Borneo International Journal of Biotechnology (BIJB)
Vol. 2 (December 2022), 14 – 21

e-ISSN 2716-697X

Evaluation of antioxidant activity and total phenolics of selected mangrove plants in Sabah

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https://doi.org/10.51200/bijb.v2i.3330

Received: 5 January 2022 | Revised: 24 February 2022 | Accepted: 28 June 2022 | Published: 1 December 2022

ABSTRACT

In recent years, research on medicinal plants has attracted much attention due to their wide range of pharmacological significance. Mangroves are biochemically unique, producing a wide array of natural products with unique bioactivity due to their ability to survive in stressful conditions of high salinity, and low air humidity as well as strong variations therein. Six species of mangrove (*Avicennia marina, Bruguiera gymnorrhiza, Ceriops tagal, Rhizohora apiculata, Rhizophora mucronata* and *Xylocarpus granatum*) from different parts of the leaves, stem and roots were extracted successively with ethanol and water. This study aims to measure quantitatively the total phenolic content and assess the radical scavenging activity of the 26 mangrove extracts. All the extracts were subjected to the Follin-Ciocalteu assay for their phenolic content and the DPPH scavenging assay for the antioxidant activity respectively. Based on the results, the highest phenolic content was observed in ethanol extracts of *C. tagal* leaves (471.78 ± 0.056 mgGAE/g) while the lowest amount of phenolic was observed in water extract of *A. marina* root (20.40 ± 0.001 mgGAE/g). Interestingly, the ethanolic extract of *C. tagal* leaves also exhibited the strongest antioxidant activity with an IC_{50} value of 9.37 ppm. Among the six species investigated, *C. tagal* leaves extracts showed high total phenolic content and strong antioxidant activities and may be used as a potential source of natural antioxidants against free radical-associated diseases.

Keywords: mangroves, total phenolic content, antioxidant activity

INTRODUCTION

Mangroves have long been a source of interest to scientists and astonishment to laymen. Mangroves are usually found only in tropical climates, as they need consistently warm conditions for development and survival (Kathiresan & Bingham, 2001; Bandaranayake, 2002). Although mangroves constitute less than 0.4% of the world's forests, they play an important role use in providing habitats for thousands of marine and pelagic species and serving the local communities with food, medicine, fuel as well as building materials (Kanniah et al., 2015). Mangroves are known for their capability to produce a lot of secondary metabolites under stressful conditions and potential sources of lead compounds (Buatong et al., 2011). In these regions, mangroves have been known since the folk era to be highly useful and active against various diseases and are potent sources of bioactive compounds including antioxidants, anti-diarrheal, anti-inflammation, anti-diabetic, and anticancer compounds (Bandaranayake, 2002; Das et al., 2015). A large number of bioactive compounds of pharmaceutical importance have also been reported from mangrove ecosystems (Bandaranayake, 2002).

It is interesting to note that nearly three-quarters of mangrove plants in Southeast Asia are located in Malaysia (0.5 million ha) and Indonesia (2.7 million ha) (Richards & Friess, 2016). In Malaysia, mangrove regions in Sabah account for about 60% of the country's entire and 7.6% of the worldwide total. However, to our knowledge, studies on mangrove plants in Sabah are still scarce and limited. The potential phytochemical characteristics and antioxidant activity of mangrove plants from Sabah have not been reported to date. This study aims to investigate the total phenolic content and antioxidant activity of the selected mangrove plants from Sabah. This study hopes to provide initial data on the total phenolic content and antioxidant activity of mangrove plants from Sabah.

MATERIALS AND METHODS

Plant Material and Extract Preparation

Six species of mangroves (*Avicennia marina, Bruguiera gymnorrhiza, Ceriops tagal, Rhizophora apiculata, Rhizophora mucronata* and *Xylocarpus granatum*) from different parts of the leaves, stem and roots were collected in Sulaman Wetland Sanctuary, Tuaran and identified by Mr Johnny Gisil, a botanist from Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. The collected sample was washed with tap water followed by distilled water to remove the dirt and any undesirable material. The washed sample was oven-dried at 40°C, powdered and exhaustively extracted with ethanol and water in the ratio of 1:5 (w/v) maceration using a magnetic stirrer at room temperature overnight. The samples were then filtered by using filter paper and a

vacuum filter. For ethanolic extract, the solvent phase was removed from the filtrate by a rotary evaporator (R215, Buchi, US11502920). Both extracts were stored in the freezer at −80°C before it was then freeze-dried using a freeze dryer (Freezone plus, Labconco, USA) for three days. The samples were then stored at −80°C for further studies.

