

# GC–MS Analysis of Bioactive Compounds in Lime Leaf Ethanol Extract (*Citrus amblycarpa* (Hassk.) Ochse), and Its Potential as a Traditional Medicine Agents

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**Abstract:** Lime (*Citrus amblycarpa* (Hassk.) Ochse) has been widely used in traditional medicine. However, only some studies have comprehensively revealed the content of leaves, including their use as candidate medicinal ingredients. This study aimed to analyze the active compounds of lime leaves through GC–MS analysis and their benefits in traditional medicine. Experimental research with a laboratory approach is used. The implementation of research by analyzing the bioactive content of lime leaf ethanol extract using GC–MS along with a comprehensive analysis related to its benefits as a traditional medicine agent. Data analysis using the Willey 7 Library database and descriptive analysis. Using GC-MS showed that lime leaf ethanol extract contains 75 compound components dominated by 11 principal components, including citronellol, caryophyllene, hexadecanoic acid, ethyl ester, 1-heptatriacotanol, Phytol, ethyl 9,12,15-oate; methyl glycocholate, 3TMS derivative; 3,7-dimethyloct-6-en-1-yl stearate; ethyl iso-allocate; Rhopin; and tricyclo [20.8.0.0 (7,16)] triacontane, 1(22),7(16)-diepoxy-. Based on the findings of the content evaluation, the compound constituents of lime leaves have the potential to be developed as traditional remedies for both degenerative and infectious diseases. However, further research is needed precisely to identify the therapeutic effects exerted by using compounds on lime leaves.

**Keywords:** *Citrus amblycarpa*; Ethanol extract; GC-MS; Lime leaf; Traditional medicine

## Introduction

*Citrus amblycarpa* (Hassk.) Ochse, often known as lime leaves or kaffir lime, is a West Java-endemic endemic shrub. It is a hybrid citrus lime species with the formula *Citrus micrantha* and *Citrus reticulata* (Ollitrault et al., 2020; Soetjipto & Martono, 2017; Talon et al., 2020). *Citrus amblycarpa* (Hassk.) Ochse, sometimes known as the lime leaf, is a citrus plant native to Southeast Asia. It is planted extensively in Indonesia, Thailand, Malaysia, and other tropical nations. This plant's spines are extraordinarily sharp and rigid. The fruit is tiny, has a thick skin, and tastes exceptionally sour. Like other

limes, the lime has dark green, rectangular leaves, white, fragrant blooms, and green fruits with a rough peel and juicy flesh.

In Indonesia, lime leaf is frequently utilized as an aromatic and flavor-enhancing fruit. Fruits and lime leaves are utilized more frequently as flavor enhancers in cooking (Budiarto et al., 2022; Budiarto & Sholikin, 2022). Lime leaves are frequently used as seasoning in traditional Southeast Asian cuisine. Due to their chemical composition, lime leaves have potential benefits in traditional medicine and their culinary purpose (Budiarto et al., 2021b; Lubinska-Szczygieł et al., 2018; Panakkal et al., 2021). The bioactive

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components found in lime leaves include essential oils, flavonoids, alkaloids, and coumarins. Several volatile chemicals, including citronellal, linalool, limonene, and -pinene, make up the essential oil of lime leaves. These chemicals are responsible for lime leaves' distinctive scent and flavor. The essential oil content of lime leaves varies by cultivar, harvesting period, and processing technique (Budiarto et al., 2021b; Dertyasasa & Tunjung, 2017; Hien et al., 2020; Siti et al., 2022; Suresh et al., 2021).

Lime leaves have been used in traditional medicine to cure various diseases for millennia. In Indonesia, lime leaves are frequently employed to cure fever, cough, and the common cold. Additionally, lime leaves cure digestive issues such as bloating, gas, and nausea. It is believed that lime leaves have a cooling impact on the body, which makes them helpful in treating fever and other inflammatory disorders (Dertyasasa & Tunjung, 2017; Harmayani et al., 2019; Siti et al., 2022). In traditional medicine, lime leaves have been used to treat various diseases for millennia. In Indonesia, lime leaves are extensively employed to treat fever, cough, and cold. The leaves of the lime tree are also used to treat digestive issues such as bloating, flatulence, and nausea.

According to popular belief, lime leaves have a cooling impact on the body, making them beneficial for treating fever and other inflammatory diseases (Putra et al., 2018). Several studies have demonstrated that the active ingredient lime has biopesticide potential against mosquitoes (Abdullah & Othman, 2018; Budiarto et al., 2021a; Kasman et al., 2021; Kasman, 2020; Adnyana et al., 2021), potentially overcoming hypertension (Kusumawati et al., 2021), and as an antidiabetic agent by inhibiting the activity of the enzyme  $\alpha$ -glucosidase (Tambunan et al., 2020). There is limited information on the health benefits and side effects of lime leaf consumption. However, the related plant *kaffir lime* leaves have been found to have several health benefits, including reducing stress, improving oral health, fighting bacteria, promoting healthy skin and hair, aiding digestion, and relieving piles (Anuchapreeda et al., 2020; Lim, 2012; Suresh et al., 2021; Wulandari et al., 2019).

