Pharmacognostic and antioxidant activity of bark and its hydro-ethanolic extract of *Rhizophora mucronata*- A true mangrove

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Abstract

Objective: *To evaluate the pharmacognostic parameters of the raw and hydro-ethanolic bark extract of Rhizophora mucronata (3:1) (BERM). Based on the evaluation, the standardization can be done for the drug formulation. To evaluate the free radical capacity of BERM, DPPH and SOD radical scavenging activity was carried out.*
Materials and Methods: *Divided into 4 phases: organoleptic characters viz, colour, texture, smell, taste and shape observed by the sensory organs. The physical parameters viz pH, bulk density, tapped density; etc has been performed based on the standard protocol. DPPH and SOD radical scavenging was performed to check its anti-oxidant capacity.*
Results: *The results thus obtained were: for raw bark: colour: outer dark brown, inner: reddish brown, texture: smooth inside but rough from outside, size: 5 to 7 mm in thickness and fracture- uneven. Similar organoleptic characters observed for the extract. The fluorescence analysis clearly gives an idea to differentiate the plant from other species. DPPH assay of BERM revealed, percent inhibition of 86.17%± 0.01 and for SOD assay: 67.64± 2.43 respectively.*
Conclusion: *For the first time, this plant has been undertaken for its pharmacognostic evaluation, which forms the basis for its standardization and drug formulation in future. Anti-oxidant results clearly indicates that the extract has the capacity to scavenge wide range of free radicals, protect from ROS and give a strong defence to the organism.*

Keywords: DPPH, organoleptic, fluorescence analysis, physical parameters

1. INTRODUCTION

Mangrove forest is a form of vegetation family includes wide range of salt-tolerant plants which usually grows in the intertidal areas and between the estuarine line between land and sea. It is considered to be one of the most productive wetlands on the earth.[1] Traditionally, local people in mangrove ecosystem, utilizes the forest for fuel wood, timber, medicines from mangrove, food and feed and other natural resources.[2] Mangroves have been known since folk era and have been considered to be therapeutic against wide range of

ailments and possesses highly bioactive compounds that include anti-diarrhoeal, antioxidant, anti diabetic, antimalarial, anti inflammation and anticancer compounds.[3]

One such mangrove plant is *Rhizophora mucronata*, a true mangrove, perennial and found in salt marsh areas. *Rhizophora*, commonly called as 'red mangrove', belonged to the *Rhizophoraceae* family and found in Indo Pacific region on the banks of rivers and on the edge of the sea. It is the only mangrove species to be found in East Africa.[4] In Tamil Nadu, this plant is considered to be the densest and extensively found both in Pitchavaram and Muthupet mangrove forest.[5]

Rhizophora mucronata plant and its parts have been used as medicine since ancient times. In folklore medicine its bark and leaf extracts has been used as anti-septic, astringent and homeostatic with antibacterial, anti viral, anti-ulcerogenic, anti cancerous, anti proliferating and anti inflammatory activities.[6,7] The leaf extract found to possess a polysaccharide capable of controlling immunodeficiency virus.[8]

Previous studies on this plant, the phytochemical analysis revealed the presence of carbohydrates, polyphenols, alkaloids, terpenoids, flavones, flavonoids, anthrocyanidins, inositols, gibberellins, carotenoids, lipids, saponins, etc. These compounds may be responsible for the diverse therapeutic values of the plant *Rhizophora mucronata.* Preliminary phytochemical analysis of hydro-ethanolic extract (3:1) of *R. mucronata* found to contain alkaloids, terpenoids, carboxylic acids, tannins, flavonoids, saponins, phenols and phytosterols respectively.

Anti-oxidants are the substances which are capable to scavenge ROS and fight against disease to protect the body. Almost all the living species are gifted with protective defense systems to protect from ROS.[9] However, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been restricted due to their proliferative effects.[10] Thus, anti-oxidants from plants have been extensively studied and isolated.

In the context of above findings, the pharmacognostic evaluation of the bark and the hydro-ethanolic extract of *Rhizophora mucronata* have been undertaken as it support and authenticates the findings of pharmacological and biological studies. This study was a first attempt at this plant part (Bark) to recognize the potent bioactives and standardize the parameters to formulate the compounds. The Pharmacognostic evaluation included, organoleptic characteristics, physical parameters, fluorescent analysis and anti-oxidant capacity has been analysed. Further, DPPH and SOD radical scavenging activity was performed to check the ability of BERM for its free radical scavenging and neutralise the oxidative stress.

