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Phytochemical screening and GC-MS analysis of local durian (*Durio zibethinus*) leaf extract from Criwik, Rembang, Central Java, Indonesia

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Abstract. *Sawitri AD, Yuniastuti E, Purwanto E, Parjanto. 2025. Phytochemical screening and GC-MS analysis of local durian (*Durio zibethinus*) leaf extract from Criwik, Rembang, Central Java, Indonesia. Biodiversitas 26: 55-61.* Durian (*Durio zibethinus*), an important tropical fruit in Indonesia, is widely known for its unique flavor and potential health benefits. Despite extensive research on the nutritional and pharmacological properties of its fruit, the bioactive potential of its other plant parts, particularly the leaves, remains limited. This study explored the phytochemical profile and bioactive compounds in the leaf extract of Criwik durian, a local variety from Rembang, Central Java, Indonesia. Phytochemical screening confirmed the presence of flavonoids, tannins, alkaloids, saponins, and phenolic compounds, all known for their diverse pharmacological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. Importantly, this is the first study to report the presence of phytochemical in the durian leaf extract. Additionally, gas chromatography-mass spectrometry analysis was conducted to characterize the bioactive components, which identified 11 distinct compounds. These included squalene, ethyl iso allocholate, neophytadiene, and phytol, all associated with health remedies and pharmacological effects. These results provide a detailed chemical profile of Criwik durian leaves, highlighting their potential as a valuable resource for the development of natural health products and herbal remedies. These findings enhance our understanding of the bioactive potential of durian leaves and encourage further research into their medicinal applications. Thus, Criwik durian leaves could serve as a promising source for the future exploration of natural health product development and sustainable medicinal practices.

Keywords: Bioactive compounds, Criwik durian, *Durio zibethinus*, natural health products, secondary metabolites

INTRODUCTION

Durian (*Durio zibethinus* Murray), which belongs to the Malvaceae family, is a fruit indigenous to Southeast Asia and is widely distributed across the region (Subhadrabandhu and Ketsa 2001; Small and Catling 2006). This exotic fruit is easily recognized by its distinct characteristics: a strong, pungent odor that can be detected from a distance and a tough, thorn-covered rind that may appear intimidating to those encountering it for the first time (Small and Catling 2006). The odor of durian is primarily attributed to volatile esters and sulfur compounds, which often provoke mixed reactions (Peng 2019). Nonetheless, its unique flavor is highly valued by enthusiasts as a component hallmark of regional cuisine (Small and Catling 2006; Bender 2017). Revered as a delicacy across East and Southeast Asia, durian holds considerable cultural and culinary significance (Ryan 2024).

In Indonesia, durian is often referred to as the "King of Fruits," owing to its significant economic and commercial importance (Thorogood et al. 2022). According to data from the Central Bureau of Statistics of Indonesia, nationwide durian production increased steadily from 2021 to 2023 (Ministry of Agriculture 2024). Production volumes increased from 1,353,037 tons in 2021 to 1,582,172 tons in 2022 and further to 1,852,045 tons in 2023, representing a significant growth rate. This surge positioned durian among the top five most-produced fruits in Indonesia. The highest-producing provinces included East Java (0.48 million tons), Central Java (0.20 million tons), West Java (0.18 million tons), South Sumatra (0.14 million tons), and South Sulawesi (0.09 million tons).

Indonesia is home to 20 different durian species that are distributed across regions such as Kalimantan, Sumatra, Java, Bali, Sulawesi, and Maluku (Sudarmono et al. 2023). Among these varieties, the Criwik durian, originating from Rembang, Central Java. is distinct from the other local varieties because of its round shape and relatively smaller size (Figure 1). This variety has been described by botanist team (i.e Endang Yuniastuti, Nandariyah, Andini Desi Sawitri, and Siswanto) and is officially registered with the Center for Plant Variety Protection and Agricultural Licensing as a local variety with registration number 590/PVL/2018.

The fruit of durian, well-known for its unique aroma and taste (Xiao et al. 2022), has also been widely studied for its nutritional properties, particularly its pharmacological potential (Arsa et al. 2021). Additionally, recent studies have highlighted the health-promoting properties of different parts of durian plants, including leaves, wood bark (Adeniyi et al. 2019), and seeds (Adeniyi et al. 2024). Further studies are needed,

particularly on the nutritional content of other parts of the durian plant, such as the leaves, which have traditionally been used to treat various ailments, including fever and malaria (Mohd-Ali et al. 2020).