Despite the potential pharmacological properties of lime leaves, there is a need for more research, mainly using gas chromatography–mass spectrometry (GCMS). GCMS is a powerful analytical technique that can identify and quantify lime leaf volatile and nonvolatile compounds. GCMS can provide a more comprehensive understanding of the chemical composition and potential pharmacological properties of lime leaves. This study aimed to analyze the active compounds of lime leaves through GCMS analysis and their benefits in traditional medicine. Hopefully, this research can contribute ideas related to compounds in lime leaves with potential as candidates for traditional medicine. A

comprehensive analysis of the content is sought to provide an overview of the importance of using lime leaves as candidates for medicinal ingredients.

## Method

This experimental research with a laboratory approach was used in this study (Darwin et al., 2021). This research analyzes the content and benefits of lime leaves (*Citrus amblycarpa* (Hassk.) Ochse). The study was conducted for one month in December 2021. Lime leaf samples were collected from Denpasar Bali, and then lime leaf ethanol extract was made at the Laboratory of the Biology Study Program, Hindu University of Indonesia. This research flow is presented in Figure 1.

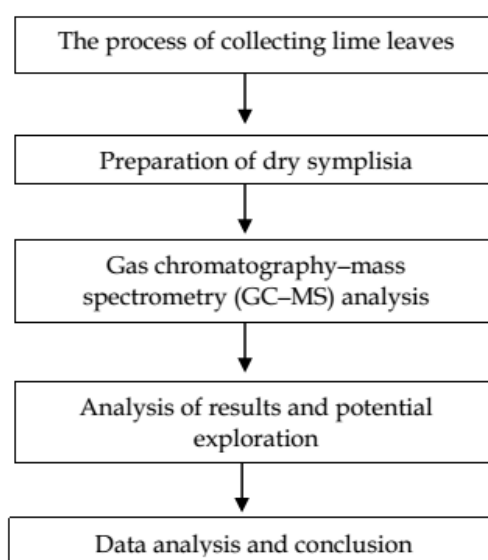


Figure 1. The flow of research

The lime extract was obtained through a maceration method with a 96% ethanol solvent (Astiti & Ramona, 2021). The simplicial setup process utilizes lime leaves that are thoroughly washed, cut into small pieces, blended, dried for 24 h, and filtered using a flour sieve. After five days of drying, the material is transformed into dry *Simplicia*. The lime leaf *Simplicia* was macerated in 96% ethanol for 48 hours before filtration using filter paper. The filtrate was then concentrated in a rotary evaporator vacuum at 45 °C to produce a viscous extract. The active component was examined using GCMS (Shimadzu GC-210 Plus). An HP-5MS UI capillary column (30.0 m × 0.25 mm × 0.25 m) was used in the GC MS instruments. Helium was used as the carrier gas at a flow rate of 1 mL per minute. The GC temperature was set as follows: the injector temperature was 230 °C, the column beginning temperature was 60 °C, the temperature rise rate was 10 °C/min, and the oven final temperature was 280 °C.

The compound was identified by matching it against the Willey7 Library database. An integrated research and testing lab performed the GCMS analysis for the study at Gadjah Mada University in Yogyakarta. The gathered data are then descriptively examined, and literature searches are conducted about using chemicals derived from lime leaves as candidates for traditional medicine in Bali. Images and tales are utilized to present the findings.

## Result and Discussion

### Gas Chromatography–Mass Spectrometry Evaluation of Lime Leaf Ethanol Extract

The Gas chromatography–mass spectrometry (GC–MS) analysis approach was qualitatively carried out to identify active compounds by looking at peak areas and

retention times. Based on the results of chromatograms, there are 75 components of compounds (Figure 2), including 11 principal components, most of which are essential oils. Additionally, there are components of active compounds that have the potential to act as antioxidants, anticancer agents, anti-inflammatory agents, and flavor boosters for food. Fragmentation of this principal component can be seen from the peak on the mass spectrum of lime extract in Table 1 and Figure 3. Each of the compounds in the compound chromatogram in Table 1 is a significant component of lime leaf ethanol extract, each with potential benefits. The main components are terpenoids. Among these components, some belong to the essential oil category. This can be seen in the lime leaf ethanol extract, which resembles a deep black paste after evaporation.

