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Research Article

Chemical Screening and Antioxidant Activity of Ethyl Acetate Fraction and Residue from *Lansium domesticum* Ethanolic Extract

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Abstract

Langsat (*Lansium domesticum*) is a plant that thrives in the tropics. The search for photoprotective compounds derived from natural ingredients has been intensively conducted to develop cosmetic formulations to obtain safe and protective products against UV rays. This study aims to identify chemical compounds in the ethyl acetate fraction and residues of ethanol extract from *L. domesticum* bark using tube and gas chromatography-mass spectrophotometry tests. At the same time, the antioxidant activity and SPF values were assessed with the DPPH and FRAP methods. The qualitative GC-MS test obtained the percentage peak value and molecular weight. Furthermore, the antioxidant activity was tested using the compound DPPH measured with an ELISA reader at a wavelength of 515.5 nm with UV/vis spectrophotometer and FRAP method using $FeCl_3$ and TPTZ compounds at 615 nm. The SPF value was assessed using Mansur's formula with UV/Vis spectrophotometer at 290 to 320 nm. The results obtained in the ethyl acetate fraction showed terpenoids, while the residue contains phenolics, flavonoids, tannins, and saponins. The GC-MS tests found that the ethyl acetate fraction had 47 types of chemical compounds, including terpenoids and fatty acids group; in the residue, four classes were found, with terpenoid and fatty acid groups being more predominant. Based on the results, the ethyl acetate fraction has an IC_{50} value of 341.25±26.45 µg/ml and 436.3±10.8 µg/ml; the residue was 94.72±34.22 µg/ml and 2602.79±11.8 µg/ml. Additionally, the SPF values for both were 2.87 and 3.9.

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INTRODUCTION

Langsat or *Lansium domesticum* is a plant native to Southeast Asia, capable of growing naturally, and cultivated in Indonesia, Malaysia, Thailand, and the Philippines. The name differs in each country, for example: Burma (*duku*, *langsak*); Philippines (*lanzone*, *lanzon*, *lanson*, *lansone*); Indonesian (*duku*, *kokosan*, *langsat*); Malay (*langseh*, *langsep*, *lansa*); Thai (*duku*, *longkong*, *langsat*); Vietnamese (*bon-bon*); Chinese (*lansa*); Japan (*ransa*); and Spain (*arbol de lanza*)[1]. The botanical characteristics include 30 m height with a 75 cm diameter trunk, but the tree height only reaches 25 m when cultivated. The bark is gray and orange with milky white spots, wrinkled, and has resin or sap with hairy twigs[2]. A chemical content analysis conducted by Abdallah *et al.*[3] reported that *L. domesticum* bark contained anthraquinones, alkaloids, flavonoids, coumarins, cardiac glycosides, tannins, saponins, and iridoids. Meanwhile, according to Apridamayanti *et al.*[4], the content includes alkaloids, saponins, tannins, flavonoids, and triterpenes. *Lansium domesticum* bark is empirically used as an antimalarial and dysentery drug in Java, Kalimantan, and Malaysia; spleen and fever medicine in Kenya; and an antifertility drug in Kalimantan[3]. Previous studies reported that it has antimalarial, antibacterial, antioxidant, and antipyretic activities[4-6].

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Meanwhile, ultraviolet radiation that reaches the earth's surface is about 5%. In 2019, there were reportedly more than 287,723 cases of malignant melanoma (MM) associated with UV radiation, with a mortality ratio of 21%, as well as 1,042,056 cases of non-melanoma skin cancer (NMSC) with a mortality ratio of 6%. The development of photoprotector-based cosmetic products can protect against sunburn, which causes suppression of the immune system and health problems caused by damage to intracellular DNA, increased production of reactive oxygen species (ROS), and oxidative stress due to premature skin aging[7]. Previous study[2] on developing photoprotector products stated that dry hydroethanolic extract of *L. domesticum* fruit could be used as cosmetics. The extract is dissolved in propylene glycol and used as a skincare product for skin depigmentation and moisturizing. Furthermore, clinical trials conducted on 30 women aged 32-52 years for four weeks found that *L. domesticum* extract can increase skin moisture and the melanin index.