Research on leaf extracts has been primarily driven by the presence of phytochemical compounds with potential functions and therapeutic properties (Lim et al. 2023). Durian leaves contain various phytochemical compounds, including flavonoids, phenolics, alkaloids, and terpenoids, with potential health benefits (Manurung et al. 2022). Leaf extracts from *D. zibethinus* demonstrate significant antidiabetic effects in both in vivo and in vitro studies (Chigurupati 2021). The flavonoids and steroids in the ethanol extracts of *D. zibethinus* can prevent diabetes and its complications. The leaf extracts can lower glucose uptake and improve glucose tolerance (Aruan et al. 2019). Moreover, the antioxidant and bioactive compounds found in durian plants provide opportunities for the development of herbal remedies and other health-related products. Consequently, understanding phytochemical content is essential for the development of natural health products.

To date, no studies have been conducted to determine the chemical composition of Criwik durian. This study aimed to identify the secondary metabolites and bioactive compounds in the leaves of Criwik durian through phytochemical screening and Gas Chromatography-Mass Spectrometry (GC-MS). It is hoped that this research will contribute to the development of medicinal plants, especially in Rembang District, Central Java, Indonesia.

MATERIALS AND METHODS

Collection of leaf samples

Leaves from the Criwik variety of durian (Figure 1.C) were collected from Criwik District, Rembang District, Central Java, Indonesia (-06.70627960, 111.49510450) on May 15, 2024. Only healthy, intact, spotless leaves were collected. The collected leaves were stored in plastic containers, and their extraction and analysis were performed at the Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada, Yogyakarta, on May 29, 2024.

Preparation of plant extracts

Plant extracts were prepared following the method described by Manurung et al. (2022). The collected durian leaves were thoroughly washed with clean water and air-dried in the shade for two weeks. The dried leaves were then chopped and ground into powder, yielding 500 g of powder.

Figure 1. Characteristics of the Criwik durian. A. Tree morphology; B. Bark texture; C. Leaf structure; D. Whole fruit; E. Opened fruit displaying flesh; F. Seed morphology

Screening of bioactive compounds

Tannin

The powdered leaf sample (100 mg) was combined with 50% ethanol and vortexed. A 20 µL aliquot was spotted onto a silica gel plate and placed in a chamber saturated with the mobile phase ethyl acetate-acetic acid-formic acid-water (100:5:5:5). The plate was eluted until the solvent front travelled to the desired limit, dried, observed under UV light, and sprayed with ferric chloride reagent (Merck, MA, USA). The UV wavelengths used were 254 nm and 365 nm. A visible grey-blue spot indicated the presence of tannins.

Alkaloids

The powdered sample (100 mg) was mixed with 10% ammonia and extracted with chloroform. The chloroform phase was collected, and 20 µL of the extract was spotted onto an F254 silica gel plate. The plate was placed in a chamber saturated with mobile phase methanol: ammonia (100:1.5) and eluted until the solvent front travelled to the desired limit. The dried plate was removed and sprayed with Dragendorff's reagent (Merck, MA, USA). The UV wavelengths used were 254 nm and 365 nm. A visible orange spot indicated the presence of alkaloids.

Flavonoid

The powdered sample (100 mg) was mixed with 1 mL of ethanol, vortexed, and sonicated for 60 min. The samples were then macerated for 24 hours. After centrifugation, the supernatant was collected, and 10 µL of the extract was spotted onto a silica gel plate, included with quercetin as the comparator. The plate was placed in a chamber saturated with the mobile phase n-hexane/ethyl acetate/formic acid (6:4:0.1) and eluted until the solvent front travelled to the desired limit. The dried plate was observed under UV light. The UV wavelengths used were 254 nm and 365 nm. A visible yellow spot indicated the presence of flavonoid.

Phenolic compounds

The powdered sample (100 mg) was mixed with 1 mL ethanol, vortexed, and sonicated for 60 min, followed by maceration for 24 h. The supernatant was obtained after centrifugation, and 10 µL of the extract was spotted onto a silica gel plate, included with gallic acid as the comparator. The plate was placed in a chamber saturated with the mobile phase of 10% methanol and formic acid (95:5) and eluted until the solvent front travelled to the desired limit. The dried plate was observed under UV light and sprayed with a ferric chloride reagent (Sigma Aldrich, Missouri, USA). The UV wavelengths used were 254 nm and 365 nm. A visible gray-black spot indicated the presence of phenolic compounds.

