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Phytochemical, GC-MS analysis and antioxidant activities of leaf methanolic extract of Lai (*Durio kutejensis*), the endemic plant of Kalimantan, Indonesia

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Abstract. *Manurung H, Susanto D, Kusumawati E, Aryani R, Nugroho RA, Kusuma R, Rahmawati Z, Sari RD. 2022. Phytochemical, GC-MS analysis and antioxidant activities of leaf methanolic extract of Lai (*Durio kutejensis*), the endemic plant of Kalimantan, Indonesia. Biodiversitas 23: 5566-5573.* Lai (*Durio kutejensis* Hassk. Beck) is a fruit plant species endemic to Kalimantan, Indonesia. The leaves are traditionally used to treat several diseases. Currently, there is no scientific validation of the phytochemical content, bioactive compounds, and biological activities of leaf extracts of this plant from the East Kalimantan Province of Indonesia. So, this study aimed to determine the phytochemical compounds, bioactive compounds, and antioxidant activity of crude leaf methanol extract of *D. kutejensis*. Gas chromatography-mass spectrometry (GC-MS) analysis was performed to determine the bioactive compounds of lai leaf methanol extract. DPPH (2,2-diphenyl-1-picrylhydrazyl) was used to evaluate antioxidant activity. Screening of phytochemical compounds of the leaf methanol extract showed the presence of alkaloids, flavonoids, phenolics, saponins, and steroids. The leaf extract of lai contains total phenolic (104,55 µg GAE g^{-1} extract) and total flavonoid (281,41 µg QE g^{-1}extract). The extract has weak antioxidant activity with an IC_{50} of 163,849 ppm. GC-MS analysis confirmed the presence of 43 identified compounds in the crude leaf methanolic extract of *D. kutenjesis* such as; 2-(1,1-dimethyl ethyl)-Phenol; 4-(3-hydroxy-1-propenyl)-2-methoxy-Phenol (Coniferyl alcohol); E7-Decenylacetate; Octadecanoic acid (Stearic acid); 1,3,6-Octatriene, 3,7-dimethyl-, (Z)-; Palmitaldehyde, diisopentyl acetal; Ledol; Estran-3-one, 17-(acetyloxy)-2-methyl-, (2.alpha.,5.alpha.,17.beta.)-. This study revealed that the leaf methanol extract of *D. kutejensis* is a good resource of many bioactive compounds that justifies the traditional usage of this species.

Keywords: Bioactive compounds, *Durio kutejensis*, endemic plant, GC-MS, Lai

INTRODUCTION

Indonesia is one of the largest producers of medicinal herbs and is appropriately called the botanical garden of the world. About 5,000 species of medicinal plants can be found in Indonesia's Medicinal Herb Index. Empirically, Indonesian herbal medicines are commonly known as *Jamu*, a freshly prepared plant material, usually in water extracts (Woerdenbag and Kayser 2014; Arozal et al. 2020).

East Kalimantan is one of the provinces in Indonesia located in a tropical rainforest with several potential endemic florae. Some of these endemic plants from Kalimantan have been proven to have medicinal properties, including tabat barito (*Ficus deltoidea*), dayak onions (*Eleutherine bulbosa*), pasak bumi (*Eurycoma longifolia)*, kayu kuning (*Arcangelisia flava***)**, karamunting (*Rhodomyrtus tomentosa*) (Sutomo et al. 2013; Manurung 2018), lai (*Durio kutejensis*), mekai (*Albertisia papuana*) (Priyanti 2012; Nurbani and Sumarmiyati 2015) and tanikkara (*Dillenia exelsa*) (Lisdiani et al. 2022). The use of plants as a source of medicine has been inherited and is an important component of the health care system. Plants used in traditional medicine contain a wide range of substances to treat chronic and infectious diseases.

