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Short Communication

Total Phenolic, Total Flavonoid Contents, and Radical Acavenging Activity of Different Concentrations of Ethanol Extract of *Pyrrosia piloselloides* (L) M. G. Price

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ABSTRACT

Pyrrosia piloselloides (L.) M.G. Price (PP) is known to have pharmacological activities. This study investigated the total phenolic (TPC), total flavonoid contents (TFC), and the radical scavenging activities (RSA) of different concentrations of PP ethanol extract. PP dried sample was macerated with different ethanol concentrations (30%, 50%, and 70%). The TPC and TFC were determined using Folin-Ciocalteu and $AlCl_3$ calorimetric assays, respectively while the RSA was investigated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. It was discovered that TPC (9.74 mg GAE/g), TFC (6.21 mg QE/g), and RSA (73.50%) of 50% ethanol extract of PP were higher than extracts obtained with 30% and 70% ethanol. Furthermore, a positive correlation was found between RSA vs TPC (r=0.43) and RSA vs TFC (r=0.35). It was concluded that 50% ethanol could be the best ethanol concentration to obtain PP extract with high contents of TPC, TFC, and antioxidant properties.

Keywords: Antioxidant, DPPH, Flavonoid, Phenolic, *Pyrrosia piloselloides*

Introduction

Pyrrosia piloselloides (L.) M.G. Price (PP), commonly called *Pakis Duwitan or Sisik Naga* in Indonesia, is an epiphytic plant that belongs to the genus *Pyrrosia* (family *Polypodiaceae*). This plant often grows on the trunks of any old tree by the roadside or in small jungles that are adequate humidity and temperature conditions, as well as in regions with a monsoonal, sub-tropical, or tropical climate, and it is traditionally used to treat pain, skin rash, fever, and gallstones.[1,2] Several studies have reported the pharmacological activities and phytochemicals in *P. piloselloides*. For example, it has been discovered that the methanol, dichloromethane, and hexane extracts of *P. piloselloides* possess anticancer and antioxidant activities.[3] In a study by Wulandari *et al*.,[3] it was reported that the highest antioxidant activity was found in the dichloromethane extract ($IC_{50,}$ 12.82 µg/ml) followed by methanol ($IC_{50,}$ 38.94 µg/ml), and n-hexane ($IC_{50,}$ 41.16 µg/ml) extracts. The anti-proliferative activity of the methanol and aqueous extracts of the plant against HeLa cell line has also been reported,[1] while another study found that 96% of the ethanol extract increased platelet cells in mice.[4] Sahid *et al.,* (2013)[5] explained that the phytochemical screening of the plant's methanol extract revealed that the presence of tannins, terpenoids, steroids, flavonoids, glycosides, and essential oils serve a high potential for anticancer activity.[5]

Generally, phenolic antioxidants in medicinal plants are extracted using solvent extraction approach,[6–8] due to its ease of use and low energy consumption. The polar solvents used include ethanol, methanol, and water, while the nonpolar organic solvents are chloroform, n-butanol, and ethyl acetate.

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It is important to note that ethanol is a popular choice for pharmaceutical and food processing because of its safety and low cost,[9,10] and has also been found that the extractions with aqueous alcohol mixtures result in excellent antioxidant capabilities.[11] This is possibly due to the failure to completely extract the phenolic chemicals that are relatively hydrophilic or aqueous-soluble. Therefore, the presence of water in the extraction solvent facilitates the release of hydrophilic phenolic antioxidants.[11] Recently, no study has determine the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities of different concentrations of ethanol extract of PP to help identify which of the extracts possesses the highest antioxidant potential. Therefore, the present study examines the TPC, TFC, and antioxidant activities of 30%, 50%, and 70% ethanol extracts of PP.

Materials and Methods

Plant material preparation and extraction

Pyrrosia piloselloides (Plate 1) sterile frond were collected in April 2020 from the premises of Bogor Agricultural University in Bogor, West Java, Indonesia. The plant was authenticated at Tropical Biopharmaca Research Center, IPB University (BMK0073042016), and was cleaned, and dried in an oven at 50°C, powdered to size of 40 mesh, packed in an air-tight container, before storing it in a cool, dark, and dry place.

