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Proximate composition and in *vitro* antioxidant properties of *Rhizophora mucronata* plant part extract

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ABSTRACT

Rhizophora mucronata is a species of mangrove widely distributed in Indian mangrove forest. It seems to be more tolerant of inundation than other mangrove species and often forms an evergreen fringe to mangrove areas. In present study, the methanolic extract of different parts of *Rhizophora mucronata,* their antioxidant properties along with primary, secondary metabolites and proximate composition, were tested. Better antioxidant activity was gained by methanolic extract of leaf than root and bark extract. Antioxidant potential of the extracts was analyzed as contents of total phenols and flavonoids; radical scavenging activity by the DPPH methods, NO and H_2O_2 and primary and secondary metabolites assay and the results of antioxidative activities from the *R. mucronata* plant. The total content of phenols and flavonoids in the methanol extracts of the studied species positively correlated with their antioxidant properties, confirmed their major role in antioxidant activity of these *R. mucronata*.

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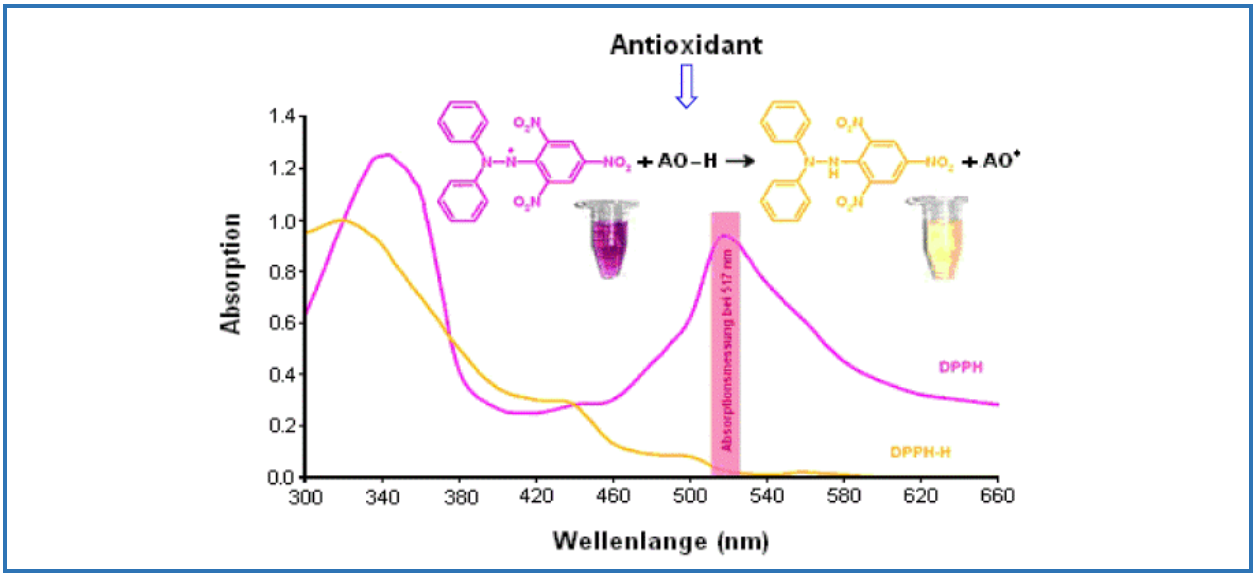
Graphical Abstract



Introduction

Indo-West Pacific stilt mangroves (IWP *Rhizophora* species) are widespread among most tropical coastal areas of the western Pacific region to east Africa. *Rhizophora* are considered as the most important ones when compared to all other mangrove genera across the Pacific tropical region. Mangroves play a vital role in shoreline protection, enhancement of water quality in nearshore environments (Including coral reefs) and in supporting estuarine and marine food chains. In most parts of the Pacific, trees are harvested for firewood, hence the trunk is the main part of the tree considered for direct use [1].

There are a number of profits delivered by *Rhizophora mucronata* to the living communities. As far as ecological benefits are concerned, *Rhizophora mucronata* act as protection barrier against storms, hurricanes and tsunamis [2–5]. It also plays a role in avoidance of coast erosion [5]. Its roots trap suspended solids assuring filtering of the upland runoff, protecting the coral reefs and sea grass beds for the negative effect of suspended particles. It also helps in reducing global warming by counteracting global heating by its high amount of carbon sequestration [6]. It provides protection to natural fish diversity in oceanic water. The extensive root systems provide the breeding place for fish and prawns, as well as the shelter for the juveniles [7].

Cautiously, mangroves are considered as a supplier of natural products such as charcoal, wild honey, timber, food and medicinal element [7]. Marine halophytes, such as mangroves and related species, are known to have many and various metabolites possessing antibacterial [8]. Antifungal [9]

antiviral [10], antidiarrheal [11], hepatoprotective [12], antifeedant [13], insecticidal [14], and antiplasmodial properties [15].

