

Article

Variation in the Chemical Profiles of Nutmeg (*Myristica fragrans* Houtt.) Essential Oils from Banda, Titawaai, and Seith

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Abstract. Nutmeg (*Myristica fragrans* Houtt.) is a leading spice commodity in Maluku that yields an essential oil containing economically valuable bioactive compounds; however, variations in its chemical profile among production areas in Central Maluku Regency have not been systematically studied. This study aimed to analyze and compare the chemical profiles of nutmeg seed essential oils from Banda, Titawaai, and Seith to determine whether major component levels differ significantly. Nutmeg seeds were sun-dried for 24 hours and steam-distilled. The oils were dried over anhydrous sodium sulfate (Na₂SO₄) and analyzed by Gas Chromatography–Mass Spectrometry (GC-MS). Oil yield was compared among locations using one-way ANOVA. Essential oil yield differed significantly among locations ($p < 0.05$), with Titawaai producing the highest yield (0.70%), followed by Banda (0.30%) and Seith (0.29%), indicating significant differences among locations ($p < 0.05$). Sabinene was the predominant component in samples from all three locations, with relative abundances of 24.69%, 29.34%, and 33.69% in Banda, Titawaai, and Seith, respectively. The relative abundances of α -pinene were 17.03%, 17.52%, and 24.37%, and those of β -pinene were 14.08%, 14.48%, and 16.35% in Banda, Titawaai, and Seith, respectively. The relative abundance of myristicin was 10.29%, 6.43%, and 5.41% in Banda, Titawaai, and Seith, respectively. The observed variations in yield and chemical composition indicate that geographical origin strongly influences nutmeg essential oil characteristics. The findings support quality standardization and the development of Central Maluku nutmeg oil as a pharmaceutical raw material.

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Universitas Pattimura, Ambon, IndonesiaEmail: dengel.engel@lecturer.unpatti.ac.id**1. Introduction**

Nutmeg (*Myristica fragrans* Houtt.) is one of Indonesia's leading spice commodities, with high economic value and an important role in the global spice trade. The Banda Islands in Central Maluku Regency, Maluku Province, are recognized as the origin of nutmeg and have long been a major production area of high-quality nutmeg. In addition to its culinary uses, nutmeg provides several economically valuable products, including seeds, mace, and essential oil. Nutmeg essential oil contains bioactive monoterpenes, including sabinene, α -pinene, and β -pinene, and phenylpropanoids including myristicin, elemicin, and safrole. These compounds have been reported to have antioxidant, antimicrobial, anti-inflammatory, and analgesic activities; therefore, nutmeg essential oil has been widely used in the food, cosmetic, pharmaceutical, and aromatherapy industries [1–4]. Among these compounds, myristicin is an important component reported to have antioxidant, antitumor, and monoamine oxidase enzyme inhibitory activities [5]. These bioactive compounds contribute to the potential use of nutmeg essential oil as a raw material for various products. Ideally, the chemical profile of essential oil from each nutmeg production area should be comprehensively characterized and documented to identify regional differences, assess product quality, and support its development as a high-value industrial raw material.

However, comprehensive chemical characterization across nutmeg production areas remains incomplete. The chemical composition of nutmeg essential oil is influenced by factors such as plant variety, environmental conditions, climate, altitude, soil type, and geographical origin [6]. Recent studies have demonstrated substantial variation among nutmeg-producing regions in Indonesia. Nutmeg seed essential oil from Aceh has been reported to be dominated by terpinen-4-ol and myristicin, with proportions that differ from nutmeg from Maluku [7]. Similarly, environmental and climatic conditions have been reported to significantly affect its chemical composition [8]. Central Maluku Regency itself has several nutmeg-producing areas spread across islands with differing environmental characteristics, including Banda, Titawaai, and Seith. These differences in geographical and ecological conditions may affect secondary metabolite biosynthesis, resulting in variation in the chemical composition of nutmeg essential oil among regions [9]. However, studies specifically comparing the chemical profiles of nutmeg seed essential oil from Banda, Titawaai, and Seith remain limited. Consequently, variations in major chemical components associated with geographical and ecological differences within Central Maluku Regency have not been well mapped.