Lai (*Durio kutejensis* Hassk Becc) is an endemic plant that grows very well in Kalimantan, including East Kalimantan. It belongs to Malvaceae (Bombaceae) family. All parts of the plant *D. kutejensis*, including fruit, bark, and leaves, can be explored for their pharmacology and bioactivity. Local people (the Dayak tribe) use the lai fruit as a food source, directly eaten or processed food such as lempok or dodol durian. The previous study revealed that lai fruit extract had been shown to have antioxidant and anti-melanogenesis activity (Arung et al. 2015), fruit peel extract shows antibacterial activity (Muhsin et al. 2016), fruit flesh, besides being consumed directly, it can be processed as fruit juice drinks (Yuliana et al. 2016). In addition, the bark extract of lai contains secondary metabolites, including triterpenes, quinones, and coumarins, that can be used as inhibitors of melanin

formation to reduce skin hyperpigmentation (Rudiyansyah and Garson 2006).

Young leaves of *D. kutejensis* were used as fresh vegetables and cosmetic ingredients by local people in Kalimantan. Boiled water from lai leaves is also used to treat diarrhea. However, these natural compounds from this plant are still under investigation and are not recommended as a replacement for standard pharmacotherapies. Nevertheless, using natural compounds of the plant is common in Indonesia. The community widely uses medicinal plants, even though rigorous clinical trials have not thoroughly assessed their effectiveness and safety (Woerdenbag and Kayser 2014).

The availability of lai is abundant throughout the seasons in Kalimantan. Traditionally, local people use lai leaves to treat diarrhea and fever and as a cosmetic ingredient. However, research on the phytochemicals, bioactivity, secondary metabolites content, and active compounds in lai leaves has not been carried out yet. In recent years, gas chromatography-mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species (Khantal et al. 2014). GC-MS instrument separates chemical mixtures (the GC component) and identifies the components at a molecular level (the MS component). The GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule. So far, no reports related to lai's (*D. kutejensis*) chemical components. The present study's purpose was to identify secondary metabolites of lai leaf and their bioactivities.

MATERIALS AND METHODS

Collection of plant

The *D. kutejensis* leaves were collected in June 2021 from the Universitas Mulawarman area and authenticated by a botanist from the Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Mulawarman, Samarinda, East Kalimantan, Indonesia.

Preparation of plant extracts

The mature leaves of *D. kutejensis* were collected, washed with clean water, and air-dried in a shaded room for two weeks. The dried leaves are chopped into small pieces and then pulverized into powder using an electric blender. A total of 500 grams of lai leaf powder was macerated using 98% methanol, incubated, and shaken at 150 rpm for 3 days. The leaf extract was filtered using Whatman no.1 filter paper. The filtrate was evaporated using a rotary evaporator at 40^{o}C so that the crude extract was obtained 12.275 g crude extract, stored at 4^{o}C for further use.

The screening of phytochemical

One gram of leaves was dissolved in 100 mL methanol and subjected to preliminary phytochemical screening, i.e., alkaloids, tannins, saponins, phenolic, flavonoids, steroids, triterpenoids, glycosides, and carotenoids following standard methods (Iqbal et al. 2015) with slight modification.

Screening of bioactive compounds of the crude extracts

Total phenolic content

The Folin-Ciocalteu protocol was used to determine the total phenolic content (TPC) of crude methanolic extract of *D. kutejensis* leaves (Manurung et al. 2019). Gallic acid (0-100 µg/mL) was used as a reference standard for the calibration curve. First, methanolic extract (1mg) was diluted with 10 mL DMSO. Then 1 mL was taken out and mixed with 0.4 mL distilled water, 0.25 mL Folin-Ciocalteu reagent (10%, v/v), 1.25 mL sodium carbonate (7.5%, w/v) and stirred vigorously using a vortex. The solution was incubated in a dark room for 60 minutes, after which the absorbance was measured at 760 nm using UV-VIS 1200 spectrophotometer. The absorbance of the sample was incorporated into the linear regression curve of the gallic acid standard, and the TPC was expressed as mg/g gallic acid equivalent (GAE) of dry extract.

Total flavonoid content

Total flavonoid content was estimated by using the Aluminum chloride colorimetric technique (ACCT) method by the reported procedure of Manurung et al. (2017) with slight modification. Quercetin was used as a standard to establish the calibration curve. One mg of quercetin was dissolved in 10 mL DMSO and then diluted to the concentration of 2,4,6,8,10 µg/mL. An extract solution was made by dissolving a-1 mg methanolic extract with 10 mL DMSO. One mL extract solution was added to 0.7 mL distilled water, 0.1 mL NaNo2 5%, 0.1 mL AlCl3 10% and 0.5 mL 1M NaOH and then incubated for 10 minutes in a dark room temperature. The absorbance was read at 510 nm by using UV-VIS spectrophotometer against a blank. The total flavonoid content of *D. kutejensis* leaves methanolic extract was calculated from the standard curve as quercetin equivalent per milligram of crude extract.

