Pharmacy Education (2023) 23(2) 174 - 178
https://doi.org/10.46542/pe.2023.232.174178

IAI SPECIAL EDITION

RESEARCH ARTICLE

Antioxidant and toxicological activities of *Pyrossia lanceolata* (L.) Farw. extracts

Ika Ria Indriani, Hilwan Yuda Teruna, Rudi Hendra

Department of Chemistry, Faculty of Mathematics and Natural sciences, Universitas Riau, Riau, Indonesia

Keywords
Antioxidant
Pyrrosia lanceolata (L.) Farw
Toxicity

Correspondence
Rudi Hendra
Department of Chemistry, faculty of Mathematics and Natural Sciences
Universitas Riau
Indonesia
Rudi.hendra@lecturer.unri.ac.id

Abstract

Background: *Pyrrosia lanceolata* (L.) Farw. is one of the polypodiaceae species that is commonly used as a traditional medicinal plant. **Objective:** The purpose of this study was to determine the antioxidant and toxic levels in the extracts of the species. **Method:** The aerial portion of the specie was extracted with methanol, followed by a liquid-liquid extraction with *n*-hexane, dichloromethane, and ethyl acetate. DPPH radicals and the brine shrimp lethality test, were used to assess its antioxidant activity and toxicity. **Result:** The *n*-hexane extract exhibited no activity with IC_{50} values exceeding 500µg/mL, whereas the ethyl acetate extract exhibited a high level of antioxidant activity with an IC_{50} value of 12.08±0.27µg/mL. Moreover, when tested against Artemia salina leach, the toxicological level of the extracts was greater than 1000µg/mL. **Conclusion:** These findings lay the groundwork for further investigation into the process of isolating and evaluating the biological activity of secondary metabolites that were discovered in the extract.

Introduction

A free radical is a molecule or compound with one or more unpaired electrons. This molecule is easily attracted to a magnetic (paramagnetic) field and is highly reactive due to one or more unpaired electrons. Stable molecules in the environment will be attacked by free radicals, which will then acquire electrons. The substance whose electrons are removed will transform into new free radicals, causing a chain reaction that results in cell damage. Unpaired free radical electrons do not influence the electrical charge of their molecules; they can be positively charged, negatively charged, or neutral. (Phaniendra *et al.,* 2015)

Numerous antioxidants present in diverse plant species can neutralise the damage caused by free radicals in the body. To improve the quality of public health at a relatively low cost, it is necessary to use natural ingredients as antioxidants. Antioxidants are electron-donor compounds that stimulate oxidation reactions by inhibiting radical formation. Most of the structure of antioxidative compounds derived from plant secondary metabolite compounds consists of phenol or phenolic aromatic rings, and phenol's aromatic ring will contribute to its antioxidant properties. (Imelda *et al.*, 2022). Indonesia has various plants, ranging from low-level plants (fungi, lichens, ferns) to high-level plants (multiple angiosperms) that inhabit different habitat types. It is estimated that there are 10,000 species of spike plants in Indonesia. In the meantime, there are nearly 1,200 species in Indonesia alone. This plant is commonly found in humid environments with tropical climates. (Sadono *et al*., 2020)

As an epiphytic fern, *Pyrrosia lanceolata* (L.) Farw. is often found attached to aged, large trees. Several reports show that the genus from *Pyrrosia* has been used as traditional medicine, such as a diuretic (Masuda *et al.*, 1997), to ease pains in labour and cough (Hovenkamp, 2003). In addition, various species of this genus, such as *P. lingua, P. sheareri,* and *P. longifolia*, exhibited high antioxidant activity when tested with multiple antioxidant assay methods. (Ding *et al.,* 2008; Gan *et al.,* 2010; Khodijah *et al.,* 2022). *P. lanceolata* (L.) Farw is a plant that is used in traditional medicine in a number of countries, including Malaysia, South Africa, and Mexico. In India, the leaf is ground up with pepper to make a paste and then taken orally to treat sore throats. (Sathiyaraj *et al.,* 2015) However, studies on the phytochemicals of this species and its pharmacology

effect, including antioxidant activities, have not yet undergone extensive investigation. In this study, we reported the toxicological and antioxidant activities of various extracts derived from this species.

