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Secondary Metabolite Profile of Essential Oils of Kaffir Lime, Kalamansi, and Lime Fruit Peels from South Sumatra

Astri Indah Lestari[1], Salni[2], Laila Hanum[3]
[1]*(Biology Master Study Program Student, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indonesia)*
[2,3]*(Lecturer in Biology Master's Study Program, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indonesia)*

* Corresponding Author. E-mail: astriindah.ai@gmail.com

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Abstrak
Penelitian ini bertujuan untuk mengetahui kandungan metabolit sekunder minyak atsiri kulit buah jeruk purut, kalamansi, dan nipis dari Sumatera Selatan. Penelitian ini terdiri dari beberapa tahapan yaitu tahap identifikasi tanaman jeruk purut, kalamansi, dan nipis, tahap persiapan alat dan bahan, tahap distilasi minyak atsiri kulit buah jeruk purut, kalamansi, dan nipis serta tahap identifikasi komponen kimia minyak atsiri menggunakan metode GC-MS. Based on research, it can be concluded that the main essential oil content of kaffir lime peel, calamansi and lime from South Sumatra are citronellal (24.55%), limonene (97.12%) and limonene (35.24%) respectively.

Kata Kunci: *Profit Metabolit Sekunder, Minyak Atsiri, Kulit Buah Jeruk Purut, Kulit Buah Jeruk Kalamansi, Kulit Buah Jeruk Nipis*

Abstract
This study aims to determine the secondary metabolite content of the essential oils of kaffir lime, kalamansi and lime peels from South Sumatra. This research consisted of several stages, namely the stage of identifying kaffir lime, calamansi and lime plants, the stage of preparing tools and materials, the stage of distillation of the essential oils of kaffir lime, calamansi and lime peels and the stage of identifying the chemical components of essential oils using the GC-MS method. Based on research, it can be concluded that the main essential oil content of kaffir lime peel, calamansi and lime from South Sumatra are citronellal (24.55%), limonene (97.12%) and limonene (35.24%) respectively.

Keywords: *Profit Secondary Metabolites, Essential Oils, Kaffir Lime Peel, Kalamansi Orange Peel, Lime Peel*

Introduction

Essential oils are secondary metabolites that are produced by many aromatic plants through complex metabolic processes. Essential oils consist of a mixture of volatile compounds, which are mainly produced by the mevalonate pathway, consisting of terpene compounds including monoterpenes and sesquiterpenes, besides that they are also produced through the shikimate pathway, namely phenolic compounds (Dhifi et al., 2016). Essential oils can be sourced from various plant organs, such as leaves, flowers, fruit, fruit skin, seeds, stems, bark, roots and rhizomes (Andila et al., 2020). Essential oils have various health benefits, several preclinical studies have reported that essential oils have antioxidant, antimicrobial, anti-inflammatory and anticancer activity (Sharifi-Rad et al., 2017). Plants that produce essential oils include oranges (A. Kurniawan et al., 2008).

Oranges are one of the plants that are widely cultivated and consumed by Indonesian people, this is because oranges contain nutrients and vitamins that are beneficial for health (Andrini et al., 2021; Wirahadi, 2017). People generally use citrus fruit for direct consumption or as an additive to food and drinks, while the peel of the fruit is not used and is often thrown away as waste (T. W. Kurniawan & Deglas, 2019). In fact, orange peel contains essential oils which have potential as natural antioxidants and antibacterials (Lestari, 2023). Several types of oranges that are easy to find in South Sumatra are calamansi (Citrus madurensis Lour.), lime (Citrus aurantifolia (Christm.) Swingle, orth.), and kaffir lime (Citrus hystrix DC.).

Based on the literature study that has been carried out (Lestari, 2023)stated that the essential oils of kaffir lime peel, calamansi and lime have antibacterial and antioxidant activity. The essential oil in orange peel consists of terpenoid compounds. Terpenoids have antibacterial activity with their mechanism of action disrupting bacterial cell walls, damaging transmembrane proteins, and reducing the permeability of bacterial cell walls (Husni et al., 2021). The essential oils of kaffir lime peel, calamansi, and lime have been known to have antibacterial activity against several gram-negative and gram-positive bacteria (Costa et al., 2014; Husni et al., 2021; Sreepian et al., 2019). The essential oils of kaffir lime peel, calamansi and lime have also been known to have inhibitory activity against DPPH free radicals, characterized by a decrease in the activity (absorbance) of DPPH free radicals, this activity can be associated with the content of monoterpenoid hydrocarbon components which act as antioxidants (Chen et al., 2017; Lin et al., 2019; Warsito et al., 2017). (Palma et al., 2019) reported that calamansi orange peel essential oil has high cytotoxicity against human breast tumor cell lines (MCF-7) with an IC50 value of 7.98 µg/mL via the microtetrazolium (MTT) test.