Saponin

To 100 mg of the powdered sample in a flask, 10 mL of 4 N sulfuric acid was added. The mixture was hydrolyzed and refluxed with reverse cooling for 30 min, after which 5 mL of chloroform was added for extraction. The chloroform phase was collected. The solvent was evaporated using nitrogen to a final volume of 500 µL. A 50 µL aliquot was spotted on a 60 F254 silica gel plate and eluted until the solvent front travelled to the desired limit in the chamber containing saturated mobile phase chloroform: methanol (95:5). After drying, the plate was observed under UV light and sprayed with sulfuric acid anisaldehyde reagent (Sigma Aldrich, Missouri, USA) followed by heating at 110°C to achieve maximum spotting. The UV wavelengths used were 254 nm and 365 nm. The presence of saponins was indicated by a visible purple-blue spot.

Gas chromatography-mass spectrometry analysis

GC-MS effectively separates chemical mixtures through its Gas Chromatography (GC) components and identifies volatile compounds at the molecular level via Mass Spectrometry (MS). GC is based on the principle that heating a mixture decomposes it into its components. Furthermore, MS functions by passing the separated substances through its inlet, where they are identified based on their molecular mass. It is feasible because only small particles from GC are introduced into MS (Manurung et al. 2022).

The powdered leaf (1 mg) sample was dissolved in 1.5 mL MeOH in a microtube, vortexed until homogeneous, and centrifuged at 9500 rpm for 5 min. The supernatant collected after centrifugation was transferred to a GC vial for injection. GC-MS analysis was conducted using Thermo Scientific™ TRACE 1310 GC equipped with Thermo Scientific™ ISQ LT Single Quadrupole MS (Thermo Fisher Scientific, Waltham, MA, USA), with HP-5MS-UI column 30 m in length and a maximum temperature of 325-350°C. The GC-MS conditions were as follows: helium was used as the carrier gas; the injector temperature was 230°C; the split flow was maintained at 50 mL/min with a split ratio of 1:50. The oven temperature was set between 60-280°C at a ramp rate of 10°C/min. Data were analyzed using NIST (National Institute of Standards of Technology) Library software.

When multiple peaks with similar potential compound identities were detected, the average concentration of these compounds was calculated using the following method,

$$\text{Percentage Content (\%)} = \left(\frac{\text{Total Peak Area of Target Compound}}{\text{Total Peak Area of All Compounds}}\right) x\ 100$$

Where, Total Peak Area of Target Compound: The sum of the intensities of all occurrences of the target compound within the specified retention time range; Total Peak Area of All Compounds: The sum of the intensities of all detected compounds within the same retention time range.

RESULTS AND DISCUSSION

Phytochemical screening

A phytochemical study of the local durian leaf extract (*D. zibethinus*) from the Criwik region revealed the presence of several bioactive compounds with potential pharmacological effects. Qualitative tests revealed the presence of flavonoids, tannins, alkaloids, saponins, and

phenolic compounds (Table 1). These findings suggest that durian leaf extract could serve as a natural source of bioactive ingredients with potential health benefits.

Phytochemical screening of the ethanol extract of durian leaf, reported by Aruan et al. (2019), identified secondary metabolites, such as flavonoids, terpenoids/steroids, and glycosides, while alkaloids and saponins were not detected. In contrast, the present study identified similar secondary metabolites in the leaves of Criwik durian varieties, along with the additional presence of alkaloids and saponins. Moreover, phytochemical screening of leaf extracts from Durian Lai (*D. kutejensis* (Hassk.) Becc.) from Kalimantan revealed no tannin (Manurung et al. 2022). In contrast, tannin was detected in the local durian Criwik. These findings highlight distinct differences among durian varieties.

Previous studies on other parts of the durian plant reported similar findings. For example, phytochemical screening of durian fruit rind from the R16 cultivar in Tien Giang, Vietnam, indicated the presence of flavonoids (Nguyen et al. 2024). In contrast, the Soya variety from Maluku, Indonesia, contained alkaloids, flavonoids, saponins, phenols, and tannins (Ahmad et al. 2024). Similarly, the analysis of methanol extracts from durian wood bark collected from local farms in Nigeria revealed the presence of phenols, alkaloids, steroids, tannins, terpenes, saponins, and flavonoids (Adeniyi et al. 2019). Additionally, flavonoids have been identified in ethanol extracts of durian seeds of a local durian variety from the Simalungun District, North Sumatra (Aisyah et al. 2024).

