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The effect of extraction methods towards antioxidant activity of ethanol extract of *Litsea cubeba* Lour. barks

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Abstract

Antioxidants are molecules that can inhibit or prevent the oxidation reactions of other molecules that produce free radicals. Antioxidants can be synthesized in the body (endogenous) or obtained from food intake (exogenous). Attarasa (*Litsea cubeba* Lour.) is a plant from the Lauraceae family which is rich in essential oil compounds. This study was aimed to analyse antioxidant activity, total phenolic content and total flavonoid content in ethanol extract (EE). The powder was extracted by maceration, percolation and reflux method with absolute ethanol. Antioxidant activity was analysed with 1,1-diphenyl-2-picrylhydrazyl (DPPH). Total phenolic content was measured using the Folin-Ciocalteau method and total flavonoid content was measured using $AlCl_3$ reagent with colorimetric method. The best antioxidant activity and highest total phenolic content were shown by reflux extraction method with IC_{50} 31.79±0.73 µg/mL and 357.18±0.84 mg GAE/g, and highest level of total flavonoid content was shown by percolation method (16.09±0.77 mg QE/g). In conclusion, the ethanol extract of *Litsea cubeba* Lour. barks can be further developed as an antioxidant and functional food.

1. Introduction

Oxidative stress causes degenerative diseases that lead to death, such as cancer, atherosclerosis, diabetes, and heart disease (Valko *et al*., 2007; Sen *et al*., 2010). Oxidation reactions release free radicals, which can cause cell damage. Free radicals are charged molecules that assault our cells, disrupt cell membranes, and cause damage to the body's nucleic acids, proteins, and enzymes as a natural result of human metabolism. Oxidative stress refers to any type of free radical damage that might harm a cell's structure and function (Aher *et al*., 2011). Free radicals are produced by cellular metabolism, radiation, ozone, cigarette smoke, hyperoxia, and heavy metal exposure. Free radicals in excess can destroy macromolecules including DNA, lipids, and proteins (Birben *et al*., 2012). Antioxidants can be utilized to treat diseases induced by oxidative stress (Al-Dalaen and Al-Qtaitat, 2014). Antioxidants are chemicals that can block or prevent other molecules from oxidizing and producing free radicals (Partap and Pandey 2012; Dalimunthe *et al*., 2016). Antioxidants can be made in the body (endogenous) or consumed through diet (exogenous). Antioxidants can be divided into two categories: enzymatic and non-enzymatic antioxidants. Superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase are three enzyme antioxidants that play a key function (CAT). Many natural exogenous antioxidants are now being produced to suit the body's antioxidant needs. This is due to the fact that natural antioxidants have fewer adverse effects than synthetic antioxidants. Non-enzymatic antioxidants, such as glutathione, vitamin C, vitamin E, carotenoids, uric acid, thiols, and polyphenols, are examples of natural antioxidants found in plants (Gomes *et al*., 2012; Pacome *et al*., 2014).

Indonesia is a country with a lot of spice plant resources. Attarasa (*Litsea cubeba* Lour.), which is frequently found in the North Tapanuli area, is one form of spice that can be developed. Essential oils can discover in the fruit, stems, roots, and leaves of this plant. This gives the entire plant a pleasant odor (Feng *et al*., 2009; Trisonthi *et al*., 2014; Dalimunthe *et al*., 2017). Attarasa (*Litsea cubeba* Lour.) is a Lauraceae plant with a high concentration of essential oil components. Antidepressants, anti-inflammatory, antioxidant, pesticide, antibacterial, anticancer, and

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neuropharmacology are all traditional uses for attarasa essential oil (Dalimunthe *et al*., 2021). Flavonoids, alkaloids, tannins, and saponins are abundant in Litsea plants (Piyapat *et al*., 2013; Piyapat *et al*., 2014; Dalimunthe *et al*., 2019).

Extraction technique is a method for obtaining high yield extracts by reducing the difficulty of the extraction process (Quispe-Condori *et al*., 2008). Using an extraction method with a ratio of time and solvent requirements greatly determines the efficiency of extraction, in addition to the use of temperature, which has a significant impact on the quality (biological activity in vitro or in vivo) and quantity (amount or yield) of the extract obtained (Latiff and Ghan, 2011; Hmidani *et al*., 2019). Cold extraction (maceration and percolation) and hot extraction (reflux and soxhletation) are the most popular extraction processes (Ministry of Health, 2013; Desmiaty *et al*., 2019). Based on the background we want to analysis the effect of extraction methods towards antioxidant activity, total phenolic content and total flavonoid content of ethanol extract of *Litsea cubeba* Lour. barks.

2. Materials and methods

2.1 Preparation of extract

The air-dried and powdered *Litsea cubeba* Lour. barks (100 g) were extracted by maceration, percolation and reflux extraction method with absolute ethanol (Merck). The filtrate was collected and then evaporated under reduced pressure to give a viscous form and then dried to dry on a water bath (Dalimunthe *et al*., 2018).

