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RESEARCH ARTICLE

Antioxidant activities of medang lendir (*Litsea glutinosa*) stem bark

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Abstract

Background: Medang lendir (*Litsea glutinosa*), a member of the Lauraceae family, is typically found in the Kampar Regency area of Riau Province, Indonesia. This plant is frequently used in traditional medicine to treat rheumatism, diarrhea, and dysentery. **Objective:** To evaluate the antioxidant activities of *L. glutinosa* stem bark extracts. **Method:** *L. glutinosa* stem bark was macerated in methanol to obtain methanol extract, followed by partitioning with ethyl acetate to obtain ethyl acetate extract. The antioxidant activities of these extracts were determined using the NO radical scavenging and the DPPH radical scavenging methods. The Folin-Ciocalteu method was also used in this study to determine total phenolic content (TPC). **Result:** The antioxidant activities of ethyl acetate and methanol extracts of *L. glutinosa* stem bark against NO radicals resulted in a small percentage of inhibition (%I < 0). Meanwhile, for DPPH radical scavenging, the antioxidant activity of the ethyl acetate extract (IC_{50} = 222.03 ug/mL) was greater than the methanol extract (IC_{50} = 232.81 ug/mL), and the TPC value of the ethyl acetate extract (23.88 mgGAE/g) was greater than the methanol extract (23.16 mgGAE/g). **Conclusion:** These results show that the antioxidant activity based on scavenging DPPH radicals for both extracts was active; however, it was inactive on NO radicals. The ethyl acetate extract possessed a higher TPC value compared to the methanol extract.

Introduction

A free radical is a molecule with one unpaired electron in its outermost orbit that is relatively unstable. Free radical molecules' capacity to oxidise other substances may contribute to oxidative injury in the body. Reactive oxygen species (ROS) are a well-known type of free radical. In the human body, ROS can interact with and damage macromolecules like proteins, lipids, and nucleic acids (Uttara *et al.*, 2009). Oxidative stress will occur if the damage can't be prevented. Large amounts of free radicals and a lack of antioxidants may worsen the condition (Sukweenadhi *et al.*, 2020).

The term "*antioxidants*" refers to a class of natural or synthetic compounds that work to prevent or delay oxidative damage by preventing the oxidation process or inhibiting the reaction that is sped up by oxide or peroxide species. The free radicals produced during cell metabolism get neutralised and stabilised by antioxidants (Sies, 1997). Effective antioxidants inhibit the chain reaction of free radicals and serve as radical scavengers. Therefore, antioxidants have a crucial stabilising role in maintaining biological activity (Sies, 1997).

Medang lendir (*L. glutinosa*) is a plant that can be found in forests throughout Riau Province, Indonesia. It belongs to Lauraceae family. In some areas, people use this plant as a mosquito repellent. It was reported that a secondary metabolite from its stem bark possessed an anti-plasmodial activity (Lestari *et al.*, 2021).

Various parts of this plant have long been used in traditional medicine. Its leaf extract is used as a mild astringent and pain reliever to treat diarrhoea and dysentery (Pradeepa *et al.*, 2011). The fruits produce oil that is used to cure rheumatism, the leaves' juice is used to treat respiratory diseases, and the roots are used to heal sprains, bruises, and wounds (Pradeepa *et*

al., 2013). Secondary metabolites found in abundance in this plant include tannins, b-sitosterol, actinodaphine, boldine, norboldine, laurothetanin, *n*-methyl laurothetanin, *n*-methyl actinodaffeine, quercetin, sebiferrin, and litseferrin. There are also large amounts of the aporphine alkaloids such as litseraglutine A and B (Pradeepa *et al.,* 2011; Pradeepa *et al.,* 2013). Likewise, bioactivities from the genus have been evaluated, including antioxidants, antidiabetic agents, anticancer agents, antimicrobials, and other activities (Kamle *et al.*, 2019).

There is currently no study that specifically looks at the antioxidant activity of the stem bark of *L. glutinosa*. Hence, studying the possible antioxidant capabilities of the *Litsea* plant components is crucial due to the numerous potential bioactivities of their components.

Methods

Plant materials

The stem barks of *L. glutinosa* were collected from Kampar Regency, Riau Province, Indonesia. The morphology and anatomy of *L. glutinosa* were identified in the Botanical Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Riau. The stem bark of *L. glutinosa* was dried for one week, finely ground into powder, and stored for further analysis.