Tannins are widely distributed across the plant kingdom and are commonly found in the bark, leaves, seeds, and stems of plants. These compounds play a protective role by deterring herbivory and also regulate plant growth (Barbehenn and Constabel 2011). Tannins are also present in various food products, including tea and wine (Ashok and Upadhyaya 2012). They can be extracted using a variety of solvents and methods, including water, methanol, ethanol, acetone, and ionic liquids (Das et al. 2020). High tannin content correlates with high antioxidant activity, as tannins are polyphenolic compounds with free radical-scavenging properties. Tannins, as active secondary metabolites, possess anti-diarrheal, antibacterial, astringent, and antioxidant properties.

Saponins are a diverse group of amphiphilic glycosides derived from steroids and triterpenes found in various plants and marine organisms such as seaweeds, starfish, sea cucumbers, and small fish (Feroz 2018; Xiao et al. 2019; Smith et al. 2024).

Table 1. Phytochemical screening of Criwik local durian leaves

Phytochemical constituent	Result
Flavonoid	+
Phenol	+
Alkaloid	+
Saponin	+
Tannin	+

Saponins are composed of two components, namely hydrophilic (glycone) and hydrophobic (aglycone) (Kamyab et al. 2018) and exhibit a wide range of biological and pharmacological activities because of the structural diversity of their sugar chains and aglycones. They serve as key active constituents in traditional remedies, particularly in traditional Chinese medicine (Yang et al. 2014). Saponins possess a variety of biological functions and medicinal benefits, including hemolytic, anticancer, anti-inflammatory, antifungal, insecticidal, antibacterial, antiviral, cytotoxic, and molluscicidal effects. In addition, saponins have demonstrated cholesterol-lowering properties in both animal and human studies (Mohamed et al. 2019).

Flavonoids are a diverse class of polyphenolic compounds which serve as secondary metabolites produced in plants and certain microorganisms (Pandey et al. 2016). They encompass several subclasses, including anthocyanidins, isoflavones, flavanones, flavonols, and flavones, and are known for their wide range of biological activities (Dias et al. 2021). These include anti-inflammatory, antimicrobial, anticancer, antiviral, antioxidant, cardioprotective, and neuroprotective effects (Frent et al. 2024). The growing recognition of their biological properties has driven their increasing use in various industrial applications, including medicines, pharmaceuticals, cosmetics, food, and nutraceuticals, where their functional and therapeutic potentials are highly valued (Dias et al. 2021; Liga et al. 2023).

Phenolic compounds are secondary metabolites with diverse biological activities such as antidiabetic, antioxidant, anti-allergic, anti-inflammatory, anti-atherosclerotic, antimicrobial, prebiotic, and antimutagenic effects. Additionally, new sources of phenolic compounds are continually being explored (Nurzyńska-Wierdak 2023). Alkaloids, another significant class of secondary metabolites, are basic compounds containing nitrogen atoms in their structure, with amino acids serving as the building blocks for alkaloid biosynthesis. Alkaloids exhibit a wide range of physiological properties, including anti-inflammatory, antibacterial, local anesthetic, antitumor, antimitotic, analgesic, hypnotic, and psychotropic effects (Kurek 2019).

In this study, we focused on detecting the presence of flavonoids, tannins, alkaloids, saponins, and phenolic compounds. Therefore, further investigation regarding the total content of these compounds is necessary. Investigations in this area will extend our understanding of the chemical properties of the local Criwik durian leaf extract, addressing the limitations of the present study.