The use of bioactive compounds found in plant parts in cosmetic formulations to meet skincare needs is increasing in line with the development of raw materials from new sustainable sources[8]. Plant extracts have been the subject of several studies in the beauty industry sector[9]. However, the potential use of tropical plants' bark in formulating cosmetic preparations has yet to be explored adequately. Therefore, this study aims to determine the chemical compounds contained in the ethyl acetate fraction and the water fraction of *L. domesticum* stem bark using gas chromatography-mass spectrophotometry (GC-MS), as well as to measure the antioxidant activity using the DPPH and FRAP methods, and the SPF value.

MATERIALS AND METHODS

Materials

Lansium domesticum was obtained from the forest area of Dusun Sekajang, Tembawang Village, Entikong District, Sanggau Regency, West Kalimantan, Indonesia. The chemicals used were ethanol (Merck and Brataco), ethyl acetate (Brataco), distilled water, methanol (Merck), H_2O_2 (Merck), HCl (Merck), $AlCl_3$ (Merck), $FeCl_3$ (Merck), Mg, H_2SO_4 (Merck), KOH (Merck), 2,4,6-tripyridyl-s-triazine or TPTZ (Merck), and 1,1-diphenyl-2-picrylhydrazyl or DPPH (Merck). The equipment used includes a rotary evaporator (Butchi), ultrasonicator (Branson 1510), spectrophotometer UV/Vis (Shimadzu), ELISA reader (ThermoSci), and GC-MS (ThermoSci).

Methods

Extraction and fractionation

Lansium domesticum bark was taken and determined in the Laboratorium of Biology, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura, on 27th September 2016. Simplicia was made using stem bark and sifted using a 40-mesh sieve (**Figure 1**). Simplicia was macerated with ethanol 86% and evaporated with a rotary evaporator, and the thick *L. domesticum* bark extract was obtained. The ethanol extract was fractionated using ethyl acetate and water solvent to get the ethyl acetate fraction and residue (water fraction).

a **b**

Figure 1. (a) simplicia and (b) powder from *L. domesticum* bark

Phytochemical screening

Phytochemical screening was carried out on the ethyl acetate fraction and residue of the ethanol extract of *L. domesticum* stem bark[4].

1. In the alkaloid test, chloroform ammonia was added to the extract and fraction, shaken, and filtered, then 1 mL of 2 N sulfuric acid was added. The acid layer (top layer) was pipetted and put into a test tube containing Mayer's, Dragendorff's, and Wagner's reagents.
2. For the phenol test, the extract and fraction were dissolved in ethanol, then a few drops of 1% $FeCl_3$ were added.
3. In the flavonoid test, the extract and fraction were dissolved in hot water, 0.5 mg of Mg powder was added, three drops of concentrated HCl were added, then five drops of amyl alcohol were added.
4. For terpenoid test, extract and fraction were dissolved with *n*-hexane, and Liebermann-Burchard reagent was added.
5. For the anthraquinone test, 1 g of the extract and fraction was boiled for 2 minutes with 2 mL of 0.5 N KOH and three drops of hydrogen peroxide. After cooling, the suspension was filtered, the filtrate was added with acetic acid to a pH of 5, and 3 mL of benzene was added. The top layer was separated with a pipette and put into a test tube, then 0.5 N potassium hydroxide was added.
6. In the tannin test, 1 g of extract and fraction was heated with 10 mL of water for 30 minutes in a water bath. The solution was filtered, and the filtrate was added with 5 mL of 1% gelatin solution.
7. In the saponin test or foam test, 1 g of extract and fraction in a test tube was added to 10 ml of distilled water, then closed and shaken vigorously for 30 minutes. The tube was then left in an upright position for 30 minutes.

GC-MS analysis

The chemical compounds were examined using GC-MS analysis based on Giri and Rajbhandari[10] methods with slight modifications. The sample fraction used was ethyl acetate, and the residue from the ethanol extract was dissolved using 3 ml of chloroform. The solution was sonicated for a minute, and chloroform was added to 5 mL in a measuring flask. GC-MS analysis was performed using an Agilent J&W GC column DB-5MS type (30 m x 0.25 mm). A total of 1 µL of sample solution was injected into Agilent Gas Chromatography GC:7890A (G3440A) for 40 minutes, with an injection port temperature of 230°C, while helium gas was used as a carrier. The mass spectroscopy was operated in electron collision mode with an ionization energy of 70 eV, while mass spectrum scans were performed at 30 to 380 m/z. The detected compounds were identified by processing the raw GC/MS data with ChemStation software and comparing the results with a mass spectral database.

