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Phenol content, antioxidant and tyrosinase inhibitory activity of mangrove plants in Micronesia

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ABSTRACT

Objective: To find out and compare the *in vitro* antioxidant and tyrosinase inhibitory activities of two species of mangrove plants. **Methods:** Mangrove samples were harvested at the shoreline on the island of Weno, Chuuk State in Micronesia. The phenol content, antioxidant activity (based on DPPH-free radical scavenging) and tyrosinase inhibitory activity in different tissues (leaves, barks and roots) of *Rhizophora stylosa (R. stylosa)* and *Sonneratia alba (S. alba)*, collected from the island of Weno. **Results:** Total phenol content ranged from 4.87 to 11.96 mg per g of freeze dried samples. The highest antioxidant activity was observed in *R. stylosa* bark (85.5%). The highest tyrosinase inhibitory activity was found in *S. alba* bark. Also, total phenol content and antioxidant activity were higher in methanol extracts than in aqueous extracts. **Conclusions:** Taken together, the results of this study proved that mangroves can be excellent sources of antioxidant compounds.

1. Introduction

Mangroves are woody plants that are widely distributed in tropical and subtropical regions. In tropical areas, the leaves or the bark of mangrove trees are used as folk remedy for diarrhea, indigestion, nose bleeding, inflammation, sore throat and wounds[1]. Water extracts of mangroves showed anti-bacterial activity[2], and promoted post-surgery recovery[3]. The medicinal effects of mangrove extracts are associated with the tannin which is contained in the tree. The bark of Rhizophora trees contains about 10% to 36% phenolic content, including tannin[4], a kind of polyphenols. Polyphenols are a group of compounds which have multiple phenolic hydroxyl (-OH) groups in their molecular structure, which are functional groups which are prevalent in plants[5,6]. The antioxidant effects of mangrove plants, which may be up to 20 times that of α-tocopherol, a powerful antioxidant, are stemmed from phenolic hydroxyl groups. Polyphenols are capable of suppressing cholesterol levels, the incidence of pathogens, blood pressure levels, halitosis and allergic rhinitis. Its strong free radical scavenging activities and subsequent antimutagenic activity prove effective for preventing various life style diseases in adults, including antiatherogenic effects, gastric ulcer, colorectal cancer, cataract and diabetic complications[7].

Rhizophora stylosa (*R. stylosa*) and *Sonneratia alba* (*S. alba*) are dominant mangrove species growing over the coast of Chuuk, Micronesia. Phenolic properties of mangrove plants and their strong antioxidant activity have been discussed in many studies[8-10]. However, there has been no study which investigates total phenol content and antioxidant activity of *R. stylosa* and *S. alba*. This study was aimed to investigate the total phenol content, antioxidant activity and tyrosinase inhibitory activity of extracts of different parts of mangrove plants, and to evaluate their use as drugs, food additives and natural antioxidants.

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2. Materials and methods

2.1. Materials

Mangroves were collected from the island of Weno, Chuuk State of Micronesia (Figure 1). The leaves, trunks and roots of *R. stylosa* and *S. alba* were sampled into vinylbags and were carried to the lab. Collected samples were washed with tap water in the lab and were then freeze-dried. Bark was separated from the trunks during the drying process. Dried samples were ground and filtered to a fine powder prior to analysis of total phenol content and biological activity. Gallic acid, tyrosinase, DPPH(1,1-diphenyl-2-picrylhydrazil) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Other chemicals used were of 99% or greater purity.

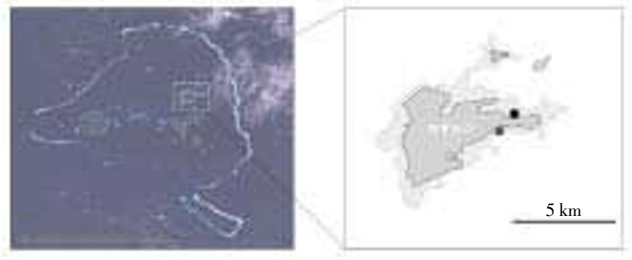

Figure 1. Chuuk lagoon image and station for mangrove sampling.

2.2. Preparation of the mangrove tissue extracts

The freeze-dried mangrove tissue powders (5 g) were extracted by stirring them with 200 mL of D.W (65 ℃) and methanol at 25 ℃ at 150 rpm for 24 h and isolated by centrifugation (5 000 rpm, 5 min, Sorvall, USA). Mangrove extracts were then rotary evaporated at 40 ℃ to dryness and kept in the dark at 4 ℃. Extract yields (% dry weight of mangrove tissue) of the *R. stylosa* and *S. alba* tissues were 23.05% and 19.25%, respectively. Samples were immediately analyzed for determination of phenolic contents, antioxidant activity and tyrosinase inhibitory activity.

