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In vitro anti-inflammatory activity of mangrove plant *Rhizophora mucronata* Lam. (Malpighiales: Rhizophoraceae)

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Abstract. In the present study, analysis of *in vitro* inflammatory showed whole plant of *Rhizophora mucronata* Lam. (Malpighiales: Rhizophoraceae) can be the potent source. The data from this study showed that the *R. mucronata* leaf, bark and root extract could serve as an important anti-inflammatory agent. Moreover, among the three extracts, the stilt root and leaves extract showed highest anti inflammatory. In vitro anti-inflammatory activity of the selected plant extracts was evaluated using albumin denaturation, membrane stabilization and proteinase inhibitory assays. As part of the investigation on the mechanism of the anti-inflammation activity, ability of extract protein denaturation was studied. Maximum inhibition (296.26%) was observed from root extract followed by bark (259.48%) and leaf (237.62%). The extracts inhibited the heat induced hemolysis of RBCs to varying degree as show in table below. The maximum inhibition 284.17% was observed from bark extract followed by root (265.05%) and leaf (232.61%). 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: Bioactivity; Marine halophytes; Anti-inflammation activity; Lysosomal enzymes.

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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 (Vadivu and Lakshmi, 2008). 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. (Sree Kumara et al., 2015).

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 (Syed Ali et al., 2013). Marine halophytes, such as mangroves and related species, are known to have many and various metabolites possessing antibacterial and antifungal, antiviral, antidiarrhoeal, hepatoprotective, antifeedant, insecticidal, cytotoxicity and antiplasmodial properties (Ravikumar et al., 2012).

The present study deals with invitro anti-inflammatory activities of methanolic extracts (Leaves, bark and stilt root) of true mangrove *Rhizophora mucronata* Lam. (Malpighiales: Rhizophoraceae).

Materials and methods

Collection and extraction of mangrove plants

The fresh leaves, bark and stilt root of *R. 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 (leaves, bark and stilt root were washed and spread out. They were shade dried, coarsely powdered and the extraction was done with methanol by Soxhlet apparatus. Crude was kept at 4 °C for further use.

In vitro anti-inflammatory activity

Inhibition of albumin denaturation. Method of was followed with minor modifications. The reaction mixture was consisting of test samples (100-500 µg/mL) and1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1 N HCl. The samples were incubated at 37 °C for 20 min and then heated at 51 °C for 20 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug of varied concentration 100-500 µg/mL. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

$$\% \boldsymbol{Inhibition} = \frac{\boldsymbol{Absorbance\ of\ control - Absorbance\ of\ sample}}{\boldsymbol{Absorbance\ of\ control}} \times \mathbf{100}$$

Where: Abs control is the absorbance of the DPPH radical + solvent,
Abs sample is the absorbance of DPPH radical + sample extract/standard.

Heat induced hemolysis. The reaction mixture (2 mL) consisted of 1 mL of test solution and 1 mL of10% RBCs suspension, instead of drug only saline was added to the control test tube. Aspirin was taken as a standard drug in the concentration ranges from 100-500 µg/mL. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56 °C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. There action mixture was centrifuged at 2,500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates. Percent of HRBC membrane stabilization or protection was calculated as follows:

$$\% \boldsymbol{of\ protection} = (\mathbf{100} - \boldsymbol{O.D\ of\ drug\ treated\ sample - O.D\ of\ control}) \times \mathbf{100}$$

Proteinase inhibitory action. The test was performed according to the modified method of 2 mL of reaction mixture containing 0.06 mg trypsin, 1 mL 20 mM Tris HCl buffer (pH 7.4) and 1 mL test sample of different concentrations (100-500 µg/mL) were mixed together. The mixture was incubated at 37 °C for 5 min and then 1 mL of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 mL of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. Aspirin was taken as standard drug (100-500 µg/mL). The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

$$\% \boldsymbol{of\ proteinase\ inhibitory} = \frac{\boldsymbol{Absorbance\ of\ control - Absorbance\ of\ sample}}{\boldsymbol{Absorbance\ of\ control}} \times \mathbf{100}$$