Antioxidant activity with DPPH assay

Antioxidant activity was tested using a DPPH assay to identify the sample's free-radical-scavenging activity. The technique was based on previous studies[4,11], with slight modifications. About 1 mL of ethyl acetate and residue fraction, each with various concentrations of 100-500 ppm and 1-300 ppm, was added to 3 mL of 1 mM DPPH and 1 mL methanol. It was incubated in the dark for 15 minutes at room temperature, and absorbance was measured at 515.5 nm, in which the experiments were carried out in triplicate.

Antioxidant activity with FRAP assay

The antioxidant activity test with FRAP was performed according to Nur *et al.*[12] with slight modifications. About 30 µL each of ethyl acetate and residue fraction with various concentrations of 100-500 ppm and 1-300 ppm was added with 30 µL $FeCl_3$ solution, i.e., 3 mM in 5 mM citric acid, and 240 µL 1 mM TPTZ in 0.05 M HCL in 96 microplate wells. Incubation was performed for 20 minutes at room temperature, and absorbance was measured at 615 nm. The results were presented in triplicate.

SPF value

The ethyl acetate fraction and residue were dissolved in ethanol at 250 µg/mL concentration and scanned across the 290 to 320 nm range at 5 nm intervals. Screening of sun protection activity was measured by determination *in vitro* of SPF, based on the equation proposed by Mansur *et al.*[13] The absorbance sample was measured three times and used for SPF calculation, as shown in **Equation 1**.

$$SPF = CF\ x\ \sum_{290}^{320} EE(\lambda) x\ I(\lambda) x\ Abs(\lambda) \quad [1]$$

Statistical analysis

Data were presented as means±standard deviation, while statistical analysis was performed using SPSS software, with a value of $p < 0.05$ considered significant.

RESULTS AND DISCUSSION

A qualitative examination of the chemical content contained in the langsat bark is presented in **Table I**. It is widely known that the ethanol extract of *L. domesticum* barks contains flavonoids, alkaloids, terpenoids, and tannins. The ethyl acetate fraction was confirmed to have terpenoids, while the residue contains phenolics, flavonoids, tannins, and saponins. A study conducted by Worang *et al.*[5] on the *L. domesticum* bark stems found alkaloids, flavonoids, phenols, and terpenoids in methanol extract, while the chloroform and *n*-butanol fractions contain alkaloids, terpenoids, and flavonoids.

Table I. Phytochemical content of secondary metabolites in *L. domesticum* stem bark extract and fraction

Secondary Metabolite	Ethanol extract	Ethyl acetate fraction	Residue
Phenols	-	-	+++
Flavonoids	+	-	++
Alkaloids	+	-	-
Terpenoids	+	+	-
Steroids	-	-	-
Anthraquinone	-	-	-
Tannins	+	-	+
Saponins	-	-	+

Note: - absent; +: present in small quantity; ++: present in moderate quantity; +++: present in large quantity

In this study, testing the ethyl acetate fraction and the residue was focused on preparing it as an active ingredient in cosmetic formulas; knowing the functional content of chemical compounds can be the basis for making cosmetic formulas. The chemical compounds detected by GC-MS in the ethyl acetate fraction and residues from the ethanol extract of *L. domesticum* bark are shown in **Tables II** and **III**. Forty-seven chemical compounds were found in the ethyl acetate fraction, including terpenoids and fatty acids. Meanwhile, four types of chemical compounds were confirmed in the residue, especially the terpenoid and fatty acid groups. The results of chromatogram separation using GC-MS are shown in **Figures 2** and **3**, with 35- and 60-minutes separation times for the ethyl acetate fraction and residue.

