

Research Article

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Chemical composition of essential oils reviewed from the height of Cajuput (*Melaleuca leucadendron*) plantations in Buru Island and Seram Island, Maluku, Indonesia

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Abstract: Maluku, Indonesia, has essential oil (EO) producing plants, namely eucalyptus (*Melaleuca leucadendron*), which are spread throughout the island. This study aims to determine the effect of geographical location on EO's yield and chemical composition. The eucalyptus oil isolation method uses steam distillation and chemical composition analysis using gas chromatography–mass spectrometry. The results showed that the yield of EO from wilted leaf samples on Buru Island for point B1 = 1.35%; B2 = 1.19; B3 = 1.52; B4 = 1.88%; B5 = 2.13% and from Seram Island for point S1 = 0.71%; S2 = 1.02%; S3 = 0.78; S4 = 0.8%; and S5 = 1.01%. The yield of EO in the lowlands is higher on Buru Island in the lowlands (23.04 masl) and on Seram Island (46 masl). The levels of 1,8-cineole in EO for both island plains ranged from 26.89 to 47.32% and α -pinene levels between 3.84 and 43.45%. Minor compounds found on both islands are α -limonene, β -pinene, and cymene. There are minor compound components that are only found on each island. The geographical conditions of eucalyptus plants affect the quality and quantity of EOs. At higher locations, temperatures tend to be lower, which affects plant metabolism and produces less oil. The eucalyptus plantation area on Seram Island has high humidity, so the yield of EO is lower.

Keywords: chemical composition, *eucalyptus*, *Melaleuca leucadendron*, essential oil

1 Introduction

Indonesia is one of the countries producing essential oil (EO), but the quality of the EO produced has not met international market standards [1]. EO plants in Indonesia contain active compounds for the perfume and pharmaceutical industries [2]. EO has been used as an alternative antimicrobial product because of its strong and broad-spectrum activity against microorganisms, besides being environmentally friendly and safe for humans [3–5]. Eucalyptus is a plant that plays a vital role in the forestry industry and has ecological, social, and economic impacts [6,7]. Sustainable eucalyptus cultivation can prevent deforestation and maintain forest sustainability, but clearing new land does not support biodiversity [8]. Exploration of eucalyptus EO opens up opportunities for further research on chemical content, mechanisms of action, potential, and utilization. Analysis of the uncertainty of eucalyptus leaf biomass availability and seasonal changes affect oil content and EO price fluctuations [9]. Eucalyptus EO has various biological activities, namely antibacterial properties that are effective against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Listeria monocytogenes*, *Bacillus cereus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Escherichia coli* [10–12]. Eucalyptus EO has antiviral properties to help treat mild viral infections [13] and contains antioxidant compounds that protect body cells from damage caused by free radicals [14]. The chemical compound components contained in EO provide its biological and pharmacological activities [15]. EO comes from *Melaleuca cajuputi*, formerly known as *Melaleuca leucadendron*. This species comes from the Maluku Islands, especially Ambon Island, Buru Island, and Seram Island [16]. The

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potential for eucalyptus commodities on Buru Island is the largest in Indonesia [17]. Buru Island and Seram Island are areas where many eucalyptus plants grow and are community plantation areas. The soil and climate conditions make it a fertile land for the growth of eucalyptus plants. The growing locations of these two areas are different, showing the differences in EO's quality and quantity. The EO-producing plant's type and geographical location affect its chemical composition [18]. Other influencing factors are the harvest season, environment, soil quality, age of the plant, and oil extraction method [19–21]. Therefore, an exploration of eucalyptus plants from the Buru Island Mainland and the Seram Island Mainland, Maluku, Indonesia, was carried out, and the quality of EO was identified.

locations and eucalyptus EO production centers on Buru Island and Seram Island, Maluku. Five sample locations are in plantations in Wamlana village, Tatanggo village, Jamilu village, Sawa village, and Jikumerasa village. Sampling points for Seram Island are in Loun village, Kotania village, Wael village, Taman Jaya village, and Pelita Jaya village (Figures 1 and 2). Each sampling point took 5 kg of wet samples, and the sampling location was recorded using the global positioning system to determine the point and height. Eucalyptus leaf samples were aired for 5 days to remove their water content so that dry eucalyptus leaves (wilted) were obtained. Wilting, which is the process when leaves lose moisture and become limp, can increase EO extraction by breaking down cell walls and facilitating oil release [22,23].