GC-MS analysis

The bioactive compounds of crude methanol extract were identified by GCMS (Shimadzu QP 2010S) analysis as described by Maharani et al. (2016) and Wiley/NIST library software used for the data analysis. The analysis was performed using Rtx-column type 5 ms, 30 m length (Restek Corp). The injector and detector temperature were maintained at 250^{o}C, while the operating temperature was 50-300^{o}C. The column temperature was programmed at 50-120^{o}C, with a 4^{o}C increase per min which was maintained for 1 min, and then it was programmed at 120-300^{o}C, with a 6^{o}C increase per min, and held on for 5 min, with retention time (Rt) total for 80 min. Helium was used as a carrier gas in 50-500 atomic mass units (AMU). Electron ionization (EI) was carried out at 70 eV.

Antioxidant activity

The antioxidant activity of crude methanolic extract of *D. kutejensis* leaves was investigated by the DPPH method (Manurung et al. 2019). First, three mg of crude extract was dissolved in 1 ml ethanol and used as a stock solution. Dilution was made to obtain 200, 100, 50, 25, 12.5, and 6.25 ppm concentrations. Then, 33 µL of each diluted solution was added to 467 µL of ethanol and 500 µL of 27% DPPH solution and incubated for 20 minutes in the darkness. Ascorbic acid was used as a positive control. The blank was prepared similarly without extract or ascorbic acid. The absorbance was measured using a UV-VIS spectrophotometer at 517 nm. The percentage of antioxidant activity was calculated using the equation:

$$\%\ antioxidant\ activity = \frac{abs\ control - abs\ sample}{abs\ control}\ x\ 100\ \%$$

The IC_{50} value was calculated using linear regression of plots where the abscissa represented the concentration of extract solution, and the ordinate was the percent of the antioxidant activity.

Data analysis

The results of phytochemical and antioxidant studies were expressed as mean values ± standard deviation of three replicates. In addition, the antioxidant activity value was calculated using linear regression analysis based on the IC_{50} value.

RESULTS AND DISCUSSION

Phytochemical screening

The preliminary phytochemical study revealed that the methanolic extract of *D. kutejensis* leaves contains alkaloids, flavonoids, phenolics, saponins, and steroids, as presented in Table 1.

Recently, phytochemical compounds in plants have become the center of attention due to their considerable health benefits, especially for treating several human diseases (Zelotek et al. 2016). However, research on the phytochemical content of various plants is still being carried out to determine the active natural compounds for medicines (Bagawan et al. 2022). For example, research on the methanol extract of *D. kutejensis* leaves proves that this plant contains several phytochemical compounds that might have bioactivities. Local people in East Kalimantan, Indonesia, use *D. kutejensis* leaves as a medicine for diarrhea, to treat fever, and as a cosmetic ingredient.

Alkaloids are important chemical compounds that serve as a rich source for drug discovery. Alkaloids have pharmacological activities, such as increasing blood pressure, reducing pain, and fighting microbial infections. Alkaloids possess antibacterial (Yan et al. 2021), anticancer (Mondal et al. 2019), anti-inflammatory (Bai et al. 2021), and antimalarial activity (Dua et al. 2013). In plants, alkaloids are toxic substances against insects or plant-eating animals because of their bitter taste. Phenolic is one of the antioxidant compounds. Phenolic compounds play an important role in electron transport in photosynthesis, cytokinin activity, and growth promoters. Phenolics absorb UV light and have anti-inflammatory activity. Plant phenolics are secondary metabolites that have gained importance as potential anti-cancer by promoting apoptosis, reducing proliferation, and targeting various aspects of cancer (angiogenesis, growth and differentiation, and metastasis) (Abotaleb et al. 2020). In addition, phenolic compounds have high antioxidant activity (Zhao et al. 2014; Suryani et al. 2022). Saponins have antipathogenic and antimicrobial activity (Ghosh et al. 2010). Saponins increase seed germination and inhibit tumor cells in plants and animals.

