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Full Length Research Paper

Scientific evaluation of aqueous extracts of fresh and dried leaves from *Rhizophora mucronata* lamk (Rhizophoracea) in Rats

M. Babuselvam[1]*, K. Kathiresan[2], S. Ravikumar[3], M. Uthiraselvam[1] and E. Rajabudeen[4]

[1]Department of Microbiology, Dr.Zakir Husain College, Ilayangudi 630702, Sivagangai Dist, Tamil Nadu, India.
[2]Centre for Advanced Study in Marine Biology, Annamalai University, Portnova, Tamil Nadu, India.
[3]Department of Marine Pharmacology, School of Marine Sciences, Alagappa University, Thondi campus, Thondi, Ramanathapuram District, Tamil Nadu, India.
[4]Department of Botany, Dr. Zakir Husain College, Ilayangudi 630702, Sivagangai Dist, Tamil Nadu, India.

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The present study investigated the efficacy of fresh and dried leaves extracts of *Rhizophora mucronata* in experimental animal models and reveals that the fresh leaf extract showed remarkable improvement in the level of counts of red blood cells (RBC), white blood cells (WBC), platelet count, differential count and the level of hemoglobin and packed cell volume (PCV) and also the level of total protein, albumin, globulin, cholesterol and urea with the exception of a transient rise in total bilurubin, serum glutamic oxaloacetic transaminase (SGOT) and urea, but serum glutamic pyruvic transaminase (SGPT) rise only in dried leaves extract of *R. mucronata*. It is also confirmed by the present study that some of the important phytochemical constituents, such as carboxylic acids, flavonoids, proteins and amino acids, steroids and tannin are predominant in fresh leaves than in dried leaf extract. Hence, the extract from fresh leaves of *R. mucronata* extract dose of 500 mg kg^{-1} by oral is safe and non-toxic.

Key words: *Rhizophora mucronata*, toxicity, hematological parameters, biochemical parameters, mangroves.

INTRODUCTION

Medicinal plants are potential source of drugs, playing an important role in the world's economy. Over 60% of world human population, 80% of in developing countries depends directly on plants for their medicinal purposes. Herbal medicine provides rational means for the treatment of diseases that are obstinate and incurable in other system of medicine. They are gaining popularity because of several advantages such as often fewer side effects, better patient tolerance, relatively less expensive and acceptance. Mangroves are widespread in tropical and subtropical regions, growing in the saline intertidal zones of sheltered coastlines. The wide variety of traditional products from mangroves produced and utilized by coastal communities are well documented (Rollet, 1981; Tomlinson, 1986; Chan and Salleh, 1987; Vannucci, 1989; Field, 1995). It is also reported that, mangrove is a folk remedy for angina, diabetes, diarrhea, dysentery, hematuria and haemorrhage (Duke and Wain, 1981). However, these remedial medicines have not been presently used by the humankind due to lack of scientific proof. Hence, reverse pharmacological studies is much more necessary to evaluate the traditional medicines for scientific validation. Generally, traditional practitioners used fresh mangrove plant parts for the preparation of medicines but currently dried plant samples have been extensively used for the extraction of bioactive compounds. Hence, the present study has been undertaken to find out the efficacy of the samples to be used for the treatment of human illness. Among the mangrove plant species, the barks of *Rhizophora mucronata* have long been used for tanning and dyeing and the leaves are the source of a black or chestnut dye (Burkill, 1966). It is also have long been traditionally used

*Corresponding author. E-mail: babu31993@gmail.com. Tel: +91 04564265124. Fax: +91 04564265124.

Table 1. Ash content in fresh and dried leaves extract of *R. mucronata.*

Samples	Ash content (% w/w)			
	Total ash	Water soluble ash	Acid soluble ash	Sulphated ash
Fresh leaves extract	1.17	3.67	0.70	0.50
Dry leaves extract	3.98	0.64	0.78	0.27

for the treatment of elephantiasis, haematoma, hepatitis, ulcers and febrifuge (Bandaranayake, 2002; Ravikumar et al., 2005) and it is also been scientifically proved to have antiviral activities (Padmakumar and Ayyakkannu, 1997), but the nature of the plant parts (fresh or dried) efficient for the treatment has not been studied. Experimental screening method is important in order to ascertain the safety and efficacy of traditional and herbal products and also to establish the active component of the herbal products. Hence, the present study has undertaken to evaluate the efficacy of fresh and dried leaves of *R. mucronata* plant by using rat animal model.

MATERIALS AND METHODS

Preparation of plant extract

Mangrove leaves of *R. mucronata* Lamk., were collected from Pichavaram mangrove forest (Southeast coast of India, Lat. 11°27' N; Long. 79°47' E). The collected fresh and shade dried leaf samples were finely ground with sterilized distilled water. After grinding, the filtrate was collected after filtration with Whatman No.1 filter paper and evaporated at 40°C for 2 days. The residue was maintained for experimental analysis.

Preliminary phytochemical screening

The extract from fresh and dried leaves of *R. mucronata* extracts were screened for the phytochemical constituents following by the method of Trease and Evans (1972).

Treatments

Adult Wistar strain rats of either sex (250 to 300 g) were used in all experiments. Animals were maintained on 12 h light and 12 h dark cycle at approximately 22 ± 2°C, relative humidity 60 to 70% and allowed food and water *ad-libitum*. All treatments were conducted between 8:00 and 9:00 a.m. to minimize variations in animal response due to circadian rhythm. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water throughout. Plant extracts were administered for 1 week at doses of 500 mg/kg^{-1} by oral treatment. The control animal received 0.5 ml of the vehicle alone. Toxic manifestations and mortality were monitored daily. Experiments were performed according to the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India.