HRBC Membrane Stabilization Method. The human red blood cell (HRBC) membrane stabilization method was used for the study of *In vitro* anti-inflammatory activity. The blood was collected from healthy human volunteer under aseptic conditions who was not taken any Non-Steroidal Anti-inflammatory Drugs for 2 weeks prior to the experiment and mixed with equal volume of AL sever solution (2% dextrose, 0.8% sodium citrate,0.5% citric acid and 0.42% NaCl). It was centrifuged at 3,000 rpm and the packed cells were

washed with isosaline (NaCl, pH 7.2) and a 10% suspension was made. To 0.5 mL of test samples, 1 mL of phosphate buffer (0.15 M, pH 7.4), 2 mL hyposaline (0.36% NaCl) and 0.5 mL of HRBC suspension were added. The solution was incubated at 37 °C for 30 min and centrifuged at 3,000 rpm for 20 min. The content of the supernatant solution was absorbed spectrophotometrically at 560 nm. Control was taken without the test sample. Diclofenac (100-500 µg/mL) was used as reference standard.

$$\textbf{Percentage}\ (\%)\ \textbf{of protection} = \frac{\textbf{100} - \boldsymbol{O.D\ of\ the\ drug\ treated\ sample}}{\boldsymbol{O.D\ of\ the\ control}} \times \textbf{100}$$

Result

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. 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. Denaturation of proteins is well documented cause of inflammation.

As part of the investigation on the mechanism of the anti-inflammation activity, ability of extract protein denaturation was studied. Maximum inhibition (296.26%) was observed from root extract followed by bark (259.48%) and leaf (237.62%) (Table 1 and 2, Figure 1).

Table 1. Inhibition of albumin denaturation of *R. mucronata* plant parts.

Concentration µg/mL	Std OD	Leaf OD	Root OD	Bark OD	Control
100	0.812 ± 0.001	0.756 ± 0.014	0.838 ± 0.221	0.789 ± 0.164	1.017 ± 0.002
200	0.654 ± 0.005	0.592 ± 0.102	0.694 ± 0.132	0.634 ± 0.175	
300	0.448 ± 0.001	0.387 ± 0.135	0.489 ± 0.135	0.422 ± 0.287	
400	0.251 ± 0.001	0.199 ± 0.184	0.297 ± 0.187	0.239 ± 0.189	
500	0.083 ± 0.002	0.038 ± 0.138	0.193 ± 0.169	0.099 ± 0.109	

Table 2. IC_{50} value of Inhibition of albumin denaturation of *R. mucronata* plant parts.

Concentration µg/mL	Std %	Leaf %	Root %	Bark %
100	20.16 ± 0.058	25.66 ± 0.143	17.60 ± 0.521	22.42 ± 0.149
200	35.69 ± 0.100	41.79 ± 0.641	31.76 ± 0.165	37.66 ± 0.125
300	55.95 ± 0.866	61.95 ± 0.012	51.92 ± 0.264	58.51 ± 0.164
400	75.32 ± 0.100	80.43 ± 0.132	70.80 ± 0.168	76.50 ± 0.138
500	91.84 ± 0.115	96.26 ± 0.158	81.02 ± 0.154	90.27 ± 0.217
IC_{50} values	268.348	237.622	296.262	259.482

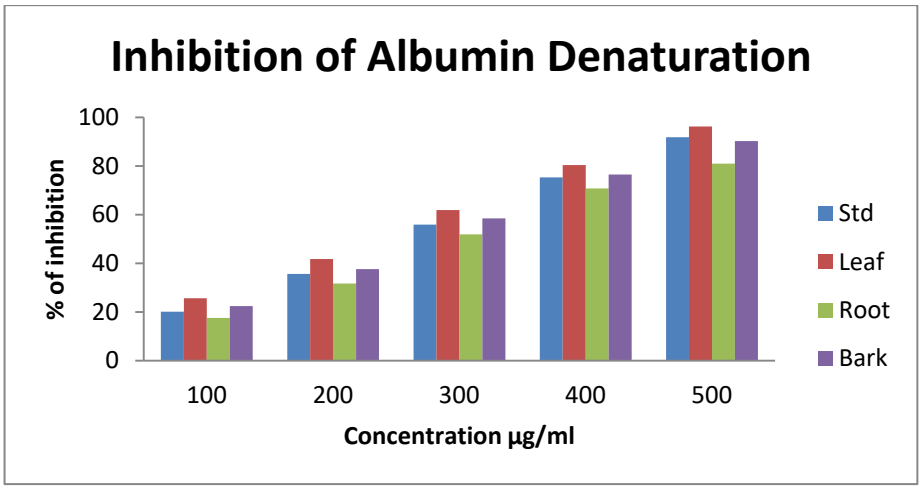

Figure 1. Inhibition of albumin denaturation assay of *R. mucronata* plant parts.