Saponins also have antifungal, anticancer, anti-inflammatory, hemolytic, antiprotozoal, and hypocholesterolemic properties (Pranoothi et al. 2014). In addition, steroids have cardiotonic, antibacterial, and insecticidal activity (Alexei et al. 2009). Plant tannins are polyphenols widely found in terrestrial plants and some marine plants (phloroglucinol). Plant tannins have been used as additives in animal production for many years. Tannins convert raw animal skins into ready-made skins (tanning). Tannins function as repellents for plant-eating animals because of their astringent taste. Tannins have been reported to function as antibacterial and have antitumor and antiviral activity (Kumari and Jain 2012). Tong et al. (2022) reported that tannins inhibit the growth of pathogenic bacteria and fungi, have antibacterial, antioxidant, and diuretic properties, and act as an insecticide.

Phenolics and Flavonoids contents

The total phenolic content of the methanolic leaves extract of *D. kutejensis* was 104,55 µg GAE/g extract, while the total flavonoid content was 281,41 µg QE/g extract (Table 2).

Phenolics and flavonoids are redox compounds. Phenolic compounds are natural antioxidants that can reduce oxygen-derived free radicals by donating hydrogen atoms or electrons to these free radicals. Phenolic compounds can scavenge free radicals facilitated by their hydroxyl groups.

Table 1. Phytochemical screening of *D. kutejensis* leaves methanol extract

Phytochemical constituent	Result
Alkaloid	+
Flavonoid	+
Phenolic	+
Saponin	+
Triterpenoid	-
Steroid	+
Tannin	-
Glycoside	-
Coumarine	-
Carotenoid	-

Note: +: Detected, -: Not detected

Table 2. Total phenolics and flavonoids content of methanolic extract of *D. kutejensis* leaves (n=3)

Methanolic extract	Amount
Total phenolics (µg GAE/mg extract)	104.55±0.002
Total flavonoids (µg QE/mg extract)	281.41±0.001

Meanwhile, the ability of flavonoids to reduce free radicals depends on the free OH group, especially the 3-OH group. Previous studies reported that phenolic compounds have anti-inflammatory (Brezani et al. 2018), anti-carcinogenic (Usman et al. 2022), and anti-atherogenic activities (Othman et al. 2020). Kumar and Pandey (2013) revealed that flavonoid compounds have anti-inflammatory, anti-cancer, anti-coronary heart disease, and hepatoprotective activities. The *D. kutejensis* extract contains phenol and flavonoid content of 104.55 µg GAE/mg extract and 281.41 µg QE/mg extract, respectively. This result shows that *D. kutejensis* extract can be used as a source of natural antioxidants to scavenge free radicals and supports its use to treat various diseases. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection. Flavonoids consist of a large group of polyphenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in plants. The phenylpropanoid pathway synthesizes them. Flavonoids also act as a secondary antioxidant defense system in plant tissues exposed to different abiotic and biotic stresses. Flavonoids are located in mesophyll cells' nuclei and within ROS generation centers. They also regulate plant growth factors such as auxin (Agati et al. 2012). Many flavonoids are shown to have antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory (Nguyen et al. 2017), and anticancer agent (Kopustinskiene et al. 2020), while some flavonoids exhibit potential antiviral activities (Badshah et al. 2021).

GC-MS analysis

The active ingredient compounds contained in leaves methanolic extract of *D. kutejensis* were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). GCMS analysis of *D. kutejensis* methanolic leave extract showed the presence of 43 identified chemical compounds (Table 3). The results revealed the presence of phenol, aromatic oil, fatty acid, aldehyde, and sesquiterpenoid. Figure 1 shows 69 peaks, and 43 of them can be identified. The major identified compounds were Phenol, 2-(1,1-dimethyl ethyl)-; Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy- (Coniferyl alcohol); E7-Decenylacetate; 11-Tetradecen-1-ol, acetate; Pentadecanoic acid, 14-methyl-, methyl ester; Palmitaldehyde, diisopentyl acetal; Heptane, 2,3-dimethyl-; 2,4-Diisopropenyl-1-Methyl-1-Vinyl-Cyclohexane; Ledol; and Estran-3-one, 17-(acetyloxy)-2-methyl-, (2.alpha.,5.alpha.,17.beta.)-. Several compounds in the extract have been reported to have bioactivities such as antioxidant, antibacterial, antimicrobial, antifungal, herbicide, and depigmentation agents.

