.46
2	10.70	81	1H-Pyrrole, 1-pentyl-	1.95
3	10.86	134	1-Methyl-1-phenyl-1-silacyclobutane	1.69
4	11.34	43	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-	0.46
		43	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-	
5	11.59	95	Borneol	1.15
		95	Borneol, heptafluorobutyrate (ester)	
		95	Bicyclo[2.2.1]hepten-2-ol, 1,7,7-trimethyl-, (1S-endo)-	
6	13.52	81	2-n-Heptylfuran	0.07
7	15.31	162	Boron, diethyl[3-imino-2-(1-iminoethyl)butanenitrilato-N2,N3]-, (t-4)-	3.73
8	16.08		Not identified	2.46
9	17.11	133	(-)-Caryophyllene-(I1)	0.81
		161	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4. alpha., 7.beta.)]-	
		161	Guaiol	
10	17.84	133	(-)-Caryophyllene-(I1)	0.97
		105	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4. alpha., 7.beta.)]-	
		189	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphtalene	
11	18.04	119	Di-epi-.alpha.-cedrene	0.57
		119	Tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl-	
		93	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	
12	18.29	161	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4. alpha., 7.beta.)]-	0.21
		107	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1. alpha.,7. alpha.,8a.alpha.)]-	
		161	1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene ,[1S-(1. alpha., 3a.beta., 4.alpha., 8a. Beta.)]-	
13	18.49	161	(-)-Isoledene	0.35
		105	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1. alpha.,4a.alpha.,8a. Alpha.)-	
		161	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.4a.alpha.,8a.alpha.)-	
14	19.02	161	Epizonarene	0.60
		204	Ethanone, 1-[5-[(5-methyl-2-furanyl)methyl]-2-furanyl]-	
		189	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1. alpha.,4a.alpha.,8a. Alpha.)-	
15	20.28	203	2,3,4,6,8,9,10,11-Octahydro-6-oxo-1H-pyrido(3,2-a)aquinolizine	4.67
16	22.41	43	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1 ar-(1a. Alpha., 4a.alpha., 7.beta.,7a.beta.,7b.alpha.)]-	0.10
		43	(-)Spathulenol	
		41	Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl-	
17	22.53	109	Humulane-1,6-dien-3-ol	0.17
		43	(-)-Globulol	
		109	1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo[5.3.1.0(4,11)]undec-8-ene	
18	22.65	43	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1 ar-(1a. Alpha., 4.beta., 4a. Beta.,7.alpha.,7a.beta.,7b.alpha.)]-	0.09
		109	6.beta.Bicylo[4.3.0]nonane, 5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-dimethyl-,	
		161	Guaiol	
19	22.81	59	2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-octahydro-. Alpha.,4a,8-tetramethyl-, [2R-(2.alpha.,4a.beta.,8.beta.)]-	0.03
		149	2,4,7,14-Tetramethyl-4-vinyl-tricyclo[5.4.3.0(1,8)]tetradecan-6-ol	
		59	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,.4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	
20	23.12	119	Cubenol	0.16