This study systematically isolated nutmeg seed essential oils from Banda, Titawaai, and Seith by steam distillation, a method that recovers volatile compounds while reducing high-temperature degradation [10]. Subsequently, the chemical components were characterized using Gas Chromatography–Mass Spectrometry (GC-MS), which enables the separation and identification of volatile components [11].

The chemical characteristics of nutmeg essential oil are important because several components possess biological activities but may also raise safety concerns. Myristicin, for example, has been reported to exhibit antioxidant, antitumor, and monoamine oxidase inhibitory activities [5]; however, excessive exposure may cause toxic effects. Information on the composition and relative abundance

of major components is important for characterizing nutmeg essential oil and evaluating its potential safety concerns. Beyond its pharmacological aspects, such information can help assess raw material quality, guide the development of essential-oil-based products, and support more targeted use of local nutmeg resources.

Therefore, this study was conducted with a focus on identifying the variation in the relative abundances of the major components, particularly sabinene, α -pinene, β -pinene, myristicin, and terpinen-4-ol in nutmeg seed essential oils from different locations in Central Maluku Regency. This study also examined variations in monoterpene and phenylpropanoid profiles and explored components with potential as chemical markers of geographical origin. The results are expected to expand scientific data on the chemical characteristics of nutmeg essential oil from Central Maluku Regency and provide information relevant to raw material standardization and derivative product development.

2. Experimental Section

2.1 Materials and Equipment

The materials used in this study included fresh nutmeg (*Myristica fragrans* Houtt.) seeds collected from three locations in Central Maluku Regency: Banda Island, Titawaai Island, and Seith Island. The chemicals used included anhydrous sodium sulfate (Na_2SO_4), distilled water, and hydrogen gas as the carrier gas for GC-MS analysis.

The equipment included a 15-L laboratory-scale steam distillation apparatus consisting of a distillation kettle, condenser, and collection flask; a hot plate; a thermometer; an analytical balance (Ohaus Pioneer); a stainless-steel knife; stainless-steel trays; a separatory funnel; and general laboratory glassware. The chemical profiles of the essential oils were analyzed using gas chromatography–mass spectrometry (GC–MS).

2.2 Sample Preparation

Fresh Banda variety nutmeg (*Myristica fragrans* Houtt.) seeds were collected from three locations in Central Maluku Regency, namely Banda, Titawaai, and Seith. Approximately 1.15–1.50 kg of seeds were collected from each location. The seeds were separated from their shells, then washed with clean water to remove surface impurities. The nutmeg seeds were then sun-dried for 24 hours until reaching a moisture content of $\leq 10\%$. The dried nutmeg seeds were then chopped into approximately 1×1 cm pieces to increase surface area and improve essential oil extraction efficiency. The prepared samples were subsequently used for essential oil isolation using the steam distillation method.

2.3 Essential Oil Isolation by Steam Distillation

Nutmeg seed essential oil was isolated using the steam distillation method adapted from Kapelle *et al.* [12] with several modifications. A total of 1.15–1.50 kg of prepared nutmeg seed samples was placed into a 15-L distillation kettle containing water up to three-quarters of its capacity. The mixture was heated on a hot plate to generate a continuous flow of steam. The steam carrying the volatile essential oil components was passed through a water-cooled condenser, and the resulting distillate was collected in a receiving vessel.

Distillation was continued until no additional oil layer formed on the surface of the resulting distillate. The collected essential oil was separated from the aqueous phase using a separating funnel. The essential oil was then dried by adding anhydrous sodium sulfate (Na_2SO_4) to remove residual moisture. Anhydrous Na_2SO_4 was used as the drying agent because it absorbs residual water without reacting with essential oil components [13].