Ren et al. (2019) reported that 2-(1,1-dimethyl ethyl)-phenol has anti-phytopathogenic. Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy- (coniferyl alcohol) at high concentrations is toxic to plant cells (Väisänen et al. 2015). E7-Decenylacetate is a pheromone compound in *Agrotis segetum*. Pentadecanoic acid, 14-methyl-, methyl ester is a palmitic acid methyl ester (Vijisaral and Arumugam 2014) which has antifungal, antimicrobial (Akpuaka et al. 2013), larvacidal (Elaiyaraja and Chandramohan 2016), antioxidant, pesticide, nematicide, and antiandrogenic (Vijisaral and Arumugam 2014). Octadecanoic acid (stearic acid) is used as a cosmetic raw material (as an emulsifier, emollient, pelican, and skin softener (da Silva et al. 2022) and has antibacterial properties (Ivanova et al. 2017). Palmitaldehyde-diisopentyl acetal exhibits anticancer activity (Ashmawy et al. 2020). Ledol is a sesquiterpenoid. Chadwick et al. (2013) reported that sesquiterpenoids play an important role in human health due to their potential for treating cardiovascular disease and cancer.

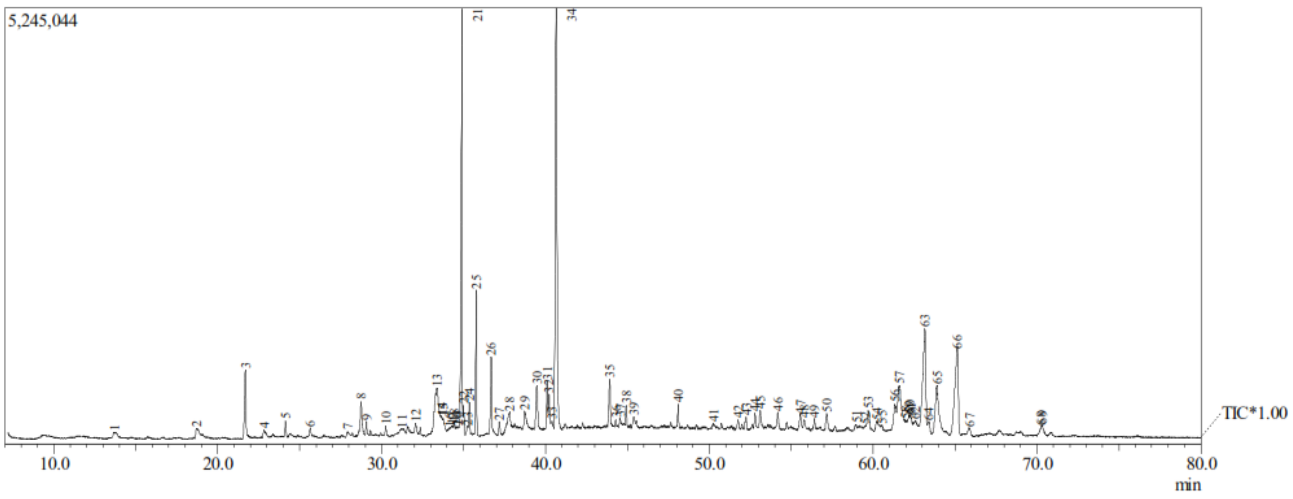

Figure 1. GC-MS chromatogram of methanolic extract of *Durio kutejensis* leaves

Table 3. Identified components of methanolic leaves extract of *Durio kutejensis* by GC-MS