2 Materials and methods

2.1 Sample preparation

Eucalyptus leaf samples (*M. leucadendron*) were obtained from 10 locations, which are eucalyptus leaf plantation

2.2 Isolation of EOs from eucalyptus leaves

2.5 kg of dry leaf samples (withered) were placed on the distillation kettle filter, and steam distillation was carried out for ± 6 h. The results of the eucalyptus leaf distillation were collected into a separating funnel, and a distillate

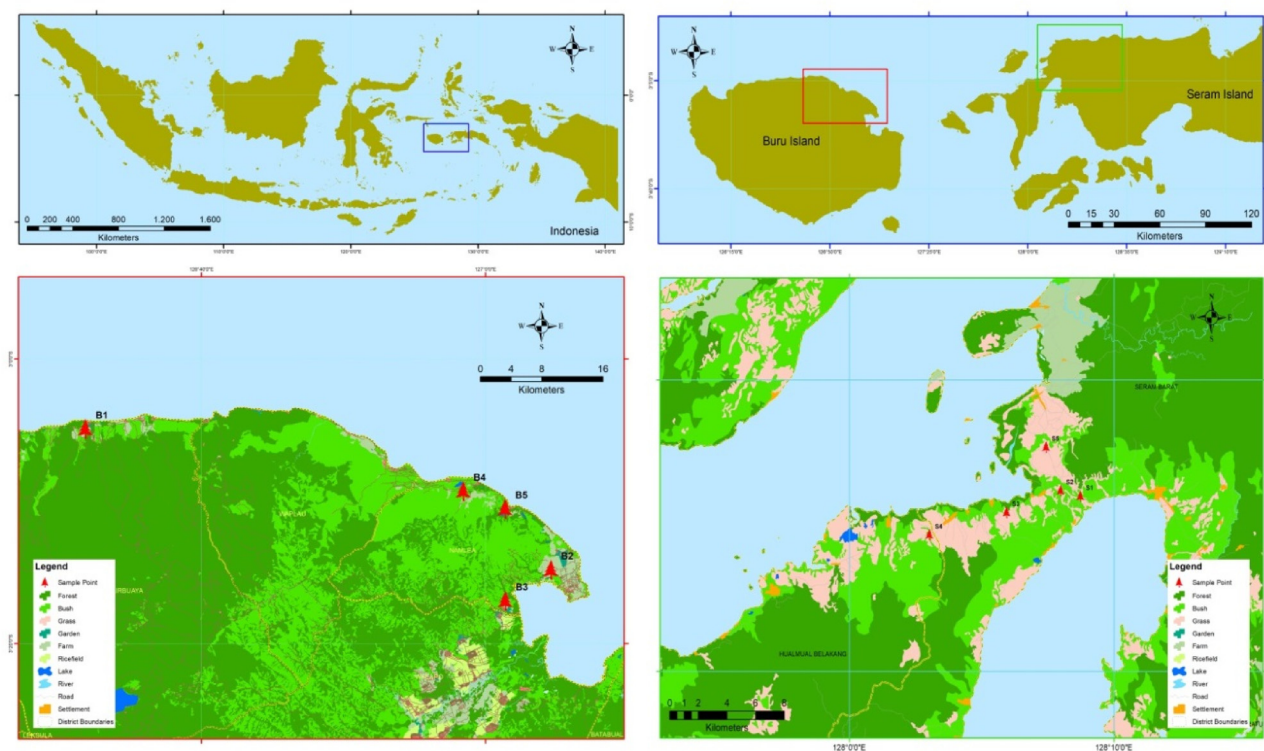


Figure 1: Map of sampling locations (maps created using ArcGIS 10.8 [<https://www.esri.com/en-us/arcgis/products/arcgis-pro/>] with geospatial from the BIG).