After drying over anhydrous Na_2SO_4 for 30 minutes, the essential oil was filtered to remove the drying agent and weighed to calculate yield. The entire isolation procedure, from sample preparation to yield determination, was performed in triplicate for each sampling location (Banda, Titawaai, and

Seith) to ensure consistency and reproducibility of yield measurements. Essential oil yield was reported as the mean $\pm$ standard deviation (mean $\pm$ SD, $n = 3$). The essential oil was subsequently stored in a tightly sealed dark glass bottle at 4 °C until chemical component analysis was performed using GC-MS.

2.4 Chemical Component Analysis by GC-MS

The chemical profile of the essential oil was analyzed by a Gas Chromatography–Mass Spectrometry (GC-MS) instrument (Shimadzu QP-5050A Series II, Class-5000 Ver. 2.2) fitted with a DB-10 capillary column (30 m length; 0.25 mm internal diameter). Hydrogen gas was used as the carrier gas at a flow rate of 1.6 mL/min. The instrument operated at an initial column temperature of 60 °C, an injector temperature of 280 °C, a detector temperature of 300 °C, and an interface temperature of 320 °C. The column pressure was maintained at 100 kPa.

GC-MS analysis was performed as a single analytical run for each essential oil sample from each location, without analytical replication. The analysis aimed to characterize and compare chemical profiles among locations rather than assess analytical variability within the same sample. Accordingly, the chemical composition data were interpreted descriptively and represent a single GC–MS analysis for each location.

Compound identification was performed by comparing the mass spectrum of each chromatographic peak with reference spectra in the WILEY 229, NIST62, and PESTICID libraries. A compound was considered identified when the similarity index was $\geq 90\%$ compared with the reference spectra in the selected libraries. The relative abundance of each compound was determined from its chromatographic peak area as a percentage of the total peak area of all detected peaks, without applying a response correction factor.

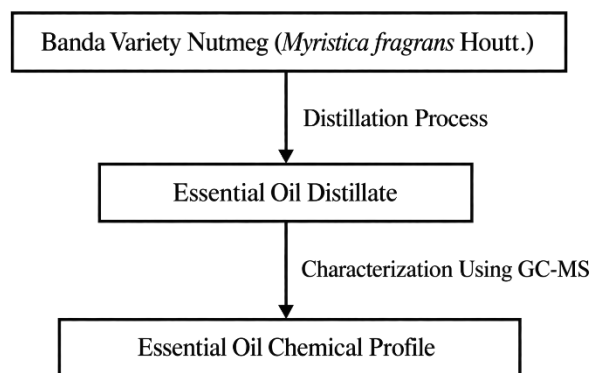


Figure 1. Flow Diagram of the Research Method

2.5 Statistical Analysis

Essential oil yield data from the three replicate isolation procedures at each location were analyzed using a one-way ANOVA to determine whether yield differed significantly among the sampling locations (Banda, Titawaai, and Seith). When the ANOVA showed a significant overall difference ($p < 0.05$), Tukey's HSD post hoc test was performed for pairwise comparisons among locations. Statistical significance was set at a 95% confidence level ($\alpha = 0.05$).

2.6 Source of Environmental Parameter Data

This study did not directly measure environmental parameters in situ at the sampling locations during the sampling period. Data on air temperature, rainfall, humidity, and sunshine duration for Banda, Titawaai, and Seith were obtained from secondary records provided by the Meteorology, Climatology,

and Geophysics Agency (BMKG) of Maluku Province for October–November 2025 [14]. Soil characteristics and agroecological conditions at each location were obtained from published literature on nutmeg cultivation in the Maluku Islands [2], [15].

Data for Banda were obtained from the Bandaneira Meteorological Station (WMO ID 97790), data for Titawaai from the Amahai Meteorological Station (WMO ID 97722), and data for Seith from the Pattimura Meteorological Station in Ambon (WMO ID 97724). These stations were used as the nearest official BMKG stations because no official BMKG observation stations are available on Titawaai or Seith Island. These data represent short-term meteorological observations covering the two-month study period rather than long-term climatological data (≥ 10 years), which are typically used in comprehensive agroecological studies. Further research involving direct measurement of environmental parameters at the sampling locations during the sampling period is recommended to strengthen the validity of the association between environmental factors and the chemical characteristics of nutmeg essential oil.

