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Antioxidant Activity of Rhizophora Mucronata Collected from Thoothukudi Coastal Area, India

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ABSTRACT

Introduction: Mangrove forests yield the coast lines of several tropical countries. Apart from ecological importance, plants hold important therapeutic uses. One among the mangrove species is Rhizophora mucronata.Antioxidant properties of medicinal plants have attracted the attention of many researchers. The antioxidant compounds terminate the attack of free radicals and reduce the risk of neurological disorders.

Aim: The aim of the present study was to evaluate the antioxidant potential of *Rhizophora mucronata* .

Materials and Methods: The fresh leaves of *Rhizophora mucronata* were collected from Tuticorin coastal area, Tamilnadu. The dried mangrove powdered leaves were used to prepare methanol extract of *Rhizophora mucronata*. The extract was used to evaluate the total antioxidant activity. Total reducing activity and free radical scavenging activity using DPPH assay and compared with the standard ascorbic acid.

Results and Discussion : The antioxidant potential of *Rhizophora mucronata* by DPPH assay showed a maximum scavenging activity of 93.86% at highest concentration (150 µg/ml)while the standard ascorbic acid showed 98.6% at high concentration.The maximum concentration of 150 µg/ml of *R.mucronata* extract was equivalent to 136.85±1.28 concentration of ascorbic acid in total antioxidant activity and the maximum concentration of 150 µg/ml of *R.mucronata* extract was equivalent to 73.24±1.22 concentration of ascorbic acid in total reducing power.

BThe present study concludes that *Rhizophora mucronat*a has very good antioxidant activity. Hence, the extract can be used for its antioxidant property in disease conditions such as heart diseases, diabetes and certain cancers after further *invivo* studies.

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INTRODUCTION

Medicinal plants have a potential role in development of different drugs with vast therapeutic uses which were obtained from different plant species.[(1)] Plant based extracts as an alternative to a chemical compound are commonly used in pharmaceutical and food industries. Antioxidant properties of medicinal plants have attracted the attention of many researchers. Plants are demonstrated to be rich sources of antioxidant compounds and contain large amounts of secondary metabolites like phenolic and flavonoid compounds.[(1,2)] Flavonoid is a well-known phenolic compound which has potent antioxidant properties. The antioxidant compounds terminate the attack of free radicals and reduce the risk of neurological disorders.[(3)] Presently much attention is given to using natural antioxidants to protect the human body, especially brain tissues from oxidative damage by free radicals. Similar studies showed that polyphenols scavenge active oxygen species and prevent oxidative cell damage.[(4)]

The tropics and subtropics is one among the important ecosystems in the realm where land meets the ocean. Mangrove forests yield the coast lines of several tropical countries.[(5)] Apart from ecological importance plants hold important therapeutic uses. One among the mangrove species is Rhizophora mucronata. Mangrove plants are used both as food and medicine and mangrove forests are important sources of biodiversity hotspots with marine fungi.[(6)] Previous studies have been done on bioaccumulation of metals in mangroves and salt marshes collected from Tuticorin coast to prevent the biosphere.[(7)]

KEYWORDS:
Antioxidant activity, Innovation, Natural source Mangrove plant, *Rhizophora mucronat*a.

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Many studies have been conducted on different activities of plants like antidiabetic, anti inflammatory and anti cancer activities.[8-14] Our team has extensive knowledge and research experience that has translated into high quality publications.[15-19] [20])

Rhizophora mucronata is a true mangrove distributed along delta regions, the bark, trees, leaves, fruit of Rhizophora mucronata is traditionally used as medicine.[21] Rhizophora mucronata is used traditionally for diabetes, diarrhea, inflammation and cognitive functions.[22] The unique ecology of traditional medicinal plants attracted the attention of many researchers and results increased significantly.[23] Which led us to many researches on marine species.[24-28] The aim of the study was to analyse the antioxidant potential of *Rhizophora mucronata* from tuticorin coastal areas.

MATERIALS AND METHODS

Study setting: The study was conducted in the Marine biomedical and Environmental Health research lab - Blue Lab, Saveetha Dental College.

Sample collection and preparation: The fresh leaves of *Rhizophora mucronata* were collected from Tuticorin coastal area, Tamilnadu. The leaves were washed thoroughly with tap water then shade dried on table tissue paper for 2-3weeks and turn into a fine power.