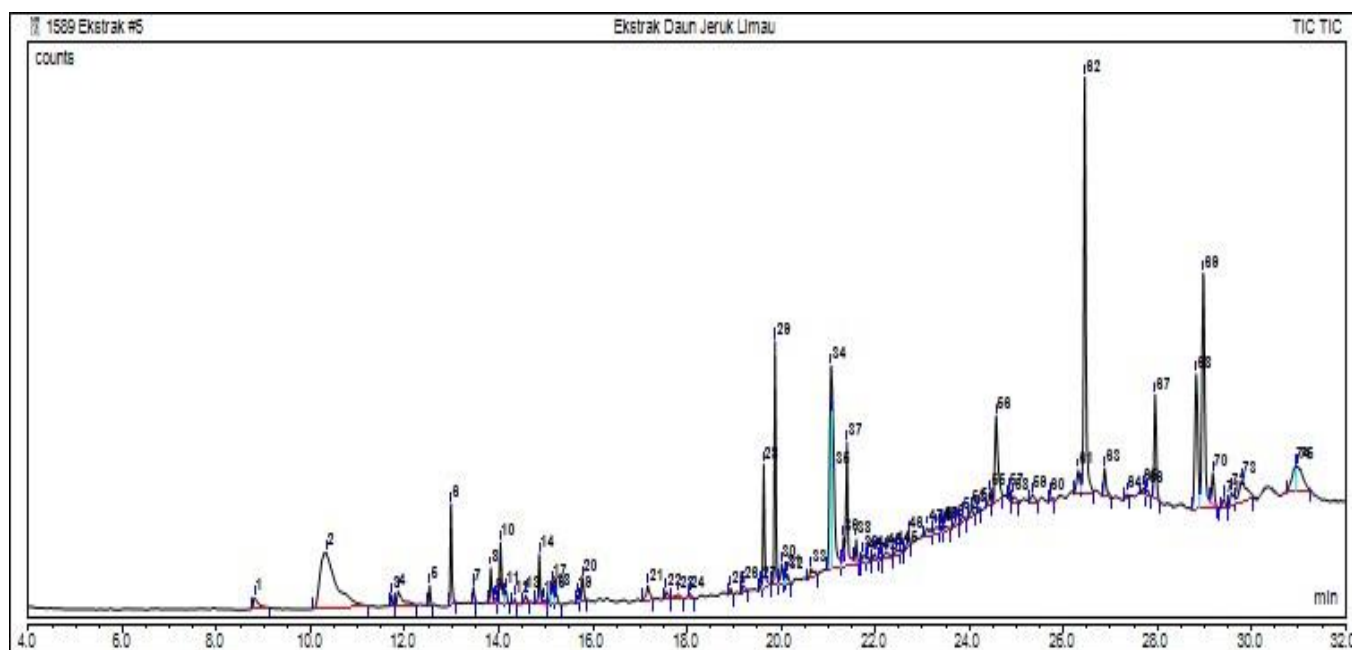
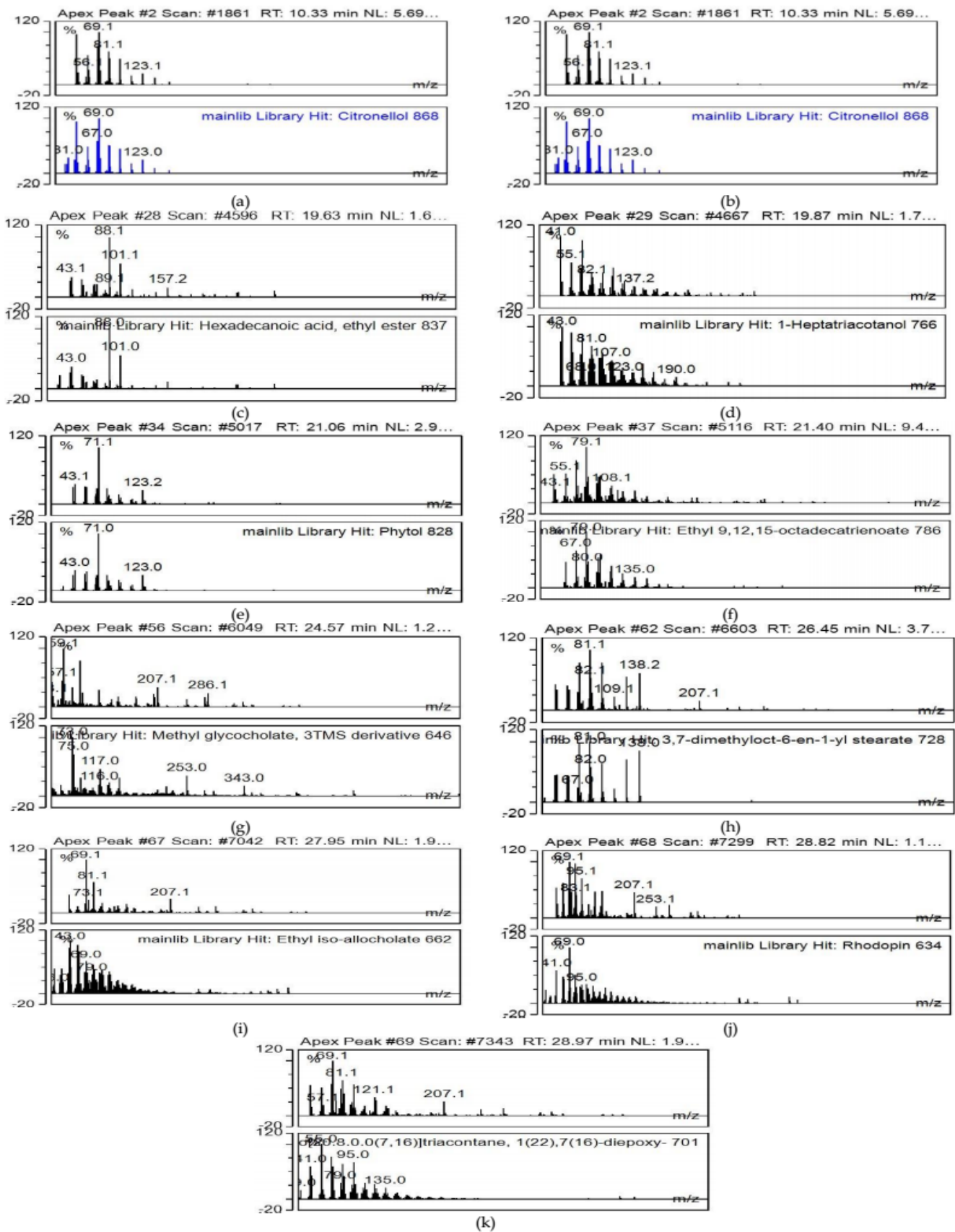