Extraction of plant materials

The dried stem bark powder was macerated with methanol for 72 hours and then partitioned with ethyl acetate to obtain an ethyl acetate extract. The extracts were collected and dried *in vacuo* at 40^{o}C (BUCHI R-114) to obtain crude extracts of methanol and ethyl acetate. These extracts were labelled as LG-M and LG-EA for methanol extract and ethyl acetate extract respectively.

NO free radical scavenging assay

In this nitric oxide (NO) assay, samples of LG-M and LG-EA extracts were prepared to obtain series concentrations by two-fold dilution from 1000 ppm to 62.5 ppm in a 96-well microplate, and ascorbic acid was used as the positive control. Griess reagent was prepared according to Muflihah *et al.* (2021). Each LG-M solution was pipetted for 50 mL and mixed with 100 mL of sodium nitroprusside, and then the microplate was incubated in a dark place for 20 minutes. After incubation, the extracts were mixed with 100 mL of Griess reagent. The absorbance of the solution was measured at 550 nm using a microplate reader (Berthold, Germany). The same procedure was used for the LG-EA solutions.

The following equation was used to figure out the percentage of inhibition (%I) of NO radical scavenging:

$$\%I = [(A_0-A_1)/A_0] \times 100$$

A_0 refers to the absorbance of the negative control, and A_1 refers to the absorbance of the samples.

The absorbance was measured at a wavelength of 520 nm to determine the level of NO radical scavenging, which formed a pink colour.

DPPH radical scavenging assay

The antioxidant activity of methanol and ethyl acetate extracts was evaluated by *1,1-diphenyl-2-pycrilhidrazyl* (DPPH) radical scavenging, according to Almurdani *et al.* (2020) and Dobrinas *et al.* (2021). Samples were prepared with a two-fold dilution, starting from 1000 ppm to 31.25 ppm in a 96-well microplate. Exactly 50 mL of the LG-M solution was pipetted and mixed with 80 mL of DPPH in a microplate. The microplate was then incubated in a dark place for 30 minutes. A microplate reader (Berthold, Germany) was used to measure the absorbance of samples at 520 nm. A similar procedure was also employed for the LG-EA solutions. The absorbance of ascorbic acid as a positive control was also measured with the same method. Reactions between antioxidants and DPPH usually change the colour of the solution from purple to yellow.

The following equation was used to determine the percentage of inhibition (%I) of DPPH:

$$\%I = [(A_0-A_1)/A_0] \times 100$$

A_0 refers to the absorbance of the negative control, and A_1 refers to the absorbance of the samples. The IC_{50} value was determined from the graph that was plotted between the %I value and the concentration of samples.

Determination of total phenolic content (TPC)

The total phenolic content of each extract was measured by the Folin-Ciocalteu method with a slight modification (Aryal *et al.,* 2019; Johari *et al*., 2019). Briefly, 5 mg of LG-M was diluted with 2 mL of ethanol. This solution was diluted ten times with deionised water before being mixed with the 0.25 N Folin-Ciocalteu reagent. After 5 minutes, 7.5% Na_2CO_3 was added and incubated in a dark place for 30 minutes at room temperature. Afterwards, the absorbance was measured using a UV-Vis spectrophotometer (Genesys 10S®) at 765 nm. Gallic acid with a variation in concentration (20–5 ppm) was used to make a

calibration curve for the standard. The total phenolic content was revealed as mg/g of gallic acid equivalents in mg/g of dry samples (mg GAE/g). All samples were run in triplicates. The same steps were also applied for the LG-EA solutions.

Results

NO Radical Scavenging Activity

The scavenging activity of NO radicals from *L. glutinosa* stem bark showed that the percentage inhibition values for LG-M and LG-EA were negative (%I<0), indicating that the extracts did not inhibit but rather increased NO radical activity. The NO radical scavenging activity of LG-EA and LG-M is depicted in Figure 1. It showed no colour change during the test. Therefore, they are considered to be inactive.

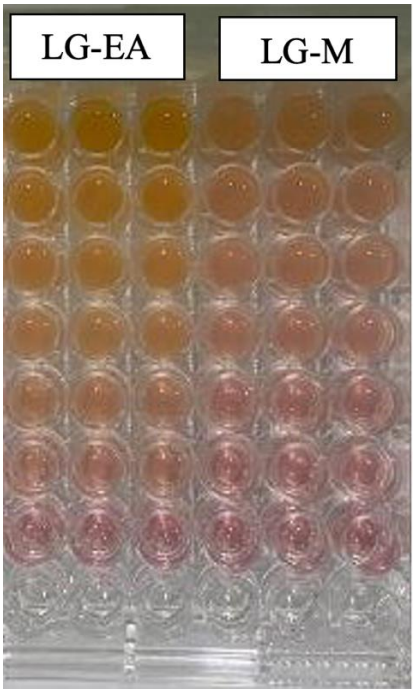

Figure 1: NO radical assay, showing no scavenging activity of ethyl acetate and methanol extracts