2.3. Analysis of phenol content

The phenol content was measured using Folin-Ciocalteu reagent (FCR) according to the method of Capannesi and Palchetti[11]. A 0.5 mL mangrove extract was mixed with FCR, and 1 mL of 7.5% Na_2CO_3 was added to the mixture. The solution was then diluted with 8 ml of distilled water and left to stand at 65 ℃ for 20 min. The blue color of the reaction was measured using a UV spectrophotometer at 765 nm. Gallic acid was used as the standard. The analysis of mangrove extracts was performed three times, and the phenol content was expressed as gallic acid equivalent (GAE).

2.4. DPPH-free radical scavenging capacity

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging capacity was measured based on the modified method described by Lu and Foo[12]. DPPH is a stable free radical which can turn into a stable diamagnetic molecule by accepting electron or hydrogen radicals[13]. The prepared solution of DPPH turned a deep violet color (517 nm), which becomes lighter with the presence of antioxidant substances. This means that antioxidant molecules can quench DPPH free radicals by electron donation and convert them to a colorless product[14]. DPPH solution was prepared by dissolving 100 mM of DPPH in 80% methanol. Then, 0.1 mL of mangrove extracts was added to the freshly prepared solution and was mixed well. The mixed solution was left to stand in a dark place at 25 ℃ for 10 min, after which the absorbance was measured at 517 nm. The inhibition rate was then calculated using the following formula, where the control group is included.

DPPH free radical scavenging capacity (%) = [(A0-A1)/A0] × 100 (A0 = Control group's absorbance A1 = experiment group's absorbance).

Experiments were conducted three times and the results were expressed as the average value,

2.5. Analysis of tyrosinase inhibitory activity

Tyrosinase inhibitory activity was measured by the method described by Kim *et al*[15]. Tyrosinase (100 units/mL), 60 mM potassium phosphate buffer (pH 6.8) and 0.4 mL of 10 mM DOPA (dihydroxyphenylalanine) were mixed together. Then, 0.2 mL of mangrove extracts was added to the mixed solution, after which the absorbance was measured at 475 nm. The inhibitory activity was then calculated according to the following formula:

Inhibition (%) = [(A0-A1)/A0] × 100

Where A0 is the control group's absorbance at 475 nm and A1 is mangrove extracts' absorbance.

2.6. Statistical analysis

Statistical analysis was performed using SPSS software. The data are expressed as the mean value±SE. Normality and homogeneity of the data was verified by ANOVA. The differences in experimental groups were analyzed using one-way ANOVA and Duncan's multiple range test.

3. Results

3.1. Phenol content in different tissues of mangrove plants

Total phenol content is expressed in μg per gram of freeze dried sample based on a standard curve generated with gallic acid. No significant difference was observed in total phenol content between water extracts and methanol extracts of mangrove trees. Total phenol content was higher in barks of both *R. stylosa* and *S. alba* (11.96 and 10.58 mg/g), compared to the roots (8.17 and 5.23 mg/g). The barks of *R. stylosa* and *S. alba* contained almost twice the phenol content of green tea extract (Table 1).

Table 1
Total phenolic contents of water and methanolic extracts from different mangrove tissues.

Group		WE	ME
R. stylosa	Leaf	9.32±1.10	9.74±0.75
	Stem	11.46±0.56	11.96±0.86
	Root	7.30±0.76	8.17±1.27
S. alba	Leaf	7.60±0.82	8.27±1.13
	Stem	10.52±0.47	10.58±0.78
	Root	4.87±0.69	5.23±0.87
Green tea		5.57±0.74	6.29±0.62

3.2. Antioxidant activity of different tissues of mangrove plants

Free radical scavenging capacity was analyzed using DPPH method. DPPH activity was determined in root, bark and leaf samples of both species. A higher DPPH activity was found in methanol extracts, compared to that in water extracts. DPPH activity was highest in the bark of *R. stylosa* (85.5%) and *S. alba* (80.8%), while it was the lowest in water extract (38.2%) from *S. alba* root (Table 2). And the levels of DPPH activity in bark and leaves of these two species were higher than that of green tea (62%). Figure 2 shows the correlation between total phenolic content and antioxidant activities in the mangrove tissue extracts. These results indicate the strong association between total phenolic content and antioxidant activities, suggesting that phenolic compounds play an important role in the antioxidant activities of those mangrove plants.