3. Results and Discussion

The Banda nutmeg plant (*Myristica fragrans* Houtt.) used in this study originated from Central Maluku Regency, Maluku Province, Indonesia, and was collected from three sampling locations: Banda Island, Titawaai Island, and Seith Island (Figure 2). Table 1 presents the results of nutmeg seed essential oil isolation (*Myristica fragrans* Houtt.) from Banda, Titawaai, and Seith. The essential oil was pale to bright yellow with a characteristic nutmeg aroma. Visually, the essential oil from Seith appeared clearer than the samples from Banda and Titawaai. This difference in physical characteristics likely relates to variation in essential oil chemical composition identified through GC-MS analysis.



Figure 2. Banda Variety Nutmeg (*Myristica fragrans* Houtt.) Seed Samples from a) Banda; b) Titawaai; c) Seith

Essential oil yields varied by location: $0.70 \pm 0.01\%$ for the Titawaai sample, $0.30 \pm 0.01\%$ for the Banda sample, and $0.29 \pm 0.01\%$ for the Seith sample (Table 1). These results indicate that the geographical origin of the samples influences the amount of essential oil produced. These yield differences are influenced by environmental factors, soil conditions, climate, fruit maturity at harvest, and plant genetic factors [2]. To evaluate differences in yield among locations, data from three replicates per location were analyzed by one-way ANOVA at $\alpha = 0.05$. The results showed a significant overall difference in essential oil yield among the three sampling locations ($p < 0.001$; $p < 0.05$). Tukey's HSD post hoc test showed that essential oil yield from Titawaai differed significantly from both Banda and Seith ($p < 0.001$; $p < 0.05$), whereas Banda and Seith showed no significant difference in yield ($p = 0.232$). These results confirm that the observed yield differences reflect real variation among locations rather than measurement error.

Drying nutmeg seeds before distillation is an important step for improving essential oil isolation efficiency. Reducing moisture content to an optimal level ($\leq 10\%$) can improve steam penetration into plant tissue and facilitate essential oil release from the oil glands during distillation [16]. Excessively

high moisture content can reduce distillation efficiency because more thermal energy is required to evaporate water within the plant tissue [17-18].

Table 1. Essential Oil Yield of Banda Variety Nutmeg (*Myristica fragrans* Houtt.) Seeds from Three Locations in Central Maluku Regency, Maluku Province, Indonesia

No.	Sampling Location	Sample Weight (kg)	Essential Oil Weight (g) (mean $\pm$ SD, n=3)	Essential Oil Yield (%) (mean $\pm$ SD, n=3)
1	Banda Island	1.15	3.37 $\pm$ 0.06	0.30 $\pm$ 0.01
2	Titawaai Island	1.20	8.40 $\pm$ 0.12	0.70 $\pm$ 0.01
3	Seith Island	1.50	4.29 $\pm$ 0.11	0.29 $\pm$ 0.01

In addition to drying, differences in essential oil yield among locations may also be influenced by the agroecological conditions of each region. Variations in soil type, rainfall, light exposure, and altitude affect secondary metabolite biosynthesis in essential-oil-producing plants [6], [14]. Banda, the historical center of nutmeg production, has mineral-rich volcanic soil and environmental conditions that support nutmeg growth [15]. However, this study's highest yield came from Titawaai, indicating that essential oil yield may reflect the combined effects of soil conditions and other environmental and physiological factors. Fruit maturity at harvest may also affect essential oil yield, as nutmeg harvested at an optimal maturity stage generally contains more essential oil [19].