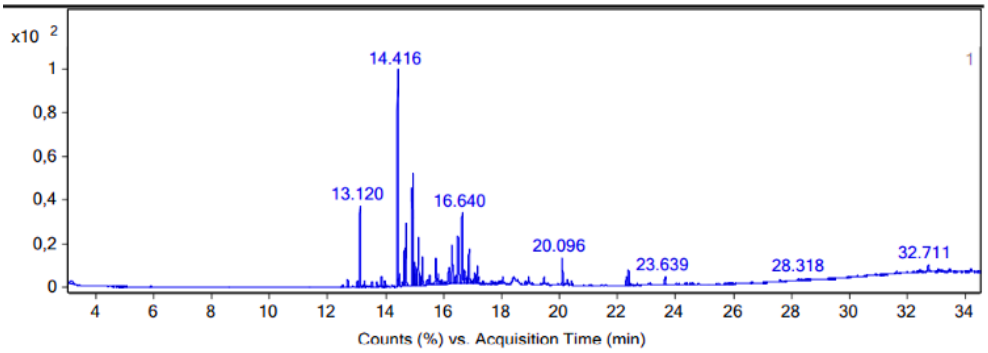

Figure 2. GC-MS results for ethyl acetate fraction of *L. domesticum*

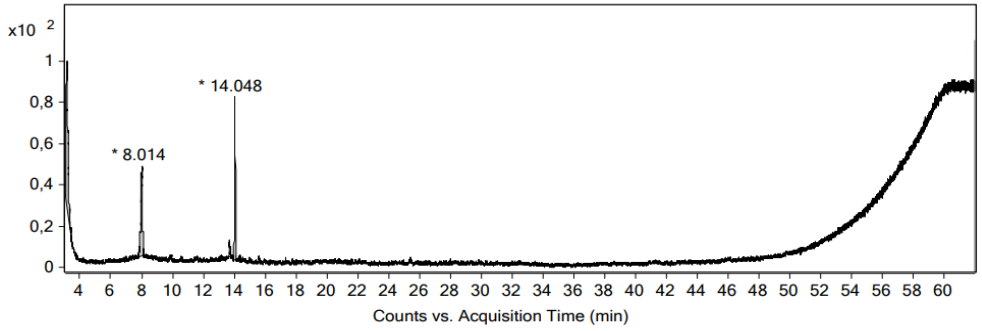

Figure 3. GC-MS results for the residue of *L. domesticum*

Table II. GC-MS results for the ethyl acetate fraction of *L. domesticum* stem bark