Figure 2: The samples of eucalyptus trees (*M. leucadendron*) are from Buru Island and Seram Island.

consisting of two layers was obtained, namely the upper layer in the form of eucalyptus oil and the lower layer in the form of water. The oil and water were separated, then anhydrous Na_2SO_4 was added to bind the remaining water. The oil was filtered, and water-free eucalyptus EO was obtained. Furthermore, the EO was weighed, and its yield was calculated [24].

2.3 Analysis of the chemical composition of EO

Determination of EO content using gas chromatography–mass spectrometry (GC–MS Simadzu QP-5050 A series II, Class-5000 Ver 2.2) equipped with a DBMS detector with a DB10 capillary column 30 m long and 0.25 mm in diameter,

using hydrogen carrier gas (1.6 ml/min). Column temperature 60°C, injector temperature 280°C, detector temperature 300°C, interface temperature 320°C, column pressure 100 kPa. Equipment conditions are set to identify EO groups. The program time lasts for 39 min. The percentage of EO obtained is the percentage of oil injected (relative percentage). The components separated through GC analysis will exit the column, enter the MS, and then be identified based on their molecular weight. Identification of the chemical composition of EO using GC–MS produces two types of data, chromatograms from gas chromatography (GC) analysis and mass spectra from mass spectroscopy (MS) analysis. Molecular profiles in EO were obtained by comparing the chromatograms that appeared through the GC–MS digital detector with the molecular chromatograms found in the WILLEY 229, NIST62 LIB, and PEST-ICID.LIB library sources. The percentage of EO molecular content obtained was relative [24].

Table 1: General conditions of sampling locations and EO yield percentage

No	Station code	Location	Position	Altitude (masl)	Yield (%)
1	B1	Wamlana village	3°4'54"LS, 126°31'50"BT	13	1.35
2	B2	Tatanggo village	3°14'48"LS, 127°4'36"BT	71	1.19
3	B3	Jamilu village	3°16'59"LS, 127°1'24"BT	16	1.52
4	B4	Sawa village	3°9'16"LS, 126°58'26"BT	16	1.88
5	B5	Jikumerasa village	3°10'31"LS, 127°1'23"BT	23	2.13
6	S1	Loun village	3°03'59.36"LS, 128°08'44.18"BT	80	0.71
7	S2	Kotania village	3°03'47.65"LS, 128°07'59.52"BT	46	1.02
8	S3	Wael village	3°04'32.27"LS, 128°05'57.15"BT	8	0.78
9	S4	Taman Jaya village	3°05'18.64"LS, 128°03'02.03"BT	22	0.8
10	S5	Pelita Jaya village	3°02'18.07"LS, 128°07'26.70"BT	32	1.01

3 Results and discussion

The locations where eucalyptus leaves were sampled on Buru Island and Seram Island showed differences in geographical location, as presented in Table 1. The eucalyptus leaf samples used have the same shape, a *Melaleuca leucadendra* characteristic. The eucalyptus leaf sample is green with a hairy leaf surface; if the leaves are crushed or squeezed, the eucalyptus oil will be smelled [16]. The process of isolating eucalyptus leaf oil using the steam distillation method produces two layers: oil (top layer) and water (bottom layer). The histogram of eucalyptus oil yield data and the height of the sampling location are shown in Figure 3. The eucalyptus oil yield from Buru Island (B1–B5) has a value of 1.19–2.13%, and samples from Seram Island (S1–S5) have a 0.71–1.02% value. The oil yield obtained from the two islands in Maluku has a higher yield when compared to the study conducted by Alfian [25] for *Eucalyptus grandis* leaf EO with a yield of 0.4%. The type of eucalyptus used can affect EO yield: *Eucalyptus camaldulensis* leaves with a yield of 0.61%, *Eucalyptus citriodora* leaves with 1.16% [26]. EO yields range from 1.2–3.0% for various *Eucalyptus* species [27].

