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INTRODUCTION *Lansium domesticum* Coor. (synonym: *L. parasiticum*) is a fruit species from the Meliaceae family, which is a tropical plant native to Southeast Asia. Local citizens call it langsat, longkong, or duku and have used it as traditional medicine (Manosroi et al., 2012; Tilaar et al., 2018). Oil from the peel of *L. domesticum* fruit is used to treat diarrhea, dysentery, and malaria (Khoo et al., 2016; Yapp & Yap, 2003). The seeds are used as a fever remedy, the bark is used as medicine to treat scorpion sting (Tilaar et al., 2008), and the leaves are used to repel mosquitoes (Klungsupya et al., 2015). Besides that, some studies showed that *L.*

*domesticum* has antimalarial (Saewan et al., 2006), anti-proliferation (matrix metalloproteinase-2 inhibition on human oral epidermal carcinoma) (Manosroi et al., 2013), anti-oxidative, and analgesic activity (Apridamayanti et al., 2018). In previous studies, it has been reported that the peel of *L. domesticum* contains several types of terpenoids (Klungsupya et al., 2015). Its seeds contain terpenoids and phenolic compounds such as flavonoids (Klungsupya et al., 2015; Nur et al., 2017), while its bark contains alkaloid metabolites, saponins, tannins, flavonoids, and triterpene (Apridamayanti et al., 2018).

Secondary metabolic compounds of polyphenols derived, such as flavonoids, tannins, stilbene, coumarin, and lignin, are abundant in its leaves, stems, flowers, and fruit, having an essential role in counteracting free radicals (Pandeya et al., 2018). These polyphenols' antioxidant properties are due to the polyphenol compound's redox properties, which acts as a reducing agent by donating its hydrogen (Piluzza & Bullitta, 2011). The body needs antioxidants because they can delay substrate oxidation by inhibiting initiation and propagation (Pham-Huy et al., 2008; Widodo et al., 2020).

Synthetic antioxidants, such as beta-hydroxy acid (BHA), tert-butyl hydroquinone (TBHQ), propyl gallate (PG), and butylated hydroxytoluene (BHT), have been used extensively in the world. However, these synthetic antioxidants have side effects, such as carcinogenic and cytotoxic effects on the heart and lungs (de Oliveira et al., 2010; Sulastri et al., 2018). Additionally, BHA and BHT also have low solubility and moderate antioxidant activity (Sannigrahi et al., 2010).

Therefore, current research focused on discovering new antioxidant compounds from natural products that exhibit high activity and lower toxic effects than synthetic compounds (Rohman et al., 2010). Natural products with antioxidant properties have been reported, such as *L. domesticum* fruit (Manosroi et al., 2012), *Moringa oleifera* extract (Fitriana et al., 2016), and peel of *Nephelium lappaceum* (Mistriyani et al., 2018). Ethyl acetate fraction and tetranortriterpenoid compounds isolated from the dichloromethane fraction of *L. domesticum* showed antimalarial activity against

*Plasmodium falciparum* (Klungsupya et al., 2015; Saewan et al., 2006).

Extracts and fractions from *Persea americana* peel have also shown antimalarial activity. This research aims to determine the potential of antiradical activity from extracts and fractions of *L. domesticum* seeds. MATERIALS AND METHODS Extraction *Lansium domesticum* seeds were obtained from fruit traders in Fruit Market Wua-Wua, Kendari, Southeast Sulawesi, Indonesia. The seeds of *L. domesticum* are then crushed into a powder and dried. Furthermore, *L. domesticum* seed powder was macerated using methanol for 3 x 24 hours. Every 24 hours, the macerate was filtered, and the solvent was replaced. Then, the macerate was concentrated using a rotary evaporator at 40°C to obtain crude extract.

Fractionation As much as 40 g of *L. domesticum* seed crude extract was partitioned using the gradient elution liquid-liquid fractionation method. First, the seed extract was partitioned using a separatory funnel with n-hexane, followed with ethyl acetate, and water fraction as the remaining fraction. Each fraction was evaporated with a rotary evaporator into crude fractions. The working method in a diagram is presented in Figure 1. / Figure 1. Fractionation scheme of *L.*

*domesticum* seeds extracts and fractions Phytochemical screening Phytochemical screening was conducted to determine the profile of secondary metabolites in *L. domesticum* seeds extracts and fractions. Phytochemical screening methods were performed based on previous research by Yamin et al. (2020). Alkaloid test *Lansium domesticum* seeds' extract and fractions were inserted separately into 1 mL test tubes and added three drops of Dragendorff's reagent. The formation of brown precipitate indicated the presence of alkaloid. Flavonoid test The extract and fractions of *L. domesticum* seeds were inserted separately into test 1 mL tubes and added with 0.2 g of magnesium powder and 2 mL of concentrated HCl.