Methods

The *P. lanceolata* was taken from the trunk of a tree on the Universitas Riau campus, Indonesia (Figure 1), and the Head of the Botany laboratory in the department of Biology at Riau University subsequently identified the species. After being air-dried, the aerial part of the species was blended to obtain a fine powder, which was then stored in the refrigerator until further analysis could be conducted. The species' 3 kg of fine powder was extracted using methanol, followed by liquid-liquid extraction, according to Hendra (Hendra, 2020).

Figure 1: Picture of Pyrrosia lanceolata (L.) Farw in Universitas Riau

Antioxidant activity

Extracts were prepared with a specific methanol concentration. Approximately 100µL of the sample was injected into microplate row A. To achieve a concentration of 31.25g/mL, successive rows of the extracts were diluted by a factor of two. A total of 5µL of Diphenylpicrylhydrazyl (DPPH) was added to each sample well at an 80 µg/mL concentration. The microtiter plate was then vortexed and incubated in a dark room for 30 minutes. As a control, ascorbic acid was subjected to an identical procedure. (Hendra *et al.,* 2020; Afham *et al.,* 2022)

The % Inhibition value is calculated by the following formula:

% Inhibition = $(A_0\text{-}A_s)/A0\times100\%$

GraphPad Prism 9 was used to perform the IC_{50} analysis. A_0 represents the absorbance of the DPPH radical solution without a sample and represents the absorbance of the model in the DPPH revolutionary solution. The test was performed in triplicate, and the results were expressed as the mean and standard deviation.

Antioxidant Activity Index (AAI)

$$\mathrm{AAI} = \frac{\mathrm{DPPH}\ (\mu\mathrm{g/mL})}{\mathrm{IC50}\ (\mu\mathrm{g/mL})}$$

(DPPH = 2,2-Diphenyl-1-picrylhydrazyl)

Toxicity activity

The extracts' toxicological activity was determined using the Brine shrimp lethality test (BSLT) (Jasril *et al.,* 2019; Hendra *et al.,* 2021). The experiment was performed in triplicate, and the results were reported as the mean and standard deviation. By plotting the median mortality percentage against the log of concentration, we could determine the concentration (LC_{50}) at which fifty per cent of the population died directly from exposure to the extracts.

Results

Antioxidant activity from various species extracts was measured with a free radical scavenging assay using DPPH as the radical. The results showed a wide range of antioxidant movement, as seen in Table I. The *n*-hexane extract showed no activity with IC_{50} values greater than 500g/mL. In contrast, the ethyl acetate extract demonstrated a high level of antioxidant activity with an IC_{50} value of 12.08±0.27 µg/mL. In addition, the free radical scavenging activity exhibited by ethyl acetate is significantly comparable to that of ascorbic acid, which serves as the standard. Moreover, when tested against Artemia salina leach, the toxicological status of the extracts showed an LC_{50} of more than 1000µg/mL.

Table I. Antioxidant and toxicological activities of *Pyrrosia lanceolata* (L.) Farw extract

Sample	IC_{50} (µg/mL)	Antioxidant Index	Toxicity (µg/mL)
n-Hexane	>500	0.14	>1000
Dichloromethane	48.55±0.54	1.65	>1000
Ethyl acetate	12.08±0.27	6.62	>1000
Water	35.83±0.76	2.23	>1000
Ascorbic acid	11.05±0.12	7.24	-

Discussion

Choosing an appropriate extraction solvent for the analysis is necessary to separate the secondary metabolites from the plant. According to Tong and colleagues, the organic solvents utilised affect both the variety of natural products that can be extracted and, as a direct consequence of this, the biological activity of the crude extract that is produced (Tong *et al.,* 2014). In this investigation, the species was extracted using organic solvents with varying degrees of polarity (*n*-hexane, dichloromethane, and ethyl acetate) to distinguish between hydrosoluble molecules that were liposoluble.

