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RESEARCH ARTICLE

Antioxidant and α-Glucosidase inhibition of *Pyrrosia longifolia* extracts

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Abstract

Background: *Pyrrosia longifolia* is a fern species belonging to the Polypodiaceae family. Three *Pyrrosia* species, *P. lingua, P. sheareri*, and *P. petiolosa*, are included in the Chinese Pharmacopeia as sources of traditional medicine for ailments such as for patients with diabetes mellitus. **Objectives:** This study examined the inhibitory activity of α-glucosidase in different *P. longifolia* extracts, as well as their antioxidant activity and toxicity levels. **Methods:** This species was extracted via maceration in methanol and partitioning according to polarity levels (n-hexane, dichloromethane, ethyl acetate, and water). Antioxidant activity was measured by scavenging free radicals against the DPPH radical, antidiabetic activity was determined using an *in vitro* α-glucosidase inhibitory test, and toxicity was determined using the Brine Shrimp Lethality Test (BSLT). **Results:** Antioxidant activity using radical DPPH showed ethyl acetate extract had the highest inhibitory activity with IC_{50} 28.22 ppm and α-glucosidase inhibitory activity showed that all the extracts showed very weak activity at concentrations of 500 ppm. Additional toxicity analysis revealed that none of the extracts was harmful to Artemia salina. **Conclusion:** This study demonstrates that this species has strong antioxidant activity, and that additional analysis is required. It also identifies the chemicals that are responsible for the antioxidant activity.

Introduction

Ferns are one of the plants that have medicinal properties. Traditional medicine has used ferns as antibacterial, anti-malarial, laxative, postnatal, and anti-inflammatory agents (Cardelús *et al*., 2010). For instance, methanol extract from *Acrostichum aureum* Linn showed antibacterial activity towards *Pseudomonas aeruginosa*, a strain resistant to Amoxicillin and Chloramphenicol (Thomas, 2012). Other ferns with antioxidant potential include *Nephrolepis radicans, Viscum articulatum*, and *Davallia solida* (Nugraha *et al*., 2020).

It has long been known that secondary metabolite compounds found in fern species can be used medicinally. Numerous fern species exhibit this property, but only a few have been studied and their bioactivity established. In comparison to other plant types, research on the secondary metabolite content of fern species is still uncommon (Nugraha *et al.,* 2020). Additionally, another fern species, the family Polypodiaceae, still lacks information about their secondary metabolites, which could have many bioactivities. *Pyrrosia sheareri, P. lingua, and P. petiolosa* are all parts of various medicinal plants used in China to treat acute pyelonephritis, chronic bronchitis, diabetes mellitus, and bronchial asthma (Cui *et al.,* 2016). While *P. piloselloides* is used in Southeast Asia to treat smallpox, dysentery, urinary tract infection, and headache (Nugraha *et al.,* 2020).

P. longifolia is one of the fern species which belongs to the family Polypodiaceae. This species is often found attached to old trees, usually on the branches (Piggott, 1998). This species has a history of being used as pain relief during labour in several areas within Indonesia and the Pacific regions (Hoven-kamp, 2003). Although *P. longifolia* has not been widely reported as traditional medicine, chemotaxonomically, this species most likely possesses the same bioactivity as the species mentioned previously. Thus, this study will be conducted to determine the bioactivity of some extracts of this species, including testing antioxidants, α-Glucosidase inhibition, and toxicity.

Materials and method

Collection of plant material

The ferns *Pyrrosia longifolia* were collected from District 50, Riau Province. The plants were identified by the Department of Biology, Faculty of Mathematics and Natural Sciences, Riau University.

Extraction and partition

The air-dried sample (5 kg) was blended and soaked in methanol for 2x24 hours with two repetitions; the filtrate was then collected and separated from the solvent using a 40°C rotary evaporator. The crude extract was dissolved in a 9:1 solution of methanol and water and partitioned with n-hexane to obtain an *n*-hexane extract (EHMP). The residue was diluted with water to 40% (v/v) and partitioned with dichloromethane to obtain dichloromethane extract (EDMP). The remaining methanol in the residue was evaporated and partitioned with ethyl acetate to yield ethyl acetate (EEMP) and water (EAMP) extracts (Hendra *et al*., 2020).

Antioxidant activity

The antioxidant activity was determined using free radical scavenging activity by using DPPH (Hendra *et al.,* 2020). Each extract, as well as quercetin as a positive control, was prepared in a 100 g/mL solution and diluted to 50, 25, 12.5, 6.25, and 3.125 µg/mL. A 50µL solution of the extract was poured into a 96-well polystyrene microplate. A 100 µg/mL DPPH solution was added in a volume of up to 80 µL and incubated for 30 minutes. The assay was repeated three times. The absorbance value was determined using a microplate reader (Berthold, Germany) set to 520 nm.

In vitro α-glucosidase inhibition assay

Inhibition of α-glucosidase activity was assessed using the method from El Ridhasya and colleagues (2020) with some modifications. α-Glucosidase (0.2 U/mL in phosphate buffer) was added to 40L of phosphate buffer (500 mM, pH 6.8) and 10µL of extract. Pre-incubation at 37°C for 30 minutes. *p*-nitrophenyl-D-glucopyranoside (20mM in phosphate buffer) was added to the enzyme reaction mixture, and it was then incubated for 30 minutes at 37°C. 100 µL Na_2CO_3 was used to stop the enzyme's reaction activity (0.1 M, pH 10). A 1000 µg/mL extract was made alongside a 500 g/mL extract, and a 250 µg/mL extract. A microplate reader measured enzyme activity at 405 nm. Other reagents and enzymes except the test sample were used as blanks, while the control mixture did not contain samples or enzymes.