The formation of red, orange, and green solutions indicated the presence of flavonoid. Terpenoid test The extract and fractions of *L. domesticum* seeds were inserted separately into 1 mL test tubes and added with 0.5 mL of acetic acid anhydride and 2 mL of concentrated sulfuric acid. The formation of green, bluish, and brown solutions indicated the presence of terpenoid. Tannin test The extract and fractions of *L. domesticum* seeds were inserted separately into 1 mL test tubes and added with 1 mL of 1% ferric chloride solution. The formation of blue to black solution indicated the presence of tannin. Saponin test The extract and fractions of *L.*

*domesticum* seeds were inserted separately into 1 mL test tubes and added with 2 mL of hot water, then cooled and shaken for ten seconds. It was declared positive for saponin



contained flavonoids, alkaloids, tannins, and terpenoids. Meanwhile, the results of the saponin test on the extract and fractions were negative.

These results are consistent with those reported by Nur et al. (2017). The result of phytochemical screening is shown in Table I. Table I. Phytochemical screening of L. domesticum extract and fractions

Testing	Extract	fraction	Methanol	n-hexane	Ethyl acetate	Water	Flavonoid	Alkaloid	Tannin	Terpenoid	Saponin
	+	+	+	+	+	+	+	+	+	+	-

(+) : presence; (-) : absence of phytochemicals

Measurement of antioxidant activity using DPPH radicals in this study was carried out after 30 minutes of incubation. This treatment allows all species involved in the reaction of antioxidants with radicals to have reacted entirely.

The parameter used to determine the antioxidant activity was IC<sub>50</sub> from extract and fractions of L. domesticum seeds. The IC<sub>50</sub> is defined as the concentration of antioxidants in inhibiting radicals by 50% (Olugbami et al., 2014). The smaller IC<sub>50</sub> value indicated the potent antioxidant in extract or fractions (Maisuthisakul et al., 2007). The standard antioxidant used in this study was vitamin C. Table II showed the IC<sub>50</sub> values of L. domesticum seed extract and fractions. The data in the table exhibited extract and fractions of L. domesticum seeds are classified in the category of very strong antioxidants, as stated by Molyneux (2004).

According to the data of IC<sub>50</sub> shown in Table II, ethyl acetate fraction made a very strong contribution as an antioxidant compared to the n-hexane fraction, water fraction, and methanol extract, whose values were  $8.938 \pm 0.031$ ;  $8.938 \pm 0.031$ ;  $13.898 \pm 0.81$ ; and  $14.624 \pm 0.456$   $\mu\text{g/mL}$ , respectively. These were in line with research that stated that the ethyl acetate fraction had strong antioxidant power compared to other solvents' fractions. Several ethyl acetate fractions with such strong antioxidant activity have been reported on Enhydra fluctuans Lour (Sannigrahi et al., 2010), Pandanus conoideus Lam (Rohman et al., 2010), Oroxylum indicum Linn (Trang et al.,

2014), Polygala sabulosa, Cyathea phalerata (Brighente et al., 2008), as well as the stem bark from Dracontomelon dao(Blanco) Merr (Yamin et al., 2020). Table II. The IC<sub>50</sub> value of extract and fraction from L. domesticum seeds

Sample	IC <sub>50</sub> value ( $\mu\text{g/mL}$ )
Methanol extract	$14.624 \pm 0.456$
n-hexane fraction	$11.012 \pm 0.094$
Ethyl acetate fraction	$8.938 \pm 0.031$
Water fraction	$13.898 \pm 0.81$
Vitamin C	$4.721 \pm 0.046$

The DPPH is a free radical widely used to examine radical scavenging activity of plant extracts (Jamuna et al., 2012), pure compounds, food ingredients, and others (Koleva et al., 2002).