Table 2
DPPH radical scavenging activity of water and methanolic extracts from different mangrove tissues.

Group		WE	ME
R. stylosa	Leaf	68.9±3.5	75.3±4.6
	Stem	72.5±3.2	85.5±5.2
	Root	40.3±4.3	41.3±3.9
S. alba	Leaf	62.2±5.0	69.6±4.9
	Stem	72.4±4.9	80.8±5.4
	Root	38.2±3.6	40.7±3.2
Green tea		52.7±2.9	62.0±5.7

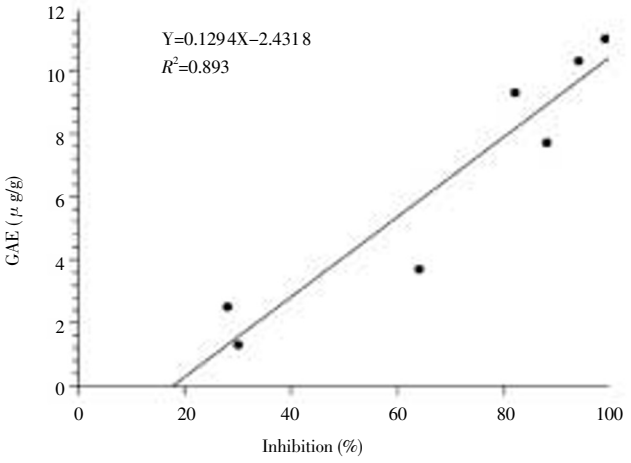

Figure 2. Correlation between total phenolic contents and antioxidant activities of the water and methanolic extracts from different mangrove tissues.

3.3. Tyrosinase inhibitory activity in different tissues of mangrove plants

The tyrosinase inhibitory effects of *R. stylosa* and *S. alba* extracts were investigated by recording the change in absorbance at 475 nm due to dopachrome formation from L-tyrosine or L-DOPA, and their tyrosinase inhibitory activities were compared with those of green tea. Tyrosinase inhibitory activity was higher in bark extracts than in extracts from leaves and roots of *R. stylosa* and *S. alba*. The level of tyrosinase inhibitory activity (89.7% and 82.4%, respectively) in bark extracts of two mangrove plants was almost twice as high as that (51.7%) of green tea extract. However, a lower tyrosinase inhibitory activity was present in the root extracts of both species, compared to that in green tea extract. A higher tyrosinase inhibitory activity in mangrove trunk and leaves signifies that mangrove trees have higher phenol content, and by association, a wider range of antioxidant activities, compared to green tea.

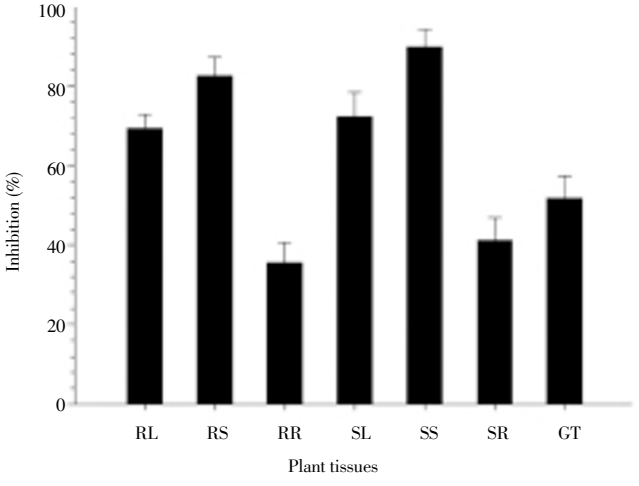

Figure 3. Tyrosinase inhibition activities of water extracts from different mangrove tissues.
RL, leaf extract of *R. stylosa*; RS, stem extract of *R. stylosa*; RR, root extract of *R. stylosa*; SL, leaf extract of *S. alba*; SS, stem extract of *S. alba*; SR, root extract of *S. alba*; GT, green tea extract.

4. Discussion

Environmental stress factors affect plant growth[16,17]. Among the effects of environmental stress is the creation of reactive oxygen species (ROS), which cause damage to cell membranes, nucleic acid and chloroplast pigments[18,19]. ROS accumulation is induced by the imbalance between ROS and antioxidants. Removal of active oxygen in cells is controlled byenzymatic and non-enzymatic antioxidants. The former include catalase, peroxidase and superoxide dismutase, and the latter include glutathione, carotenoids, ascorbate and polyphenol. Antioxidants provide an important function in maintaining oxidative resistance[20,21]. The levels of reactive oxygen species are controlled by enzymatic antioxidants under normal conditions[22], however, ROS rise under stress and stimulate the activity of antioxidant enzymes.