Any compound significantly slowing or preventing the oxidation process is considered an antioxidant. An indirect indicator of antioxidant activity is the rate at which the presence of an antioxidant slows oxidation processes. (Shahidi *et al.,* 2015) The DPPH free radical scavenging method was used to evaluate the antioxidant activity of each extract. When measuring a compound's antiradical activity or section, DPPH is frequently used. DPPH is an organic stable radical that exists in crystalline form and solution. It is common practice to link a substance or extract's ability to act as an antioxidant with its capacity to neutralise the effects of free radicals. (Karimi *et al.*, 2010; Kedare *et al.,* 2011)

The lack of standardisation of the DPPH method results makes it difficult to compare the antioxidant potency of plant extracts and pure compounds. Scherer and Godoy proposed an antioxidant index (AAI). The AAI was calculated based on the mass of DPPH and the sample used in the reaction, resulting in a constant for each sample. When AAI is less than 0.50, the sample has low antioxidant activity. AAI between 0.5 and 1.0 indicates moderate antioxidant activity. An AAI between 1.0 and 2.0 characterises high antioxidant activity. (Scherer & Godoy 2009) Following these criteria, the water, ethyl acetate, and dichloromethane extracts ought to be considered to possess high antioxidant activity. In comparison, antioxidant activity by using DPPH radical between *Pyrrosia* species, the quotes from *P. lanceolata* showed high activity compared to *P. longifolia*, P. petioles extract and comparable with P. lingua extracts. (Hsu, 2008; Akhmadjon *et al.,* 2020; Khodijah *et al.,* 2022)

These results might be due to the presence of phenolics such as flavonoids. Flavonoids have several properties, the most important of which is unquestionably related to their capacity to neutralise the damaging effects of free radicals and work as antioxidants. The antioxidant action mechanisms of flavonoids can include the following. (a) Direct scavenging of ROS. (b) Inhibition of ROS formation through the chelation of trace elements. (c) Activation of antioxidant defences (Dias *et al.,* 2021). These species contain flavonoids, including quercetin and its glucoside, kaempferol, and its glucoside, naringenin, trifolin, and astragalin, according to several previous reports on the phytochemical studies of *Pyrrosia sp* (Xiao *et al.*, 2017; He *et al.,* 2019; Pan *et al.*, 2021). Therefore, the antioxidant activity of this current study might be due to the presence of flavonoids in the extracts. Hirano and colleagues reported that flavonoids like quercetin and kaempferol possessed antioxidant properties against the DPPH radical at IC_{50} values of 2.35 and 5.50 µM, respectively (Hirano *et al.,* 2001).

The extracts were tested for toxicity using brine shrimp. The test determines the toxic potential of bioactive substances or sections, and it's based on test compounds' ability to kill shrimp, *Artemia salina* Liech (Wu, 2014). Meyer and his colleagues (1982) found that plant extracts were toxic with an LC_{50} of less than 1000 µg/ml but highly toxic to shrimp at less than 30 µg/ml (Meyer *et al.,*1982). All of the extracts were found to have an LC_{50} value that was more significant than 1000 µg/ml, indicating that they were not toxic, as seen in Table I.

Conclusion

The results of this study revealed that extracts of *P. lanceolata* have potent antioxidant activity against DPPH radicals, as well as non-toxic action against tested animals. These findings lay the groundwork for further research into isolating and analysing secondary metabolites found in the extract.

Acknowledgement

The authors want to take this opportunity to thank the Faculty of Mathematics and Natural Sciences at Universitas Riau, in addition to the Center for Research and Community Development at the University, for the assistance they have provided.