No.	RT	m/z	Compound name	Area (%)
		161	1-Naphthalenol,1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyk-4-(1-methylethyl)-, [1R-(1.alpha.,4.beta.,4a.beta.,8a.beta.)]-	
		109	1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo[5.3.1.0(4,11)]undec-8-ene	
21	23.43	161	1-Naphthalenol,1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyk-4-(1-methylethyl)-, [1R-(1.alpha.,4.beta.,4a.beta.,8a.beta.)]-	0.11
		161	.tau.-Cadinol	
		43	.tau.-Muurolol	
22	23.67	43	1-Hexacosene	0.42
		84	Cyclopentanone, 2-(2-octenyl)-	
23	29.26	69	1,6,10,14-Hexadecatetraen-3-ol,3,7,11,15-tetramethyl-, (E,E)-	0.14
		93	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	
		43	1-Heptatriacotanol	
24	29.94	93	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	0.03
		69	1,6,10,14-Hexadecatetraen-3-ol,3,7,11,15-tetramethyl-, (E,E)-	
		43	cis-Z--.alpha.-Bisabolene epoxide	
25	30.48	41	Kaur-16-ene	0.03
		94	Kaur-15-ene	
		272	Podcarpa-6,13-diene, 13-isopropyl-	
26	30.76	275	1H-Naphto[2,1-b]pyran, 3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-, [3R-(3,alpha.,4a.beta.,6a.alpha.,10a.beta.,10b.alpha.)]-	0.32
		275	1H-Naphto[2,1-b]pyran, 3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-, [3S-(3,alpha.,4a.alpha.,6a.beta.,10a.alpha.,10b.beta.)]-	
		81	1-Naphthalenemethanol , decahydro-5(5-hydroxy-3-methyl-3-pentenyl)-1,4a-dimethyl-6-methylene-, [1S-[1.alpha.,4a.alpha.,5a.alpha.(E).,8a.beta.]]-	
27	31.46	41	Kaur-16-ene	2.03
		257	Kaurene	
		257	Atis-16-ene, 5.beta.,8.alpha.,9.beta.,10.alpha.,12.alpha.)-	
28	32.56	71	Phytol	0.21
		81	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	
		71	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	
				100.000

Notes: RT = retention time, m/z= mass-to-charge ratio.

The second highest relative abundance compound was 2,3,4,6,8,9,10,11-Octahydro-6-oxo-1H-pyrido(3,2-a)aquinolizine, reaching 4.67%. This compound was categorized as a heterocyclic compound. Heterocyclic compounds have functions as drugs, vitamins, pigments, and other natural chemicals. The third compound was boron(t-4)-diethyl[3-imino-2-(1-iminoethyl)butanenitrilato-N2,N3] reaching 3.73%. The boron-contained compound is widely used in organic chemistry and health (Kohlpaintner et al. 2023). There is a content of 1-pentyl-1H-pyrrole, which reaches 1.95%. These compounds are abundant in essential oils, providing flavor and antibiotic properties (Hu et al. 2023).

The terpenoid compounds that give aroma to the perfume industry are guaiol, borneol, cedrene, and cubenol in nutmeg leaf essential oil. While forming a green color, there are phytol compounds, and for phytohormones, there are kaurene compounds. According to Prayogo et al. (2022), the guaiol content in the mindi tree (*Melia azedarach*) includes phenolic compounds that are beneficial as antioxidants.

All the active compounds contained in nutmeg leaf essential oil are antioxidants. It is best to store essential oils in dark glass bottles and fill them completely. If the storage is not full, there will be changes in the composition and physico-chemical properties that increase oxidation. Storage in clear bottles will be easily exposed to light, and then monoterpene compounds will

easily degrade, thus changing the composition of essential oils (Khayyat and Roselin 2018). According to Kapelle et al. (2022), the ethanol extract of nutmeg leaves has strong antibacterial properties against *Staphylococcus aureus* with an inhibition zone diameter of 20.31 mm and 23.56 mm. In addition, nutmeg leaf extract showed antifungal properties with 3–4% inhibition against *Aspergilus niger,* compared to nutmeg seed extract (Adibuduge 2023).

3.2. Quality of Nutmeg Essential Oil

Quality analysis shows that the nutmeg essential oil produced has fulfilled most of the requirements of SNI 06-2388-2006 (**Table 2**). The color, smell, bias index, solubility in alcohol, and evaporation residue met the criteria stated in SNI 06-2388-2006. However, the specific gravity and optical rotation of nutmeg leaf essential oil did not follow the SNI 06-2388-2006 (BSN 2006).

