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IDENTIFICATION OF ESSENTIAL OIL COMPONENTS FROM THE PEELS OF LIME (*Citrus aurantifolia*) AND KAFFIR LIME (*Citrus hystrix*) BASED ON GROWING ALTITUDE USING GC-MS

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ABSTRACT

Essential oils are oils whose components are generally volatile and are obtained from various types of plants and are the largest demand commodity in marketing circles. One of the essential oil-producing plants, namely lime (*C. aurantifolia*) and kaffir lime (*C. hystrix*) so that they are widely used by industry as raw materials for making medicines, cosmetics, and perfumes because they contain various components in them. However, the community only uses the fruit as a spice for cooking and drinks, while the skin is thrown away so that it becomes waste. This study aims to determine the effect of the height of the growing location on the essential oil components of the fruit peels of *C. aurantifolia* and *C. hystrix* as measured using GC-MS. The sampling method was carried out by simple random sampling, which was taken from several trees randomly at an altitude range of 0-100, 400-500, and 1,200-1,300 masl. The extraction method used is steam-water distillation at 100°C for 6 hours. The results of GC-MS identification showed that the essential oil from the skin of *C. aurantifolia* fruit contains 3 main components, namely dl-Limonene, 2-β-Pinene, and γ-Terpinene and the highest component is obtained at an altitude range of 400-500 masl. Meanwhile, *C. hystrix* contains 5 main components in the form of 2-β-Pinene, Sabinene, dl-Limonene, Citronella, and β-Citronellol and the highest component is obtained at an altitude range of 0-100 masl. GC-MS analysis showed that the volatile oil components of the fruit peels of *C. auratifolia* and *C. hystrix* were affected by the altitude of the growing location including temperature and humidity.

Keywords : Essential Oil, *C.aurantifolia*, *C.hystrix*

ABSTRAK

Minyak atsiri adalah minyak yang komponennya secara umum mudah menguap dan diperoleh dari berbagai jenis tanaman serta menjadi komoditas permintaan terbesar di kalangan pemasaran. Salah satu tanaman penghasil minyak atsiri yaitu jeruk nipis (C. aurantifolia) dan jeruk purut (C. hystrix) sehingga banyak dimanfaatkan oleh industri sebagai bahan baku pembuatan obat, kosmetik, dan parfum karena mengandung berbagai komponen di dalamnya. Namun di kalangan masyarakat hanya memanfaatkan bagian buahnya sebagai bumbu masakan dan bahan minuman, sedangkan bagian kulitnya dibuang begitu saja sehingga menjadi limbah. Penelitian ini bertujuan untuk mengetahui pengaruh ketinggian lokasi tumbuh terhadap komponen minyak atsiri dari kulit buah C. aurantifolia dan C. hystrix yang diukur dengan menggunakan GC-MS. Metode pengambilan sampel dilakukan dengan cara simple random sampling yaitu diambil dari beberapa pohon secara acak pada rentang ketinggian 0-100, 400-500, dan 1.200-1.300 mdpl.

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Metode ekstraksi yang digunakan adalah destilasi uap-air dengan suhu 100°C selama 6 jam. Hasil identifikasi GC-MS menunjukkan minyak atsiri dari kulit buah C. aurantifolia mengandung 3 komponen utama yaitu dl-Limonene, 2-β-Pinene, dan γ-Terpinene serta diperoleh komponen terbanyak pada rentang ketinggian 400-500 mdpl. Sedangkan C. hystrix mengandung 5 komponen utama berupa 2-β-Pinene, Sabinene, dl-Limonene, Citronella, dan β-Citronellol serta komponen terbanyak diperoleh pada rentang ketinggian 0-100 mdpl. Analisis GC-MS menunjukkan bahwa komponen minyak atsiri dari kulit buah C. auratifolia dan C. hystrix dipengaruhi oleh ketinggian lokasi tumbuh yang meliputi suhu maupun kelembaban.