Experimental

Collection and extraction of mangrove plants

The fresh leaves, bark and stilt root of *Rhizophora mucronata* were collected from Pichavaram mangrove forest, Tamil Nadu, India. The samples (leaves, bark and stilt root) were carefully examined. Old, insect damaged and fungus infected leaves were removed. Healthy samples were washed and spread out. They were shade dried, coarsely powdered and the extraction was done with methanol in Soxhlet apparatus. The collected samples were kept at 4 °C for further use.

Quantification of primary and secondary metabolites

Primary metabolites like carbohydrates, protein, chlorophyll, lipids and secondary metabolites like total phenolic, tannin and total flavonoids were assessed by the method followed by *Bhuvana* et al. [16].

Proximate composition

The methanolic leaves, bark and stilt root extract of *Rhizophora mucronata* were examined for moisture content, protein, crude fat, crude fiber, ash content and extractive values using the methods described by AOAC (1990), *Bhuvana* et al.

In vitro antioxidant activity

The total antioxidant and radical scavenging activity of the methanolic extracts of leaves, bark and stilt root from the species, *Rhizophora mucronata* were determined by standard protocol. In *vitro* assays include total phenolic, total flavonoid, DPPH assay, hydrogen peroxide activity and nitric oxide scavenging assay based on the procedure followed by *Bhuvana* et al. [16].

Results and discussion

Rhizophora mucronata has multiple uses. It is used to help prevent coastal erosion and in restoration of mangrove habitats [1]. The timber is used for firewood and in the construction of buildings, as poles and pilings, and in making fish traps. The fruits can be cooked and eaten or the juice extracted to make wine, and the young shoots can be consumed as a vegetable. The bark is used in tanning and a dye can be extracted from both bark and leaves. Various parts of the plant are used in folk medicine [2].

Table 1. Quantification of primary metabolites of *R.mucronata* plant parts

S.NO	Primary metabolites	Leaf weight (mg/g dw)	Root weight (mg/g dw)	Bark weight (mg/g dw)
1	Carbohydrates	62.36 ± 0.53	24.68 ± 0.83	14.78 ± 0.25
2	Chlorophyll	10.13 ± 0.19	1.24 ± 0.12	0.94 ± 0.48
3	Protein	5.48 ± 0.01	3.21 ± 0.32	1.21 ± 0.27
4	Lipids	0.78 ± 0.16	0.84 ± 0.51	0.91 ± 0.62

Dw=Dry weight

Table 2. Quantification of secondary metabolites of *R.mucronata* plant parts

S.NO	Secondary metabolites	Leaf Weight (mg/g dw)	Root Weight (mg/g dw)	Bark Weight (mg/g dw)
1	Total phenolic	3.15 ± 0.13	2.28 ± 0.43	1.54 ± 0.28
2	Tannin	2.23 ± 0.11	1.11 ± 0.82	1.01 ± 0.11
3	Total flavonoids	1.38 ± 0.45	0.91 ± 0.01	0.46 ± 0.13

Dw=Dry weight

Table 3. Proximate composition of *R.mucronata* plant

S.NO	Composition	Leaf % Dry weight	Root % Dry weight	Bark % Dry weight
1	Moisture content	34.91 ± 0.41	19.71 ± 0.13	4.91 ± 0.24
2	Ash content	1.81 ± 0.13	2.35 ± 0.23	0.78 ± 0.25
3	Crude protein	1.32 ± 0.35	0.98 ± 0.35	0.52 ± 0.31
4	Crude fiber	0.78 ± 0.65	0.73 ± 0.37	0.91 ± 0.26
5	Fat content	0.29 ± 0.20	0.11 ± 0.18	0.09 ± 0.56

Dw=Dry weight

Table 4. Extractive values of *R.mucronata* plant

S.NO	Parameters	Leaf (Values)	Root (Values)	Bark (Values)
1	Alcohol soluble extractive %	12.49 ± 0.31	9.58 ± 0.01	18.79 ± 0.21
2	Water soluble extractive %	2.07 ± 0.25	5.83 ± 0.11	4.07 ± 0.37
3	Ether soluble extractive %	0.73 ± 0.76	0.45 ± 0.18	1.93 ± 0.89

It is thought that many stresses inherent in the modern life style may cause an increased incidence of diseases such as cancer, diabetes, heart diseases, inflammatory and hypertension [15]. The rising

incidence of such diseases is alarming and becoming a serious public health problem. Many synthetic drugs confer protection against oxidative damage but they have adverse side effects. An alternative solution to the problems was to consume natural antioxidants from food supplements and traditional medicines [14].

Recently, many natural antioxidants have been isolated from different medicinal plants [17]. In the present study, we have analysed *R. mucronata* evaluated in pharmacognostic study such as powder study, physiochemical analysis (Moisture content, loss on drying, ash values, extractive values), phytochemical analysis, scavenging activities and anti-inflammatory assay are enlisted along with their importance.

During the present research work, leaf, bark and root of the plant were evaluated quantitatively for the analysis of primary metabolites (Chlorophyll, carbohydrate, protein and lipids.) and secondary metabolites (Total phenol, tannin, total flavonoids). Keeping in view the importance of these primary metabolites, the present studies for biochemical evaluation of primary metabolites from different parts of *R. mucronata* were undertaken (Table 1 and 2).

