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RESEARCH ARTICLE

Antioxidant activity of *Loranthus ferrugineus* twigs extracts

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Abstract

Background: A coffee parasite, *Loranthus ferrugineus*, is a type of parasitic plant that is widely used as a medicinal plant. It has many activities, including as an antioxidant agent. **Objective:** This study is to determine the antioxidant activity in the twig extracts of *L. ferrugineus*. **Method:** The twigs of *L. ferrugineus* were collected from Pekanbaru, Riau Province, Indonesia. Extraction was carried out by maceration of the dry twigs using methanol, and then liquid-liquid extraction was carried out to obtain ethyl acetate and methanol fractions. The antioxidant activity test was carried out using the radical DPPH and NO free radical scavenging methods by calculating the IC_{50} values, and the total phenolic content (TPC) was measured. **Result:** The antioxidant activity assay of the DPPH method on ethyl acetate and methanol extracts revealed IC_{50} values of 20.85 µg/mL and 18.83 µg/mL, respectively. Meanwhile, the NO free radical method on ethyl acetate and methanol extracts showed IC_{50} values of 78.52 µg/mL and greater than 1000 µg/mL. The total phenolic content of the ethyl acetate extract was 26.78 mgGAE/gram, while it was 24.85 mgGAE/gram in the methanol extract. **Conclusion:** The ethyl acetate and methanol extracts could inhibit free radicals from DPPH, which was classified as very strong antioxidant sources. Meanwhile, the inhibition of the extracts against the NO free radical was very weak.

Introduction

Our body is exposed to free radicals every day, either from the environment or the food and drinks we consume. Long-term exposure to these will increase levels of pro-oxidant factors that can cause structural defects at the mitochondrial DNA level, as well as the functional alteration of several enzymes and cellular structures, leading to aberrations in gene expression. Oxidative stress plays an essential role in the pathogenesis of chronic diseases such as cardiovascular diseases, diabetes, neurodegenerative diseases, and cancer (Sharifi-Rad *et al.*, 2020). To prevent cell damage due to exposure to free radicals, the body needs an adequate intake of antioxidants that can inhibit or prevent oxidative damage.

Medicinal plants have been used in healthcare since ancient times. Studies have been carried out globally to verify their efficacy, and some of the findings have led to the production of plant-based medicines. The global market value of medicinal plant products exceeds $100 billion per annum (Sofowora *et al.*, 2013). Natural resources in the form of medicinal plants are Indonesian assets that need to be explored, developed, and optimised.

Parasite plants are a group of hemiparasitic plants that attach to the branches and twigs of trees. The benefits of this plant are to cure cough, hypertension, cancer, smallpox, ulcers, skin infections, and many other illnesses. *Loranthus ferrugineus* (family of Loranthaceae), or mistletoe, is a medicinal herb used for a variety of human ailments. Traditionally, decoctions of this parasitic shrub have been mainly used to treat high blood pressure and gastrointestinal complaints. These uses were supported by experimental-based pharmacological investigations (Ameer *et al.*, 2015).

Several studies have been conducted showing that the family of this plant, Loranthaceae, possesses antibacterial and antifungal activity (Osadebe &

Akabogu, 2016). Leaf extract of *L. ferrugineus* has very strong antioxidant activity, and it was found that one of its secondary metabolites, quercitin, possessed anticancer activity (Yulian & Safrijal, 2018). It has also been reported that *L. ferrugineus* extract has anti-diabetic activity (Teruna *et al.*, 2022).

Based on the description above, it is necessary to conduct further research to test the antioxidant activity of the twigs of the plant. In this study, activity tests were carried out on ethyl acetate and methanol extracts using the DPPH and NO free radical methods to determine the total phenolic content (TPC).

Method

Collection of plant materials

L. ferrugineus was collected in Pekanbaru, Riau Province, Indonesia. The plants were identified in the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Riau. Twigs of this plant were separated from other parts of the plant.

General Experimental

An assay for antioxidant activity was conducted using a multimode microplate reader, TriStar LB 941 (Berthold Technologies). In the DPPH method, the absorbance was recorded at 520 nm, and for NO free radical scavenging, it was recorded at 550 nm. To determine total phenolic content (TPC), we used a UV-visible spectrophotometer (Genesys 10S UV-Vis) at 765 nm.

Sample Preparation

The twigs were extracted by the maceration method. The samples were dried for ten days. The samples were ground prior to maceration using methanol for 24 hours and filtered after ultrasonication for 30 minutes. These processes were repeated three times. The filtrate was mixed with water to make 70% methanol. Then it was subjected to liquid-liquid extraction to obtain the ethyl acetate and methanol extracts. These extracts were then evaporated *in vacuo*.