Stabilization of RBCs membrane was studied for further establishes the mechanism of anti-inflammatory action of different leaf, root and bark extract of *R. mucronata.* These results provide evidence form membrane stabilization as an additional mechanism of their anti-inflammatory effect. This effect may possibly inhibit the release of lysosonal content of neutrophils at the site of inflammation. The extracts inhibited the heat induced hemolysis of RBCs to varying degree as show in table below. The maximum inhibition 284.17% was observed from bark extract followed by root (265.05%) and leaf (232.61%) (Table 2 and 3, Figure 2).

Table 2. Membrane stabilization test: Heat induced hemolysis.

Concentration µg/mL	Std OD	Leaf OD	Root OD	Bark OD	Control
100	1.435 ± 0.002	1.373± 0.134	1.456± 0.346	1.511± 0.175	1.752 ± 0.002
200	0.915 ± 0.002	0.869± 0.132	0.938± 0.286	0.996± 0.186	
300	0.702 ± 0.003	0.611± 0.123	0.730± 0.137	0.786± 0.287	
400	0.495 ± 0.002	0.378± 0.154	0.519± 0.186	0.572± 0.153	
500	0.213 ± 0.002	0.179± 0.108	0.238± 0.061	0.288± 0.264	

Table 3. IC_{50} value of Membrane stabilization test: Heat induced hemolysis.

Concentration µg/mL	Std %	Leaf %	Root %	Bark %
100	18.09 ± 0.153	21.63 ± 0.186	16.89± 0.275	13.76± 0.151
200	47.77 ± 0.100	50.40± 0.271	46.46± 0.224	43.15± 0.715
300	59.93 ± 0.200	65.13± 0.122	58.33± 0.264	55.14± 0.162
400	71.75 ± 0.100	78.42± 0.153	70.38± 0.186	67.35± 0.175
500	87.84 ± 0.115	89.78± 0.264	86.42± 0.208	83.56± 0.124
IC_{50} Values	256.716	232.619	265.051	284.176

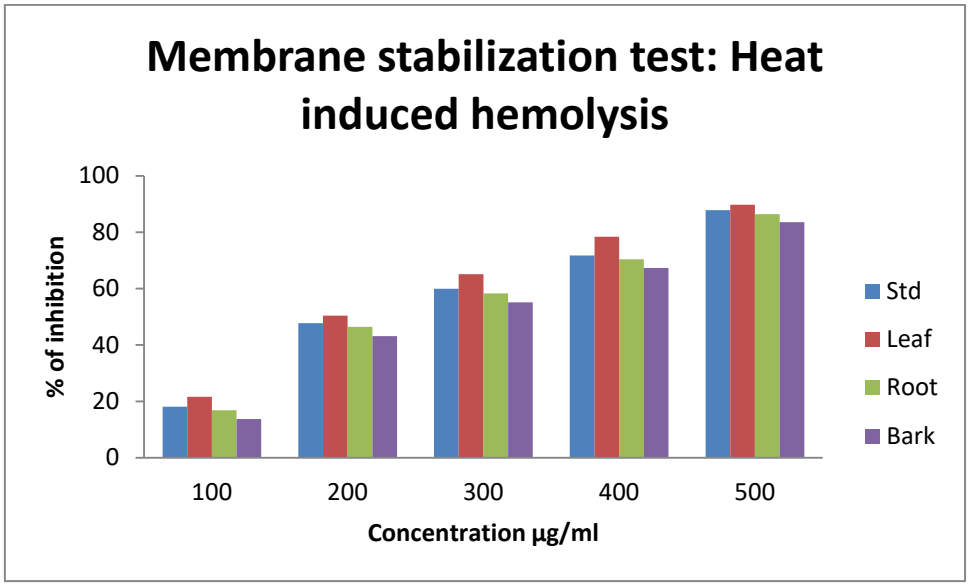

Figure 2. Membrane stabilization test: Heat induced hemolysis.

R. mucronata leaf, bark and root extracts at different concentrations (100, 200, 300, 400, 500 µg/mL) showed significant stabilization towards HRBC membranes. Among all extracts bark showed 312.48% protection of HRBC followed by root (295.07%) and leaf (284.85%) (Table 3 and 4, Figure 3).

Table 3. HRBC membrane stabilization test.

Concentration µL	Std OD	Leaf OD	Root OD	Bark OD	Control
100	1.765 ± 0.187	1.578 ± 0.186	1.812 ± 0.175	1.657 ± 0.261	2.108 ± 0.001
200	1.441 ± 0.173	1.286 ± 0.146	1.521 ± 0.297	1.386 ± 0.175	
300	0.987 ± 0.162	0.756 ± 0.162	1.006 ± 0.157	0.895 ± 0.142	
400	0.572 ± 0.134	0.399 ± 0.197	0.786 ± 0.173	0.653 ± 0.151	
500	0.312 ± 0.012	0.198 ± 0.201	0.589 ± 0.123	0.458 ± 0.117	

Table 4. IC_{50} value of HRBC membrane stabilization test.