Figure 2. Chromatogram of lime leaf ethanol extract, peak numbers to the chemicals listed in table 1

Table 1. Main Components of Lime Leaf Ethanol Extract

Top	Retention Time	Chemical Components	Area	BM	Molecular Formula
2	10.33	Citronellol	12.03	156	C <sub>10</sub> H <sub>20</sub> O
6	12.98	Caryophyllene	2.04	204	C <sub>15</sub> H <sub>24</sub>
28	19.63	Hexadecanoic acid, ethyl ester	2.66	284	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>
29	19.87	1-Heptatriacotanol	4.58	536	C <sub>37</sub> H <sub>76</sub> O
34	21.06	Phytol	7.50	296	C <sub>20</sub> H <sub>40</sub> O
37	21.40	Ethyl 9,12,15-octadecatrienoate	3.83	306	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>
56	24.57	Methyl glycocholate, 3TMS derivative	3.59	695	C <sub>36</sub> H <sub>69</sub> NO <sub>6</sub> Si <sub>3</sub>
62	26.45	3,7-dimethyl oct-6-en-1-yl stearate	11.45	422	C <sub>28</sub> H <sub>54</sub> O <sub>2</sub>
67	27.95	Ethyl iso-allocholate	3.02	436	C <sub>26</sub> H <sub>44</sub> O <sub>5</sub>
68	28.82	Rhodopin	5.36	554	C <sub>40</sub> H <sub>58</sub> O
69	28.97	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	9.24	444	C <sub>30</sub> H <sub>52</sub> O <sub>2</sub>



**Figure 3.** GC–MS spectra citronellol mass (a); Caryophyllene (b); Hexadecanoic acid, ethyl ester (c); 1-Heptatriacotanol (d); Phytol (e); Ethyl 9,12,15-octadecatrienoate (f); Methyl glycocholate, 3TMS derivative (g); 3,7-dimethyloct-6-en-1-yl stearate (h); Ethyl iso-allocholate (i); Rhodopin (j); Tricyclo[20.8.0.0(7,16)]triacotane, 1(22),7(16)-diepoxy- (k).

## *The Composition of Lime Leaf Ethanol Extract and Its Traditional Medicinal Benefits*

### *Citronellol*

Citronellol is the chemical that gives lime leaves their characteristic scent. This chemical has a retention time of 10.33 minutes and a relative peak of 12.03% on chromatograms. Citronellol belongs to the terpenoid (monoterpenoid) class, is volatile and is present in numerous essential oils, particularly leaf components. The terpenoid chemicals are antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, anticancer, and anti-inflammatory. This category of compounds is also insoluble in water. By reducing oxidative stress, citronellol lowers endothelial dysfunction and slows the course of atherosclerosis in mice and is considered a possible treatment for atherosclerosis in human studies (Lu, 2019). Citronellol has been shown to have anticancer properties by causing apoptosis in the triple-negative breast cancer cell line MDA-MB-231 by inhibiting the anti-apoptotic protein Bcl-2, which activates the pro-apoptotic protein Bax and caspase-dependent apoptosis. Activation of the Caspase-3 protein. As a result, the pace of proliferation of cancer cells and their capacity to travel and survive will be slowed (Ho, 2020). In addition, citronellol was discovered to be harmful to MCF-7 breast cancer cells and to inhibit proliferation by causing apoptosis in Huh7 hepatocellular cells. However, the precise mechanism by which citronellol slows tumor cell development remains unknown and deserves further study (Juliasih & Adnyana, 2023; Yu et al., 2019).

