INVITRO ANTI-INFLAMMATORY AND ANTI-ARTHRITIC PROPERTY OF *RHIZOPORA MUCRONATA* LEAVES

C. Sree Kumari[1*], N. Yasmin[1], M. Raffiq Hussain[2] and M. Babuselvam[2]

[1] P.G. & Research Department of Zoology, Muslim Arts College,
Thiruvidhancode – 629174, Tamil Nadu, India

[2]P.G. and Research Department of Zoology, Dr. Zakir Husain College,
Ilayangudi- 630702, Tamil Nadu, India.

ABSTRACT

The present investigation was carried out the evaluation of the anti-arthritic property of *Rhizophora mucronata* by three different methods *viz.*, membrane stabilization assay, protein denaturation assay and albumin denaturation assay. Four different concentration of test (100, 200, 300 and 400 mg) were using in this study. Action was observed in dose dependent manner. In protein denaturation method at 400 mg of chosen extract showed maximum protection (97.56%) and standard drug provided 99.2% protection. Similarly, in membrane stabilization test, the selected extract at concentration of 400 mg showed maximum protection (95%) and standard drug provided 97% protection. Moreover, albumin denaturation test showed maximum protection (90.12%) at concentration of 400 mg. We concluded that, methanolic extract of *Rhizophora mucronata* shows strong anti-arthritic activity at different concentration when compared to standard drug of Diclofenac sodium. In addition, phytochemical analysis of *Rhizophora mucronata* showed the presence of saponins, flavanoids, tannins, anthracene, phenols, aminoacids and sugars. It reveals that these phytochemical constituents are responsible to maximum protection of protein denaturation, albumin denaturation and membrane stabilization assay. The future work will be determination of anti-inflammatory and anti-arthritic activities by *in vivo* models.

Keywords: Human red blood cells; inflammation; *in vitro* anti-inflammatory; *in vitro* antiarthritic property; membrane stabilization assay

***Corresponding author**

INTRODUCTION

Inflammation is the reaction of living tissues to injury, infection or irritation. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are assumed to be responsible for certain pathological conditions as heart attacks, septic shocks, and rheumatoid arthritis etc. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane [1]. HRBC or erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of human red blood cell membrane (HRBC) by hypotonicity induced membrane lysis can be taken as an *in-vitro* measure of anti-inflammatory activity of the drugs or plant extracts.

Rheumatoid arthritis is a systemic autoimmune disease that causes chronic inflammation of connective tissue primarily in the joints that involves synovial proliferation and cartilage destruction. The first joint tissue to be affected is synovial membrane which lays the joint cavity [2]. Researchers have found many new mediators *viz*, TNF- á, IL-2 and enzymes which take part directly or indirectly in perpetuation of RA[3,4,5]. Mangrove plants are specialised plants that grow in the tidal coasts of tropic and subtropic regions of the world. Their unique ecology and traditional medicinal uses of mangrove plants have attracted the attention of researchers over the years, and as a result, reports on biological activity of mangrove plants have increased significantly in recent years. Inflammation is the reaction of previous reports indicated that ethanolic extract of *Excoecaria agallocha* mangrove plant showed anti-inflammatory and anti-arthritic property by *in-vivo* model [6]. The analgesic activity of *Excoecaria agalloca* mangrove plant also studied. It reveals that the *Excoecaria agallocha* mangrove plant showed significantly decreased the number of writhes in 20 minutes and also increased the percentage of inhibition in acetic acid writhing test in test animals. *Rhizopora mucronata* commonly known as Asiatic mangroves, widely distributed along the coastal tropical and sub-tropical region has been reported to possess several medicinal properties. In countries like Burma, India, and China bark of *Rhizopora mucronata* has been used as traditional medicine in the treatment of diarrheoa, dysentery, blood in urine, and fever. The

present investigation is to scientifically evaluate for *in vitro* anti-inflammatory on human red blood cell membrane and anti-arthritic activity on bovine serum albumin and egg albumin assay suggest about their mechanism for therapeutic activity.

MATERIALS AND METHODS

Plant material

The plant material (leaves) was collected from south East coast of India (Lat. of $79^0$44'10''N and Long. of 79^0 10' 45'' E) and was authenticated at official agencies. The fresh leaves were washed under running tap water to remove adhere dirt, followed by rinsing with distilled water, shade dried and pulverised in a mechanical grinder to obtain coarse powder.

Extraction of bioactive compounds

The coarse powder of 500gm of *Rhizopora mucronata* was subjected to maceration for 6 days by using methanol. The solvents were recovered by distillation of the extracts at 75^0c to 80^0c. The extracts were dried under desicator and the percentage yield was calculated. The methanolic extract was used for the preliminary phytochemical investigation, anti-inflammatory, anti-arthritic activity.