The presence of active compounds contained in the essential oils of kaffir lime peel, calamansi and lime can make them a source of natural medicine. Kaffir lime, calamansi and lime plants are easy to cultivate in all places, both in the lowlands and highlands (Sarwono, 1993; Widiastuti, 2012). Differences in plant types, growing habitat, genetics, climate, soil nutrient composition, soil pH, climate, and harvest age can influence the percentage and type of compounds contained in essential oils (Mulyani et al., 2020; Yanti et al., 2017). Therefore, this study aims to determine the secondary metabolite content of the essential oils of kaffir lime, kalamansi and lime peels from South Sumatra.

Method

This research consists of several stages, namely the identification stage of kaffir lime, calamansi and lime plants; tool and material preparation stage; the distillation stage of the essential oils of kaffir lime peel, kalamansi, and lime; and the identification stage of the chemical components of essential oils using the GC-MS method.

Identify Kaffir Lime, Calamansi, and Lime Plants

The kaffir lime, calamansi and lime plants used in this research were identified in the Biosystematics Laboratory, Biology

Department, Faculty of Mathematics and Natural Sciences, Srwijaya Indralaya University. The purpose of this identification is to find out the correct identity of the plants used in this research (Sari et al., 2022).

Stages of Preparation of Tools and Materials

At this stage the tools and materials used in the research are prepared first. The kalamansi, lime and kaffir limes used in this research were ripe fruits. Ripe orange fruit contains a higher yield of essential oils than unripe oranges (Sukarta et al., 2022). This statement is supported by research (Voo et al., 2012) that the essential oils contained in orange peel develop with the level of fruit maturity. The kaffir lime, kalamansi and lime fruits used in this research came from South Sumatra Province. Calamansi orange fruit is obtained from Banyuasin Regency, while lime and kaffir lime fruit are obtained from Lahat Regency. The test bacteria used in this research were pure strains of Staphylococcus epidermidis bacteria obtained from the Palembang Health Laboratory Center (BBLK).

Preparation of Kaffir Lime, Kalamansi and Lime Fruit Peel Simplisia

5 kg of kaffir lime, calamansi and lime fruit each, washed under running water until clean, then drained. The orange fruit is then peeled to remove the skin and reduced in size by cutting into pieces (Yuriah & Kartini, 2022). Reducing the size aims to simplify the distillation process because the surface contact area of the simplicia with water vapor increases (Mulyani et al., 2020). Simplisia is fresh orange peel that has been reduced in size, then the essential oil distillation process is carried out (Muthoharoh et al., 2022).

Distillation of the essential oils of kaffir lime peel, calamansi and lime

The distillation process for the essential oils of kaffir lime peel, calamansi and lime in this study used water and steam distillation methods. This method can minimize damage to essential oil components because the temperature is not too high and the simplicia is not in direct contact with boiling water, so the quality of the essential oil obtained will be better (Wijayati et al., 2023). A total of 2 kg of fresh simplicia, kaffir lime peel, kalamansi, and lime each was distilled in stages until no more essential oil dripped into the separator (Gunawan & Kurniaty, 2021). The condensation results in the form of condensate (hydrosol and essential oils) will be collected in a separator and separated based on specific gravity (Agustiana et al., 2015; Wijayati et al., 2023). The hydrosol and essential oil are then separated. During the distillation process, the hydrosol will be removed little by little by opening the tap on the separator until the distillation process is complete and the essential oil is collected in the vial (Simanjuntak et al., 2021). The essential oil that has been obtained is added with anhydrous Na2SO4 in a ratio of (10:1) and filtered using Whatman No. 1 paper (Adiyasa et al., 2014; Angin, 2015). The purpose of adding anhydrous Na2SO4 is to draw out the water remaining in the essential oil (Palma et al., 2019). Next, the yield of each essential oil of kaffir lime peel, kalamansi, and lime is determined using the formula:

$$\text{Rendemen (\%)} = \frac{\text{volume of distilled essential oil (ml)}}{\text{weight of orange peel sample (g)}} \times 100\%$$

(Farahmandfar et al., 2020)

Analysis of Essential Oil Components Using the GC-MS Method

A total of 3 ml of each of the essential oils of kaffir lime peel, kalamansi, and lime obtained was subjected to essential oil component analysis using the GC-MS method, at the Integrated Laboratory of the Islamic University of Indonesia. The GC-MS method was carried out to separate and identify the essential oil components of kaffir lime, calamansi and lime peels (LPPT, 2018).