Sample B5 produced the highest EO yield, while sample S1 produced the lowest. The histogram of samples from mainland Buru Island shows a pattern of the higher the EO yield, the lower the location of the eucalyptus plant growth. The EO yield was higher at altitudes below 23.04 masl (1.35–2.13%) compared to samples at an altitude of 71.05 masl (1.19%). The same thing happened in samples from mainland Seram Island; at altitudes below 46 masl, the EO yield was higher (0.8–1.0%) than in samples above 80 masl (0.7%). EO results can vary based on the freshness of the leaves, while dry leaves have higher results. The place where eucalyptus oil grows affects the quality of eucalyptus oil, the distinctive odor of eucalyptus oil, the color of the oil, and the specific refractive index value [28].

The height of the plant's growth location affects the yield and quality of the EO produced. Differences in the height of the growing location can cause variations in microclimate, such as air temperature, soil temperature, air humidity, and soil water content. At higher elevations, temperatures tend to be lower, which affects plant metabolism and the secondary metabolites produced. Plants produce less oil in response to cooler conditions [29]. Higher elevations have higher light intensity, which increases photosynthesis. Water availability may be lower at higher elevations due to variable rainfall and faster drainage. Water deficits can cause plants to produce less EO [30] and affect EO components [31].

Climate has a significant effect on eucalyptus oil production. Climate change can alter the composition of secondary metabolites [32]. Temperatures that are too low or too high can inhibit plant growth and reduce EO production [33]. Soils at higher elevations have different nutrient contents than soils at lower elevations. P levels affect plant growth, while lower N levels reduce chlorophyll content, but EO yield is not affected by N and P levels. Both N and P levels affect the main components of EOs and minerals. These factors collectively can affect the amount and quality of EO plants produced [34].

The results of GC analysis of 10 samples are shown in Figure 4. Variations in sampling points from ten different locations can affect the quality of compounds in eucalyptus plants. Several factors affect the environment: leaf age, soil structure, soil temperature, leaf storage, and climate. The peak of the chromatogram and mass spectrum shows that Buru Island has 11 components and Seram Island has 19 components. The chemical compound components identified for all sampling locations include α -pinene at a retention time of 3.440 min and 1,8-cineole at a retention time of 7.118 min. The mass spectrum of 1,8-cineole is $m/z = 55, 63, 71, 81, 93, 108, 125, 139, \text{ and } 154$ ($C_{10}H_{18}O$). The mass

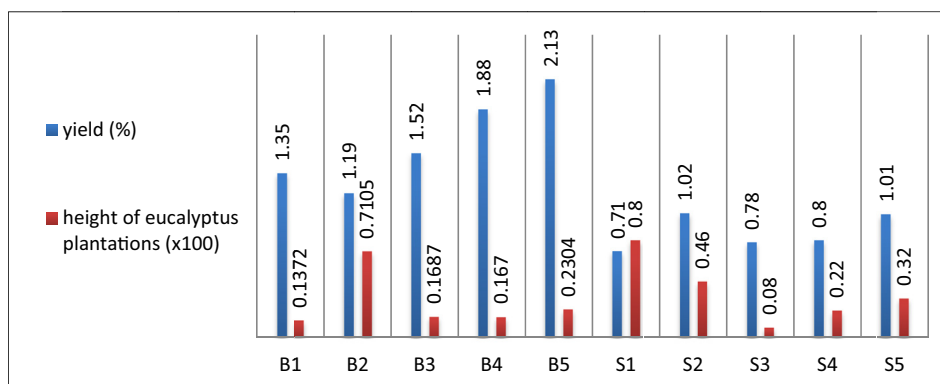


Figure 3: Histogram of eucalyptus oil yield and eucalyptus plantation heights.

spectrum provides a peak of the molecular ion M^+ 154, which is the molecular weight of $C_{10}H_{18}O$. The release of CH_3 produces the fragment $[C_9H_{15}O]^+$ with $m/z = 139$, the release of CH_2 produces the fragment $[C_8H_{13}O]^+$ with $m/z = 125$. Furthermore, the release of OH produces the fragment $[C_8H_{12}]^+$ with $m/z = 108$, and the release of C_3H_2 produces the fragment $[C_6H_9]^+$ with $m/z = 81$, which is the base peak.