Kata kunci : *Minyak Atsiri, C. Aurantifolia, C. Hystrix*

Introduction

Essential oils are volatile compounds that are insoluble in water and are obtained from various types of plants, becoming the largest demanded commodity in the marketing sector (Kurniawan et al., 2008). There are 150 types of essential oils traded in the international market, with 40 types of them being producible in Indonesia (Zulnely et al., 2015). Essential oils are characterized by their distinct aroma according to the type of plant and their volatility. They are widely utilized in cosmetics, perfumes, food and beverage industries, and even pharmaceuticals (Suardhika et al., 2018). Plant parts capable of producing essential oils include fruits, peels, leaves, stems, flowers, seeds, and roots (Noverita et al., 2014). One of the plants capable of producing essential oils belongs to the Rutaceae family, specifically the Citrus genus (Mayasari et al., 2013).

Lime (*Citrus aurantifolia*) and kaffir lime (*Citrus hystrix*) are commonly among the plants producing essential oils in Indonesia. Both of these plant species belong to the Rutaceae family and thrive in subtropical and tropical regions. In Indonesia, *C. aurantifolia* and *C. hystrix* are extensively utilized by industries as raw materials for medicine, cosmetics, and perfume aroma due to their diverse chemical components. However, in society, only the fruit part is utilized as a cooking spice and beverage ingredient, while the peel is discarded, thus becoming waste (Ramdhan et al., 2020; Noverita et al., 2014). Both waste peels of these citrus fruits can be processed to produce high-value essential oils. Processing citrus fruit peels to produce essential oils can be done using isolation techniques such as extraction with organic solvents or distillation. Generally, steam distillation methods are more commonly used because they offer advantages such as lower cost, faster processing, and the steam lowers the boiling point of compounds in the essential oil, thereby preventing the decomposition of the oil constituents (Astuti et al., 2014).

Several studies on the components of essential oil compounds from the peels of *C. aurantifolia* and *C. hystrix* have been reported, such as the research conducted by Wibaldus et al. (2016) on the essential oil components of *C. aurantifolia* peels using GC-MS, which resulted in 5 major compounds including limonene, β-citral, β-pinene, citral, and β-phellandrene. Hairunisa et al. (2019) identified the main components as d-limonene and β-pinene. Meanwhile, research on the essential oil components of *C. hystrix* peels by Noverita et al. (2014) yielded major compounds including *citronella*, *cyclohexene*, *β-citronella*, *β-phellandrene*, and *citronellyl acetate*. Warsito (2017) stated that the main components of the essential oil from *C. hystrix* peels include *sabinene*, *β-pinene*, *limonene*, *citronellal*, and *terpinen-4-ol*. Other studies have also

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been conducted by Iryani and Deka (2018), identifying 27 types of compounds with the highest peaks including *limonene, sabinene, β-citronellol*, and *citronellal*. Based on these studies, it is evident that the peels of *C. aurantifolia* and *C. hystrix* contain various components of essential oil compounds with similar major constituents but varying percentages.

The chemical components of essential oils are suspected to be influenced by the altitude of the cultivation location, both in lowland and highland areas. Generally, higher altitudes correspond to lower average air temperatures, and conversely, lower altitudes correspond to higher air temperatures (Dacosta et al., 2017). Plants grown in water-deficient soils tend to produce more essential oils compared to those grown in adequately watered soils, resulting in differences in the composition of the essential oils (Khalid, 2016; Ahmadian et al., 2011). Research conducted by Astuti et al. (2014) on mango ginger rhizomes (*Curcuma mangga*) originating from lowland areas revealed a greater variety of essential oil compounds compared to those from highlands, possibly due to lower rainfall in lowland areas compared to highlands. GC-MS analysis results obtained by Dacosta et al. (2017) showed that the components of lemongrass essential oils from lowland areas had larger peak areas compared to those from highlands. These GC-MS results indicate a correlation between peak area and compounds, where a larger peak area corresponds to a greater quantity of compounds. Chandra and Proborini (2018) stated that the GC-MS method can be used to analyze the components of essential oils in citrus peels. Some advantages of the GC-MS method include long-term usability, high sensitivity, good separation, and rapid identification.