Furthermore, Lopez-Romero et al. (2015) stated that citronellol is the most effective chemical against *Escherichia coli* and *Staphylococcus aureus*, followed by citronellal, carveol, and carvone, according to the study. After exposure to citronellol compounds, changes in hydrophobicity, surface charge, and membrane integrity with subsequent *E. coli* and *S. aureus* leakage were detected. Thus, citronellol has the potential to be conventionally developed as an antibacterial and antimicrobial agent. Felim et al. (2021) stated that *Citrus amblycarpa* (Hassk.) Ochse is highly connected with numerous pharmacological effects, according to previous studies. The safety of Ochse seed ethanol extract (CASEE) at 12.5 and 75 g/mL was demonstrated. CASEE at a concentration of 75 g/mL exhibited a mean reaction of 312.75 to TNF-, 155.19 to IL1-, and 524.41 to PGE-2, whereas CASEE at a concentration of 12.5 g/mL exhibited a mean response of 450.08 to TNF-, 317.26 to IL1-, and 729.66 to PGE-2. The study concluded that CASEE is a promising anti-inflammatory agent due to its efficacy and safety.

Citronellol is a naturally occurring acyclic monoterpene alcohol found in the essential oil of numerous plants, such as lime leaves (*Citrus amblycarpa*

(Hassk.) Ochse). It is often used in perfumes and aromatherapy due to its flowery fragrance. Citronellol has been utilized in traditional medicine for its anti-inflammatory, analgesic, and antibacterial effects (Santos et al., 2019). The medical benefits of lime leaves, which are high in citronellol, include treating fever, cough, and respiratory infections. According to previous studies, citronellol has diverse pharmacological activities, including anti-inflammatory, analgesic, antipyretic, and anticancer activities. It is believed that these features result from its capacity to decrease the activity of cyclooxygenase (COX) enzymes, which are responsible for the production of prostaglandins, which are mediators of inflammation and pain (Desai et al., 2018). The concentration of citronellol in lime leaves varies by species and extraction technique. However, it is typically in relatively high concentrations, making it a plentiful supply of this vitally crucial active chemical.

### *Caryophyllene or (-)-β-Caryophyllene*

Caryophyllene, or (-)-β-caryophyllene, is a natural, whispy sesquiterpene compound in many essential oils. This compound is seen on chromatograms with a retention time of 12.98 minutes and a relative peak of 2.04%. Caryophyllene is a natural sesquiterpene in many plants, including lime leaves (*Citrus amblycarpa* (Hassk.) Ochse). It is often used in perfumes and flavorings because of its spicy, woody scent. Caryophyllene has been utilized for its anti-inflammatory, analgesic, and anxiolytic effects in traditional medicine (Johnson et al., 2020). Caryophyllene has been associated with several positive pharmacological actions, including antioxidant, anti-inflammatory, anticancer, cardioprotective, hepatoprotective, gastroprotective, nephroprotective, antibacterial, and immunomodulatory properties (Machado, 2018). Caryophyllene has also been reported to improve wound healing in a variety of ways (Koyama, 2019; Parisotto-Peterle, 2020) and can be considered a potential candidate for hyperoxaluria-induced renal complication therapy (Xu & Yan, 2021), premature lipid accumulation in preadipocyte 3T3-L1, and improved glucose absorption in myotube C<sub>2</sub>C<sub>12</sub> (Geddo, 2019).

The medical benefits of lime leaves, which are high in caryophyllene, include treating fever, cough, and respiratory infections. According to previous studies, caryophyllene has numerous pharmacological activities, including anti-inflammatory, analgesic, and antioxidant characteristics. It is believed that these features are a result of its capacity to interact with the endocannabinoid system (ECS), a complex cell-signaling system that regulates a variety of physiological and cognitive processes, including pain, mood, and appetite (Gallego-Landin et al., 2021; Stasiulewicz et al., 2020; Zou & Kumar, 2018). Caryophyllene is exceptional

among terpenes because it can interact with the CB2 receptor of the ECS, primarily expressed in immune cells and tissues. This connection is believed to activate the CB2 receptor, which controls the release of different cytokines and other pain and inflammation mediators. In addition to its effects on the ECS, caryophyllene contains antibacterial characteristics that may be useful in treating different illnesses (Handayani et al., 2019; Lopez-Romero et al., 2015; Mulyani, 2014; Shetty et al., 2016).