Besides, this method is fast, reliable, reproducible, requires less energy, does not require

sophisticated instruments, the reagents needed in this method are inexpensive (Jamuna et al., 2012, Koleva et al., 2002). An antioxidant compound's intrinsic ability to donate hydrogen atoms or electrons to homogeneously reactive radical compounds can be determined. This method is based on a decrease in the solubility of methanolic DPPH, which is caused by antioxidant compounds that donate their hydrogen (Rohman et al., 2010).

The antioxidant activity of extract and fractions is affected by the phenolic and flavonoid contents. This phenomenon is caused by the presence of a hydroxy group from those compounds. The strength of antioxidants by flavonoid compounds depends on the number of hydroxyl groups attached to ring B. The more hydroxyl groups are attached to ring B, the stronger the compound is in counteracting radicals. This is because the hydroxyl groups in ring B play a role in stabilizing the aryloxy radical (Cao et al., 1997).

Besides, the existence of ortho-hydroxyl substitution in ring B or ring A is important in the inhibition of radicals, while substitution in other positions does not show a clear role in stabilizing radicals (Yokozawa et al., 1998). Table III showed the phenolic and flavonoid contents in the extract and fractions of *L. domesticum* seeds. The levels of phenolic and total flavonoid contents were ethyl acetate fraction > n-hexane > water fraction > crude extract, with the total phenolic contents of  $58.25 \pm 0.501$ ;  $44.315 \pm 1.737$ ;  $39.454 \pm 0.446$ ; and  $31.028 \pm 0.605$  mg GAE/g dry samples, respectively.

Meanwhile, the values of total flavonoids were  $75.123 \pm 0.175$ ;  $59.626 \pm 0.268$ ;  $58.866 \pm 0.202$ ; and  $56.175 \pm 0.175$  mg QE/g dry samples, respectively. Table III. Total phenolic and flavonoid contents from *L. domesticum* seeds extract and fractions

Sample	Total phenolic content (mg GAE/g sample)	Total flavonoid content (mg QE/g sample)
_Methanol extract	$31.028 \pm 0.605$	$56.175 \pm 0.175$
_Ethyl acetate fraction	$58.25 \pm 0.501$	$75.123 \pm 0.175$
_n-hexane fraction	$44.315 \pm 1.737$	$59.626 \pm 0.268$
_Water fraction	$39.454 \pm 0.446$	$58.866 \pm 0.202$

Phenolic and flavonoid compounds are the most responsible for antioxidants activity.

This is due to the hydroxy groups present in phenolic and flavonoid compounds in free radical scavenging (Saxena et al., 2012; Aryal et al., 2019). Based on the IC<sub>50</sub> values in Table II, the total phenolic and flavonoid contents in the *L. domesticum* seeds are presented in Table III. It is known that the antioxidant activity of a material correlated with the phenolic and flavonoid contents in that material. The higher the total phenolic and flavonoid levels in the sample, the stronger the sample will be as an antioxidant. Correlation of total phenolic and flavonoid contents to radical activity (IC<sub>50</sub> values) in *L.*

*domesticum* seeds is showed in Figure 2 and Figure 3, respectively. The relationship

between radical activity (y) with total phenol (x) revealed a coefficient of determination (R<sup>2</sup>) of 0.9182, whereas total flavonoid content (x) has an R<sup>2</sup> of 0.7658. The results suggested that phenolic compounds and flavonoids compounds contributed to 91.82% and 76.38% to free DPPH radical scavenging of extract and fraction of *L. domesticum* seeds. Also, it can be stated that the scavenging effect of extracts/fractions is not limited to phenolic and flavonoid compounds.

The activity may also come from other antioxidant secondary metabolites in the extracts such as volatile oils, carotenoids, and vitamins (Javanmardi et al., 2003; Rohman et al., 2010; Mistriyani et al., 2018). / Figure 2. Correlation between of phenolic contents with the free DPPH radical activity (IC<sub>50</sub>) value of extract and fractions of *L. domesticum* seeds / Figure 3. Correlation between of flavonoid contents with the free DPPH radical activity (IC<sub>50</sub>) value of extract and fractions of *L. domesticum* seeds CONCLUSION The ethyl acetate fraction of *L.*

*domesticum* seeds have a very strong activity as an antioxidant using the DPPH method, and the compounds most responsible as antioxidants are phenolic compounds and flavonoids. Ethyl acetate fraction can be further isolated to find out which compounds are most responsible as antioxidants.

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