Based on the aforementioned factors, research on the essential oil components of the peels of *C. aurantifolia* and *C. hystrix* based on the altitude of the growing location, whether highland, midland, or lowland, is necessary to be conducted. This research aims to provide information for selecting the altitude of the growing location that yields a greater quantity of essential oil components from the peels of *C. aurantifolia* and *C. hystrix.*

Methods

The tools used in this research include a set of steam distillation apparatus, a set of GC-MS equipment (QP2010 Ultra SHIMADZU), a thermometer, a pycnometer bottle (Pyrex), a refractometer (Atago), a watch glass, vial bottles, an analytical balance, knives, measuring beakers (Pyrex), chemical glassware (Iwaki), and Erlenmeyer flasks (Duran).

The materials used in this research are lime peel (*C. aurantifolia*), kaffir lime peel (*C. hystrix*), water, ice cubes, aluminum foil, and anhydrous Na_2SO_4.

The fruits of *C. aurantifolia* and *C. hystrix* were collected from different altitude variations. These variations are located at elevations ranging from 0-100 masl in Simpang Tiga District, Pidie Regency, 400-500 masl in Tangse District, Pidie Regency, and 1.200-1.300 masl in Bebesen District, Central Aceh Regency. This research is experimental in nature. The sampling method employed was simple random sampling, where samples were randomly taken from several trees at each of the three locations. The three altitude locations can be seen in the figure below.

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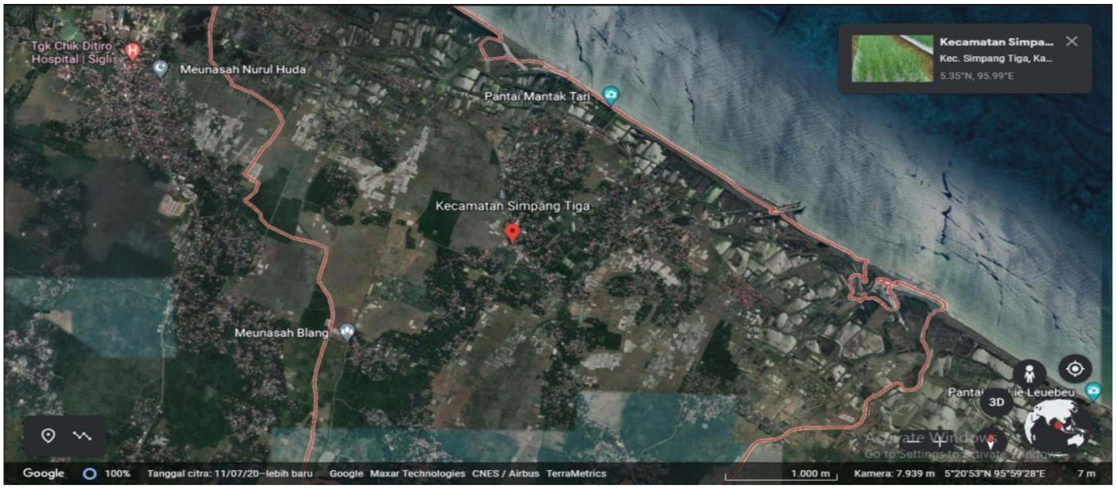

Figure 1. Location Map of Lowland Areas (0-100 masl), Simpang Tiga District, Pidie Regency