References

Afham, M., Teruna, H. Y., & Hendra, R. (2022). IAI SPECIAL EDITION: The potential of Mimosa pudica L as an α-glucosidase inhibitor and antioxidant agent. *Pharmacy Education,* **22**(2), 1-4. https://doi.org/10.46542/pe.2022.222.14

Akhmadjon, S., Hong, S. H., Lee, E.-H., Park, H.-J., & Cho, Y.-J. (2020). Biological activities of extracts from Tongue fern (Pyrrosia lingua). *Journal of Applied Biological Chemistry,* **63**(3), 181-188. https://doi.org/10.3839/jabc.2020.025

Dias, M. C., Pinto, D. C., & Silva, A. M. (2021). Plant flavonoids: Chemical characteristics and biological activity. *Molecules,* **26**(17), 5377. https://doi.org/10.3390/molecules26175377

Ding, Z. T., Fang, Y. S., Tai, Z. G., Yang, M. H., Xu, Y. Q., Li, F., & Cao, Q. E. (2008). Phenolic content and radical scavenging capacity of 31 species of ferns. *Fitoterapia,* **79**(7-8), 581-583. https://doi.org/10.1016/j.fitote.2008.01.011

Gan, R.-Y., Kuang, L., Xu, X.-R., Zhang, Y., Xia, E.-Q., Song, F.-L., & Li, H.-B. (2010). Screening of natural antioxidants from traditional Chinese medicinal plants associated with the treatment of rheumatic disease. *Molecules,* **15**(9), 5988-5997. https://doi.org/10.3390/molecules15095988

He, K., Fan, L.-L., Wu, T.-T., & Du, J. (2019). A new xanthone glycoside from Pyrrosia sheareri. *Natural Product Research,* **33**(20), 2982-2987. https://doi.org/10.1080/14786419.2018.1514398

Hendra, R., Gurning, S. N., Panjaitan, U. P. A., & Teruna, H. Y. (2020). *Antioxidant activity of an Epiphyte Fern in Palm Oil Tree.* Paper presented at the Journal of Physics: Conference Series. https://doi.org/10.1088/1742-6596/1655/1/012031

Hendra, R., Khodijah, R., Putri, R., Amalia, R., Haryani, Y., Teruna, H. Y., & Abdulah, R. (2021). Cytotoxicity and Antiplasmodial Properties of Different Hylocereus polyrhizus Peel Extracts. *Medical Science Monitor Basic Research,* **27**, e931118-931111. https://doi.org/10.12659/MSMBR.931118

Hendra, R., Masdeatresa, L., Abdulah, R., & Haryani, Y. (2020). *Red dragon peel (Hylocereus polyrhizus) is an antioxidant source.* Paper presented at the AIP Conference Proceedings. https://doi.org/10.1063/5.0001391

Hirano, R., Sasamoto, W., Matsumoto, A., Itakura, H., Igarashi, O., & Kondo, K. (2001). The antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. *Journal of Nutritional Science and Vitaminology,* **47**(5), 357-362. https://doi.org/10.3177/jnsv.47.357

Hovenkamp, P. H. (2003). *Pyrrosia* Mirbel. In W. P. De Winter & V. B. Amaroso (Eds.), *Plant resources of South-East Asia No 15(2): Ferns and Fern Allies* (pp. 170-174). Leiden: Backhuys

Hsu, C.-Y. (2008). Antioxidant activity of Pyrrosia petiolosa. *Fitoterapia,* **79**(1), 64-66. https://doi.org/10.1016/j.fitote.2007.07.007

Imelda, E., Idroes, R., Khairan, K., Lubis, R. R., Abas, A. H., Nursalim, A. J., & Tallei, T. E. (2022). Natural Antioxidant Activities of Plants in Preventing Cataractogenesis. *Antioxidants,* **11(**7), 1285. https://doi.org/10.3390/antiox11071285

Jasril, J., Teruna, H. Y., Aisyah, A., Nurlaili, N., & Hendra, R. (2019). Microwave Assisted Synthesis and Evaluation of Toxicity and Antioxidant Activity of Pyrazoline Derivatives. *Indonesian Journal of Chemistry,* **19**(3), 583-591. https://doi.org/10.22146/ijc.34285