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of each extract was determined spectrophotometrically by the Folin-Ciocalteu method of Singleton et al. (1999) with some modifications. Briefly, 200 µL of crude extract (1 mg/ml) was mixed thoroughly with 1.5 ml of Folin–Ciocalteu reagent for 5 mins, followed by the addition of 1.5 ml of sodium carbonate. After 90 mins of incubation at room temperature, the absorbance of the mixture was measured at 750 nm against a blank. The results of the total phenolic were expressed as mg of gallic acid equivalent (GAE) per g of dry extract (Zahin et al., 2009) and calculated as follows:

$$TPC = (C \times V)/M$$

Where, TPC is total phenolic content (mg/g extract in GAE), C is the concentration of gallic acid determined from the standard curve (mg/ml), V is the volume of the extract (ml) and M is the mass of the plant extract (g). All samples were done in triplicate.

Determination of Antioxidant Activity

The radical scavenging activity of the extracts was measured by a modification method by Singh et al. (2002). Briefly, 300 µl of each extract (1 mg/ml) was mixed thoroughly with 3 ml of DPPH solution and incubated at room temperature in the dark for 10 mins. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as the positive control. IC_{50} value represents the concentration of extract needed to inhibit 50% of DPPH activity which can be calculated from the calibration curve by linear regression. The ability of the extract to scavenge DPPH radical was calculated as follows:

$$\text{Inhibition (\%)} = [(A_B - A_S) / A_B] \times 100$$

Where A_B is the absorption of blank samples and A_S is the absorption of extract solution. All samples were done in triplicate.

Statistical Analysis

All experimental measurements were carried out in triplicate and are expressed as the average of three analyses ± standard deviation.

RESULTS AND DISCUSSION

Total Phenolic Content

Phenolic compounds are the largest group of phytochemicals that have been recorded from every plant part (Dai et al., 2010). It has been reported that phenol compounds are responsible for biological activities including antioxidants, anti-cancer, antibacterial, antiviral and anti-inflammatory (Zheng et al., 2001). In this experiment, mostly, the sample from the ethanol extract has a higher phenolic content compared with the sample from the aqueous extract. This trend is compatible with that reported by Rosas-Burgos et al. (2017), whereby their study also shows that ethanolic extract exhibited a higher total phenolic content than aqueous extract. This is also in agreement with Jing et al. (2015) who suggested that ethanol is the best and preferred solvent to extract phenolic compounds from plants.

Table 1 Total phenolic content (TPC) of selected mangroves from ethanol and water extracts (mg GAE/g)

Species Name	Part	Total phenolic content (mg GAE/g)	
		Aqueous	Ethanol
Avicennia marina	Leaf	34.90 ± 0.001	54.92 ± 0.004
	Root	20.40 ± 0.001	26.11 ± 0.003
Bruguiera gymnorrhiza	Leaf	70.40 ± 0.006	128.33 ± 0.005
	Stem	147.02 ± 0.012	231.53 ± 0.024
	Root	22.05 ± 0.001	344.02 ± 0.025
Ceriops tagal	Leaf	259.47 ± 0.012	471.78 ± 0.056
Rhizophora apiculata	Leaf	39.67 ± 0.004	242.95 ± 0.009
	Stem	54.32 ± 0.001	308.70 ± 0.018
	Root	49.66 ± 0.009	194.85 ± 0.024
Rhizophora mucronata	Leaf	213.95 ± 0.140	365.47 ± 0.008
	Stem	83.21 ± 0.017	299.64 ± 0.001
	Root	33.64 ± 0.001	138.96 ± 0.013
Xylocarpus granatum	Leaf	35.61 ± 0.005	177.20 ± 0.004

Based on the result shown in Table 1, the highest phenolic content was observed in ethanol extracts of *C. tagal* leaves (471.78 ± 0.056 mgGAE/g) while the lowest amount of phenolic was observed in water extract of *A. marina* root (20.40 ± 0.001 mgGAE/g). As a comparison, Shamsuzzaman et al. (2021) reported that the methanolic extract of *C. tagal* leaves is 101.52 mgGAE/g which is approximately four times lower than the present study. According to Kowalczyk et al. (2013), ethanol extracts consist of high polarity compared to other solvents as it affects the production of polyphenol enriched. The amount of phenolic also was higher in leaves compared to other parts of plants. This is probably because leaves are more exposed to stressors as their role in defence against microbial pathogens and UV light, and require protection due to their vital function in photosynthesis (Banerjee et al., 2008; War et al., 2012).

