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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.

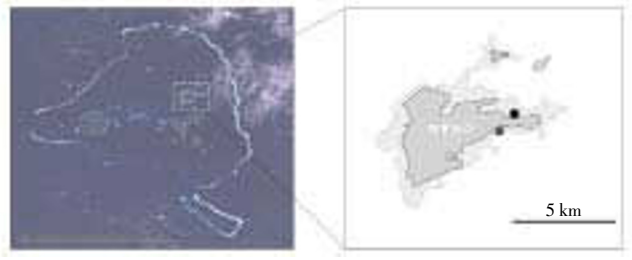

Figure 1. Chuuk lagoon image and station for mangrove sampling.

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.