The results of the GC–MS analysis in Table 2 show the main components of eucalyptus EO from the mainland of Buru Island and the mainland of Seram Island with differences in chemical composition even though they come from the same species. The levels of 1,8-cineole in EO ranged from 26.89 to 47.32%, the highest in sample S3 and the lowest in sample B5. The levels of α -pinene ranged

from 3.84 to 43.45%, the highest in sample B5 while the lowest in sample S4. In addition to 1,8-cineole and α -pinene, eucalyptus oil samples contain *D*-limonene (1.14–6.78%), β -pinene (1.10–5.25%), and cymene (1.184.94%). The relationship between the chemical components of EO and the secondary metabolic processes that occur in plants is influenced by the ecosystem and natural conditions, such as climate, weather, and soil conditions [34].

Although the dominant components that makeup EO are the same, other components will affect the quality of EOs. All EOs contained α -pinene, 1,8-cineol, and pinocarveol-trans for all eucalyptus species [27]. The results of eucalyptus EO analysis showed 12 components, namely α -pinene (45.21%), camphene (1.38%), β -pinene (1.11%), camphogen

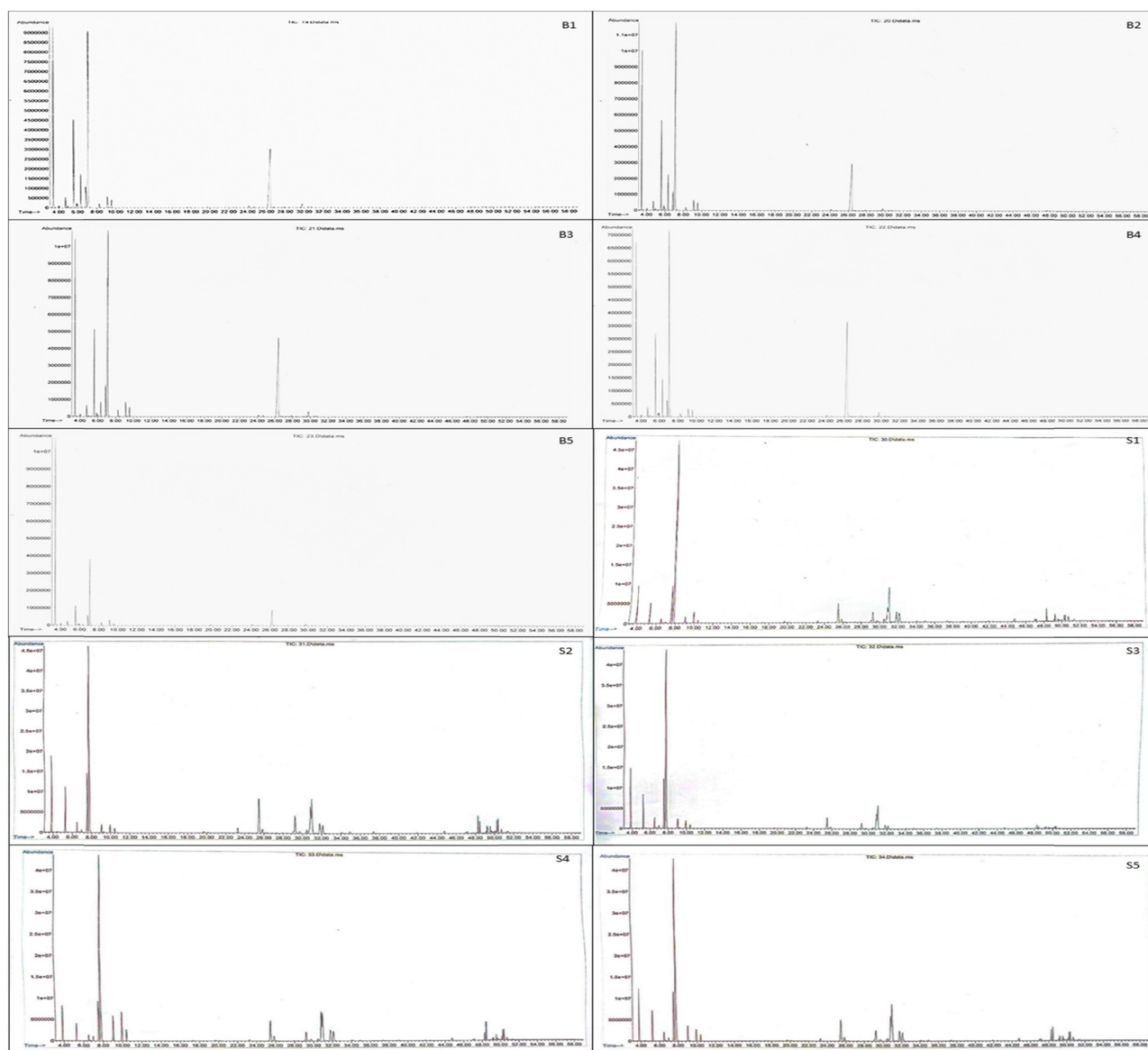


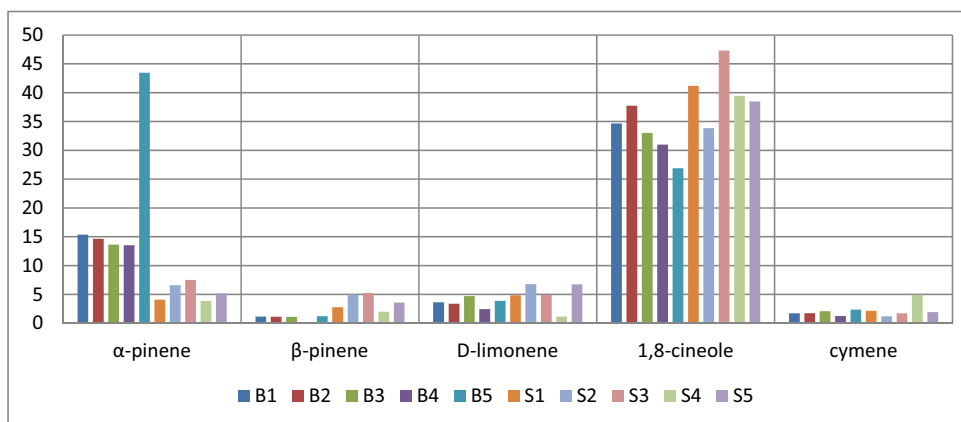
Figure 4: Chromatogram of eucalyptus oil from Buru Island and Seram Island.