Table 2. Quality of nutmeg essential oil

Parameter	Nutmeg leaf essential oil	SNI 06-2388-2006
Color	Clear yellow	Colorless–pale yellow
Odor	Special nutmeg oil	Special nutmeg oil
Specific gravity (g/mL)	0.87	0.8800–0.9100
Bias index	1.48	1.470–1.497
Solubility in alcohol	1:3	1:3 next clear
Evaporation residue (%)	1.27%	Maximum 2
Optical rotation (%)	-9.48°	+8° to 25°

On the parameters of color and odor, organoleptic tests of 20 respondents were used to see the correct color and smell results. Eighteen out of 20 respondents stated that the color of the nutmeg leaf essential oil was clear yellow. In contrast, 18 respondents stated that the smell was typical of nutmeg oil, and 2 respondents stated that it was very typical of nutmeg oil. This result followed SNI 06-2388-2006, namely in color, where it was colorless to pale yellow, and in terms of the characteristic odor of nutmeg oil. Similarly, Rahardiyan (2020) reported that nutmeg essential oil has a clear yellowish color and a characteristic odor of nutmeg, but what is usually distilled is nutmeg oil obtained from the nutmeg seeds and nutmeg mace.

The refractive index of nutmeg leaf essential oil was 1.48, fulfilling the SNI 06-2388-2006 requirements of 1.47–1.49. The refractive index is the ratio between the speed of light in air and the speed of light in essential oils at a specific temperature; the lower the refractive index value, the smaller the heavy fraction contained in the oil (Rangkuti 2018). The number of compound components in the essential oil can influence the refractive index value. When the carbon chain is longer, and there are more double bonds, the density of the essential oil is higher. This results in a larger refractive index, indicating better quality (Latifah et al. 2023).

The specific gravity test of nutmeg leaves yielded a result of 0.87, which falls below the SNI 06-2388-2006 requirement of 0.88–0.91. A lower specific gravity indicated a lesser weight fraction of essential oil present. Specifically, specific gravity represents the ratio between the weight of oil and the weight of water that occupies the same volume and temperature (Rangkuti 2018).

The results of the solubility test conducted on nutmeg leaf essential oil in 90% alcohol were consistent with the SNI 06-2388-2006 standard, which was 1:3. The solubility in alcohol referred to the proportion of essential oil that dissolved entirely in alcoholic solvents. The solubility of

essential oil in alcohol is inversely proportional to the ease of essential oil dissolving in alcohol, with lower values indicating higher quality of essential oils (Rangkuti 2018). Oxygenated terpenes presented in essential oils were more soluble in alcohol when compared to non-oxygenated terpenes.

The evaporation residue of nutmeg leaf essential oil was 1.27%, meeting the SNI 06-2388-2006 maximum limit of 2%. The length of the distillation process causes the high value of evaporation residue. It might be due to the components not evaporating when heated above 100°C and being distilled (Polii 2018). The optical rotation was calculated using a polarimeter. Optical rotation is the total optical rotation resulting from the chemical compounds comprising the essential oil. The plus (+) sign represented the right rotation optic, for instance, Limonene compounds (+96° until +104°) (Ciriminna et al. 2014). In contrast, the left rotations were usually designated with a minus sign (-), such as compounds β-pinene (-20 until -16°) (Latifah et al. 2023). The optical rotation result of the essential oil derived from nutmeg leaves is -09.48°. It is worth noting that the test results differ from the SNI 06-2388-2006, which usually measures the optical rotation of nutmeg oil made from its seeds, with a range of +8° to +25°.

4. Conclusions

The study revealed that the nutmeg leaf essential oil from Pesawaran contained 28 active compounds. These compounds have potential uses in the pharmaceutical, chemical, and food industries. The essential oil contains terpenoid compounds, including borneol, cedrene, and cubenol, contributing to its aroma. Additionally, it contains major antioxidant and antibacterial compounds such as streptamine, aquinolizine, boron-containing compounds, and pyrrole. The quality test found that the nutmeg leaf essential oil meets the standards set in the SNI 06-2388-2006, with a difference in optical rotation of (-) 9.48° compared to nutmeg seeds with a (+) result. Hopefully, this research will develop into a valuable product and increase the potential for refining nutmeg leaves in the Lampung Province area.

Acknowledgments

The authors thank the University of Lampung for providing research funding and journal publication.

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