The dried powdered sample (50 g each) was macerated with 500 mL of 30%, 50%, and 70% ethanol for 24 hours under room temperature and each resulting mixture was filtered by using Whatman filter paper. The residue was further extracted two more times with the same extraction solvent and each filtrate was concentrated using a rotary evaporator at 50°C to obtain 0.22 g, 0.37 g, and 1.16 g extracts, which were labelled as 30%, 50%, and 70% crude ethanol extracts of PP, respectively.

Determination of TPC

The total phenolic content of PP extracts obtained from 30%, 50%, and 70% ethanol was determined using the Folin-Ciocalteu method described by Calvindi *et al*.,[12] where 20 µL of each extract (1 mg/mL) was mixed with 100 µL of 50% Folin-Ciocalteu reagent in 96-well

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microplate, and each resulting mixture was incubated for 5 min. To the mixture was further added 80 µL of 7.5% Na_2CO_3 and incubated for 2 hours in the dark. Subsequently, a microplate reader (Epoch BioTek, USA) was used to measure the absorbance at 750 nm, while TPC was measured as mg gallic acid equivalent per g extract (mg GAE/g) by using gallic acid as the standard.

Determination of TFC

The total flavonoid content of 30%, 50%, 70% ethanol extracts of PP was determined using the $AlCl_3$ method described by Nurcholis *et al*.[13] In a 96-well microplate, 50 µL of each extract (1.2 mg/mL) was mixed with 10 µL of 10% $AlCl_3$, 10 µL of 1 M sodium acetate, and 130 µL of 96% ethanol, while the resulting mixture was incubated in the dark for 40 min. Subsequently, Epoch BioTek was used to measure the absorbance at 415 nm, and the TFC was measured as mg quercetin equivalent per g extract (mg QE/g) using quercetin as the standard.

Determination of antioxidant activity

Antioxidant activity evaluated by the radical scavenging activity (RSA) of each PP extract was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method as described by Batubara *et al*.,[14] where 100 µL of each extract (400 µg/mL) was mixed with 100 µL of 125 µM DPPH in 96-well microplate, and each resulting mixtures was incubated for 30 min in the dark at room temperature. Subsequently, the absorbance was measured at 517 nm, and the antioxidant capacity of each extract was reported as % inhibition of DPPH scavenging activity.

Plate 1: Habitat of *Pyrrosia piloselloides* growing on a tree (A) and plant parts used (sterile frond) in this study (B).

Statistical analysis

A one-way ANOVA and the Tukey's test were used for statistical analysis. The statistical analysis was conducted using SPSS version 25 and Prism 9. The data were expressed as the mean and standard deviation (SD) of three independent readings. $P < 0.05$ was regarded as significant.

Results and Discussion

Figure 1 shows the TPC, TFC, and RSA of 30%, 50%, and 70% ethanol extracts of *P. piloselloides*. Generally, natural antioxidants are associated with polyphenol metabolites found in plants,[15,16] hence, TPC and TFC were used in the present study to evaluate polyphenol content of *P. piloselloides* sterile frond.

Figure 1A shows that the TPC obtained was 4.41, 8.74 and 9.74 mg GAE/g for 70%, 30%, and 50% ethanol extracts of PP, respectively while Figure 1B shows that the TFC was 5.43, 6.08, and 6.21 mg QE/g for 70%, 30%, and 50% ethanol extracts of PP, respectively. Previously, the qualitative presence of phenolic and flavonoid compounds in ethanol and aqueous extracts of PP has been studied.[17] Figure 1C shows that the RSA of PP was 35.25%, 46.76%, and 73.50% for the 30%, 70% and 50% ethanol extracts, respectively. A further study by Wulandari *et al*.,[3] presented that the highest antioxidant activity was found in the dichloromethane ($IC_{50,}$ 12.82 µg/mL), methanol ($IC_{50,}$ 38.94 µg/mL) and n-hexane ($IC_{50,}$ 41.16 µg/mL) extracts.

Recently, study on plants rich in polyphenolic antioxidants has increased and it has been discovered that these compounds improved the quality and nutritional value of food, and also inhibit the degradation of lipid oxidation in food industries.[18] Meanwhile, in the pharmaceutical industry, it is used as drugs to treat various diseases such as diabetes mellitus, cholesterol, and cancer.[19–21] Some plants that have been reported to contain polyphenol antioxidants are cardamom, *Curcuma aeruginosa*, and Java tea.[13,14,22] Therefore, 50% ethanol extract of PP is an additional alternative source of new polyphenol antioxidants from medicinal plant.