In addition to yield, the chemical profiles of the essential oils were characterized to compare the volatile components among locations. GC-MS analysis of nutmeg seed essential oil (*Myristica fragrans* Houtt.) from Banda, Titawaai, and Seith identified distinct chemical components in each sample. The GC chromatograms showed relatively similar patterns, with differences in the number of peaks, peak intensity, and relative concentration of the detected compounds. The Banda sample yielded 25 chromatographic peaks, whereas the Titawaai and Seith samples yielded 20 and 21 peaks, respectively (Figure 3). Table 2 presents the major chemical components identified in each sample.

Based on the analysis results, sabinene was the predominant component in all nutmeg seed essential oil samples. The Seith sample had the highest sabinene relative abundance (33.69%), followed by Titawaai (29.34%) and Banda (24.69%). This bicyclic monoterpene contributes to nutmeg's characteristic fresh aroma. The predominance of sabinene in all three samples is consistent with the characteristics of Banda variety nutmeg essential oil reported in previous studies [2]. In addition to sabinene, α -pinene and β -pinene were also identified as major components with relatively high relative abundances. The Seith sample had the highest relative abundances of α -pinene and β -pinene (24.37% and 16.35%, respectively), indicating the predominance of the monoterpene group in the essential oil from that location.

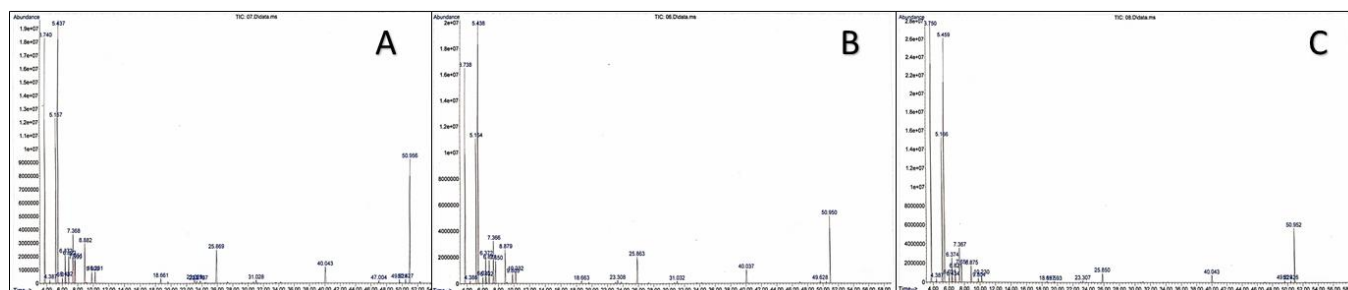


Figure 3. GC Chromatogram of Banda Variety Nutmeg (*Myristica fragrans* Houtt.) Seed Essential Oil from a) Banda; b) Titawaai; c) Seith

Table 2. Major Components Identified in Banda Variety Nutmeg (*Myristica fragrans* Houtt.) Seed Essential Oils from Three Locations in Central Maluku Regency

No	Chemical Component	Banda		Titawaai		Seith	
		RT (min)	Relative Abundance (%)	RT (min)	Relative Abundance (%)	RT (min)	Relative Abundance (%)
1	α -Pinene	3.740	17.03	3.740	17.52	3.751	24.37
2	β -Pinene	5.156	14.08	5.156	14.48	5.168	16.35
3	Sabinene	5.436	24.69	5.436	29.34	5.459	33.69
4	β -Myrcene	6.374	2.47	6.374	2.73	6.374	2.56
5	Terpinolene	6.823	2.46	6.823	2.47	6.823	1.45
6	D-Limonene	7.371	4.73	7.365	4.88	7.365	4.01
7	Eucalyptol	7.598	2.19	-	-	-	-
8	β -Phellandrene	7.651	2.19	7.651	3.63	7.651	2.19
9	γ -Terpinene	8.881	4.21	8.880	4.25	8.875	2.13
10	o-Cymene	9.807	1.27	9.807	1.27	-	-
11	Limonene	10.233	1.37	10.233	1.61	-	-
12	Terpinen-4-ol	25.871	5.61	25.865	5.24	25.848	1.89
13	Safrole	40.040	3.01	40.035	2.99	40.040	1.76
14	Myristicine	50.958	10.29	50.952	6.43	50.952	5.41