#### *Hexadecanoic Acid, Ethyl Ester*

Hexadecanoic acid, ethyl ester, is found in lime leaves (*Citrus amblycarpa* (Hassk.) Ochs) as a fatty acid ethyl ester. The chemical compound hexadecanoic acid, ethyl ester, is found in the lime leaves of *Citrus amblycarpa* (Hassk.) ochse (Adorjan & Buchbauer, 2010). With a molecular weight of 284.4772, it is also known as palmitic acid ethyl ester. The chemical is a form of fatty acid ester found in other plants (Budiarto et al., 2017; Kim et al., 2020). While research on the medical effects of hexadecanoic acid ethyl ester is limited, studies have demonstrated that other fatty acids contained in lime leaves, such as palmitic acid, provide various health benefits (Liu et al., 2022). Stearate acid is widely used as a dietary supplement, cosmetics, and other industrial products, such as plastics, soaps, and softened rubber (Astuti & Ramona, 2021). The active component hexadecanoic acid, ethyl ester, is responsible for the larvicidal action against *Aedes aegypti* in the extract of ethyl acetate leaves of *Cassia uniflora* Mill non-Spreng (Toro, 2019). Hexadecanoic acid, ethyl ester, is also the main component in *Moringa oleifera* leaf hydroalcoholic extract and has antihelminthic activity against *Trichuris* sp. and *Ostertagia* sp. (Pedraza-Hernández et al., 2021).

Hexadecanoic acid, a saturated fatty acid that is abundant in lime leaves, has been discovered to possess anti-inflammatory, antioxidant, and antidiabetic characteristics (Adorjan & Buchbauer, 2010; Buathong & Duangsrisai, 2023; Kasman et al., 2020; Liu et al., 2022). It is hypothesized that these features result from its ability to influence multiple signaling pathways, including the NF- $\kappa$ B pathway, a critical regulator of inflammation (Cuevas-Cianca et al., 2023; Liu et al., 2021; Rinkenbaugh & Baldwin, 2016). While there is no specific information on the benefits of hexadecanoic acid, ethyl ester in lime leaves, it probably possesses the same qualities as other fatty acids in the plant. It may also possess qualities that have not yet been identified.

#### *1-Heptatriacotanol*

1-Heptatriacotanol is a type of fatty alcohol found in the leaves of *Citrus amblycarpa* (Hassk.) ochse, also known as kaffir lime or makrut lime. The compound had a retention duration of 19.87 minutes and a maximal

relative area of 4.58 percent. This chemical has been used in traditional medicine to treat fever, coughs, and sore throats, among other ailments. In traditional medicine, 1-heptatriacotanol is commonly consumed as a tea brewed from the kaffir lime plant's leaves. Some individuals also take it topically to treat skin diseases such as eczema and psoriasis. Some research suggests that 1-heptatriacotanol may possess anti-inflammatory and antibacterial characteristics, although its mechanism of action has yet to be entirely understood. It is believed to function by limiting the generation of inflammatory chemicals and preventing the growth of microbes (Dewi et al., 2022).

1-Heptatriacotanol possesses antioxidant, anticancer, and anti-inflammatory effects. Additionally, 1-heptatriacotanol is included in the ethanol extract of *Cnidioscolus aconitifolius* leaves. It has been shown to lower total cholesterol, LDL cholesterol, and triglycerides while elevating HDL. It can treat coronary heart disease (Ezinne et al., 2020). One of the essential components of *Pterocarpus cambodianus* is the antihypercholesterolemic compound 1-heptatriacotanol. Additionally, obtained from *Caralluma* retrospections exudate, 1-heptatriacotanol may have antibacterial effects (Makeen, 2020); it is also derived from *Aloe fleurentiniorum* exudate and is utilized in traditional medicine (Moni et al., 2021). It has been identified in *Nigella sativa* as an antioxidant, anticancer, and anti-inflammatory agent (Hadi, 2016).

#### *Phytol*

Phytol is a plant-based acyclic hydrogenated diterpene alcohol that is a component of chlorophyll. Phytol compounds were observed at a retention time of 21.06 with a relative peak area of 7.50%. It is one of the plant's many phytochemicals that provide various health advantages. Phytol is a valuable essential oil used as a fragrance with potential pharmacological and biotechnology applications. Phytol and its derivatives have been demonstrated to have pharmacological applications in the central nervous system, including anxiolytic and neuroprotective properties (Islam et al., 2018). In recent years, Phytol's possible health advantages have garnered increased attention. Studies indicate that it possesses antioxidant, anti-inflammatory, antidiabetic, and analgesic activities and may also have insect-repellent capabilities (Agustina et al., 2022; Tambunan et al., 2020).

In addition, it may possess antibacterial properties, making it practical for combating bacterial and other microbial illnesses (Islam et al., 2018). Phytol's antioxidant, anti-inflammatory, and analgesic effects have been demonstrated (Hassan et al., 2021). It also has insect-repellent potential. According to previous studies, phytol may help reduce inflammation in the

body, which is considered to play a role in various diseases, including arthritis and cardiovascular disease. Phytol also has analgesic properties, suggesting that it may aid in alleviating pain. This could make it a possible alternative to conventional painkillers. In addition, some research suggests that Phytol may have antibacterial characteristics, indicating that it can combat diseases caused by bacteria and other microbes (Dewi et al., 2022; Islam et al., 2018). Phytol is also reported to have the ability to act as an antimalarial (Saxena, 2018). Phytol suppresses *P. berghei* and can correct some pathological changes induced by *P. berghei* (Usman, 2021). Phytol decreases clinical symptoms in experimental autoimmune encephalomyelitis by regulating NADPH oxidase two expression (Blum et al., 2018). Phytol has been found to have anticancer capabilities by regulating molecular marker expression in human embryonic carcinoma NCCIT cells (Soltanian, 2020).