Determination of ash

About 2 to 3 g accurately weighed powdered drug was incinerated in a silica dish at a temperature not exceeding 450°C until free from carbon. It was then cooled and weighed. The percentage (w/w) of total ash, sulphated ash, alcohol soluble extractive, water soluble extractive were calculated followed by the method of Trease and Evans (1972).

Haematological analysis

Rats were anesthetized with chloroform and blood was collected from the neck using ethylenediaminetetraacetic acid as anticoagulant. Counts of red blood cells (RBC), white blood cells (WBC), level of hemoglobin (Hb), packed cell volume, platelet count and differential counts were measured by an automatic analyzer (COBAS OT, Korea).

Serum biochemical analysis

To find out the changes in the biochemical parameters, blood was collected from the neck without any anticoagulant. After 1 h, serum was separated by centrifugation and maintained at −4°C until further use. Determination of total bilirubin, urea, cholesterol, SGOT, SGPT, total protein, albumin and globulin in serum were performed by a COBAST OT automatic analyzer. Biochemical parameters were determined for three different pooled sera from at least two animals for each group.

RESULTS

The present study was carried out with the extracts from fresh and air-dried leaves of *R. mucronata* for the evaluation of phytochemical analysis and to find out the safety and efficacy of the plant extract. The present study observed that, the content of ash was within the level in the fresh leaf extract when compared with the control (Table 1). It is also interesting to notice that, most of the blood and serum parameters were found normal with the administration of fresh leaf extract than the dried leaf extract. Moreover, the dried leaf extract showed some negative sign of improvement when compared with the fresh leaf extract and control (Table 2). The positive sign of improvement in the fresh leaf extract administered rats of many of the parameters are due to the good source of some of the phytochemical parameters viz., carboxylic acids, flavanoids, protein and amino acids, steroids and tannin present in the fresh leaf extract than the dried leaf extract (Table 3).

DISCUSSION

Herbal medicines have received greater attention as an

Table 2. Effect of *R. mucronata* extract on the blood and serum parameters in rats.

Parameter		Control	Fresh leaf extract	Dried leaf extract
Glucose ($mgdl^{-1}$)		149 ± 1.63	196 ± 1.63	404 ± 2.49
Cholesterol ($mgdl^{-1}$)		33 ± 2.05	40 ± 1.63	53 ± 1.63
SGOT (IUL^{-1})		434 ± 1.63	233 ± 2.05	435 ± 2.05
SGPT (IUL^{-1})		140 ± 1.24	131 ± 1.24	215 ± 1.24
Alkaline Phosphate (IUL^{-1})		114 ± 1.63	212 ± 1.69	363 ± 1.63
Total protein (gdl^{-1})		7.89 ± 0.02	6.36 ± 0.02	7.31 ± 0.02
Albumin (gdl^{-1})		4.33 ± 0.02	3.39 ± 0.02	3.77 ± 0.02
Total bilirubin ($mgdl^{-1}$)		0.22 ± 0.02	0.25 ± 0.02	0.31 ± 0.02
Direct ($mgdl^{-1}$)		0.20 ± 0.02	0.21 ± 0.02	0.29 ± 0.02
Total Leucocytes count ($cellsm^{-1}$ mm^{-1})		2500 ± 5.2	3100 ± 5.2	2100 ± 5.2
Hemoglobin		12.7 ± 0.16	12.0 ± 1.24	10.3 ± 0.24
Red blood cells ($cellsm^{-1}mm^{-1}$)		4.01 ± 00.2	3.96 ± 0.02	3.42 ± 0.02
Platelet count (Lakhs.m.mm)		2.80 ± 0.01	0.90 ± 0.01	0.85 ± 0.01
P.C.V (%)		36 ± 1.63	35 ± 1.6	30 ± 1.63
E.S.R	30 mt (mm)	2±0.02	4 ± 0.02	2 ± 0.02
	60 mt (mm)	4 ± 0.02	4 ± 0.02	4 ± 0.02
Neutrophil (%)		22 ± 1.63	45 ± 1.24	35 ± 1.63
Lymphocyte		77 ± 1.63	55 ± 1.63	65 ± 1.63
Eosinophil		1 ± 0.09	0.5 ± 0.09	1.3 ± 0.09

Table 3. Phytochemical evaluation of the fresh and dried leaves extracts of *R. mucronata.*

Phytochemical constituents	Observations	
	Fresh leaves extract	Dried leaves extract
Alkaloids	+	++
Carboxylic acids	++++	+
Coumarins	+	+
Flavonoids	++++	+
Phenols	+	++
Proteins and amino acids	++++	+
Quinones	+	+
Resins	+	++
Saponins	+	+
Steroids/phytosterols	++++	+
Tannins	++++	+
Xanthoprotins	+	+
Sugars	+	+

alternative to clinical therapy and the demand for these remedies has currently increased. Numerous mangrove plants are been used in folklore medicine and recently extracts from mangroves and mangrove dependent species have proven activity against human, animal and plant pathogens but only limited investigations have been carried out to identify the nature of the plant parts for efficient application and the metabolites responsible for their bioactivities. Previous studies revealed that, saponins, tannins, flavonoids are referenced as anti-ulcer compounds (Lewis and Hanson, 1991). The present findings suggest that, the fresh leaf extract of *R. mucronata* are non toxic since no marked changes in haematological and biochemical were observed. Thus, at normal therapeutic doses, *R. mucronata* fresh leaves extract is considered to be safe for long term treatment in

human ailments.

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