The chemical profiles of nutmeg seed essential oils from Banda, Titawaai, and Seith contained the same major components, particularly sabinene, α -pinene, β -pinene, and myristicin, but at different relative abundances. The Seith sample had the highest relative abundances of the major monoterpenes, including sabinene (33.69%), α -pinene (24.37%), and β -pinene (16.35%). In contrast, the Banda sample had higher relative abundances of myristicin (10.29%) and terpinen-4-ol (5.61%) than the Titawaai and Seith samples and was the only sample in which eucalyptol (2.19%) was detected. Myristicin, an important phenylpropanoid component of nutmeg seed essential oil, was found at 6.43% in Titawaai and 5.41% in Seith. These differences indicate that nutmeg essential oil chemical profiles vary across geographical locations in Central Maluku Regency [20].

Terpinen-4-ol was identified in all samples, with relative abundances of 5.61% in Banda, 5.24% in Titawaai, and 1.89% in Seith. This compound has antimicrobial activity and contributes to the pharmacological properties of nutmeg essential oil [7],[21]. Safrole was also identified in all samples, with relative abundances of 3.01% in Banda, 2.99% in Titawaai, and 1.76% in Seith. Although safrole has certain biological activities, its presence warrants attention because international regulations restrict its use in some products due to potential toxic effects at high exposure levels [22-23]. However, the relative abundance of safrole observed in this study was within the range previously reported for nutmeg essential oil and provides important information for its quality evaluation and potential industrial applications [24].

Essential oil yield also varied among sampling locations. Titawaai produced the highest yield ($0.70 \pm 0.01\%$), followed by Banda ($0.30 \pm 0.01\%$) and Seith ($0.29 \pm 0.01\%$). One-way ANOVA showed a significant overall difference in yield among locations ($p < 0.05$), and Tukey's HSD post hoc test showed that Titawaai differed significantly from both Banda and Seith, whereas Banda and Seith did not differ significantly from each other ($p = 0.232$). Notably, the location with the highest essential oil yield did not have the highest relative abundances of the major monoterpenes. Titawaai produced the highest yield, whereas Seith had the highest relative abundances of sabinene, α -pinene, and β -pinene despite its lower yield. These findings show that a higher essential oil yield does not necessarily correspond to higher relative abundances of individual components and may reflect the combined

effects of postharvest conditions, growing environment, and possible genetic variation among plants [6],[9].

A similar relationship between essential oil yield and chemical composition has been reported in other aromatic plants, such as *Rosmarinus officinalis*, in which environmental factors including temperature, rainfall, light intensity, and humidity can affect both parameters. However, changes in yield and composition do not always follow the same pattern because essential oil composition can also vary with season and the phenological stage of the plant at harvest [25]. Other studies also reported that environmental factors such as light, temperature, drought, seasonal variation, and water availability can differentially affect the mevalonate, methylerythritol, and shikimate biosynthetic pathways. Consequently, an increase in total essential oil yield is not always accompanied by a proportional increase in the relative abundance of a particular group of compounds such as monoterpenes [26]. In contrast, several studies on other aromatic plants have reported a positive correlation between essential oil yield and monoterpene content, particularly when compositional variation is linked to the plant's developmental stage or harvest season. This pattern was not observed in the present study, as the highest essential oil yield and the highest relative abundances of major monoterpenes occurred in different sampling locations. This difference indicates that yield and chemical composition are distinct quality parameters and should be evaluated separately when characterizing and standardizing nutmeg essential oil.