Preparation of extraction: 25g of dried powdered mangrove leaves samples were mixed with 100ml of methanol and allowed to place for 24 hours at ambient temperature. Then the mixture was passing through whatman filter paper (No.4) then the filtrate was centrifuged at 3000rpm for 10min and further filtered by 0.45µm syringe micro filter. At last, the solvents are evaporated via vacuum rotary evaporator until samples are obtained in powder form. Then the sample was stored in a shadowy aluminum container at 4°C for further analysis.

Total antioxidant activity: Total antioxidant activity of the crude mangrove extract was determined by following method: 0.3 ml of sample was prepared in different concentrations (0.5- 3mg/ml) with 3ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95¤c for 90 minutes in a water bath. Absorbance of all sample mixtures was measured at 695 nm. Total antioxidant activity has been expressed as the number of equivalents of ascorbic acid.

DPPH Assay: The antioxidant potential of mangrove crude extract was determined on the basis of their scavenging activity of the stable 1,1- diphenyl-2-picryl hydrazyl (DPPH) free radical. Different concentrations (0.5-3mg/ml) of samples were mixed with 2.9ml diphenylpicrylhydrazyl (DPPH) solution (120µM) in methanol and incubated in darkness at 37°c for 30 minutes. The absorbance was recorded at 517 nm. Inhibition of free radical by DPPH in percentage (I %) was calculated with the following equation:

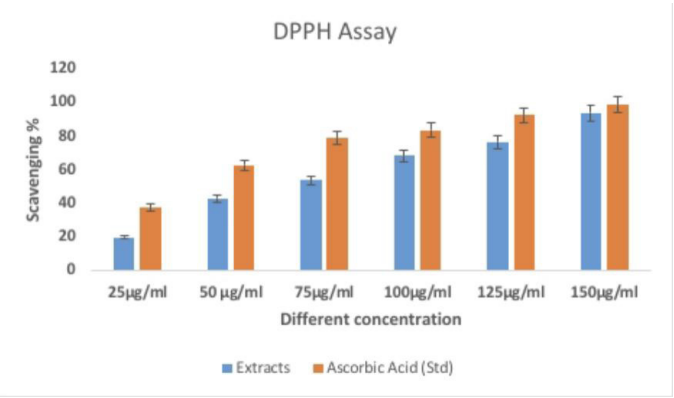
$$\text{Percentage of Inhibition (I \%)} = \frac{\text{(A blank - A sample)}}{\text{A blank}} \times 100$$

Where, A blank is the absorbance of the control reaction and A sample is the absorbance of the test compound. The values of inhibition were calculated for the various concentrations of the sample.Ascorbic acid was used as positive control (Kamala et al., 2015) and all the tests were carried out in triplicate.

Total reducing power: Reducing capacity of methanol crude extract obtained from the mangrove leaves were determined by following method: Briefly, 1ml of Benzene: chloroform (2:1) containing different concentrations of extract (0.5-3mg/ml) were mixed with 2.5 ml of benzene: chloroform and 2.5 ml potassium ferricyanide (1%) reaction mixture was incubated at 50¤C for 20 min. After incubation, 2.5 ml 10% trichloroacetic acid was added and centrifuged at 10,000 rpm for 10 min 2.5ml. The upper layer was mixed with 2.5ml distilled water and 0.5ml FeCl¤ (0.1%) and the absorbance was measured at 700 nm. The data was then represented in graphs.

RESULTS

The antioxidant activity and reducing power of *R.mucronata* was compared with the standard ascorbic acid and represented in tables and the activity of *R.mucronata* on DPPH assay was compared with ascorbic acid and represented as a bar chart. At low concentration (25µg/ml) the extract showed 19.67% scavenging activity while the standard ascorbic acid showed 37.3% scavenging activity at highest concentration of 150µg/ml the extract showed 93.86% while the standard ascorbic acid at high concentration showed 98.6% scavenging activity(Bar graph 1). The minimum concentration of 25 µg/ml *R.mucronata* extract was equivalent to 31.29±1.21 concentration of ascorbic acid. The maximum concentration of 150 µg/ml of *R. mucronata* extract was equivalent to 136.85±1.28 concentration of ascorbic acid of antioxidant activity(Table 1), while The minimum concentration of 25 µg/ml R.mucronata extract was equivalent to 10.47±1.23 concentration of ascorbic acid. The maximum concentration of 150 µg/ml of *R. mucronata* extract was equivalent to 73.24±1.22 concentration of ascorbic acid to reduce power(Table 2). These results showed that increase in concentration of extract increases the scavenging activity and the antioxidant activity and reducing activity of the extract was equivalent to standard ascorbic acid.