2. MATERIALS AND METHODS

2.1 Collection of Plant material

The bark of *Rhizophora mucronata* were collected from Pichavaram Mangrove forest, (latitude: 11° 23' to 11° 30' N and longitude: 79° 45' to 79° 50' E) is located between Coleroon and Vellar estuary in the state of Tamil Nadu, identified in the herbarium of C.A.S. in Marine Biology, Annamalai University, Parangipettai, India and authenticated by Prof Jayaraman, Botanist and Director, PARC, West Tambaram, Chennai, Tamil Nadu, India with the specimen no: PARC/2018/3854 for future reference.

2.2 Extract Preparation

Maceration procedure has been employed for the extraction. After extracting with different solvents with increasing polarities, the marc left out was dried and then it is extracted with 1500 ml of alcohol 70% and water (3:1) by maceration process for 2 weeks. After extraction it was filtered and the removal of solvent was done through filtration. The

filtrate obtained was dried at room temperature and the (20.0 gm) extract was stored at 4ºC for further use (Figure: 1).

2.3 Pharmacognostic parameters

2.3.1 Organoleptic characteristics

The raw bark and the extract EtOH: water (3:1) of *Rhizophora mucronata* (BERM) was macroscopically examined for surface characteristics viz, colour, odour, taste, shape, texture, etc.[11]

2.3.2 Physical parameters

The raw and extract Ethanol: water (3:1) of bark of *Rhizophora mucronata* was standardized for physicochemical parameters. It included: 5 parameters

2.3.2.1 pH range

The extract EtOH: water (3:1) approximately 1 gm was weighed and dissolved in 100 ml of water respectively with occasional stirring for complete dissolution. The solution was kept undisturbed for 24 hours. After 24 hours both the solution were decanted in another beaker and pH was measured with the help of calibrated pH meter.

2.3.2.2 Bulk Density

Bulk density is the ratio of given mass of powder and its bulk volume. For the extract EtOH: water (3:1), 5 gms was accurately weighed and transferred to the graduated cylinder with the aid of a funnel. The initial volume was noted. The ratio of weight of the volume it occupied was calculated.

$$\text{Bulk density} = W/V_0 \text{ g/ml}$$

where,

W = mass of the powder

V_0 = untapped volume.

2.3.2.3 Tapped Density

Tapped density for the raw and extract EtOH: water (3:1) was measured, wherein accurately weighed 5 gms of extract EtOH: water (3:1) was suspended in the graduated cylinder and tapped for specific number of times approximately 30 times for 10-15 mins. The initial volume was noted. The density can be determined as the ratio of mass of the powder to the tapped volume.

$$\text{Tapped volume} = W/V_f \text{ g/ml}$$

where,

W = mass of the powder

V_f = tapped volume.

2.3.2.4 Hausner Ratio

It usually indicates the flow properties of the powder. The ratio of tapped density to the bulk density of the powder is called Hausner ratio. The extract EtOH: water (3:1) was subjected to Hausner's ratio and results were noted.

$$\text{Hausner ratio} = \text{Tapped density/bulk density}$$

where,

h=height of the pile

r = radius of the pile

2.3.2.5 Compressibilty index

It is the propensity of the powder to be compressed. In this, the extract (EtOH: water (3:1) was subjected for compression. Based on the apparent bulk density and tapped density the percentage compressibility of the powder can be determined using the following formula.

$$\% \text{ Compressibility} = [(\text{tapped density} - \text{bulk density})] / \text{tapped density}] \times 100$$

2.4 Fluorescent analysis

Fluorescent analysis of the raw bark and extract (EtOH: H_2O) in the ratio of 3:1 was performed as per.[12] The Ethanol: water (3:1) extract of *Rhizophora mucronata* was screened for fluorescent analysis with and without chemical treatment. The change in the colour observed in visible light and under UV (short- 254 nm and Long- 366 nm) was noticed.