GC-MS analysis

The active compounds in the Criwik durian leaf extract were analyzed using GC-MS, which identified 11 compounds (Table 2). Figure 2 illustrates 21 peaks, of which 11 were identified. The major compounds identified were squalene (26%), ethyl iso allocholate (18%), neophytadiene (13%), and phytol (11%). Other compounds detected in small amounts included cholestan-3-one, cyclic 1,2-ethanediyl acetal, (5ß)-, 10-octadecenoic acid, methyl

ester, 5H-cyclopropa[3,4]benz[1,2-e]azulen-5-one, 3,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-2-chloro-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-, [1aR-(1aa,1bß,2a,3ß,4aß,7aa,7ba,8a,9ß,9aa)]-, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, ethanol, 2-(9-octadecenyloxy)-, (Z)-cyclopropane butanoic acid, 2-[[2-[[2-[(2-pentyl cyclopropyl) methyl] cyclopropyl] methyl] cyclopropyl] methyl]-, and methyl ester. Several of these compounds are known for their biological activities, including anti-inflammatory, antibacterial, and antimicrobial properties. Additionally, they serve as components of vitamins and plant metabolites. However, it is important to note that the sample amount analyzed in this study was limited; therefore, the findings should be considered preliminary and warrant further investigation.

The present study provides new insights into the chemical composition in Criwik durian leaf extract, analyzed using the GC-MS method. To date, the only prior study reporting the chemical profile of methanolic durian leaf extracts of *D. kutejensis* via GC-MS was conducted by Manurung et al. (2022). However, our findings differ from those reported in that study, which identified 43 compounds, including Palmitaldehyde, Diisopentyl Acetal, E7-Decenyl Acetate, Estran-3-one, 17-(acetyloxy)-2-methyl-, (2α,5α,17β)-, and Ledol. Notably, none of these compounds were detected in the present study. This discrepancy suggests that different *Durio* species or varieties may exhibit distinct chemical profiles, warranting further investigation to validate this hypothesis.

Furthermore, a study by Adeniyi et al. (2019) utilizing GC-MS to analyze the methanolic extract of *D. zibethinus* wood bark reported 2–4 compounds across four different fractions, none of which corresponded to the compounds identified in our study. Similarly, an analysis of the n-hexane extract from *D. zibethinus* seeds by Adeniyi et al. (2024) revealed 12 compounds, with the most abundant being Triacontanediol (34.47%), Squalene (32.75%), Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl- (29.34%), and Phytol (1.36%). Interestingly, Squalene (26.17%) and Phytol (10.85%) were also identified in the Criwik durian leaf extract in our study, indicating some chemical similarities between the leaf and seed extracts. These results highlight intriguing similarities and differences across various parts of *Durio* species.

Table 2. Identified component of leave extract from Criwik local durian

Number	Compound name	Retention Time (RT)	% Content
1	Neophytadiene	15.82	12.72
2	Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate	15.89	0.56
3	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	15.07	2.20
4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	16.25	3.49
5	Ethyl iso-allocholate	16.66-30.07	23,80
6	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentyl cyclopropyl) methyl] cyclopropyl] methyl] cyclopropyl] methyl]-, methyl ester	16.71-18.61	3.97
7	10-Octadecenoic acid, methyl ester	18.38	5.46
8	Phytol	18.54	10.85
9	Squalene	24.18	26.17
10	Cholestan-3-one, cyclic 1,2-ethanediyl acetal, (5ß)-	27.20	6.22
11	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 3,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-2-chloro-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-, [1aR-(	28.97	4.56

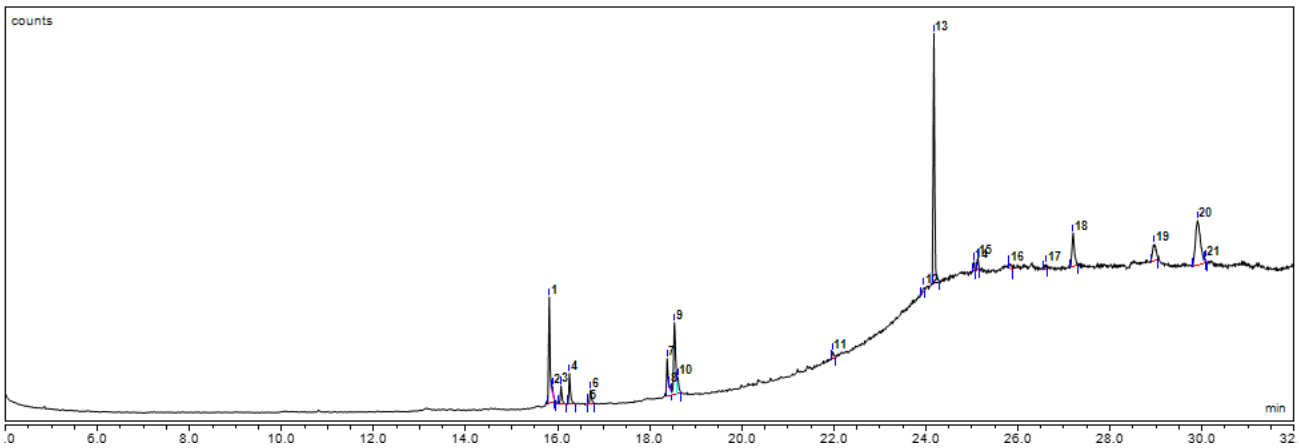

Figure 2. GC-MS chromatogram of Criwik local durian leaf extract. The names of the compounds identified at each retention time are listed in Table 2