No	Compound name	Retention time (rt)	% content
1	Mequinol	13.694	0.42
2	Benzaldehyde, 4-methyl	18.702	0.71
3	Phenol, 2-(1,1-dimethyl ethyl)-	21.688	2.34
4	Phenol, 2,6-dimethoxy-	22.834	0.40
5	1,1-diacetoxy-9,9-diformylnona-2,4,6,8-tetraene	24.134	0.38
6	Eugenol	25.634	0.28
7	Isobornylacetate	27.925	0.27
8	1,3-isobenzofurandione, 4,5,6,7-tetrahydro-4,7-dimethyl	28.751	1.57
9	Cyclopropane,1-(2-methylene-3-butenyl)-1-(1-methylenepropyl)-	29.074	0.39
10	Cyclopropane, 1-(2-methylene-3-butenyl)-1-(1-methylenepropyl)-	30.256	0.30
11	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy- (coniferyl alcohol)	33.365	5.41
12	Atropine	34.575	0.38
13	E7-decenyl-acetate	34.905	11.38
14	2-dodecanone	34.997	0.63
15	11-tetradecen-1-ol, acetate,	35.776	3.14
16	Pentadecanoic acid, 14-methyl-, methyl ester	36.690	2.33
17	Ethanedioic acid, dibutyl ester	37.183	0.38
18	Octadecanoic acid (stearic acid)	37.811	1.52
19	1,3,6-octatriene, 3,7-dimethyl-, (z)-	38.725	1.02
20	1h-pyrazole, 4,5-dihydro-3-phenyl-	38.725	1.82
21	9,12-octadecadienoic acid, methyl ester,	40.108	1.13
22	9-hexadecenoic acid, methyl ester (z)-	40.218	1.02
23	Palmitaldehyde, diisopentyl acetal	40.650	16.18
24	4-ethyl-5,6-dihydro-2-phenyl-(4h)1,3-oxazine-5-one	43.925	1.73
25	Iso-pinocampheol	44.544	0.40
26	Hexanoic acid, 2-propenyl ester	44.929	0.60
27	2,6-octadiene-1-ol, 3,7-dimethyl-	45.387	0.49
28	Di-n-octyl phthalate	48.099	0.55
29	Tetrahydroionone	51.752	0.43
30	2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-	52.255	0.51
31	4-undecene, 7-methyl-	52.791	0.55
32	4-undecene, 7-methyl-	53.111	0.73
33	Dipentyl sulfone	54.184	0.54
34	Heptane, 1-bromo-6-methyl-	55.542	0.69
35	Heptane, 1-bromo-6-methyl-	55.785	0.44
36	Nerolidol-epoxyacetate	56.410	0.47
37	Alpha-tocopherol (Vit E)	57.157	0.76
38	Butane, 1-bromo-3-methyl-	59.699	0.97
39	6,10,14-trimethyl-5,9,13-pentadecatrien-2-one / farnesyl acetone	60.533	0.30
40	2h-pyran, 2-(7-heptadecynyloxy)tetrahydro-	61.336	1.51
41	Heptane, 2,3-dimethyl-	61.592	4.51
42	Globulol	62.223	0.51
43	2,4-diisopropenyl-1-methyl-1-vinyl-cyclohexane	63.157	7.44
44	Cholesta-8,24-dien-3-ol, 4-methyl- (3.beta.,4.alpha.)-	63.390	0.66
45	Ledol (kelompok seskuiterpenoid)	63.884	4.26
45	Estran-3-one, 17-(acetyloxy)-2-methyl-, (2.alpha.,5.alpha.,17.beta.)-	65.120	7.00
46	Beta.-cedrenoxide	65.857	0.47

Table 4. Percentage inhibition of methanolic crude extract of *Durio kutejensis* and ascorbic acid against DPPH free radical at 517 nm

Crude methanol extract		Ascorbic acid	
Concentration (µg/mL)	**% inhibition**	**Concentration (µg/mL)**	**% inhibition**
6.25	0.372	1	6.111
12.5	9.497	1.5	10.883
25	11.918	2	13.889
50	12.663	2.5	20.000
100	32.402	3	24.167
Note: IC_{50} value: 163,849 ppm		Note: IC_{50} value: 5,865 ppm	