#### *Ethyl 9,12,15-Octadecatrienoate*

The chemical compound ethyl 9,12,15-octadecatrienoate belongs to the family of fatty acid esters. It is also known as ethyl linoleate and is typically found in linseed and soybean, among other plant oils. Ethyl 9,12,15-octadecatrienoate, also called ethyl linoleate, is a long chain of ethyl ester fatty acids made when the carboxy group of linolenic acid combines with the hydroxy group of ethanol. Ethyl 9,12,15-octadecatrienoate is a polyunsaturated fatty acid with multiple double bonds in its carbon chain. Specifically, it contains three double bonds at positions 9, 12, and 15 in the carbon chain. Ethyl 9,12,15-octadecatrienoate, a polyunsaturated fatty acid, has demonstrated anti-inflammatory and neuroprotective characteristics, which may lessen the risk of chronic diseases such as heart disease, stroke, and Alzheimer's disease (Devassy et al., 2016; Ganesh & Mohankumar, 2017; Giacobbe et al., 2020; Sianipar et al., 2016). Additionally, it may boost mood and cognitive performance. In addition, some research suggests that ethyl 9,12,15-octadecatrienoate may have anticancer potential (Márquez-Fernández & Camargo, 2019). In animal cancer models, linolenic acid, a polyunsaturated fatty acid comparable to ethyl 9,12,15-octadecatrienoate, was reported to have antitumor effects in one study. Additional research is needed to completely comprehend the potential advantages and mechanisms of action of ethyl 9,12,15-octadecatrienoate as a traditional medicine when found in lime leaves.

#### *Methyl glycocholate, 3TMS Derivative*

Methyl glycocholate, a 3TMS derivative, is a chemical molecule discovered in the ethanol extract of lime leaves of *Citrus amblycarpa* (Hassk.) (Budiarto et al., 2017; Kasman et al., 2020). Methyl glycocholate compound, a 3TMS derivative, belongs to the group of

terpenoids with antimicrobial activity. The compound had a retention time of 24.57 minutes and a 3.59% area peak. Methyl glycocholate, a 3TMS derivative, has also been identified from green coffee bean hexane extract, which can act as an antioxidant (Rosiak, 2020). In addition, methyl glycocholate, a 3TMS derivative of lime leaf ethanol extract, has potential as a larvicide and bioinsecticide since it supplies contact and respiratory toxins to the insect body. Due to the acidic nature of this leaf, fragrant aromatics emit clearly, which has ramifications for mosquito mortality and irritation; as a result, it is efficiently utilized as an insecticide agent, but more research is required (Kasman et al., 2020).

#### *3,7-dimethyl oct-6-en-1-yl stearate*

3 The chemical compound 3,7-dimethyloct-6-en-1-yl stearate belongs to the class of fatty acid esters. It consists of a molecule of stearic acid (a long-chain saturated fatty acid) and a group of 3,7-dimethyloct-6-en-1-yl (a branched unsaturated hydrocarbon chain) (Sammi et al., 2016). Compound 3,7-dimethyloct-6-en-1-yl stearate is a fragmentation of citronella seen at a retention time of 26.45 with a relative area peak of 11.45%. Another name for this compound is citronellyl stearate. Due to their propensity to combine with water and oil, fatty acid esters are frequently employed in the food and cosmetic industries as emulsifiers, thickeners, and stabilizers (Watanabe et al., 2017). It is well known for its emollient and conditioning characteristics, which enhance the texture and look of the skin. This chemical has been isolated from various natural sources, including lime leaves (*Citrus amblycarpa* (Hassk.) ochse), utilized in traditional medicine for possible health advantages. In addition, they may have therapeutic qualities, such as anti-inflammatory and antibacterial activities (Sammi et al., 2016). However, the exact effects of 3,7-dimethyloct-6-en-1-yl stearate still need to be fully understood, and additional research is required to evaluate its possible applications in traditional medicine.

#### *Ethyl iso-allochololate*

The chemical compound ethyl iso-allochololate belongs to the class of sterols. It has a similar structure to other sterols, such as sitosterol and stigmasterol, and is derived from cholesterol. Ethyl iso-allochololate has been discovered in various natural sources, including lime (*Citrus aurantifolia*) leaves, utilized in traditional medicine for their possible health advantages. However, there needs to be more investigation on the possible advantages and mechanisms of action of this chemical as a traditional medicine. According to several studies, ethyl iso-allochololate is a steroid derivative that acts as an antibacterial, diuretic, anti-inflammatory, and anti-asthmatic agent (Muthulakshmi et al., 2012).