Neophytadiene is a class of diterpenes that is present in the extracts of *Crataeva nurvala* Buch.-Ham. and *Blumea lacera* (Burm.fil.) DC. Neophytadien exhibits anxiolytic activity, suggesting its use to prevent anxiety as well as its anticonvulsant properties to prevent and treat seizures (Gonzalez et al. 2023). Moreover, neophytadiene from *Turbinaria ornata* (Turner) J.Agardh extract demonstrated significant anti-inflammatory potential. Lipopolysaccharide-induced neophytadiene possesses antioxidant, anti-inflammatory, and cardioprotective properties (Bhardwaj et al. 2020). A laboratory-scale assay using the leaf extracts of *Froriepia subpinnata* (Ledeb.) Baill. and *Grewia bulot* Gagnep., which contain neophytadiene, demonstrated that this antioxidant agent may hold anticancer properties (Pham et al. 2023; Rostamabadi et al. 2023).

Phytol is an isoprenoid alcohol linked via an ester bond to chlorophyll, the predominant photosynthetic pigment in plants. Significant quantities of phytols are released during chlorophyll degradation during leaf senescence (Gutbrod et al. 2021). Phytol is commonly utilized in the fragrance, pharmaceutical, and biotechnological industries (Islam et al. 2018). They exhibit antioxidant, anti-inflammatory, cytotoxic, and antimicrobial properties (Taj et al. 2021). Additionally, phytol exhibits a range of effects, including anxiolytic, metabolism-modulating, autophagy- and apoptosis-inducing, antinociceptive, and immune-modulating (Islam et al. 2018).

Ethyl iso-allocholate is proposed to be a sterol compound, exhibiting various properties including antibacterial, antioxidant, and antitumor, and serves as a pesticide and chemopreventive agent (Tyagi and Agarwal, 2017). Moreover, it functions as an antimicrobial agent and has the potential to treat bacterial infections (Malathi et al. 2016). In addition, ethyl iso acetylcholine from Trigonella foenum graecum extract induced apoptosis in A546 lung cancer cells by activating the caspase signaling pathway (Thakur and Ahirwar 2019). Ethyl iso-allocholate was detected nine times within the Retention Time (RT) range of 16.66–30.07 minutes. In a related study on rice Karungkavuni (Malathi et al. 2016), ethyl iso-allocholate was observed at RT 42.13, suggesting that the RT range for ethyl iso-allocholate in the current study remains within detectable limits.

Squalene is a naturally occurring organic compound commercially extracted from shark liver oil. It is also present in various plant-based sources, including olive oil, wheat germ oil, amaranth oil, palm oil, and rice bran (Lippi et al. 2010). The multifunctional roles and biological activities of squalene have been demonstrated both in vivo and in vitro (Huang et al. 2009). It is widely recognized for its applications as a skin moisturizer, anticancer and antiviral agent, skin immunity enhancer, and drug delivery carrier. Moreover, the consumption of squalene-rich oils decreases the risk of heart disease (Ardhyni et al. 2022). Ultrasonic-assisted extraction is a suitable method for obtaining crude extracts with maximum antioxidant activity and squalene content from durian leaf waste (Kam 2017).

In conclusion, this study revealed, for the first time, the key bioactive compounds in Criwik durian leaf extract, highlighting their potential therapeutic benefits. Phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, saponins, and phenolic compounds. Furthermore, GC-MS analysis identified 11 notable compounds, including squalene, ethyl iso allocholate, neophytadiene, and phytol. These findings suggest that Criwik durian leaf extract may serve as a valuable natural source of bioactive compounds with potential health applications. Therefore, further studies are warranted to evaluate and quantify these bioactive compounds and to investigate their specific pharmacological potential through clinical trials for the development of healthcare products.

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