In vitro anti-inflammatory activity

Membrane Stabilization Property

Preparation of Red Blood Cells (RBCs) Suspension

Fresh whole human blood (10 ml) was collected and transferred to the heparin zed centrifuged tubes. The tubes were centrifuged at 3000rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

Heat Induced Hemolysis

The 2ml reaction mixture is consisted of 1ml of test extract at various concentration and 1ml of 10% RBCs suspension, instead of drug only saline was added to the control test tube. Diclofenac sodium was taken as a standard drug. All the centrifuged tubes containing reaction mixture were incubated in a water bath at 56^0C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500rpm for 5 min and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicate. % of membrane stabilization activity was calculated by the formula mentioned below:

$$\text{Percent inhibition} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{treated}}}{\text{Abs}_{\text{Control}}} \times 100$$

In vitro anti-arthritic activity:

Inhibition of protein denaturation method

The reaction mixture (0.5ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *Rhizopora mucronata* extract at different concentration. The samples were incubated at 37^0C for 30 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660nm for control test 0.05 ml distilled water was used instead of extracts while product control test lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.

$$\text{Percent inhibition} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{treated}}}{\text{Abs}_{\text{Control}}} \times 100$$

The control represents 100% protein denaturation. The results were compared with acetyl salicylic acid (250mcg/ml) treated samples.

Inhibition of Albumin Denaturation

The 5ml of reaction mixture was comprised of 0.2ml of eggs albumin (from hens egg), 2.8ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentration of extracts. Similar volume of double distilled water served a control. Then the mixture was incubated at 37^0 C in BOD incubator for about 15 mins and then heated at 70^0 C for 5 mins. After cooling, their absorbance was measured at 660nm by using pure blank. Diclofenac sodium (standard drug) was used as reference drug and treated as such for determination of absorbance [7]. The percentage inhibition of protein denaturation was calculated as mentioned in membrane stabilization assay.

Phytochemical analysis

The dried powdered samples were subjected to qualitative tests for the identification of phytochemical constituents according to standard procedures [8, 9].

RESULTS AND DISCUSSION

Literatures are now full of scientific documentation today regarding medicinal plants and they have potential to cure various human diseases [10]. Thus, this suberb future further encourage to manufacture a pharmaceutical products procured from medicinal plants as they are safe and dependable as compare to synthetic drugs, that are not only costly but also have adverse effects [11]. The naturally isolated anti-arthritic agents function by suppressing the different types of inflammatory mediators involved in inflammation process [12]. So in this study three different methods were adapted to evaluate the anti-arthritic property of *Rhizophora mucronata* (mangrove) extract with this belief that the treatment of extracts releases various bioactive substances that could play a role in generating a particular pharmacological activity. Similarly protein denaturation method, membrane stabilization and albumin denaturation test was done for this purpose. Protein denaturation is well documented method for this analysis and membrane stabilization also reflects the effect of extracts on cellular membrane like red blood cells. Since HRBC membrane are similar to lysomal membrane components. The prevention of hypotoxicity induce HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drugs. The methanolic extract of *Rhizophora mucronata* source significant anti-inflammatory activity at the concentration of 500 mg which is comparable to the standard drug diclofenac sodium. The anti-inflammatory activity of the extracts were concentration the activity is also increased.

The production of auto antigens in certain arthritic disease may be due to denaturation of protein and membrane lysis action. The maximum % inhibition of protein denaturation, albumin denaturation and membrane stabilization action were observed as 97.56%, 90.12% and 95% at 500 mg respectively, as shown in Table 1-3.

Table 1. Effect of methanolic extract of Rhizophora mucronata on heat induced hemolysis for anti-inflammatory activity

Extract	Concentration (mg)	Absorbance at 560 nm	% of inhibition
Control	-	0.9	-
Rhizophora mucronata	100	0.6	33
	200	0.2	77
	300	0.1	88
	400	0.04	95
Diclofenac sodium	400	0.03	96

Table 2. Effect of methanolic extract of Rhizophora mucronata on Protein denaturation assay for anti-arthritc activity

Extract	Concentration (mg)	Absorbance at 660 nm	% of inhibition
Control	-	8.2	-
Rhizophora mucronata	100	0.6	92.68
	200	0.4	95.12
	300	0.2	97.56
	400	0.2	97.56
Diclofenac sodium	400	0.1	98.76

Table 3. Effect of methanolic extract of Rhizophora mucronata on egg albumin denaturation assay for anti-arthritc activity

Extract	Concentration (mg)	Absorbance at 660 nm	% of inhibition
Control	-	8.1	-
Rhizophora mucronata	100	2.6	67.90
	200	1.5	81.48
	300	1.0	87.65
	400	0.8	90.12
Diclofenac sodium	400	0.2	97.53

In addition that, dried powdered samples were subjected to qualitative tests for the identification of phytochemicals constituents according to standard procedures. The preliminary phytochemical investigation showed the presence of phytoconstituents such as carbohydrates, amino acids, phenols, saponins, alkaloids, flavanoids, tannin and anthracene were observed in *Rhizophora mucronata*. The leaves of *Rhizophora mucronata* was subjected to qualitative analytical tests for the various plant constituents (Table4).