Ledol is a toxic sesquiterpene compound that can cause paralysis and exhibits herbicide (Vardeguer et al. 2012) and antimicrobial activities (Landoulsi et al. 2020). Eugenol is a hydroxyphenyl propene, naturally occurring in the essential oils of several plants belonging to the Lamiaceae, Lauraceae, Myrtaceae, and Myristicaceae families. Eugenol has excellent antimicrobial activity, being active against fungi and a wide range of Gram-negative and Gram-positive bacteria (Marchese et al. 2017), and has high antioxidant activity (Makuch et al. 2017). Mequinol compounds were found in small amounts of *D. kutejensis* leaves. Mequinol can be used as a depigmentation agent/skin depigmentation and effectively improve the appearance of solar lentigines and related hyperpigmented lesions (Marks et al. 2019). The methanol extract of *D kutejensis* leaves contains 9,12,-octadecatrienoic acid, methyl ester-a fatty acid group; these compounds are also found in the katuk (*Sauropus androgynus*) extract (Awaluddin et al. 2020). Putranto et al. (2017) reported that applying *S. androgynus* extract increases reproductive hormones in female goats. The ethanolic root bark extract of *D. kutejensis* showed cytotoxic activity (Priyadi et al. 2021). The active compounds in the extract of *D. kutenjesis* prove and support that lai leaves can be used as raw materials for traditional medicines and cosmetic ingredients.

Antioxidant activity

The antioxidant activity of crude methanol extract of *D. kutejensis* leaves was investigated using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, and the results are presented in Table 4. The scavenging activity of leaves *D. kutejensis* leaves extract against DPPH was expressed as % inhibition and compared with the standard antioxidant, ascorbic acid. DPPH assay is the most commonly used antioxidant assay for plant extract. In this assay, a molecule or antioxidant with weak A-H bonding will react with a stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl, λmax=517 nm), causing discoloration of the molecule (Baliyan et al. 2022). DPPH radical forms intense violet color, but the presence of antioxidant compounds decreases the intensity and the absorbance. The methanolic extract of *D. kutejensis* exhibited the highest radical scavenging of 32.402% at 100 ppm. Increasing the concentration of extract increases the percentage of inhibition.

The IC_{50} value of *D. kutejensis* leaf methanol extract is 164 ppm, and the IC_{50} value of ascorbic acid is 5.865 ppm (Table 4). The lower the IC_{50} value, the higher the antioxidant activity. Conversely, the higher the IC_{50} value, the lower the antioxidant activity. The strength of the antioxidant activity categorized as strong (IC_{50} < 50ppm), active (IC_{50} = 50-100 ppm), moderate (IC_{50} = 101-150 ppm), and weak (IC_{50} = 151-200 ppm) (Cruz et al. 2020). A recent study showed that the methanolic leaf extract of *D. kutejensis* is categorized as a weak antioxidant, while the standard ascorbic acid is categorized as a strong antioxidant. Arung et al. (2015) reported that the ethyl acetate (EtOAc) extract of *D. kutejensis* fruit had an IC_{50} value of 97.4 µg/mL. The EtOAc extract of *D. kutejensis* fruit had a higher antioxidant than the methanol leaves extract. The fruit peel extract of durian has moderate antioxidant activity with an IC_{50} value of 108.87 ppm (Setyowati and Damayanti 2014). In addition, Priyadi et al. (2021) reported that the ethanol root bark extract of *D. kutejensis* had an IC_{50} value of 761.29 µg/mL. Although the antioxidant value of *D. kutejensis* is weak, this extract might have the potential as a medicinal ingredient because the secondary metabolites, i.e., phenolic, flavonoids, palmitaldehyde-diisopentyl acetal, ledol, octadecanoic acid eugenol, and mequinol have been reported to have biological activity.

Phytochemical content, secondary metabolites, and antioxidant activities of leaf methanolic extract of lai (*D. kutejensis*) as the endemic plant of Kalimantan were reported for the first time in this study. Methanolic lai leaves extract has a total phenolic content of 104.55 µg GAE/mg extract and total flavonoid content of 281.41 µg QE/mg extract. It has antioxidant activity. Based on the GC-MS analysis, there are 43 identified compounds in leaf extracts of *D. kutejensis*. The spectrum of identified compounds and the antioxidant activity is compatible with the pharmacological activity of lai and its traditional use as a remedy and dietary ingredient. Our study is a starting point for further study on lai leaves' biochemical and pharmacological properties.

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