Concentration µL	Std %	Leaf %	Root %	Bark%
100	16.27 ± 0.100	25.14 ± 0.122	14.04 ± 0.125	21.39 ± 0.174
200	36.39 ± 0.058	38.99 ± 0.186	27.85 ± 0.118	34.25 ± 0.192
300	53.18 ± 0.153	64.14 ± 0.127	52.28 ± 0.191	57.54 ± 0.101
400	72.87 ± 0.100	81.07 ± 0.118	62.71 ± 0.134	69.02 ± 0.102
500	84.20 ± 0.529	90.61 ± 0.101	72.06 ± 0.197	78.27 ± 0.120
IC_{50} values	285.018	242.261	327.913	285.902

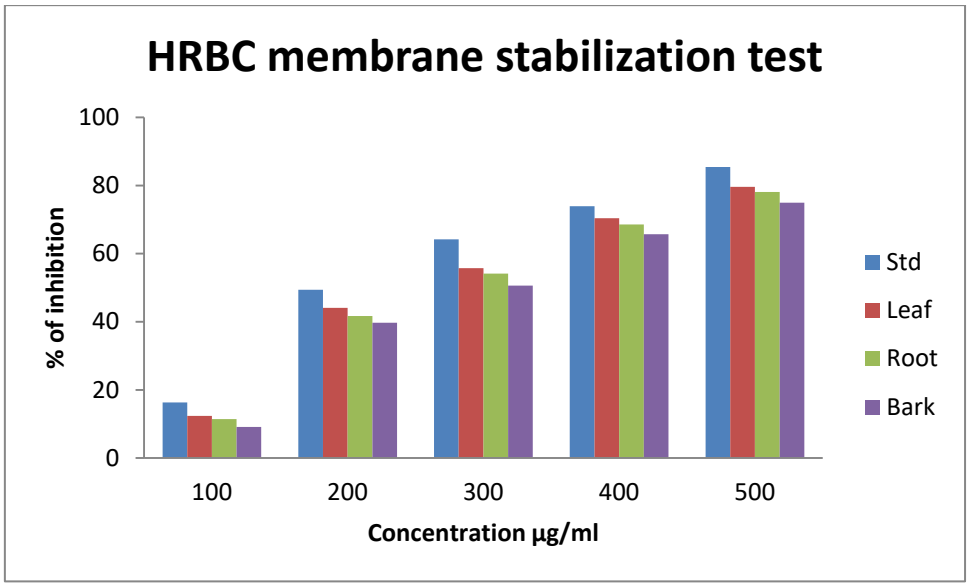

Figure 3. HRBC membrane stabilization test.

R. mucronata leaf, bark and root extracts at different concentrations (100, 200, 300, 400, 500 µg/mL) showed significant antiproteinase activity. The maximum inhibition was observed form root extract (327.91%) followed by bark (285.90%) and leaf (242.26%) (Table 4 and 5, Figure 3).

Table 4. Protein inhibition action.

Concentration µg/mL	Std OD	Leaf OD	Root OD	Bark OD	Control
100	1.281 ± 0.003	1.342 ± 0.187	1.356 ± 0.124	1.391 ± 0.186	1.531 ± 0.001
200	0.775 ± 0.002	0.856 ± 0.191	0.893 ± 0.154	0.923 ± 0.254	
300	0.548 ± 0.003	0.678 ± 0.120	0.702 ± 0132	0.756 ± 0.121	
400	0.339 ± 0.001	0.453 ± 0.192	0.481 ± 0122	0.525 ± 0.135	
500	0.223 ± 0.002	0.312 ± 0.128	0.335 ± 0.452	0.383 ± 0.521	

Table 5. IC_{50} value of Protein inhibition action.