Antioxidants are known to affect cardiovascular health, atherosclerosis, cancer and aging processes, drawing a broad spectrum of attention from a variety of biomedical fields[23]. Antioxidant compounds found in plants have free radical scavenging effects[24,25]. Many studies have been conducted to identify natural antioxidants, with the aim of replacing synthetic antioxidant and using them as an ingredient for food and drugs[26,27]. Although synthetic antioxidants like butylatedhydroxyanisole or tert-butylhydroquinone are used as an ingredients of food, the possible toxic effects of these compounds poses a concern[28]. Amid growing attention on natural antioxidants, the effort to separate antioxidants from plants and commercialize their usage continues. Spices, herbs and other plants with higher levels of polyphenol compounds are actively explored by food manufacturers due to their function in delaying the oxidation of lipids, as well as boosting the quality of food, including nutritional value[29].

R. stylosa and *S. alba* have total phenol contents which are twice that of green tea. The antioxidant activity was also determined to be higher in mangrove species growing in Chuuk State, Micronesia, compared to that of green tea (Table 2). Moreover, the correlation between total phenol content and antioxidant activity in different parts of mangrove trees was presented in Figure 2, where a strong association was observed. The antioxidative activity of phenolic compounds is therefore confirmed in mangrove plants. This finding is consistent with the results of a study in which a correlation was present between the total phenol content and the free radical scavenging effects of Swiss beta vulgaris subspecies cicla[30]. The relationship between phenol content and antioxidant activity has been identified in fruit juice, vegetables and seeds[31,32]. This result means that *R. stylosa* and *S. alba* have great potential for development into various ingredients for use as antioxidants. If a sustainable source of mangroves were to be established, then the antioxidant properties contained in the trunk or leaves of the trees could be widely applied for the development of functional food additives and cosmetics.

In conclusion, we analyzed the phenol content, antioxidant activity and tyrosinase inhibitory activities of methanol and water extracts of *R. stylosa* and *S. alba* from the Island of Weno, Chuuk State of Micronesia. Total phenol content and antioxidant activity did not show significant differences between methanol and water extracts. However, methanol extracts of *R. stylosa* exhibited a higher phenol content and greater antioxidant activity as compared to aqueous extracts. Methanol extracts of *R. stylosa* bark had almost twice the level of total phenol content (11.96 mg/g) as that of green tea (6.29 mg/g). Also, tyrosinase inhibitory activity was higher in extracts of *S. alba* bark (89.7%) than that (51.7%) of green tea extract. These findings suggest that *R. stylosa* and *S. alba* could be successfully developed as natural antioxidants and cosmetics.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Morton JF. *Atlas of medicinal plants of Middle America*. Bahamas to Yucatan, C.C. Thomas: Springfield, IL; 1981.

[2] Melchor G, Armenteros M, Fern´andez O, Linares E, Fragas I. Antibacterial activity of *Rhizophora manglebark*. *Fitoterapia* 2001; **72**: 689-691.

[3] Fernandez O, Capdevila JZ, Dalla G, Melchor G. Efficacy of *Rhizophora mangle* aqueous bark extract in the healing of open surgical wounds. *Fitoterapia* 2002; **73**: 564-568.

[4] Chapman VJ. Mangrove vegetation. *J Cramer Germany* 1976; 447.

[5] Hatano T, Edamatsu R, Mori AF, Yasuhara E. Effect of interaction of tannins with co-existing substances Ⅵ. Effect of tannins and related polyphenols on superoxide anion radical and on DPPH radical. *Chem Pharm Bull* 1989; **37**: 2016-2021.

[6] Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of HarngJyur (*Chrysanthemum morifolium Ramat*). *Lebnesmittel-Wissenschaft Technologie* 1999; **32**: 269-277.

[7] Tanaka M, Kuei GW, Nagashima Y, Taguchi T. Application of antioxidativemaillard reaction products from histidine and glucose to sardine products. *Nippon Suisan Gakkashi* 1998; **54**: 1409-1414.

[8] Rahim AA, Rocca E, Steinmetz J, Kassim MJ, MS Ibrahim, Osman H. Antioxidant activities of mangrove *Rhizophora apiculata* bark extracts. *Food Chem* 2008; **107**: 200-207.