Toxicity assay

The extracts were tested for toxicity using the Brine Shrimp Lethality Test (BSLT). The test method consisted of preparing 10 vials of 2 mL seawater, each diluted twofold to produce a number of concentrations. Each vial received an Aliquot (0.1 mL) containing approximately ten nauplii. After 24 hours, dead larvae were counted from each vial. DMSO was used as a negative control. The median mortality percentage *vs* log of concentration graph was used to determine the LC_{50} (Syahmi *et al.*, 2010; Jasril *et al*., 2019; Hendra *et al*., 2021). The assay was done in triplicate, and the data were reported as mean ± standard deviation.

Statistical analysis

The assay was done in triplicate, and the data were reported as mean ± standard deviation.

Results

In this study, the antioxidant activity of all extracts from the species was determined using free radical scavenging activity against DPPH (1,1-diphenyl-2-picryl hydrazyl) radicals. The free radical scavenging activity of various extracts is depicted in Table I. The ethyl acetate extract was more active than quercetin, with an IC_{50} value of 28.22 ± 0.015 µg/mL. Dichloromethane and aqueous extracts had IC_{50} values of 101.29 ± 0.027 and 90.22 ± 0.023 µg/mL, respectively, indicating moderate antioxidant activity. With an IC_{50} value greater than 500 µg/mL, the *n*-hexane extract exhibited the least antioxidant activity.

Table I: Antioxidant, α-glucosidase inhibition, and toxicity of *P. longifolia* extracts

Sample	IC_{50} DPPH (µg/mL)	α-glucosidase IC_{50} (µg/mL)	Toxicity LC_{50} (µg/mL)
n-hexane extract (EHMP)	> 500	>1000	>1000
Dichloromethane extract (EDMP)	101.29 ± 0.027	>1000	>1000
Ethyl acetate extract (EEMP)	28.22± 0.015	>1000	>1000
Aqueous extract (EAMP)	90.22± 0.023	>1000	>1000
Quercetin	47,09± 0.011	262,33± 0.01	>1000

The extracts were subjected to an antidiabetic evaluation using an in vitro α-glucosidase inhibition method. Compared to quercetin, which served as a positive control, the IC_{50} of the extracts showed more than 1000 µg/mL (Table I). Furthermore, all the extracts demonstrated toxicity levels with LC_{50} > 1000 µg/mL.

Discussion and conclusion

The methanol extract was partitioned with different organic solvents using a modified Kupchan partitioning technique, which was used in this investigation. The Kuphan partition is a simple but effective way to begin a purification protocol by separating a total crude methanol extract into four large extracts of different polarities. The polarity distributions between extracts of different compounds can be quite diverse, and the polarity distributions between extracts of different compounds can be quite diverse as well (Kupchan *et al*., 1976).

The findings of this study revealed that the extracts had a wide range of antioxidant activity. The antioxidant activity of the ethyl acetate extract was significantly higher than that of the positive control quercetin. Quercetin, a flavonoid that can be found in various parts of the plant, was found to have significant antioxidant activity (Hirano *et al*., 2001; Hendra *et al*., 2017). *Pyrrosia* species have antioxidant properties, according to research published in peer-reviewed journals. When tested against DPPH radical, the ethanolic extract of *P. petiolosa* demonstrated antioxidant activity with an IC_{50} value of 140 g/mL, while the dichloromethane extract of *P. piloselloides* demonstrated antioxidant activity with an IC_{50} value of 12.82 g/mL (Hsu, 2008; Wulandari *et al*., 2013). According to Akhmadjon and colleagues (2020), phenolic ethanol extract from *Pyrrosia lingua* exhibited high antioxidant activities against DPPH and ABTS radicals, with percentages of 75.22% and 98.86% at the concentration of 100 µg/mL, respectively, against these radicals. To the authors' knowledge, this is the first analysis of the species' antioxidant activity, and the secondary metabolites involved require further investigation.

Furthermore, in comparison to the methanol extract of *P. lingu*, which inhibited α-glucosidase with an IC_{50} value of 14.00 ± 0.770.01 µg/mL, all the extracts were inactive against α-glucosidase when compared to quercetin, which inhibited α-glucosidase with an IC_{50} value of 262,33 ± 0.01 µg/mL. It was found that several species of *Pyrrosia,* such as *P. sheareri, P. lingua, and P. petiolosa* have been used to treat diabetes mellitus in East Asia (Cui *et al*., 2016). Additionally, the extracts' toxicity was determined using the brine shrimp lethality test. The test is a simple but effective method for determining the toxicity of bioactive compounds or extracts. It is based on the ability of test samples to kill the shrimp (*Artemia salina*) (Wu, 2014). According to Meyer and colleagues, the samples exhibited toxicity at a concentration of < 1000 ppm, while the samples were classified as highly toxic to sage shrimp at a concentration < 30 ppm (Meyer *et al.,* 1982). As a result, this study concluded that all extracts were nontoxic to the tested organism.

According to the findings of this study, P. longifolia extracts contain a variety of antioxidants capable of scavenging DPPH radicals. They are, however, ineffective at inhibiting α-glucosidase. More research is needed to determine which active compounds are responsible for bioactivity.

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