Additionally, ethyl iso-allocholate was isolated from traditional rice *karungkavuni* and is a selective inhibitor of the enzyme dihydropteroate synthase in *Escherichia coli* (Malathi et al., 2016). *Phyllanthus nivosus* contains ethyl iso-allocate, which possesses anti-inflammatory properties due to its high affinity for the target protein caspase-1 (Johnson, 2020). *Ipomoea obscura* (L.) Ethyl iso-allocholate has also been isolated and is known to have the capacity to prevent the attachment of the SARS-CoV viral genome to the target proteins angiotensin-converting enzyme 2 (ACE2) and major protease (MPro) (Poochi et al., 2020).

### Rhodopin

Rhodopin has been isolated from the ethanol extract of lime leaves (*Citrus amblycarpa* (Hassk.) ochse) as a flavonoid. Flavonoids are recognized for their antioxidant and anti-inflammatory qualities and are commonly found in fruits, vegetables, and medicinal plants, among other natural sources (Adnyana & Sudaryati, 2023). Lime leaves have been utilized in traditional medicine for their potential health advantages, including as an anti-inflammatory agent, to help digestion and to ease headaches. Rhodopin's presence in the ethanol extract of lime leaves may contribute to these possible medicinal effects. Rhodopin possesses antioxidant characteristics, which may protect against oxidative stress and reduce inflammation in the body. Some studies suggest that it may limit the growth and spread of cancer cells (Cuevas-Cianca et al., 2023; Desai et al., 2018; Lim, 2012; Liu et al., 2022; Sammi et al., 2016).

Several studies have indicated that rhodopin compounds belong to the carotenoid group with antioxidant activity (Hassan et al., 2021). Carotenoids are a class of fat-soluble pigments that give various fruits and vegetables in our daily meals their beautiful color, including carrots, tomatoes, pumpkins, peppers, and dark green leafy vegetables (Xavier & Pérez-Gálvez, 2016). Rhodopin is also found in photosynthetic bacteria such as *Rhodospseudomonas palustris* (Faith-Anthony et al., 2014), *Rhodoplanes* sp. (Takaichi et al., 2012), *Thermochromatium tepidum* (Niedzwiedzki et al., 2011), and *Phaeospirillum oryzae* (Lakshmi et al., 2011). Rhodopin can act as an antioxidant and decrease the proliferation of cells with low toxicity in carcinoma cell cultures (Astiti & Ramona, 2021). While additional research is required to completely comprehend the potential advantages and mechanisms of action of rhodopin as a traditional medicine, the substance's antioxidant and anti-inflammatory qualities make it a viable option for further investigation.

*Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-*

*Tricyclo[20.8.0.0(7,16)]* The chemical compound 1(22),7(16)-diepoxy-triacontane belongs to the class of triterpenes. It is a cyclic hydrocarbon with a highly branched structure found in various natural sources. Tricyclo compounds [20.8.0.0 (7,16)] triacontane, 1 (22) and 7 (16)-diepoxy-are compounds that have antioxidant and antibacterial activity, seen at a retention time of 28.97 with a relative peak area of 9.24%. As a traditional medicine, little is known about the possible advantages and mechanisms of action of tricyclo[20.8.0.0(7,16)]triacontane,1(22),7(16)-diepoxy-. Triterpenes have been reported to have anti-inflammatory, anticancer, and antiviral effects, among other potential medicinal applications (Budiarto et al., 2021b; Budiarto & Sholikin, 2022; Khan et al., 2023).

### Conclusion

Based on research findings using gas chromatography–mass spectrometry (GCMS), it was shown that ethanol extracts of lime leaves (*Citrus amblycarpa* (Hassk.) Ochse) contains 75 compound components dominated by 11 principal components, including citronellol, caryophyllene, hexadecanoic acid, ethyl ester, 1-heptatriacotanol, Phytol, ethyl 9,12,15-oate; methyl glycolate, a derivative of 3TMS; 3,7-dimethyloct-6-en-1-yl stearate; methyl iso-allocholate; rhopin; and tricyclo [20.8.0.0(7,16) triacontane, 1(22),7(16)-diepoxy-. The results of the content evaluation show that the compound components in lime leaves have the potential to be developed as traditional medicines for both degenerative and infectious diseases. However, further research is needed precisely to identify the therapeutic effects of using compounds on lime leaves. Lime leaves in Bali have been widely used as antioxidants, anticancer agents, anti-inflammatory agents, and flavor enhancers for food. In the future, further research is needed to comprehensively evaluate the compound content of lime leaves for their potential as candidates for traditional medicine, especially in Bali.

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### Author Contributions

I.N.A: conceptualization, supervisor; N.K.A.J: resources, commentary; A.A.A.S.S.W: data curation. All authors performed initial manuscript writing, and final manuscript approval.



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## Conflicts of Interest

The authors declare that there is no conflict of interest.

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