BAR GRAPH 1 : Represents the graphical representation of Rhizophora mucronata action on DPPH assay in different concentrations. Descriptive statistics were done to compare the activity of Rhizophora mucronata and Ascorbic acid. X axis represents different concentrations of rhizophora extract and ascorbic acid, Y axis represents scavenging percentage. Blue represents the activity of Rhizophora mucronata extract and Orange represents activity of Ascorbic acid. The bar chart represents that, with increase in concentration the extract showed increased scavenging activity. The values were done in triplicate (n= 3) with mean ± SE

Table 1: Represents the total antioxidant activity of *Rhizophora mucronata* equivalent to ascorbic acid as standard where n= 3 and the values are given as mean ± SE

TOTAL ANTIOXIDANT ACTIVITY (TAA) µg/ml	*ASCORBIC ACID EQUIVALENT (AAE)*
25	31.29±1.21
50	58.47±1.3
75	71.86±1.3
100l	91.54±1.25
125	121.68±1.31
150	136.85±1.28

Table 2: Represents the total reducing power of *Rhizophora mucronata* equivalent to ascorbic acid as standard. where n= 3 and the values are given as mean ± SE

TRP µg/ml	*AAE*
25	10.47±1.23
50	28.64±1.27
75	37.53±1.24
100	42.94±2.1
125	59.43±1.3
150	73.24±1.22

DISCUSSION

The biologically active substance with antioxidant potential in *Rhizophora mucronata* extract results in the reduction of stable 2,2-diphenyl-1-picryl hydrazyl radical. The analysis showed an increase in the scavenging activity. Ascorbic acid was used for comparison, as a reference with high antioxidant potential. At low concentration (25µg/ml) the extract showed 19.67% scavenging activity while the standard ascorbic acid showed 37.3% scavenging activity. With increase in concentration of *R. mucronata* extract, it showed an increase in tendency of increased radical scavenging activity. Maximum scavenging activity was seen at highest concentration (150µg/ml) with 93.86% while the standard ascorbic acid at high concentration showed 98.6% scavenging activity. The activity of *R. mucronata* was equivalent to ascorbic acid thus the extract showed a promising result. Similarly in a study done by (29) methanolic extract exhibited the highest antioxidant activity with an IC50 value of 47.39±0.43 with DPPH assay similarly in the present study *R. mucronata* extract showed a highest radical scavenging activity.

The total antioxidant activity of *R. mucronata* extract was analysed and it was compared with the standard ascorbic acid antioxidant activity. The evaluation was done using different concentrations (25 µg/ml to 150 µg/ml) and the equivalence was observed. The minimum concentration of 25 µg/ml *R.mucronata* extract was equivalent to 31.29±1.21 concentration of ascorbic acid. The maximum concentration of 150 µg/ml of *R. mucronata* extract was equivalent to 136.85±1.28 concentration of ascorbic acid. Similar antioxidant potential was assessed in dry birch leaves extract in study by (30) where Trolox was considered as the standard and the extract possessed antioxidant potential only in acute application while in chronic application the results were poor due to biotransformation or elimination process.

The total reducing power of *R. mucronata* extract was also analysed and it was compared with the standard ascorbic acid reducing power. The evaluation was done using different concentrations (25 µg/ml to 150 µg/ml) and the equivalence was observed. The minimum concentration of 25 µg/ml R.mucronata extract was equivalent to 10.47±1.23 concentration of ascorbic acid. The maximum concentration of 150 µg/ml of *R. mucronata* extract was equivalent to 73.24±1.22 concentration of ascorbic acid. Similarly in an i*n vitro* study by[31] the reducing power was analysed using ferric reductase assay and showed a dose dependent antioxidant activity in further Secondary metabolite isolation and characterization are also done. Contradictorily in a study done by[32] ethanol extract of *G. biloba* were not effective for yeast cell protection or reinforcing the antioxidant potential of the extracts so the plant species was not considered for antioxidant potential. Many plants were evaluated for their antioxidant activity and other activities directly or after isolation of the active components or preparing nanoparticles with it.[7,33, 34] The present study was done to analyse only the antioxidant activity of one species of mangrove plants and only antioxidant potential of the plant was evaluated. Previously many studies have been done by the institution.[10, 35-51] Further studies can be done on different mangrove species and other activities like anti inflammatory, anti cancer and anti diabetic activities.

CONCLUSION

The present study concludes that *R. mucronat*a has very good antioxidant activity. Hence, the extract can be used for its antioxidant property in disease conditions such as heart diseases, diabetes and certain cancers after further *invivo* studies. Further studies can interpret other bioactive properties of the species.

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