Table 2: Main components of eucalyptus EO

No	Chemical compound	Concentration (%)									
		B1	B2	B3	B4	B5	S1	S2	S3	S4	S5
1	α -Pinene	15.39	14.65	13.62	13.51	43.45	4.09	6.60	7.50	3.84	5.16
2	β -Pinene	1.13	1.11	1.10	—	1.21	2.75	4.97	5.25	1.99	3.58
3	3-Carene	10.74	11.78	9.71	9.54	6.52	—	—	—	—	—
4	α -Terpinolene	4.53	5.16	1.84	4.81	—	—	—	—	7.71	—
5	α -Limonene	3.62	3.37	4.70	2.44	3.86	4.84	6.78	4.92	1.14	6.74
6	1,8-Cineole	34.67	37.73	33.04	31.00	26.89	41.20	33.84	47.32	39.44	38.46
7	Cymene	1.72	1.75	2.07	1.25	2.37	2.13	1.18	1.71	4.94	1.91
8	α -Terpinolene	1.27	1.39	1.31	1.09	—	—	—	—	1.97	1.08
9	Hexylene glycol	23.87	20.22	28.89	32.72	10.76	—	—	—	—	—
10	α -Terpinyl propionate	—	1.12	—	1.01	1.02	—	—	—	—	—
11	γ -Terpinene	—	1.13	—	—	—	1.16	1.16	2.00	4.05	2.34
12	Dipentene	—	—	—	—	—	7.87	9.90	11.57	7.37	9.12
13	Caryophyllene	—	—	—	—	—	5.89	8.00	3.55	5.23	5.35
14	α -Caryophyllene	—	—	—	—	—	3.22	4.20	1.74	2.37	2.81
15	β -Fenchyl alcohol	—	—	—	—	—	10.54	7.57	7.25	6.62	9.00
16	β -Selinene	—	—	—	—	—	3.32	2.50	1.46	2.84	2.67
17	α -Selinene	—	—	—	—	—	2.67	1.88	1.15	2.42	2.15
18	Ledene	—	—	—	—	—	1.63	1.66	—	—	—
19	β -Myrcene	—	—	—	—	—	—	1.21	1.87	—	1.19
20	Champaca camphor	—	—	—	—	—	—	1.03	—	1.76	1.25
21	α -Eudesmol	—	—	—	—	—	—	1.19	—	1.03	—
22	β -Eudesmol	—	—	—	—	—	—	1.37	—	1.20	—