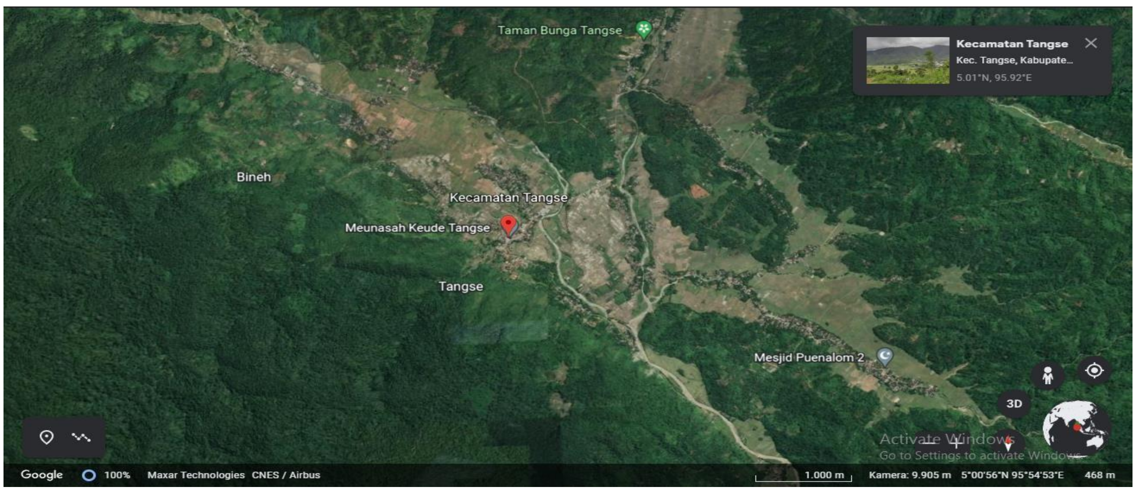

Figure 2. Location Map of Midland Areas (400-500 masl), Tangse District, Pidie Regency

Figure 3. Location Map of Highland Areas (1,200-1,300 masl), Bebesen District, Central Aceh Regency

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The peels of fresh lime (*C. aurantifolia*) and kaffir lime (*C. hystrix*) fruits were sorted wet. Subsequently, each type of peel, amounting to 3 kg, was washed with running water and drained. The peels were then finely chopped into approximately 0.5–1 cm pieces and air-dried at room temperature for 7 days. Following this, the essential oils were isolated from both types of fruit peels using steam distillation apparatus.

The isolation of essential oils from the peels of *C. aurantifolia* and *C. hystrix* refers to the study conducted by Alfianur (2017), employing steam distillation method with slight modifications. The prepared peels of *C. aurantifolia* and *C. hystrix* were placed into a flask partitioned with a sieve positioned at the top of the apparatus setup, while water was added into another flask located at the bottom of the apparatus setup. Subsequently, the flasks were connected to glass apparatus comprising a condenser and a receiver for the distillation products. Once the apparatus was assembled, the condenser water and heating apparatus were activated, and distillation was conducted for 6 hours at 100°C. The distilled oil was then separated using a separating funnel, followed by the addition of anhydrous Na_2SO_4 to remove any remaining water content mixed with the oil. The yield of the obtained essential oil was calculated, and its composition was analyzed using GC-MS.

$$Rendemen = \frac{Oil\ weight\ (g)}{Orange\ peel\ weight\ (g)}\ x\ 100\% \text{..............................} (1)$$

Results and Discussion

Sampling in this study was conducted at different altitudes, including lowland, midland, and highland areas. According to Sutopo et al. (2021), lowland refers to areas located at elevations from 0 to 400 masl, midland refers to areas situated at elevations ranging from 400 to 800 masl, while highland refers to areas located at elevations above 800 to 1,200 masl. Meanwhile, Istiawan and Katsono (2019) categorized altitude into three groups: lowland (<400 masl), midland (400-700 masl), and highland (>700 masl).

Variations in altitude above sea level can result in differences in weather and overall climate at the location, especially in temperature, humidity, and rainfall. Lowland areas are characterized by high environmental temperatures, air pressure, and oxygen levels. Conversely, highland areas significantly affect a decrease in air pressure, air temperature, and an increase in rainfall (Andrian et al., 2014). Optimum temperature is crucial for plant growth and productivity. Excessively high temperatures can hinder plant growth and even lead to plant death, while excessively low temperatures can also adversely affect plant growth. Light is a vital source of energy for plants (Maulidiya, 2015).