Antioxidant assay with the DPPH method

The ability of the extracts to scavenge DPPH free radicals was measured using the method described by Takao and researchers in 1994. The ethyl acetate extract was prepared at concentrations of 1000 µg/mL. Two-fold dilutions were applied to give the smallest sample concentration of 31.25 µg/mL. Then 80 µL of DPPH (1,1-diphenyl-2-picrylhydrazyl) solution was added to each well. Methanol was used as a negative control. The test solutions with various concentrations and negative controls were incubated without light for 30 minutes. The absorption of the solution was measured at a maximum absorption wavelength of 520 nm using a microplate reader (Berthold Technologies). The same procedure was applied to the methanol extract.

Antioxidant assay with the NO radical method

This method is based on the principle that sodium nitroprusside in ethanol solutions at physiological pH spontaneously produces nitric oxide, which interacts with oxygen to produce nitrite ions, which can be quantified using the Griess reagent.

Ten milligrams of ethyl acetate extract from twigs of *L. ferrugineus* were weighed and dissolved in one mL (1000 µg/mL). A two-fold dilution from 1000 µg/mL to 62.5 µg/mL was used to make a set of samples. Then, in each well, 100 L of sodium nitroprusside were added, and it was incubated for 20 minutes. 100 µL Griess reagent was added and incubated for two minutes. The absorbance of the solution was measured at 550 nm using a microplate reader (Berthold, Germany).

The total phenolic content test was carried out using Folin-Ciocalteu's UV-Vis spectrophotometry at 765 nm (Wootton-Beard *et al.,* 2011).

Result

The results of antioxidant testing using the DPPH and NO methods were carried out on extracts of ethyl acetate and methanol. Ascorbic acid was used as a positive control. The results are shown in Table I and Table II, respectively.

Vamanu and researchers found that the phenolic content of the extract was linked to its ability to fight free radicals. The linear regression equation used to measure the total phenolic content is Y = 0.069X + 0.057, with a correlation coefficient (*r*) of 0.997, in the range of 5–20 µg/mL. The linear calibration curve is obtained in Figure 1.

Table I: Antioxidant test results of L. ferrugineus twigs extract using the DPPH method

Type of samples	$IC_{50} \pm SD$ (µg/mL)	AAI
Ethyl acetate	20.85±1.38	3.84
Methanol	18.83±1.37	4.25
Ascorbic acid	3.46 ± 0.77	23.09

Table II: Antioxidant assay results of *L. ferrugineus* twigs extract using NO method

Type of samples	IC_{50} (µg/mL)+SD	Nitric oxide scavenged
Ethyl acetate	78.52 ± 14.06	42.04%
Methanol	>5000	35.22%
Ascorbic acid	90.23 ± 21.64	62.00%

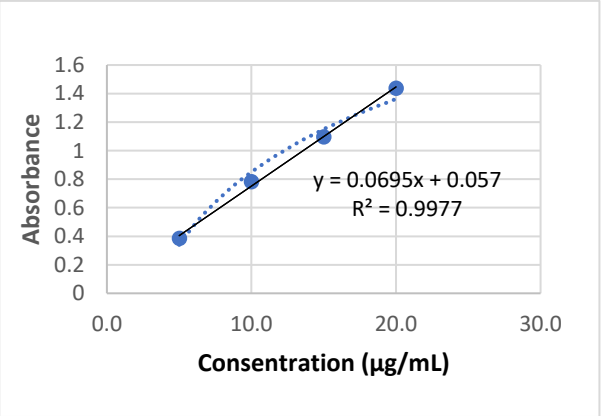

Figure 1: Gallic acid calibration curve

Discussion

The results of the antioxidant assay using the DPPH method on ethyl acetate extract had an IC_{50} value of 20.85 µg/mL with an AAI value of 3.84, which was classified as very strong. The methanol extract has an IC_{50} value of 18.83 µg/mL with an AAI value of 4.25, which is very strong. Ascorbic acid has an IC_{50} value of 3.464 µg/mL with an AAI value of 23.09, which is very strong (Table I). Antioxidants are confirmed as very strong if the antioxidant activity index (AAI) value is greater than two, AAI greater than one to two is strong, AAI greater than 0.5 to one is moderate, and AAI less than 0.5 is weak (Scherer & Godoy, 2009).

The results of the antioxidant assay using the NO radical method on ethyl acetate extract with an IC_{50} value of 78.52 µg/mL showed strong antioxidant activity with a percentage of inhibition of 42.04%. Methanol extract with an IC_{50} value greater than 1000 µg/mL has very weak antioxidant activity with a percentage of inhibition of 35.22%. Ascorbic acid has an IC_{50} value of 90.23 µg/mL which is classified as strong with a large percentage of inhibition of 62% (Table II).