(0.74%), 1,8-cineol (36.55%), α -campholine aldehyde (0.73%), pinocarvone (0.83%), α -terpineol (8.87%), β -caryophyllene (1.72%), spathulenol (0.84%), elemol (0.85%), and 1-nonadecenes (1.17%) [25]. 1,8-Cineol is the main compound in all eucalyptus species (49.07–83.59%) [27]. Cineole or 1,8-cineole is a natural cyclic ether and a monoterpene member. In this study, the eucalyptus EO sample with the highest cineole content was 43.45%, so it was classified as first-grade eucalyptus oil.

Eucalyptus EO samples from mainland Buru Island and mainland Seram Island have the same five main components: α -pinene, β -pinene, α -limonene, 1,8-cineole, and cymene (Figure 5), with varying compositions. Eucalyptus oil contains various active compounds that have important biological activities. The main component, 1,8-cineole in eucalyptus oil, has significant antimicrobial properties, especially against bacteria that cause respiratory tract infections, and functions as an expectorant that helps

**Figure 5:** Histogram of chemical compounds found in from Buru Island and Seram Island.

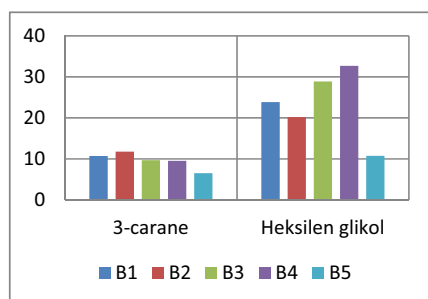


Figure 6: Histogram of chemical compounds found only in samples from Buru Island.

thin mucus and has anti-inflammatory and analgesic properties. 1,8-Cineole also has anti-inflammatory, antioxidant, and anticancer activities [35]. α -Pinene and β -pinene have antibacterial properties against Gram-positive and Gram-negative bacterial strains and are also antifungal against *Candida* species [36,37].

Compounds 3-carane and hexylene glycol are only contained in eucalyptus EO samples from Buru Island (Figure 6) with different compositions. Compounds 3-carane and hexylene glycol in EO result from complex interactions between genetic factors, environment, and processing methods. Genetic variation in eucalyptus plants causes differences in metabolic pathways, resulting in unique chemical profiles in each plant [38]. Environmental conditions such as climate, soil, and altitude also play an important role in modulating the production of secondary metabolite compounds, including 3-carane and hexylene glycol. Hexylene glycol is a relatively rare compound found in EO. Complex biochemical factors often affect its presence in plants and may not always be present in all types of eucalyptus. 3-Carane, as

one of the common monoterpenes, is more frequently found, but its amount can vary significantly.