Sample preparation for the peel of *C. aurantifolia* and *C. hystrix* involves several stages, including wet sorting, washing, draining, slicing, and drying, as illustrated in Figure 4.

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(a) (b) (c) (d)

Figure 4. Sample preparation process (a) wet sorting, (b) sample washed with running water and drained, (c) sample sliced with a knife, (d) sample air-dried at room temperature

Wet sorting of fresh fruit peels is performed to separate impurities and unusable parts of the peel. Each sample weighing 3 kg is washed with running water to remove dirt and contaminants adhering to the fruit peel (Triana, 2013). Meanwhile, the process of draining is conducted to remove surface water content from the material (Muryanto et al., 2020). Subsequently, the fruit peels are finely chopped into pieces approximately ±0.5–1 cm in size to expedite the drying process and to ensure optimal opening of oil glands, thereby facilitating the evaporation of essential oils during distillation (Suardhika et al., 2018).

After chopping, the fruit peels are air-dried at room temperature for 7 days to reduce the water content present in the peels, thus enlarging the pores of the fruit peel. The larger the open pores of the fruit peel, the easier it is for the oil stored beneath the surface of the fruit peel to evaporate (Alfianur, 2017).

The distillation method employed in this study is steam distillation. Steam distillation is a method aimed at separating a substance from its mixture which do not mix or lowering the boiling point of mixed components with high boiling points with the aid of steam. According to Hasanah (2007), steam distillation method has high economic value, hence it is widely used in various countries, especially in developing countries. The advantage of steam distillation method lies in the occurrence of hydrodiffusion phenomenon where steam enters the plant cell tissues causing the rupture of plant cell walls, thus pushing out the contained oil (Alfianur, 2017). Additionally, steam distillation method offers advantages such as lower cost, higher oil yield, better quality, faster process, and steam lowers the boiling point of compounds in essential oils, thereby preventing the decomposition of essential oil constituents (Astuti et al., 2014). The results of essential oil yield are presented in Table 1.

Table 1. Data of essential oil yield measurements for the peel of *C. aurantifolia* and *C. hystrix* fruit

Percent yield of *C. aurantifolia*			**Percent yield of C. hystrix**	
0-100 masl	400-500 masl	1,200-1,300 masl	0-100 masl	400-500 masl
0.42%	0.41%	1.31%	1.49%	1.30%

Analysis of the essential oil components from the peels of *C. aurantifolia* and C. hystrix based on the altitude of their growth locations using GC-MS aims to determine the composition of compounds contained within them. Additionally, this analysis can be utilized to ascertain the concentration of each compound. Variations in

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the concentration of each component also influence the number of identified compound types. Anita (2012) states that unidentified compounds may still be present in an essential oil. These compounds might exist but in very small quantities, making them difficult to identify accurately.

Table 2. Components of essential oil in the peel of *C. aurantifolia* fruit

Compound Name	Percentage (%)		
	0-100 masl	**400-500 masl**	**1.200-1.300 masl**
1-Phellandrene	0.39	0.22	0.42
α-Pinene	2.23	1.90	2.14
Camphene	0.14	0.12	0.11
Sabinene	3.21	2.23	2.41
2-β-Pinene*	23.44	22.39	23.84
6-Methyl-5-hepten-2-one	0.10	0.13	0.10
β-Mycrene	1.17	1.05	0.84
Octanal (CAS) n-Octanal	0.07	0.09	0.09
α-Terpinene	0.68	0.56	0.61
Benzene, methyl(1-methylethyl)-(CAS)	2.23	1.33	1.61
dl-Limonene*	46.08	47.88	43.88
Cis-Ocimene	0.14	0.11	-
1,3,6-Octatriene, 3,7-dimethyl, (E)-(CAS)	0.47	0.31	0.13
γ-Terpinene*	9.12	5.77	13.03
α-Terpinolene	0.70	0.54	0.73
Linalool	0.49	0.67	0.44
Citronella	0.11	0.07	-
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylene)	1.61	1.54	1.53
β-Fenchyl alcohol	1.05	1.38	1.46
Decanal (CAS) n-Decanal	0.30	0.33	0.34
Nerol	0.26	0.58	0.31
β-Citronellol	0.11	0.12	0.10
Z-Citral	1.83	1.60	1.73
Nerol	0.35	0.50	-
E-Citral	2.26	2.01	2.15
Neryl acetate	0.14	0.28	0.12
Linalyl acetate	0.27	0.33	0.21
trans-Caryophyllene	0.28	0.84	0.15
α-Bergamotene	0.22	0.73	0.21
E,E-α-Farnesene	0.14	1.05	0.34
β-Bisabolene	0.41	1.44	0.40
Herboxide second isomer	-	0.17	-