No	Retention time in minutes	Name of compound	Molecule formula	Molecule weight	Percentage peak value	Nature of compound
1	12.691-12.728	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)	$C_{15}H_{24}$	203.87	3.52	Triterpenoid
2	12.670-12.713	α-cubebene	$C_{15}H_{24}$	203.9	2.76	Sesquiterpene
3	13.009-13.052	Ylangene	$C_{15}H_{24}$	203.89	36.70	Sesquiterpene
4	13.098-13.141	Copaene	$C_{15}H_{24}$	204.08	2.38	Sesquiterpene
5	13.249-13.292	(S,1Z,6Z)-8-isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	$C_{15}H_{24}$	203.86	1.92	Terpenoid
6	13.702-13.764	(3R,4aS,8aS)-8a-methyl-5-methylene-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene	$C_{15}H_{22}$	201.85	2.52	Terpenoid
7	13.861-13.908	Tricyclo[4.4.0.0(2,7)]decane, 1-methyl-3-methylene-8-(1-methylethyl)-,stereoisomer	$C_{15}H_{24}$	203.92	4.80	Triterpenoid
8	13.982-14.028	Germacrene D	$C_{15}H_{24}$	203.89	100	Sesquiterpene
9	14.416-14.451	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,[1R-(1α,3aβ,4al)	$C_{15}H_{24}$	203.9	6.3	Sesquiterpene
10	14.473-14.513 14.707-14.753	γ-muurolene	$C_{15}H_{24}$	204	2.25	Sesquiterpene
11	14.583-14.610 14.915-14.928 15.435-15.466	α-muurolene	$C_{15}H_{24}$	204.14	73.65	Sesquiterpene
12	14.648-14.672	10,11-epoxycalamenene	$C_{15}H_{24}$	203.85	32.74	Sesquiterpene
13	15.009-15.047	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1α,4ab)	$C_{15}H_{24}$	204	10.47	Sesquiterpene
14	15.082-15.093	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)-	$C_{15}H_{24}$	204	30.87	Sesquiterpene
15	15.122-15.171	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)	$C_{15}H_{22}$	201.9	5.11	Sesquiterpene
16	15.195-15.231 16.408-16.435 16.497-16.597 16.875-16.926 17.161-17.188 17.209-17.230 18.042-18.101	(1R,7S,E)-7-isopropyl-4,10-dimethylenecyclodec-5-enol	$C_{15}H_{24}O$	218.91	89.78	Terpenoid
17	15.271-15.330	Guaiol	$C_{15}H_{26}O$	221.15	13.06	Sesquiterpene
18	15.527-15.569 15.815-15.861	α-calacorene	$C_{15}H_{20}$	203.96	4.46	Sesquiterpene
19	15.737-15.780 15.939-15.986	Caryophyllene oxide	$C_{15}H_{24}O$	219.93	9.68	Terpenoid
20	15.877-15.901	(-)-spathulenol	$C_{15}H_{24}O$	204.98	2.26	Spathulenol
21	16.041-16.066	(1R,2R,4S,6S,7S,8S)-8-isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decan-4-ol	$C_{15}H_{24}O$	221.92	1.5	Terpenoid
22	16.082-16.112	(-)-globulol	$C_{15}H_{26}O$	221.87	1.75	Sesquiterpene
23	16.182-16.222 16.287-16.346 22.446-22.489	Alloaromadendrene oxide-(1)	$C_{15}H_{24}O$	219.93	12.88	Terpenoid
24	16640-16689	4a(2H)-naphthalenol, 1,3,4,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S,4R,4aS,8aR)-	$C_{15}H_{24}$	203.95	35.87	Terpenoid
25	16.718-16.802	τ-cadinol	$C_{15}H_{26}O$	221.8	12.56	Sesquiterpene
	16.837-16.850	α-cadinol	$C_{15}H_{26}O$	221.97	19.14	Sesquiterpene
26	16.948-16.972	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	$C_{15}H_{18}$	183.100	2.33	Sesquiterpene
27	17.042-17.047	8-isopropyl-1,5-dimethyltricyclo[4.4.0.02,7]dec-4-en-3-one (α-copaene)	$C_{15}H_{24}$	202.920	1.49	Sesquiterpene
28		cis-Z-α-bisabolene epoxide	$C_{15}H_{24}O$	221.92	5.88	Sesquiterpene
29	17.123-17.188	Tricyclo[3.3.0.0(2,8)]octan-3-one, 4-methyl-4-(2-methyl-2-propenyl)-			9.42	Triterpenoid
30	17.188-17.228	Aristol-1(10)-en-9-yl isovalerate	$C_{20}H_{32}O_2$	219.86	2.85	Sesquiterpene
31	17.339-17.390	Ylangenal	$C_{15}H_{22}O$	217.89	2.07	Sesquiterpene
32	17.983-18.004	(1aR,4aS,8aS)-4a,8,8-trimethyl-1,1a,4,4a,5,6,7,8-octahydrocyclopropa[d]naphthalene-2-carbalde	$C_{15}H_{22}O$	217.93	1.64	Sesquiterpene
33	17.878-17.937	Bicyclo[4.3.0]nonan-1-ol, 7,9-bis(methylene)-2,2,6-trimethyl-	C14H22O	233.900	1.78	Triterpenoid
34	17.950-18.004	Trans-Z-α-bisabolene epoxide	$C_{15}H_{24}O$	206.7	4.27	Sesquiterpene