Results and Discussion

Yield of Essential Oil of Kaffir Lime, Calamansi and Lime Fruit Peel

The yield of essential oils from kaffir lime peel, calamansi and lime obtained from the water and steam distillation process is presented in Table 1 below:

Table 1. Yield of essential oils from kaffir lime, calamansi and lime peels

No.	Simplicity	Fresh Simplicia Weight (g)	Volume of Essential Oil (ml)	Yield (%)
1	Kaffir lime peel	2,000	14.6	0.73
2	Calamansi orange peel	2,000	9.2	0.46
3	Lime peel	2,000	11.8	0.59

A total of 2,000 g each of kaffir lime peel, calamansi, and lime were distilled in stages, over a period of ±5 hours. The distillation in this study aims to obtain essential oils from the peel of kaffir lime, kalamansi and lime. Table 1 shows that the essential oil of kaffir lime peel has the largest yield, namely 0.73%, followed by lime and calamansi which have yields of 0.59% and 0.46% respectively.

The yield of kaffir lime peel essential oil obtained in this study was smaller than in research (Latifah et al., 2023) namely 1.86%. Meanwhile, the yield of essential oils from kalamansi and lime peels obtained in this study was greater than in the previous study. (Tutuarima & Antara, 2020) namely (0.33%) in kalamansi and research (Stiawan et al., 2023) namely 0.42% in lime. Factors that influence the percentage of essential oil yield from the distillation process include the level of fruit maturity and the length of distillation time. Ripe orange fruit contains a higher yield of essential oils than unripe oranges (Sukarta et al., 2022). This statement is supported by research (Voo et al., 2012) that the essential oils contained in orange peel develop with the level of fruit maturity. According to (Effendi & Widjanarko, 2014)) one of the factors that influences the amount of essential oil yield from the distillation process is the length of distillation time. The longer the distillation time, the greater the percentage of essential oil yield obtained (Yuriah et al., 2023).

Analysis of Essential Oil Components Using the GC-MS Method

GC-MS (Gas Chromatograpy-Mass Spectrometry) in this study was used with the aim of separating and identifying the essential oil components of kaffir lime peel, calamansi and lime. The components of the essential oil mixture are first identified using a GC tool which produces a chromatogram, then an MS tool is used to separate and identify the compounds contained in the essential oil. Each compound is called a spectrum (LPPT, 2018; Nurhaen et al., 2016). The results of analysis of the essential oil components of kaffir lime peel, calamansi, and lime using a GC tool are shown in Figure 1, Figure 2, and Figure 3, respectively.