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Δ-Elemene	-	0.15	-
β-Elemene	-	0.35	0.08
γ-Elemene	-	0.14	-
α-Humulene	-	0.11	-
Germacrene-D	-	0.07	-
α-Selinene	-	0.13	-
trans-α-Bisabolene	-	0.11	-
Germacrene B(CAS) 1,5-Cyclodecadiene	-	0.36	0.11
4,4-Dimethyl-3-(3-methyl-3-buten-1-ylide	-	0.15	-
Cis-Farnesol	-	0.15	-

The results of the identification of the essential oil from the peel of *C. aurantifolia* fruit as presented in **Table 2** exhibit various types of compound components with differing concentrations. The essential oil within the altitude range of 400-500 masl yields a greater variety of compound types, totaling 42, compared to altitude ranges of 0-100 and 1,200-1,300 masl, which yield 31 and 30 compound types, respectively. The predominant components include 2-β-Pinene, dl-Limonene, and γ-Terpinene. This finding aligns with the research conducted by Wibaldus et al. (2016) on the essential oil from lime peel (*C. aurantifolia*), which obtained major components such as Limonene, β-citral, β-pinene, Citral, and β-Phellandrene. Similarly, Wahyudi et al. (2017) identified major components from *C. aurantifolia* as β-pinene, dl-Limonene, Terpinen-4-ol, and α-Terpineol. Likewise, Hairunisa et al. (2019) obtained the same major components, namely dl-Limonene and β-pinene.

Table 3. Components of essential oil in the peel of C. hystrix fruit

Compound Name	**Percentage (%)**	
	0-100 masl	**400-500 masl**
1-Phellandrene	0.35	0.35
α-Pinene	2.30	2.09
Camphene	0.15	0.14
Sabinene*	16.59	14.01
2-β-Pinene*	26.68	25.86
β-Mycrene	1.05	1.08
Octanal (CAS) n-Octanal	0.05	0.11
α-Terpinene	1.70	2.80
Benzene, methyl(1-methylethyl)-(CAS)	0.22	0.15
dl-Limonene*	11.34	18.72
γ-Terpinene	2.66	4.14
α-Terpinolene	1.02	1.52
Linalool	2.09	2.82
Citronella*	11.13	5.92
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylene)	6.63	9.04

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β-Citronellol*	7.88	2.47
trans-Caryophyllene	0.25	0.13
Germacrene-D	0.09	-
Rose Oxide B	0.09	-
trans-Sabinene hydrate	0.39	0.47
Isopulegol 2	0.18	0.14
Isopulegol 1	0.11	0.08
Burneol L	0.12	0.15
α-Terpineol	3.30	4.47
trans-Piperitol	0.10	-
trans-Geraniol	0.13	-
3-7-Dimethyl-Oct-6-Enoic Acide E	0.21	-
Citronellyl acetate	0.29	0.19
α-Copaene	0.34	0.30
Δ-Cadinene	0.52	0.35
Elemol	0,22	0,08
Linalool Oxide Cis	1,55	1,77
Nonanal (CAS) n-Nonanal	-	0,08
p-menth-2-n-1-ol	-	0,15