A compound is considered to be a very strong antioxidant if the IC_{50} value is less than 50 µg/mL, strong activity is between 50-100 µg/mL, moderate activity if the IC_{50} value is 101-150 µg/mL and weak activity if the IC_{50} value is 151-200 µg/mL, activity is very weak if the IC_{50} value is greater 200 µg/mL (Blois, 1958).

In the measurement of total phenolic compounds, three replications were made for data accuracy purposes. Based on the results of this study, the total phenolic content of the ethyl acetate extract of the twigs of *L. ferrugineus* was 26.78 mg gallic acid equivalent (mgGAE/gram) extract, meaning that in every gram of the ethyl acetate extract was phenolic equivalent to 26.78 mg of gallic acid. Meanwhile, the total phenolic content of methanol extract was 24.85 mgGAE/gram extract. The phenolic compounds in each extract are secondary metabolites that have the potential to be used as antioxidants in conventional medicines or herbal medicines.

Conclusion

The conclusion from the results of research on the twigs of the coffee plant parasite, *Loranthus ferrugineus*, can be stated that the antioxidant activity of ethyl acetate and methanol extracts can inhibit free radicals from DPPH, which is classified as very strong, while the inhibition of NO radicals is strong for ethyl acetate extract and very weak for methanol extract. The total phenolic content of the ethyl acetate extract of *L. ferrugineus* was 26.78 mgGAE/gram while the methanol extract was 24.85 mgGAE/gram.

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References

Ameer, O.Z., Salman, I.M., Quek, K.J. & Asmawi, M.Z. (2015). *Loranthus ferrugineus*: a Mistletoe from Traditional Uses to Laboratory Bench. *Journal of Pharmacopuncture,* **18**(1), 7–18. https://doi.org/10.3831/KPI.2015.18.001

Blois, M.S. (1958). Antioxidant Determinations by the Use of a Stable Free Radical. *Nature, 181*, 1199-1200

Osadebe, P.O. & Akabogu, I.C. (2006). Antimicrobial activity of *Loranthus micranthus* Harvested from Kola Nut Tree. *Fitoterapia*, **77**(1), 54–56. https://doi.org/10.1016/j.fitote.2005.08.013

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P. V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M. E., Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A. O., Setzer, W. N. & Sharifi-Rad, J. (2020). Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Frontiers in physiology*, *11*, 694. https://doi.org/10.3389/fphys.2020.00694

Scherer, R. & Godoy, H. (2009). Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. *Food Chemistry*, **112**(3), 654-658

Sofowora, A., Ogunbodede, E. & Onayade, A (2013). The Role and Place of Medicinal Plants in the Strategies for Disease Prevention. *African Journal of Traditional, Complementary and Alternative Medicines,* **10**(5). https://doi.org/10.4314/ajtcam.v10i5.2

Takao, T., Kitatani, F., Watanabe, N., Yagi, A & Sakata K. (1994). A Simple Screening Method for Antioxidants and Isolation of Several Antioxidants Produced by Marine Bacteria from Fish and Shellfish. *Biosci Biotechnol Biochem*, *58*, 1780-3

Teruna, H.Y., Hendra, R., & Almurdani, M. (2022). IAI Special Edition: α-Glucosidase Inhibitory Activities of *Loranthus ferrugineus* and *Peperomia pellucida* extracts. *Pharmacy Education*, **22**(2), 5–8. https://doi.org/10.46542/pe.2022.222.58

Vamanu, E. & Nita, S. (2013). Antioxidant Capacity and the Correlation with Major Phenolic Compounds, Anthocyanin, and Tocopherol Content in Various Extracts from the Wild Edible *Boletus edulis* Mushroom. *BioMed Research International,* 2013, 1–11. https://doi.org/10.1155/2013/313905

Wootton-Beard, P.C., Moran, A. & Ryan, L. (2011) Stability of the Total Antioxidant Capacity and Total Polyphenol Content of 23 Commercially Available Vegetable Juices Before and After *In Vitro* Digestion Measured by FRAP, DPPH, ABTS and Folin-Ciocalteu Methods. *Food Res Int*, *44*, 217-24

Yulian, M. & Safrijal. (2018). Antioxidant Activity Test of Coffee Parasite Leaves (*Loranthus ferrugineus* Roxb.) by 1,1-Diphenyl-2-pycrilhidrazyl (DPPH) Method. *Lantanida Journal* , 103-202