Concentration µg/mL	Std %	Leaf %	Root %	Bark %
100	16.33 ± 0.200	12.34 ± 0.115	11.43 ± 0.482	09.14 ± 0.135
200	49.38 ± 0.110	44.09 ± 0.253	41.67 ± 0.143	39.71 ± 0.035
300	64.21 ± 0.153	55.72 ± 0.155	54.15 ± 0.256	50.62 ± 0.142
400	73.94 ± 0.058	70.41 ± 0.163	68.58 ± 0.164	65.71 ± 0.154
500	85.43 ± 0.058	79.62 ± 0.128	78.12 ± 0.298	74.98 ± 0.142
IC_{50} Values	251.720	284.858	295.071	312.481

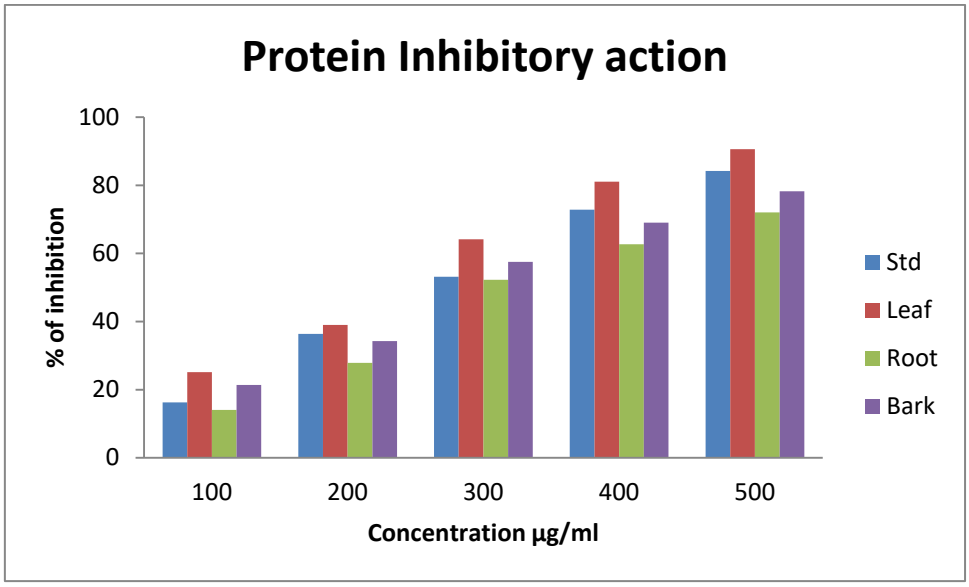

Figure 4. Protein inhibition action of *R. mucronata* plant parts.

Discussion

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 (Banerjee et al., 2008) Mangroves in Sundarbans, North India were found to contain high content of polyphenolics like tannins (Kathiresan and Bingham, 2001). Previous reports revealed that mangroves are rich in polyphenols and tannins (Ravikumar et al., 2010). Phenolics widely encountered in the plants tested as the most active radical scavengers. 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 (Anuradha et al., 2017). *R. mucronata* plant of mangrove enviroment, found on the coastal region of India and used in treatment of various diseases (Manilal, 2015). In traditional medicine its bark and leaf extracts has been used as astringent, anti-septic and hemostatic with antibacterial, anti-ulcerogenic and anti inflammatory activities.

In the present of *R. 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 *Invitro* anti inflammatory potentials of various plant parts of *R. mucronata.*

The anti-inflammatory activity, ability of extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation at different concentrations as shown in Table 1 and 2. Maximum inhibition, was observed and IC_{50} value was found to be 296.262 µg/mL at stilt root extract Aspirin, a standard anti-inflammatory drug showed the maximum inhibition, 268.348 at the concentration of 500 µg/mL. These provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect. This extract may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophil lysosomal constituents

include bactericidal enzymes and protease, which upon extracellular release cause further tissue inflammation and damage (Chou, 1997). The extract at concentration range from 100 µg/mL to 500 µg/mL protects the human erythrocyte membranes against lysis induced by hypotonic solution. At concentration of 500 µg/mL, the extract inhibited 327.91% of RBC haemolysis as compared with 285.01% produced by Aspirin at 500 µg/mL (Table 2). Since human red blood cell membranes are similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of anti-inflammatory activity of drugs. The results obtained maximum inhibition demonstrated that methanolic extract of stilt root and leaves of *R. mucromata* can significantly and dose dependently inhibit HRBC haemolysis. From the results of the study, it can be concluded extract of *R. mucronata* possessed *in vitro* anti-inflammatory 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.

Conclusion

From the study of this experiment, it may be concluded that the methanolic extract (leaves, bark and stilt root) of the *R. mucronata* mangrove plant has good membrane stability, hence good anti-inflammatory activities. Since the methanolic extract of leaves of leaves, bark and stilt root) of the *R. mucronata* shows significant anti-inflammatory properties, further laboratory study and chemical isolation of this plant parts (leaves, bark and stilt root) might confirm an effective drug molecule in pharmacologic aspects effectively, in both types of pharmaceutical arena.

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Conflicts of interest

The authors declare that have no conflict of interests.

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