Based on the data from the GC-MS analysis shown in **Table 3**, the essential oil from the peel of *C. hystrix* fruit also contains various types of compound components with differing concentrations. The essential oil yields the most abundant components within the altitude range of 0-100 masl, with a total of 32 different compound types. The primary components include 2-β-Pinene, Sabinene, dl-Limonene, Citronella, and β-Citronellol. This finding is consistent with research conducted by Hasanah (2007) on the essential oil from the peel of *C. hystrix* fruit, which identified major components such as Sabinene, β-Pinene, dl-Limonene, Citronellal, and Linalool. Similarly, Warsito (2017) identified major components from *C. hystrix* peel as Sabinene, β-Pinene, dl-Limonene, Citronellal, and Terpinen-4-ol. Likewise, Iryani and Deka (2018) found major components such as Limonene, Sabinene, β-Citronellol, and Citronellal.

The compounds dl-Limonene, 2-β-pinene, γ-Terpinene, Sabinene, Citronella, and β-Citronellol are terpenoid compounds believed to impart flavor to fruits. These compounds are utilized by industries in the production of medicine, cosmetics, food and beverage additives, as well as for their antifungal, antibacterial, antiviral, anticancer, and insecticidal properties (Cahyati et al., 2016; Dari et al., 2020). Marsadi et al. (2018) stated that terpenoid compounds are the main components of essential oils responsible for the scent and fragrance of plants, and they are useful in the production of cosmetics, medicines, and natural insect repellents.

The components of essential oil produced by a plant can be influenced by geographical differences. The altitude of a location affects changes in temperature, rainfall, air humidity, and sunlight intensity. Temperature is a vital factor, with air temperature decreasing as altitude increases. These temperature variations can lead to differences in plant metabolism, thereby affecting the synthesis of active compounds. Temperature characteristics regarding plants are divided into three categories: optimum

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temperature (plants can grow well), minimum temperature (temperature lower than this inhibits plant growth), and maximum temperature (temperature higher than this inhibits plant growth) (Anita, 2012).

Based on the GC-MS analysis of the essential oil from *C. aurantifolia* peel, the highest number of components was obtained within the altitude range of 400-500 masl. This is because at this altitude, the temperature is around 27-28°C and the humidity is approximately 74%. Sarwono (2001) stated that the optimal temperature for *C. aurantifolia* plants ranges from 25-30°C, with ideal humidity between 70-80%, for optimal growth. Therefore, the temperature at the sampling location within the altitude range of 400-500 masl falls within the optimal range, resulting in a higher yield of compound components. Conversely, the GC-MS analysis of the essential oil from *C. hystrix* peel yielded the highest number of components within the altitude range of 0-100 masl. At this altitude, the average temperature is 30-33°C and the humidity is around 76%. Bimo (2018) mentioned that *C. hystrix* plants thrive in areas with slopes of approximately 30°, with altitudes ranging from 1-400 masl and humidity levels of about 70-80%. Zamzamiyah (2020) also stated that optimal growth of *C. hystrix* plants occurs at altitudes of 1-400 masl. Based on these findings, it can be concluded that the sampling location within the altitude range of 0-100 masl is conducive to the growth of *C. hystrix* plants, resulting in a higher yield of compound components.

Conclusion

The components of the essential oil obtained from the peel of *C. aurantifolia* fruit are influenced by the altitude of the growth location. This is evidenced by the fact that the components obtained from the altitude range of 400-500 meters above sea level (masl) yield a greater number of components compared to the other two altitude ranges, namely 0-100 masl and 1,200-1,300 masl.

Similarly, the components of the essential oil obtained from the peel of *C. hystrix* fruit are also influenced by the altitude of the growth location. This is indicated by the fact that within the altitude range of 0-100 masl, the highest number of components is obtained compared to the altitude range of 400-500 masl.

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