Table 3 illustrates, the proximate analysis results of all plants parts investigated with *Rhizophora mucronata* leaf having highest moisture content (34%), followed by root (19.71%) respectively.

Table 4 explains the level of extractive values in this plant root with alcohol soluble extractive having highest (18.79%) followed by leaf (12.49%) level respectively.

Mangrove plants provide an important source for the search of novel drugs as they are stress tolerant plants and rich in bioactive compounds. The bioactive compounds of *Rhizophora mucronata* plants can be used as potent source of modern drugs against various life threatening diseases. Keeping this in mind we have attempted to make a study on antioxidant potentials of various plant parts of *R. mucronata* (Table 5, 6, 7 and 8).

During the present research work, leaf, bark and root of the plant were evaluated quantitatively for the analysis of primary metabolites of chlorophyll, carbohydrate, protein and lipids. Keeping in view the importance of these primary metabolites, the present studies for biochemical evaluation of primary metabolites from different parts of *R. mucronata* were undertaken (Table 1). In the present investigation, it was observed that maximum amount of carbohydrate and chlorophyll was found in leaf and minimum amount in bark of *R. mucronata*. Pervious study reported by [18] found maximum chlorophyll result in callus of *Triticum aestivum* and other medicinally important plants.

In this study, the mangrove, *R. mucronata* from Pichavaram, South India was found to contain appreciable content of polyphenolics. The total phenolic content higher when compared to the species (94.4 mg/g) found in Sundarbans, India [19]. Mangroves in Sundarbans, North India were found to contain high content of polyphenolics like tannins [20]. Previous reports revealed that

mangroves are rich in polyphenols and tannins [21, 22]. Phenolics widely encountered in the plants tested as the most active radical scavengers. They are present in a variety of plants utilized as important components of both human and animal diets [23, 24]. There is strong evidence on the preventive effects of phenolics on age related chronic diseases [25].

Table 5. Total Phenolic and Total Flavonoid content

S.No	Activity performed	Leaf	Root	Bark
		mg equivalent to standard drug		
1	Total Phenolic	21.25 ± 0.231	16.10 ± 0.021	26.02 ± 0.031
2	Total Flavonoid	8.02 ± 0.351	3.62 ± 0.162	14.07 ± 0.072

Table 6. DPPH scavenging effect of *R.mucronata* plant parts

Conc µl	Std %	Leaf %	Root %	Bark%
100	13.72± 0.175	22.92 ± 0.114	17.69 ± 0.164	21.03 ± 0.127
200	25.90± 0.146	40.16 ± 0.272	28.43 ± 0.134	38.36 ± 0.32l
300	38.17± 0.251	53.79 ± 0.154	41.25 ± 0.287	50.18 ± 0.127
400	44.77± 0.196	67.78 ± 0.281	52.44 ± 0.291	64.26 ± 0.175
500	64.71± 0.294	80.51 ± 0.135	71.39 ± 0.286	77.17 ± 0.283
IC 50 Values	403.815	278.768	359.052	298.553

Table 7. Hydrogen peroxide scavenging activity of *R.mucronata* plant parts

Conc µl	Std %	Leaf %	Root %	Bark %
100	23.68 ± 0.161	47.12 ± 0.127	27.55 ± 0.122	42.39 ± 01.25
200	40.50 ± 0.152	60.16 ± 0.153	41.54 ± 0.132	64.37 ± 0.113
300	54.91 ± 0.128	70.98 ± 0.179	56.24 ± 0.143	72.92 ± 0.174
400	71.08 ± 0.123	80.95 ± 0.386	72.54 ± 0.365	85.30 ± 0.124
500	84.26 ± 0.117	93.15 ± 0.126	85.44 ± 0.236	94.38 ± 0.236
IC_{50} Values	267.800	118.591	254.612	124.898

Conclusion

R. mucronata plant part extracts demonstrated significant antioxidant properties as determined by the scavenging assay, reducing power assay and total antioxidant capacity. However, the methanol

extract of *R. mucronata* plant part extracts exhibited highest phenolic content and antioxidant potential. 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. Further in *vitro* study can be performed by bioactive compound isolation from this plant parts (Leaves, bark and stilt root) which might confirm an effective pharmacologic aspects.

Table 8. NO scavenging activity of *R.mucronata* plant parts

Conc µl	Std %	Leaf %	Root %	Bark %
100	23.00 ± 0.164	18.40 ± 0.132	14.01 ± 0.115	19.62 ± 0.142
200	36.52 ± 0.172	30.25 ± 0.165	26.14 ± 0.137	31.31 ± 0.172
300	54.76 ± 0.116	42.14 ± 0.176	35.30 ± 0.241	46.58 ± 0.261
400	69.10 ± 0.121	62.91 ±0.127	49.06 ± 0.264	66.00 ± 0.272
500	82.45 ± 0.134	74.47 ± 0.121	64.45 ± 0.216	78.05 ± 0.213
IC_{50} Values	279.100	330.152	398.611	311.138

Disclosure statement

No potential conflict of interest was reported by the authors.

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