No	Retention time in minutes	Name of compound	Molecule formula	Molecule weight	Percentage peak value	Nature of compound
	21.010-21.158					
35	18.007-18.080	Isoaromadendrene epoxide	$C_{15}H_{24}O$	205	1.82	Sesquiterpene
	18.290-18.339					
36	18.355-18.425	Benzene, 1-[1,1-dimethylethyl]-4-[2-propenyloxy]-			4.33	Organic acid
37	18.530-18.587	Isopropyl myristate	$C_{17}H_{34}O_2$	227.99	4.31	Organic acid
38	18.899-18.956	4-(1,3,3-trimethyl-bicyclo[4.1.0]hept-2-yl)-but-3-en-2-one		233.85	4.31	Triterpenoid
39	20.069-20.123	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.1	14.78	Fatty acid
40		5-methoxy-2,2,6-trimethyl-1-(3-methyl-buta-1,3-dienyl)-7-oxa-bicyclo[4.1.0]heptane	C15H24O2	263.93	2.33	Triterpenoid
41	20.390-20.444	4,6,10,10-tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol	C13H22O	217.86	2.48	Triterpenoid
42	22.276-22.325	9,12-octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294.040	4.81	Fatty acid
43	22.352-22.403	9-octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	263.990	8.45	Fatty acid
44	22.659-22.704	Methyl stearate	$C_{19}H_{38}O_2$	297.98	4.04	Organic compound
45	23.618-23.666	7-hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	277.9	5.29	Fatty acid
46	28.264-28.355	Cyclotrisiloxane, hexamethyl	$C_6H_{18}O_3Si_3$	220.78	2.08	Organic compound
	31.597-31.659					
	32.396-32.480					
47	27.583-27.677	1,2-bis(trimethylsilyl)benzene	C12H22Si2	280.89	2.80	Organic compound
	32.682-32.779					

Table III. GC-MS results for the residue of *L. domesticum* stem bark

No	Retention time in minutes	Name of compound	Molecule formula	Molecular weight	Percent peak value	Nature of compound
1	7.857-8.097	Nonanal	$C_9H_{18}O$	128.7	63.49	Monoterpene
2	9.793-9.958	2-octanol, 2-methyl-	$C_9H_{20}O$	128.7	1.96	Fatty acid
3	28.264-28.355	Cyclohexanecarboxylic acid, 4-methylpentyl ester	$C_{13}H_{24}O_2$	280.83	5.62	Fatty acid
4	31.597-31.659	Cyclobutanecarboxylic acid, oct-3-en-2-yl ester	$C_{13}H_{24}O_2$	280.9	58.80	Fatty acid

The most significant chemical compound, according to the percentage of the highest peak value, was germacrene D. This is in line with previous studies[3,14], which stated that germacrene D is the most abundant compound obtained from *L. domesticum*. Germacrene is a terpenoid compound, and the sesquiterpene group is volatile. There are two types: germacrene A and D. Moreover, it is a biogenetic compound and the precursor of cadinenes and muurolenes[15]. The results also showed γ and α muurolenes compounds with peak percentages of 2.25% and 73.65%. In previous studies[3,16], germacrene D was found in the fruit and had antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, *Trichophyton mentagrophytes*, and *Aedes aegypti* with a bond energy of -9.5 kcal/mol. γ muurolene compound produced by germacrene biosynthesis have larvicidal activity against *Anopheles stephensi*. It was also reported that *L. domesticum* bark has antibacterial and antimalarial activities[5,17,18].

Studies related to antioxidant activity in medicinal plants have been widely reported. Oxidation in lipids, proteins, and DNA caused by ROS (reactive oxygen species) induces degenerative and neurogenerative diseases, wrinkled skin, DNA damage, cardiovascular disease, inflammatory conditions, and carcinogenesis[19]. Therefore, further studies are needed to produce antioxidants sourced from natural ingredients to replace synthetic types. Previous studies[4,6,20] reported that *L. domesticum* has antioxidant activity. This is further demonstrated in this study, as presented in **Table IV**. The antioxidant activity was tested by measuring the IC_{50} value after reacting the sample with the DPPH and FRAP. DPPH, as a radical compound, predicts antioxidant activity through a reduction event that occurs due to the acceptance of electrons or hydrogen radicals by antiradical. Meanwhile, the FRAP method is based on the ability of antiradical compounds to reduce Fe^{3+} to Fe^{2+} in an acidic environment. This method uses TPTZ reagents, with tripyridyltriazine acting as an iron-linking ligand and ferrozine compounds, to assess the reduction ability[21,22]. In terpenoid compounds, antioxidant activity occurs when a hydrogen atom is donated in the framework and based on its ability to inhibit lipid oxidation[23].