The compounds γ -dipentene, dipentene, caryophyllene, α -caryophyllene, β -phenyl alcohol, β -selinene, and α -selinene are only contained in eucalyptus oil samples from the mainland of Seram Island (Figure 7) with varying compositions. Eucalyptus leaves harvested in the dry season tend to have higher cineole levels than those harvested in the rainy season due to differences in leaf water content and other environmental conditions. Leaf age and soil conditions have a significant effect on eucalyptus oil yields. Older leaves tend to have higher EO content than younger leaves due to the accumulation of chemical compounds in the leaves as the leaves age.

There are significant differences between eucalyptus plants grown in the low and highlands. Depending on environmental conditions, plants grown in the highlands have different content of certain compounds, such as higher or lower cineole levels. Plants in the lowlands tend to produce higher EO due to more optimal growing conditions. The quality of EO can vary depending on the chemical composition influenced by environmental factors in each location.

The mainland of Buru Island and the mainland of Seram Island have similar climates, namely a humid tropical climate. However, altitude, location, and topography differences can cause greater temperature, rainfall, and humidity variations. Seram Island has a wider mountainous area, so it can cause greater variations in temperature and rainfall compared to Buru Island, which is relatively flatter. The climate at the Seram Island sampling location is included in the category of a humid tropical climate. This area experiences high yearly rainfall with a

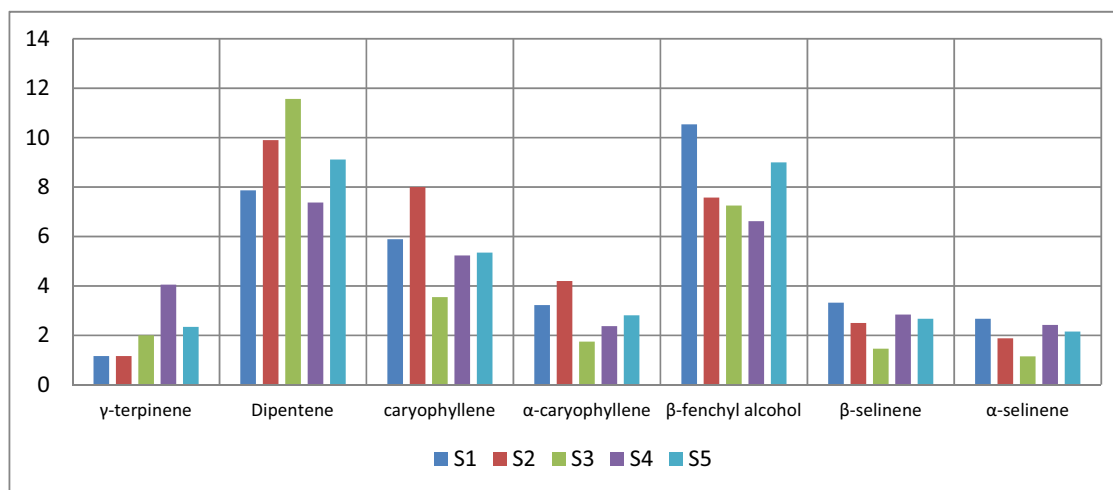


Figure 7: Histogram of chemical compounds that are only found in samples from Seram Island.

relatively short dry season. The sampling location on Buru Island has a tropical climate with two seasons, namely the dry and the rainy seasons, which are influenced by the movement of the monsoon winds. So, the high humidity on the Seram Island plains affects the yield and chemical components contained in the EO. The composition of eucalyptus EO varies according to the influencing factors but only has one chemotype, with the main component being 1,8-cineole [39].

4 Conclusion

The geographical conditions of eucalyptus (*M. leucadendron*) plants affect the quality and quantity of EO. Eucalyptus plantation areas on the mainland of Seram Island have high humidity, which results in lower EO quality. The yield of eucalyptus EO from Buru Island is higher than that of Seram Island. Eucalyptus plants planted in the lowlands have higher EO yields. Climate affects the chemical components of EO; for humid plantation areas on Seram Island, there are minor components of terpene compounds that are not found on Buru Island, but for Buru Island, there is hexylene glycol. At higher locations, temperatures tend to be lower, which affects plant metabolism and produces less EO.

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