Antioxidant Assay

The scavenging activity of the extracts can be seen from the ability of the sample to decolourize the DPPH blue colour and the decrease in its absorbance at 517 nm. To determine whether the mangrove extracts have a good scavenging activity or not, the IC_{50} value was calculated. Antioxidant activity of the plant extracts can be classified as very strong with IC_{50}<50 ppm, strong with IC_{50} value ranging between 50 – 100 ppm, moderate at 101 – 250 ppm, weak at 250 – 550 ppm and inactive at IC_{50}>500 ppm (Jun et al., 2003).

Table 2 Antioxidant DPPH scavenging activity of selected mangroves from ethanol and water extracts

Species Name	Part	IC_{50} value (ppm)	
		Aqueous	Ethanol
Avicennia marina	Leaf	CBD	CBD
	Root	CBD	CBD
Bruguiera gymnorrhiza	Leaf	652.30	35.82
	Stem	683.79	17.74
	Root	CBD	29.64
Ceriops tagal	Leaf	145.04	9.37
Rhizophora apiculata	Leaf	846.22	23.21
	Stem	849.34	17.97
	Root	534.80	28.14
Rhizophora mucronata	Leaf	154.56	9.84
	Stem	331.32	19.56
	Root	652.25	19.27
Xylocarpus granatum	Leaf	663.69	28.78

*CBD = Cannot be determined

Based on the results shown in Table 2, it could be seen that the ethanolic extract of *C. tagal* leaves has the highest antioxidant activity with an IC_{50} value of 9.37 ppm followed by the ethanolic extract of *R. mucronata* leaves (9.84 ppm) and then ethanolic extract of *B. gymnorrhiza* stem (17.74 ppm). Interestingly, all the ethanolic extracts of selected mangrove plants (except *A. marina*) can be considered very strong antioxidants as the IC_{50} value are less than 50 ppm. In the present study, the ethanolic extract of *C. tagal* leaves that has the highest total phenolic content also demonstrated the highest antioxidant activity with an IC_{50} value of 9.369 ug/ml. This positive correlation might be due to the excellent resource of phenolic compounds in *C. tagal* which possess potent antioxidant properties as reported by previous studies (Bandaranayake, 2002; Maisuthisakul et al., 2007; Golder et al., 2020). Various research has indicated that phenolic compounds scavenge the free radicals by virtue of their hydroxyl groups or

conjugated ring structures the main structural features influencing the antioxidant capacity of phenolics (Carocho & Ferreira, 2013; Mathew et al., 2015; Cory et al., 2018). An antioxidant compound such as polyphenol in plant extracts has the ability to neutralize free radicals by donating an electron or hydrogen atom (Hatano et al., 1989). The difference value of IC_{50} in each extract is due to the polarity of solvent used as well as the types and the amount of bioactive compound that acted as an antioxidant in the extract. Therefore, the present study indicates the potential of the extracts as a source of natural antioxidants with potential applications to reduce oxidative stress with consequent health benefits.

CONCLUSION

In general, it is found that the solvent extraction used significantly affected the total phenolic content and antioxidant activities. In this present study, the objective of this study is achieved with the finding that ethanol extract gives a higher phenolic content and antioxidant activity compared to the aqueous extract. Among all the 26 extracts of different parts of mangroves, *C. tagal* leaves exhibited the highest phenolic content with the strongest scavenging activity. Further study is needed to isolate the active compound from this extract which could serve as a medicine against free-radical-associated oxidative damage.

ACKNOWLEDGEMENTS

This study was supported by the Universiti Malaysia Sabah under an External Collaboration Research Grant Scheme (GKP0021-2018) "Characterization of anti-tuberculosis and dengue virus antiviral activities of mangrove plant extracts".

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