Figure 2 shows that TPC and TFC values for each PP ethanol extract were positively correlated with RSA (r=0.43 for TPC and r=0.35 for TFC). While those that are less correlated indicates that PP had other secondary metabolites that probably contributed to the antioxidant property of the plant.[17] For example, previous studies conducted revealed that 2-furancarboxaldehyde, 5-(hydroxymethyl), a non-phenolic compound that was detected in the methanol and aqueous extracts of the plant, exhibited antioxidant activity.[1,23]

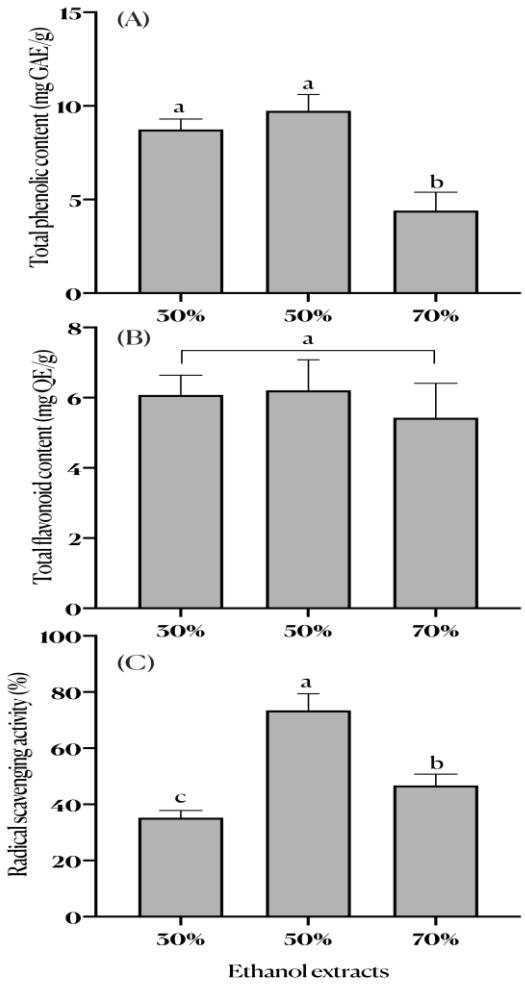

Figure 1: Total phenolic content (A), total flavonoid content (B), and percentage of radical scavenging activity (C) of different concentration of ethanol extract of PP. Different

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letters in the same data presented significant different at $p < 0.05$ using Tukey test.

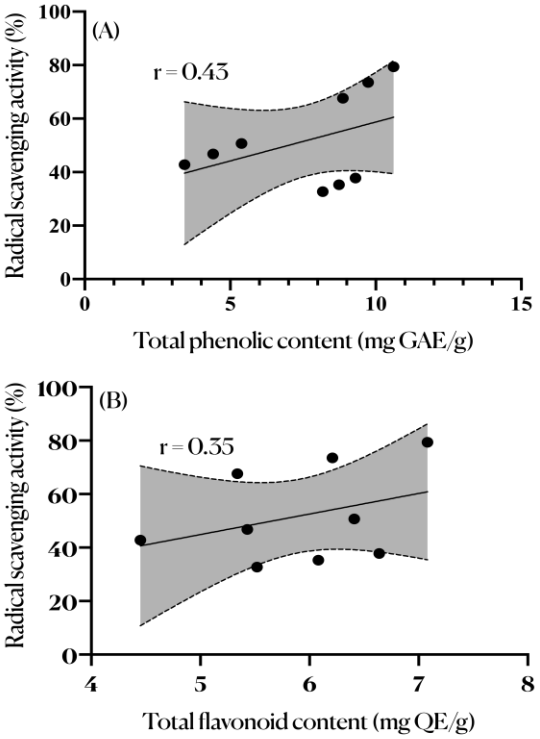

Figure 2: Pearson correlation of radical scavenging activity vs total phenolic content (A) and radical scavenging activity vs total flavonoid content (B) of different concentration of ethanol extract of PP. r = Pearson value.

Conclusion

The result shows that 50% PP ethanol extract had the highest content of total phenols and total flavonoids. This extract also displayed the most significant radical scavenging activity, when compared to 30% and 70% ethanol extracts of the plant.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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