Table 4. Preliminary phytochemical analysis of Rhizophora mucronata

S.No.	Tests	Observation
1.	Test for glycosides	-
2.	Test for saponins	+
3.	Test for flavanoids	+
4.	Test for tannins	+
5.	Test for anthracene	+
6.	Test for anthraquinones	-
7.	Detection of carboxylic acids	-
8.	Detection of coumarins	-
9.	Detection of phenols	+
10.	Detection of proteins and free aminioacids	+
11.	Detection of quinones	-
12.	Detection of steroids	-
13.	Detection of xanthaproteins	-
14.	Detection of sugars	+

(+); Presence – (-); Absence

So from the results of our study reveals that extract of *Rhizophora mucronata* are capable of controlling the production of auto antigens and inhibit denaturation of protein denaturation of albumin and membrane lysis in rheumatic disease. Our present studies indicate that extract of *Rhizophora mucronata* exhibits strong anti arthritic property could be potential source of anti- arthritic property.

The inhibition of protein denaturation, albumin denaturation and membrane stabilization was studied to establish the mechanism of anti-arthritic activity of *Rhizophora mucronata*. Therefore, our *in vitro* studies on extract of *Rhizophora mucronata* demonstrate the significant anti-arthritic activity. Hence, this mangrove plant can be used as a potent natural anti arthritic agents. The results show that the extracts of *Rhizophora mucronata* exhibited anti-arthritic activities might be due to the presence of active principles such as polyphenolic content, triterpenoids, alkaloids and flavanoids.

From the results of the study, it can be concluded extract of *Rhizophora mucronata* possessed anti-arthritic property. However, one should try to further figure out extract more as having much better activity in quest of active candidate or chemical molecule that is mainly responsible for this activity via detailed experimentation.

REFERENCES

[1] Rajendran V, Lakshmi KS, Bangladesh J Pharmacol **2008**; 3: 121- 4.

[2] Smolen J., van der Heijde D., St.Clair E., Emery P., Bathon J., Keystone E., et al. for the Active-Controlled Study of Patients Receiving Infliximab for the Treatment of Rheumatoid Arthritis of Early Onset (ASPIRE) Study Group Predictors of joint damage in patients with early rheumatoid arthritis treated with highdose methotrexate with or without concomitant infliximab: results from the ASPIRE trial. Arthritis Rheum **2006**; 54: 702–10.

[3] Mythilypriya R, Shanthi P, Sachdanandam P. Efficacy of siddha formulation Kalpaamruthaa in ameliorating joint destruction in rheumatoid arthritis in rats. Chem biol interact **2008**; 176: 243-251.

[4] Berbert AA, Kondo CRM, Almendra CL, Matsuo T, Dichi I. Supplementation of fish oil and olive oil in patients with rheumatoid arthritis. Nutrition **2005**; 21: 131-6.

[5] Nair V, Singh S, Gupta YK. Evaluation of the disease modifying activity of Colchicum luteum Baker in experimental arthritis. J Ethnopharmacol **2011** ; 133: 303-7.

[6] Babuselvam M, Ravikumar S, Mohamed Farook KA, Abideen S, Peer Mohamed M and Uthiraselvam M. Evaluation of anti-inflammatory and analgesic effects on the extracts of different parts of Excoecaria agallocha L. J. Appl Pharma Sci **2012**; 2 **(9):** 108-12.

[7] Mizushima Y., and Kobayashi, M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins, Journal of Pharmacy and Pharmacology, **1968,** 20(3), 169-173.

[8] Harborne JB. Phytochemical methods, London. Chapman and Hall, Ltd. 1973 p. 49-188.

[9] Sofowora, A.,. Medicinal plants and traditional medicinal in Africa. John wiley and Sons Ltd. **1982,** 8, pp 256.

[10] Lai, PK. and J. Rov. Antimicrobial and chemopreventive properties of herbs and spices. Curr. Med. Chem., 200; **47(2):** 234-8.

[11] Gordon MC, David JN. Natural product drug discovery in the next millennium. Pharm. Biol. 2001; **39:** 8-17.

[12] Ekambaram S, Perumal SS, Subramanian V. Evaluation of antiarthritic activity of Strychno spotatorum Linn seeds in Freund's adjuvant induced arthritic rat model. BMC Comp. Alt. Med. 2010; **10:**56.