2.5 Antioxidant assay

2.5.1 DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay

The scavenging effects of samples for DPPH radical were determined by the method.[13] Briefly, 2.0 ml of 0.16 mM DPPH methanolic solution was added to 2.0 ml of aliquot of test samples. The mixture was then vortexed for 1 min and then left to stand at room temperature for 30 min in the dark. The absorbance of all the sample solutions was measured at 517 nm. The scavenging effect (%) was calculated by using the formulae given by.[14]

Scavenging effect (%) = [1-(A sample -A sample blank)/A control] × 100

2.5.2 Superoxide Dismutase radical scavenging assay

The Superoxide Dismutase Radical Scavenging Activity was measured by,[15] Superoxide anions were generated using PMS / NADH system. The superoxide anions are subsequently made to reduce nitro blue tetrazolium (NBT) which yields a chromogenic product, which is measured at 560 nm. Test solution (20-100 mg/ml) in 0.1M phosphate buffer pH 7.4, 625 µl of 468 µM NADH solution, 625 µl of 150 µM NBT solution and 625µl of 60 µM PMS solution were added to a test tube and incubated at room temperature for 5 min. The absorbance was read at 560 nm. Linear graph of concentration vs percentage inhibition was prepared and % inhibition was calculated.

% Superoxide dismutase radical scavenging activity = control- test/ control X 100

3. RESULTS

The study is a unique one as there is no previous investigations were available for this plant for its Pharmacognostic evaluation. This study is considered to be vital as it gives a clear and concise idea about the basic parameters required for the formulation of the drug.

3.1 Organoleptic characteristics

The different organoleptic characteristics viz, Colour, odour, taste, texture, appearance and size of the raw as well as the extract (EtOH: H_2O) (3:1) is illustrated in the Table: 1.

3.2 Physical parameters

The physical parameters included, bulk density, tapped density, compressibility index, Hausner's ratio and pH range, for the bark and the extract EtOH: water (3:1) of *R.mucronata* were illustrated in the Table: 2.

3.3 Fluorescent analysis

The raw bark and Ethanol: water (3:1) extract were screened for Fluorescent analysis in visible light, UV short wave (254 nm) and UV long wave (366 nm) respectively, with chemical treatment and the results are illustrated in Table: 3

3.4 Antioxidant assay results

DPPH assay and SOD scavenging assay

BERM showed % inhibition of 86.17%± 0.01 and for SOD assay: 67.64± 2.43 respectively, which clearly indicated the presence of high antioxidant capacity in the plant.

4. DISCUSSION

The pharmacognostic evaluation is considered to be the most reliable and basis of the formulation of the drug. Literature revealed that the selected plant part was not standardized for Pharmacognostic and physicochemical parameter, thus our study is a unique and the first

attempt on this regard. According to World Health Organization (WHO) the macroscopic and microscopic evaluation of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.[16]

Organoleptic characteristics clearly indicated the extract was dark brown in colour, with a resinous smell, appeared to be solid, with uneven fracture, inner side smooth and outer bark, rough and bitter in taste. Results of the present study are in accordance with the previous results published by various investigators.[17 18]

The physical parameters included: Bulk density for raw was found to be: 0.25 g/ml and for the extract was 0.33 g/ml respectively. Similarly for the tapped density: raw bark: 0.333 g/ml and that for the extract was 0.526 g/ml respectively. The pH value was ranged between 4.5 to 5.04 for raw bark and extract. And for Hausner's ratio it was found to be 1.33% and 1.58% for raw as well as extract respectively. Our study co-related with the earlier studies.[18]

In terms of fluorescent analysis of the raw bark as well as the extract with or without treatment indicated in the table: 4, from the table it is clear that with different reagents at a fixed concentration yielded different coloured solutions. This was observed both in visible as well as UV range (short wave, 254 nm and UV long wave 366 nm) respectively. This technique of observing plant material under fluorescence light has been used as a Pharmacognostic tool to distinguish between plants and their species. Similar studies have been performed by[19] which co-related our study.