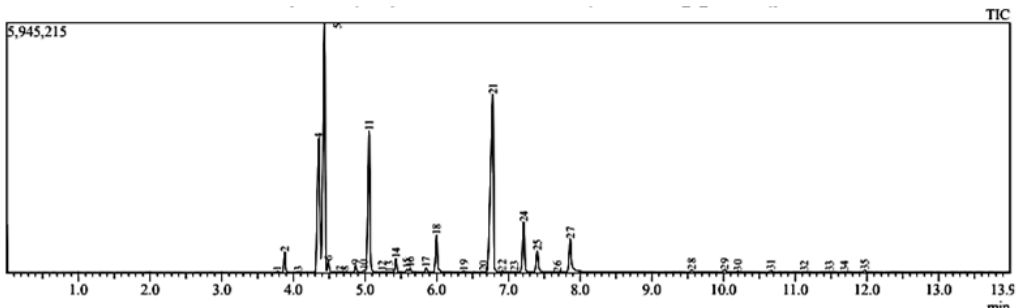

Figure 1. GC chromatogram of kaffir lime peel essential oil

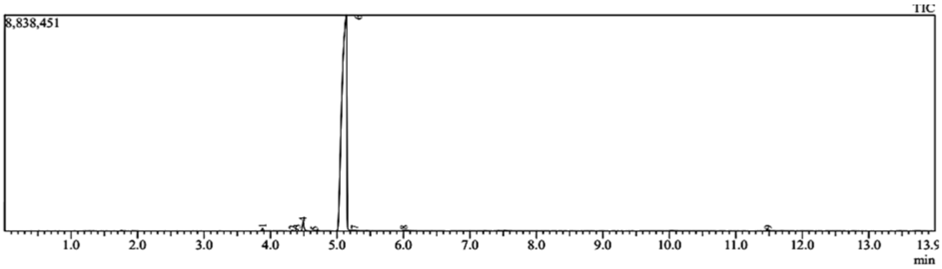

Figure 2. GC chromatogram of essential oil of calamansi orange peel

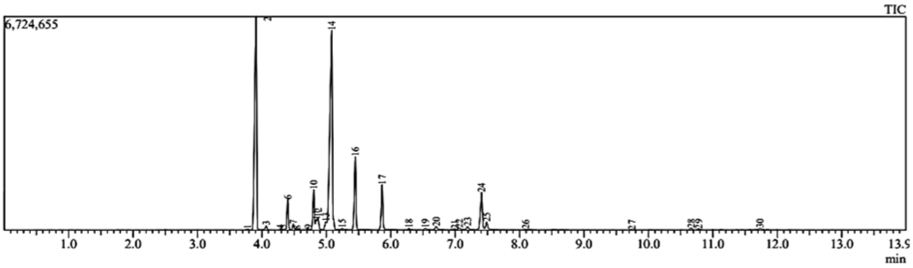

Figure 3. GC chromatogram of lime peel essential oil

Based on the results of GC analysis, the chromatogram (Figure 1) contains 35 peaks, which means that there are 35 compounds that make up the essential oil of kaffir lime peel. The chromatogram (Figure 2) contains 9 peaks, which means that there are 9 compounds that make up the essential oil of kalamansi orange peel. The chromatogram (Figure 3) contains 30 peaks, which means that there are 30 compounds that make up the essential oil of lime peel.

Each compound identified has a different retention time. The results of analysis of the essential oil composition of kaffir lime peel, calamansi, and lime using MS tools are shown in Table 2, Table 3, and Table 4.

Table 1. Components of the essential oil of kaffir lime peel

Peak	Retention time	Compound Name	Similarity Index	Area (%)
1	3,777	α-Thujene	96	0.12
2	3,878	α-Pinene	98	1.50
3	4,066	Camphene	97	0.10
4	4,354	Sabinene	98	15.33
5	**4,433**	**1-β-Pinene**	**98**	**24.07**
6	4,487	β-Myrcene	97	0.96
7	4,653	Octanal	94	0.09
8	4,718	1-Phellandrene	93	0.10
9	4,868	α-Terpinene	96	0.59
10	4,982	Benzene	95	0.20
11	5,056	l-limonene	96	13.32
12	5,240	1,3,6-Octariene	92	0.06
13	5,338	2,6-Dimethyl Hept-5-1-Al	86	0.04
14	5,430	γ-Terpeinene	97	1.11
15	5,585	Trans-Sabinene hydrate	92	0.27
16	5,628	Trans-Linaloloxide	95	0.37
17	5,852	Alloocimene	88	0.45
18	5,998	Linalol	97	3.51
19	6,375	p-Menth-3-en-1-ol	92	0.18
20	6,651	p-Menth-2-en-1-ol	91	0.09

21	**6,782**	**Citronellal**	**93**	**24.55**
22	6,912	Neoiso(Iso)Pulegol	96	0.23
23	7,079	Borneo	95	0.15
24	7,213	3-Cyclohexen-1-methanol	97	4.87
25	7,401	Linalyl propionate	96	2.34
26	7,680	Trans-Piperitol	74	0.04
27	7,861	β-Citronellol	98	3.78
28	9,554	Citronellyl acetate	97	0.31
29	10,019	α-Copaene	93	0.37
30	10,200	Germacrene D	95	0.16
31	10,662	Trans-Caryophyllene	97	0.21
32	11,127	α-Humulene	93	0.06
33	11,480	Germarcene	92	0.10
34	11,685	Bicyclogermacrene	90	0.10
35	11,972	δ-Cadinene	93	0.27
	Total		100	