Table IV shows a significant difference between the DPPH and FRAP methods. Based on the test results, the ethyl acetate fraction had an IC_{50} value of 341.25±26.45 g/mL and 436.3±10.8 g/mL, while that of the residue was 94.72±34.22 g/mL and

2602.79±11.8 g/mL. The difference in the ability to ward off free radicals can be caused by the different compounds in the two samples. The ethyl acetate fraction is known to include 47 types of compounds with terpenoids dominating, while in the residue, there are four types of compounds with fatty acids being predominant. Nevertheless, the DPPH method has the best IC_{50} value, possibly due to the presence of these compounds. Phenols and flavonoids were also detected qualitatively. This is in line with previous studies which reported differences in the results of antioxidant activity tests using the DPPH and FRAP methods[24,25,26]. This is presumably due to the compound responsible for the sample with the FRAP test, which is classified as a secondary antioxidant having a mechanism to stabilize hydroperoxidase by inhibiting the breakdown of hydroperoxides into free radicals. Combinations that can chelate metals are also included in this class of antioxidants. Secondary antioxidants bind metal ion mechanisms, capture oxygen, convert hydrogen peroxide to non-radical species, and deactivate singlet oxygen, unlike DPPH testing, where the compound responsible has a mechanism for scavenging free radicals through breaking the chain of radical reactions by giving or donating hydrogen radicals quickly[27].

Table IV. Antioxidant activity of the ethyl acetate and residual fractions of *L. domesticum*

Sample	Antioxidant activity (IC_{50}) µg/mL	
	DPPH	FRAP
Ethyl acetate fraction	341.25±26.45	436.3±10.8
Residue	94.72±34.22	2602.79±11.8

The SPF value was determined in the ethyl acetate fraction and residue. The test was conducted at 250 g/mL concentration using the Mansur formula[13] with UV/Vis spectrophotometry in the 290-320 nm wavelength range while performing absorbance measurements at every 5 nm. The measurement results are demonstrated in **Table V**. The mechanism of sunscreen as a photoprotector can be explained through molecules capable of absorbing energy from UV rays, then experiencing excitation events to a higher energy level followed by the release of energy and the return to a lower level. The UV rays absorbed by these molecules can absorb energy and function as a sunscreen because they have low power, thereby reducing the effect of UV exposure[28]. The higher the SPF value, the higher the protective effect against UV rays[29].

The measurement results show that the ethyl acetate and residual fractions have SPF values of 2.87 and 3.9, indicating a non-significant difference with the minimal protection category. The minimal protection offered was due to predominant chemical compounds: terpenoids and followed by fatty acids. According to previous studies, optimal protection against UV rays is given by the group of phenolics and flavonoids. This occurs through a conjugated double bond mechanism in the benzene nucleus which will experience resonance due to electron transfer when exposed to UV light and have a chromophore group that acts as an aromatic conjugated double bond, absorbing light at UV A and B wavelengths.

Table V. SPF results for ethyl acetate and residual fractions of *L. domesticum*

Sample	SPF value (at 250 µg/mL)
Ethyl acetate fraction	2.87±0.09
Residue	3.9±0.01

CONCLUSION

The ethyl acetate fraction of *L. domesticum* showed terpenoids, while the residues contained phenolics, flavonoids, tannins, and saponins. GC-MS tests found that the ethyl acetate fraction had 47 types of chemical compounds. While in the residue, there are four types of chemical compounds with terpenoid and fatty acid groups. Antioxidant activity test using DPPH and FRAP methods show the IC_{50} of ethyl acetate fraction of 341.25±26.45 µg/ml and 436.3±10.8 µg/ml, respectively, while the IC_{50} of the residue is 94.72±34.22 µg/ml and 2602.79±11.8 µg/ml, respectively. The SPF values for the ethyl acetate fraction and residues have values of 2.87 and 3.9. The ethyl acetate fraction and the residue of *L. domesticum* stem bark have moderate to low antioxidant activity and minimal UV protection.

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AUTHORS' CONTRIBUTION

Pratiwi Apridamayanti: Responsible for manuscript creation and data processing. **Rafika Sari**: Responsible for the results of GC-MS analysis.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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