2.2 DPPH radical scavenging assay

The free radical scavenging activity was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH•) method. 0.2mM solution of DPPH• in methanol was prepared and 100 µL of this solution was added to various concentrations of *Litsea cubeba* Lour. barks extracts. After 60 mins, absorbance was measured at 516 nm. All assays were carried out in triplicate (Satria *et al*., 2017; Rosidah *et al*., 2018).

2.3 Determination of total phenolic content

The total phenol concentration (TPC) of the sample was determined using a folin reagent. Briefly, 100 µL of EE (500 µg/mL) were mixed with 7.9 mL of distilled water and 0.5 mL of Folin-ciocalteu's reagent (1:10 v/v) and mixed with vortex for 1 min. After mixing, 1.5 mL of 20% aqueous sodium bicarbonate was added, and the mixture was allowed to stand for 90 mins within intermittent shaking. The absorbance was measured at 775 nm using a spectrophotometer. Total phenolic concentration is expressed as gallic acid equivalent in mg per gram of extract. The methanol solution was used as a blank. All assays were carried out in triplicate (Rosidah *et al*., 2008).

2.4 Determination of total flavonoid content

The amounts of total flavonoids in the extracts were measured spectrophotometrically as previously reported. Two millimetres of extract in methanol was mixed with 0.10 mL of 10% aluminium chloride ($AlCl_3.6H_2O$), 0.10 mL of sodium acetate ($NaC_2H_3O_2.3H_2O$) (1M), and 2.80 mL of distilled water. After incubation of 40 mins, absorbance was measured at 432 nm using a spectrophotometer. To calculate the concentration of flavonoids, we prepared a calibration curve using quercetin as standard. The flavonoid concentration is expressed as quercetin equivalents in mg per gram of extract. All assays were carried out in triplicate (Jamuna *et al*., 2012; Nazliniwaty *et al*., 2021).

3. Results and discussion

3.1 Antioxidant activity, total phenolic content and total flavonoid content of Litsea cubeba Lour. barks

The extract *Litsea cubeba* Lour. was found to have high antioxidant activity when tested using DPPH techniques. The DPPH technique revealed strong and moderate activity in barks extracted with ethanol and a variety of extraction procedures. The IC_{50} parameter is used to estimate the concentration of the sample necessary to capture 50% of DPPH radicals when employing the DPPH technique to determine antioxidant activity. Table 1 presents the results of antioxidant testing with an IC_{50} value of ethanol extract *Litsea cubeba* Lour using the DPPH method. Percolation method (33.41±0.52 µg/mL), maceration method (113.85±0.20 µg/mL), and reflux method (31.79±0.73 µg/mL) extraction methods were used to extract barks, with the lower the IC_{50} value, the higher the antioxidant activity (Molyneux, 2004). Very strong (IC_{50} < 50 µg/mL), strong (IC_{50} 50 - 100 µg/mL), moderate (IC_{50} 101 - 150 µg/mL), and weak (IC_{50} > 150 µg/mL) antioxidant activity was measured (Blois, 2003).

Table 1 shows the total phenol extraction methods of percolation (161.54±0.79 mg GAE/g), maceration (212.29±0.86 mg GAE/g), and reflux (357.18±0.84 mg GAE/g). Table 1 shows the total flavonoids extracted by percolation method (16.09±0.77 mg QE/g), maceration method (12.26±0.19 mg QE/g), and reflux method (11.68±0.45 mg Q/g). The type of solvent has an impact on the overall phenol concentration. Phenol is a polar chemical, which means it dissolves best in polar liquids. Polar solvents dissolve phenol more effectively, resulting in higher quantities of phenol in the extract (Moein and

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Table 1. Result of antioxidant activity (IC_{50}) with DPPH, total phenolic and total flavonoid content.

No	Extraction method	DPPH (µg/mL)	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)
1	Reflux	31.79±0.73	357.18±0.84	11.68±0.45
2	Maceration	113.85±0.20	212.29±0.86	12.26±0.19
3	Percolation	33.41±0.52	161.54±0.79	16.09±0.77

Values are presented as mean±SD.

Mahmood, 2010). Because not all of the phenol concentration in the extract is a flavonoid component, total phenol content is larger than total flavonoids. Polyphenol chemicals, which include phenolic acid, melanin, coumarin, flavonoids, lignins, and tannins, are the most abundant metabolites in plants (Srividya *et al*., 2014). Variances in the composition and quantity of secondary metabolites extracted in the extract induced differences in antioxidant activity strength. The reflux approach showed the lowest IC_{50} value compared to the maceration and percolation methods, hence it was the most active antioxidant activity in this investigation. The antioxidant activity of secondary metabolites, which have a lot of antioxidant activity in the extract, cannot be separated from the role of metabolite compounds, as shown in the reflux method of secondary metabolites, which have a lot of antioxidant activity in the extract, because the reflux method can attract compounds more efficiently and solvents can remove compounds in the sample more effectively (Wazir *et al*., 2011).

4. Conclusion

The best antioxidant activity and highest total phenolic content were shown by the reflux extraction method. The highest total flavonoid content was shown by the percolation extraction method.

Conflict of interest

The authors declare no conflict of interest in conducting this study.

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