Table 2. Components of the essential oil of kalamansi orange peel

Peak	Retention time	Compound Name	Similarity Index	Area (%)
1	3,878	α-Pinene	98	0.33
2	4,331	Sabinene	96	0.11
3	4,398	β-Pinene	97	0.30
4	4,490	β-Myrcene	97	1.43
5	4,657	Octanal	94	0.14
6	**5,138**	**L-limonene**	**96**	**97.12**
7	5,265	1,3,6-Octatriene	91	0.11
8	6,005	Linalol	96	0.26
9	11,485	Germacrene	92	0.20
	Total	100		

Table 3. Components of lime peel essential oil

Peak	Retention time	Compound Name	Similarity Index	Area (%)
1	3,778	α-Pinene	95	0.14
2	**3,909**	**α-Pinene**	**96**	**30.79**
3	4,066	Camphene	97	0.49
4	4,286	2H-Pyran	97	0.10
5	4,330	Sabinene	94	0.07
6	4,399	β-Pinene	96	2.87
7	4,486	β-Myrcene	91	0.64
8	4,540	Phellandral	45	0.02
9	4,718	1-Phellandrene	96	0.13
10	4,803	δ-3-Carene	97	4.16
11	4,845	Isocineole	93	1.02
12	4,871	α-Humulene	94	1.30
13	4,993	Benzene	95	1.03
14	**5,083**	**l-Limonene**	**95**	**35.24**
15	5,241	Trans-Ocimene	87	0.16
16	5,449	γ-Terpinene	97	7.98
17	5,866	α-Terpinolene	97	5.07
18	6,281	D-Fenchyl alcohol	96	0.16
19	6,536	p-Menth-3-en-1-ol	95	0.25
20	6,704	Cyclohexanol	94	0.51
21	6,989	β-Terpineol	93	0.12
22	7,063	Borneo	97	0.33
23	7,191	3-Cyclohexen-1-ol	96	0.47

24	7,410	3-Cyclohexen-1-methanol	97	5.20
25	7,488	γ-Terpineol	90	1.03
26	8,091	Z-Citral	93	0.06
27	9,740	Neryl acetate	82	0.02
28	10,662	Trans-Carophyllene	97	0.26
29	10,779	Trans-α-Bergamotene	93	0.18
30	11,734	β-Bisabolene	95	0.22
	Total		100	

Table 2 shows that the main component of the essential oil of kaffir lime peel from Lahat, South Sumatra is citronellal with an area of less than 50%, namely 24.55% and followed by 1-β-pinene at 24.07%. The percentage of the main component of kaffir lime peel essential oil in this research is different from the research (Srisukh et al., 2012) in his research, the main component of kaffir lime peel essential oil was limonene at 40.65%. Meanwhile, the main component of the essential oil of kalamansi orange peel from Banyuasin, South Sumatra is l-limonene with an area percentage of more than 50%, namely 97.12% (Table 3). The percentage of the l-limonene compound in the essential oil of kalamansi orange peel obtained in this research was greater than the results of the study (Chen et al., 2017) namely 87.52%. Table 4. shows that the main component of the essential oil of lime peel from Lahat, South Sumatra is l-limonene, with an area percentage of less than 50%, namely 35.24%, followed by the percentage of the second largest component, namely α-pinene. The percentage of the l-limonene compound in lime peel essential oil obtained in this study was smaller than in the previous study (Lemes et al., 2018) which contains more than 50% l-limonene compounds, namely 77.5%.

Differences in the percentage and types of compounds contained in the essential oils of kaffir lime, calamansi and lime peels in this study can be caused by many factors, including plant type, genetics, climate, differences in growing habitat, soil nutrient composition, soil pH, and harvest age (Mulyani et al., 2020; Yanti et al., 2017).

Conclusion

Based on research, it can be concluded that the main essential oil content of kaffir lime peel, calamansi and lime from South Sumatra are citronellal (24.55%), limonene (97.12%) and limonene (35.24%) respectively.

The suggestion from this research is that further research needs to be carried out regarding the influence of external factors (climate differences, growing habitat, soil nutrient composition, soil pH, and fruit harvest age) on the essential oil components of Kalamansi, Kaffir Lime, and Lime Fruit peels.

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Author Profile

Astri Indah Lestari is an active biology master's student at Universitas Sriwijaya.

Salni is an active teaching staff at the MIPA faculty at Universitas Sriwijaya.

Laila Hanum is an active teaching staff at the MIPA faculty at Universitas Sriwijaya.