Differences in essential oil yield may also be influenced by the condition of the plant material before distillation, particularly its moisture content after drying. Drying to an optimal moisture content ($\leq 10\%$) can improve essential oil release efficiency during distillation, whereas excessively high or low moisture content can reduce oil yield [16–18]. Sun drying, which was applied to samples from all three locations, may also cause variation in temperature and final moisture content among samples, potentially affecting the composition of volatile components in the essential oil [15]. The higher yield in the Titawaai sample indicates that the raw material condition during distillation may have been more optimal than in the Banda and Seith samples.

In addition to postharvest conditions, geographical and agroecological factors may also influence differences in chemical composition. Soil conditions, rainfall, temperature, light intensity, water availability, and other environmental factors can influence the biosynthesis and accumulation of secondary metabolites in aromatic plants [2],[27],[29–31]. Banda Island, with its characteristic volcanic soil, is known to be rich in nutrients that support spice plant growth [19]. The availability of certain nutrients can affect secondary metabolic pathways involved in phenylpropanoid biosynthesis, including myristicin [28–29]. These conditions may partly contribute to the higher relative abundance of myristicin observed in the Banda sample. In contrast, light exposure, temperature variation, and water availability can influence terpenoid biosynthesis through metabolic pathways involved in the formation of monoterpene precursors [32–37], which may contribute to the predominance of hydrocarbon monoterpenes, particularly sabinene, α -pinene, and β -pinene, in the Seith and Titawaai samples. Environmental conditions favorable for vegetative growth may also affect the accumulation of more complex secondary metabolites, including the phenylpropanoid group [38–41].

In addition to environmental factors, genetic differences among nutmeg plant populations may also influence variation in essential oil chemical composition across locations. The presence of eucalyptol only in the Banda sample, along with the absence of several minor components at certain locations, indicates differences in chemical profiles among plant populations. Previous studies have shown that nutmeg plants have considerable genetic diversity and can produce variation in essential oil component content among populations and cultivation regions [44–46]. Therefore, the differences in essential oil chemical profiles observed in this study may result from an interaction between environmental factors and the genetic characteristics of the plants at each location.

Further research should directly examine the relationship between environmental conditions and essential oil composition by measuring soil nutrient content, light intensity, water availability, and

microclimate temperature at each sampling location. These measurements could be combined with analyses of the expression of key genes involved in terpenoid and phenylpropanoid biosynthesis, such as terpene synthase and phenylalanine ammonia-lyase genes, using molecular approaches such as qPCR or transcriptomic analysis [42-43]. Such studies could provide a more comprehensive understanding of the environmental and genetic mechanisms underlying variation in the chemical profiles of nutmeg essential oils. Overall, these findings show that essential oil yield and chemical composition vary differently across locations. The high yield observed in Titawaai and the distinct chemical profiles of oils from Banda, Titawaai, and Seith provide a basis for developing Central Maluku nutmeg essential oils according to specific quality requirements and potential applications.

4. Conclusion

Nutmeg seed essential oil (*Myristica fragrans* Houtt.) from Banda, Titawaai, and Seith was isolated by steam distillation and characterized by GC-MS. Essential oil yield ranged from 0.29% to 0.70%, highest in Titawaai and lowest in Seith, and differed significantly among locations (one-way ANOVA, $p < 0.05$). Sabinene was the predominant component at all three locations (24.69–33.69%), followed by α -pinene (17.03–24.37%) and β -pinene (14.08–16.35%), all of which had their highest relative abundances in the Seith sample, whereas the Banda sample had the highest relative abundance of myristicin (10.29%) and was the only sample in which eucalyptol was detected (2.19%).

These findings show that essential oil yield and chemical profiles vary across Central Maluku Regency, with different patterns between yield and the relative abundances of major components. The distinctive characteristics of each location, including the highest yield in Titawaai, the highest relative abundances of major monoterpenes in Seith, and the highest relative abundance of myristicin and the presence of eucalyptol in Banda, provide useful information for the quality evaluation, standardization, and development of Central Maluku nutmeg essential oils for potential pharmaceutical, food, and cosmetic applications.

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