DPPH radical scavenging activity

DPPH is a stable, dark purple-coloured free radical that turns yellow when exposed to an antioxidant. The degree of discolouration indicates the amount of DPPH that has been scavenged. The DPPH antioxidant assay on LG-EA and LG-M demonstrated an activity. The LG-EA had the lowest IC_{50}, followed by LG-M, which had IC_{50} values of 222.026 ug/mL and 232.814 ug/mL, respectively. The DPPH radical scavenging activities of LG-EA and LG-M are shown in Figure 2. The scavenging activities of DPPH from *L. glutinosa* stem barks are also presented in Table I.

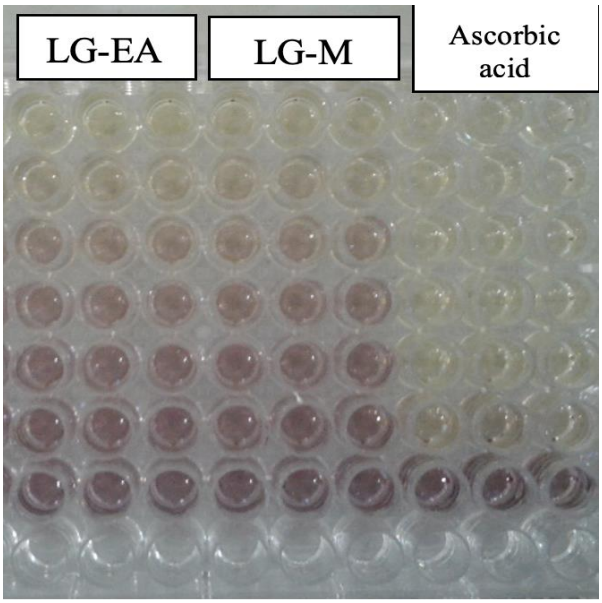

Figure 2: DPPH radical assay, showing the scavenging activity of ethyl acetate and methanol extracts as well as ascorbic acid as a positive control

Table I: IC_{50} values of the methanol and ethyl acetate extracts (ascorbic acid is a positive control)

Sample	IC_{50} (ug/mL)	SD
LG-M	245.90	20.84
LG-EA	164.84	14.81
Ascorbic acid	0,06	0.10

Total phenolic content (TPC)

The results showed that the LG-M exhibited lower TPC as compared to the LG-EA, with 23.159 mg gallic acid equivalence (mgGAE/g) and 23.884 mgGAE/g, respectively. Gallic acid with a variation in concentration (20–5 ppm) was used to make a calibration curve for standard ($y = 0.069x + 0.057$; $R^2 = 0,997$). TPC values from *L. glutinosa* stem barks are presented in Table II.

Table II: Total phenolic content of the methanol and ethyl acetate extracts

Sample	Total phenolic (mgGAE/g)
LG-M	23.159
LG-EA	23.884

Discussion

The formation of nitrite comes from nitric oxide, which is produced from the reaction of sodium nitroprusside with oxygen. As higher antioxidant activity is indicated by a lower IC_{50} value, LG-EA has a higher ability to scavenge DPPH (free radicals) compared to LG-M. The presence of phenolic compounds may be responsible for antioxidant activity (Johari & Khong, 2019; Mfotie-Njoya *et al.*, 2017). Phenolic compounds contained in plants are important secondary metabolites that have redox properties. Free radicals are usually scavenged by the presence of hydroxyl groups on phenol.

The LG-EA represents high antioxidant activity and has a positive relationship with TPC. Previous research has demonstrated that a high TPC level correlates linearly with high antioxidant activity. The amount of total phenolic components in the crude extract of the plant strongly influences the antioxidant ability (Johari & Khong, 2019).

The measurement of the TPC was based on the formation of a blue-coloured product from the oxidation of phenolic compounds by the active centre Mo(VI) in the phosphomolybdate-tungstate complex.

Conclusion

The methanol and ethyl acetate extracts of *L. glutinosa* stem bark showed good capacity as a natural source of antioxidant using the DPPH assay method, but were unable to inhibit NO radicals. The antioxidant properties of both extracts were possible due to the presence of phenolic compounds. This report can be used for further research to extract compounds that possess antioxidant activity.

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