DPPH is a purple colour dye having absorption maxima of 517 nm and upon reaction with a hydrogen donor the purple colour fades or disappears due to conversion of it to 2, 2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance and increase in the % inhibition. From the study DPPH activity was found to be 86.17%± 0.01, similar results were obtained in the previous study.[20] In a study, by[21] the different extracts of *Avicennia marina* showed concentration dependent percentage inhibition on tested free radicals (Superoxide, Hydroxyl and DPPH) which co-related our study. The free radicals are produced in different metabolic process of the body and they can damage a wide range of bio molecules such as proteins, DNA and amino acids in the body.[22]

5. CONCLUSION

The present study revealed quite interesting and promising results, and findings of the current study used to investigate and identify the plant and its parts viz bark and its extract of *Rhizophora mucronata* for manufacturers and researchers, who further interested to study this plant as a drug for different activity with potent bioactives. The anti oxidant assay results revealed the BERM to be the potent extract to scavenge wide range of ROS and prevent the oxidative stress. The present pharmacognostic evaluation of the raw bark and the hydro-ethanolic extract of *Rhizophora mucronata* and radical scavenging assay had undertaken to laid down the standards which could be helpful for the drug standardization and formulation of the plant and its parts.

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FIGURES

Figure: 1 Petri plate showing Ethanol: water (3:1) extract obtained after drying

TABLES

Table: 1 Organoleptic characters of both raw bark and the extract of *Rhizophora mucronata*

S.No	Properties	Raw bark	(EtOH: H_2O) (3:1) Extract
1.	Colour	Outer: Dark Brown Inner: Reddish Brown	Dark brown
2.	Odour	Resinous	Resinous
3.	Taste	Bitter	Bitter
4.	Texture	Inner: Smooth Outer: Rough	Smooth
5.	Appearance	Solid	Semi- Solid
6.	Size	5-7 mm thickness	-----
7.	Fracture	Uneven	-----

Table: 2 Physical parameters for both powdered bark as well as the Ethanol: H_2O (3:1) extract respectively

S.No	Physical Parameters	Powdered bark of *R. mucronata* g/ml/ (%)	(EtOH: H_2O) (3:1) Extract of *R.mucronata* g/ml/ (%)
1.	Bulk Density	0.25 g/ml	0.333 g/ml
2.	Tapped Density	0.333 g/ml	0.526 g/ml
3.	Compressibility Index	24.92%	36.7%
4.	Hausner's Ratio	1.332%	1.58%
5.	pH Value	5.04	4.50

Table- 3 Fluorescent analysis of raw bark and Ethanol: H_2O (3:1) with chemical treatment and as such

S.No	Different reagents	Powdered Bark			EtOH: H_2O (3:1)		
		Visible light	Short UV 254 nm	Long UV 366 nm	Visible light	Short UV 254 nm	Long UV 366 nm
1.	As such	Honey colour	Light orange	Dark grey	Dark brown	Light brown	Wine
2.	Powder+ 1M NaOH	Wine colour	Dark brown	Dark grey	Reddish brown	Brown	Black
3.	Powder + 1M NaOH + water	Wine colour	Dark brown	Dark grey	Reddish brown	Brown	Black
4.	Powder + 1M HCl	Lemon Yellow	Light Brick Red	Transparent	Light orange	Lemon yellow	Greyish black
5.	Powder + Dil HNO_3	Mustard yellow	Light brick red	Transparent	Dark orange	Lemon yellow	Greyish black
6.	Powder + 5 % Iodine	Ink Blue	Purple	Light Blue	Dark blue	Purple	Wine coloured
7.	Powder + 5% $FeCl_3$	Olive green	Mustard yellow	Grape colour	Black	Dark brown	Black
8.	Powder+ Dil Ammonia	Cranberry coloured	Dark brown	Dark grey	Brick red	Brown	Wine coloured
9.	Powder + 1M H_2SO_4	Light mustard	Light yellow	Transparent	Pale orange	Lemon yellow	Greyish black
10.	Powder + conc. HNO_3	Mustard yellow coloured	Lemon yellow	Dark grey	Yellow	Transparent yellow	Greyish Black
11.	Powder + $K_2Cr_2O_7$	Mustard yellow coloured	Lemon green	Grape coloured	Dark Brown	Chocolate Brown	Black
12.	Powder + EtOH	Light orange	Yellow coloured	Transparent	Honey coloured	Lemon yellow	Black
13.	Powder + toluene	Light brown	Light brick red	Transparent	Transparent	Orange with green spots	Wine coloured
14.	Powder + dil HCl	Mustard yellow	Light brick red	Transparent	Pale orange	Lemon yellow	Greyish black