[9] Ravikumar S, GnanadesiganM. Hepatoprotective and antioxidant activity of a mangrove plant Lumnitzeraracemosa. *Asian Pac J Trop Biomed* 2011; **5**: 348-352.

[10]Beula JM, Gnanadesigan M, Ravikumar B, Anand M. Antiviral, antioxidant and toxicological evaluation of mangrove plant from South East coast of India. *Asian Pac J Trop Biomed* 2011; **2**: s352-s357.

[11]Capannesi C, Palchetti I, Mascini M, Parenti A. Electrochemical sensor and biosensor for polyphenols detection in olive oils. *Food Chem* 2000; **71**: 535-562.

[12]Lu Y, Foo LY. Antioxidant and radical scavenging activities of polyphenols apple pomace. *Food Chem* 2000; **68**: 81-85.

[13]Soares JR, Dins TCP, Cunha AP, Ameida LM. Antioxidant activity of some extracts of *Thymus zygis*. *Free Radical Res* 1997; **26**: 469-478.

[14]Yamaguchi T, Takamura H, Matoba T, Terao J. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci Biotechnol Biochem* 1998; **62**: 1201-1204.

[15]Kim S, Woo S, Yun H, Yum S, Choi E, Do J-R, et al. Total phenolic contents and biological activities of Korean seaweed extracts. *Food Sci Biotechnol* 2005; **14**: 798-802.

[16]Maksymiec W. Effect of copper on cellular processes in higher plants. *Photosynthetica* 1997; **34**: 321-342.

[17]Siedlecka A, Tukendorf A, Sk′orzy′nska-Polit E, Maksymiec W, W′ojcik M, Baszy′nski T, et al. *Angiosperms (Asteraceae, Convolvulaceae, Fabaceae and Poaceae; other than Brassicaceae)*. New York: Marcel Dekker Inc; 2001, p. 171-115.

[18]Fang W-Ch, Kao CH. Enhanced peroxidase activity in rice leaves in response to excess iron, copper and zinc. *Plant Sci* 2000; **158**: 71-76.

[19]Tewari RK, Kumar P, Sharma PN, Bisht SS. Modulation of oxidative stress responsive enzymes by excess cobalt. *Plant Sci* 2002; **162**: 381-388.

[20]Xiang C, Oliver DJ. Glutathione metabolic genes coordinately respond to heavy metals and jasmonic acid in Arabidopsis. *Plant Cell* 1998; **10**: 1539-1550.

[21]Srivastava S, Tripathi RD, Dwivedi UN. Synthesis of phytochelatins and modulation of antioxidants in response to cadmium stress in Cuscutareflexa—n angiospermic parasite. *J Plant Physiol* 2004; **161**: 665-674.

[22]Asada K. Chloroplasts: formation of active oxygen and its scavenging. *Methods Enzymol* 1984; **10**: 422-429.

[23]Packer L. *The antioxidant miracle-Your complete plan for total health and healing*. New York: Wiley; 1999.

[24]Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen. *J Agric Food Chem* 1994; **42**: 6-9.

[25]Duh PD. Antioxidant activity of burdock (*Arctiumlappa* Linne) : its scavenging effect on free radical and active oxygen. *J Am Oil Chem Soc* 1998; **75**; 455-465.

[26]Ito N, Fukushima S, Hasegawa A, Shibata M, Ogiso T. Carcinogenecity of butylatedhydroxylanisole in F 344 rats. *J Nat Cancer Institue* 1983; **70**: 343-347.

[27]Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. *J Agric Food Chem* 2001; **49**: 5165-5170.

[28]Valentao P, Fernandes E, Carvalho F, Andrade PB, Seabra RM, Bastos ML. Antioxidative properties of cardoon (*Cynaracardunculus* L.) infusion against superoxide radical hydroxyl radical, and hypochlorous acid. *J Agric Food Chem* 2002; **50**: 4989-4993.

[29]Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA. Free radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem* 2004; **84**: 551-562.

[30]Pyo YH, Lee TC, Logenda L, Rogen RT. Antioxidant activity and phenolic compounds of Swiss chard (*Beta vulgaris* subspecies cicla) extracts. *Food Chem* 2004; **85**: 19-26.

[31]Park YK, Lee WY, Park SY, Ahn JK, Han MS. Antioxidant activity and total phenolic content of *Callistemon citrinus* extracts. *Food Sci Biotechnol* 2005; **14**: 212-215.

[32]Choi YM, Ku JB, Chang HB, J Lee. Antioxidant activities and total phenolics of ethanol extracts from several edible mushrooms produced in Korea. *Food Sci Biotechnol* 2005; **14**: 700-703.