Karimi, E., Oskoueian, E., Hendra, R., & Jaafar, H. (2010). Solid state fermentation effects on pistachio hull antioxidant activities. *Asia-Pacific Journal of Science and Technology,* **15(**5), 360-366. https://doi.org/10.3390/molecules15096244

Kedare, S. B., & Singh, R. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of food science and technology,* **48**(4), 412-422. https://doi.org/10.1007/s13197-011-0251-1

Khodijah, R., Teruna, H. Y., & Hendra, R. (2022). Antioxidant and α-Glucosidase inhibition of Pyrrosia longifolia extracts. *Pharmacy Education,* **22**(2), 16-19. https://doi.org/10.46542/pe.2022.222.1619

Masuda, K., Yamashita, H., Shiojima, K., ITOH, T., & AGETA, H. (1997). Fern constituents: triterpenoids isolated from rhizomes of Pyrrosia lingua. I. *Chemical and Pharmaceutical Bulletin,* **45**(4), 590-594. https://doi.org/10.1248/cpb.45.590

Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D., & McLaughlin, J. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica, **45***(1), 31-34. https://doi.org/10.1055/s-2007-971236

Pan, P., Cheng, J., Si, Y., Chen, W., Hou, J., Zhao, T., . . . Zhu, Z. (2021). A stop-flow comprehensive two-dimensional HK-2 and HK-2/CIKI cell membrane chromatography comparative analysis system for screening the active ingredients from Pyrrosia calvata (Bak.) Ching against crystal-induced kidney injury. *Journal of Pharmaceutical and Biomedical Analysis,* **195**, 113825. https://doi.org/10.1016/j.jpba.2020.113825

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry, **30***(1), 11-26. https://doi.org/10.1007/s12291-014-0446-0

Sadono, R., SOEPRIJADI, D., SUSANTI, A., WIRABUANA, P. Y. A. P., & Matatula, J. (2020). Species composition and growth performance of mangrove forest at the coast of Tanah Merah, East Nusa Tenggara. *Biodiversitas Journal of Biological Diversity,* **21**(12). https://doi.org/10.13057/biodiv/d211242

Sathiyaraj, G., Muthukumar, T., & Ravindran, K. C. (2015). Ethnomedicinal importance of fern and fern allies traditionally used by tribal people of Palani Hills (Kodaikanal), Western Ghats, South India. *Journal of Medicinal Herbs and Ethnomedicine, **1***, 4-9. https://doi.org/10.5455/jmhe.2015-07-08

Scherer, R., & Godoy, H. T. (2009). Antioxidant activity index (AAI) by the 2, 2-diphenyl-1-picrylhydrazyl method. *Food Chemistry,* **112**(3), 654-658. https://doi.org/10.1016/j.foodchem.2008.06.026

Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of functional foods,* **18**, 757-781. https://doi.org/10.1016/j.jff.2015.01.047

Tong, W., Zaadah, J. N., Tan, W., Melati, K., Latiffah, Z., & Darah, I. (2014). Antimicrobial activity of Phomopsis sp. ED2 residing in medicinal plant Orthosiphon stamineus Benth. *Annual Research & Review in Biology*, 1490-1501. https://doi.org/10.9734/ARRB/2014/8060

Wu, C. (2014). An essential player in brine shrimp lethality bioassay: The solvent. *Journal of advanced pharmaceutical technology & research,* **5**(1), 57

Xiao, W., Peng, Y., Tan, Z., Lv, Q., Chan, C.-o., Yang, J., & Chen, S. (2017). Comparative evaluation of chemical profiles of pyrrosiae folium originating from three Pyrrosia species by HPLC-DAD combined with multivariate statistical analysis. *Molecules,* ***22***(12), 2122. https://doi.org/10.3390/molecules22122122