STUDY OF ANTI-INFLAMMATORY POTENTIAL OF SOME MANGROVE BARK

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Abstract

Mangroves are plants which can protects tsunami, high winds, high waves, soil erosion, binds soil, useful to produce ecosystem in saline water. They provides income source to people live in that area. Mangroves plants are rich sources of secondary metabolites as they defend extremely adverse condition. From ancient times a variety of plants used to cure inflammation. In this study an attempt was done, to find out whether mangrove plant possess anti-inflammatory potential in them. As we know that synthetic chemicals gives instant reduction in inflammation. On the contrary it will cause many side –effects latter. We are in search of natural remedy on inflammation with no side effects. To study in-vitro anti-inflammatory activity we use aqueous and methanolic extracts were assayed, for the human red blood cells (HRBC) membrane stabilization method was used. In the present investigation the prop root and stem bark extracts of R. mucronata and S. alba inhibits hypotonicity induced lysis of erythrocyte membrane displaying the membrane stabilization effect. All the three plants have of A. officinalis, R. mucronata and S. alba shows significant stabilization property which is comparable to standard drug diclofenac.

Key words: *Anti-inflammatory, Avicennia, bark, mangrove, Rhizophora, Sonneratia*

Introduction

An Inflammation is considered as a primary physiological defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. Uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses (Kumar *et al.*, 2004). Inflammation is the part of biological reaction of vascular tissues to external harmful stimuli, such as pathogens, damaged cells, or irritants (Ferrero- Miliani *et al.*, 2007). They further reported that inflammation clinically causes redness, heat, pain of the affected region and is a complex biological response of vascular tissues to harmful stimuli including pathogens, irritants or damaged cells (Denko,1992). Henson and Murphy (1989) showed that it is defensive mechanism of the body to remove the injurious stimuli as well as promote the healing process for the tissue. Continued inflammation, leads to onset of diseases such as vasomotor rhinorrhea, rheumatoid arthritis, and atherosclerosis. Vane *et al.* (1995) viewed inflammatory response is a complex array of enzyme activation, mediator release, fluid extravasations, cell migration tissue breakdown and repair which cause host defense and usually promoted in most disease conditions. Sosa *et al.* (2002) viewed that although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases. Currently used anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary.

Materials and methods

The plants were collected from Ratnagiri district, located in the west coast of Maharashtra state, India. The external stem bark of mangrove species *Avicennia officinalis* L. from family-Avicenniaceae, prop root and stem bark of *Rhizophora mucronata* Poir from family- Rhizorhoraceae and stem bark of *Sonneratia alba* J. Smith from family-Sonneraticeae were collected from Pawas estuaries of Ratnagiri Districts during the month of December. The outer bark samples were cut into pieces, sun-dried and then oven dried at 60°C. Dried bark samples were ground into powder and stored in an air tight plastic container. Human Red Blood Cells (HRBC) membrane stabilization method of Gandhisan *et al.* (1991) was used for the determination of anti-inflammatory activity in vitro.

- **Preparation for blood (HRBC suspension)**

Collect blood from healthy volunteers. Mixed with equal volume of Alsever's solution [Dissolve Citric acid C(OH)(COOH) (CH_2.COOH) 2.H_2O 0.055g, Sodium Citrate $Na_3C_6H_5O_7.2H_2O$ 0.8g, D-Glucose $C_6H_{12}O_6$ 2.05g, Sodium chloride NaCl 0.42g. made final volume 100 mL with distilled water. Pipette out 10 ml of solution in sterilized bottles and sterilized it by autoclaving at 116°C for 10 minutes. Use slow exhaust. Allow to cool, then tighten the lids and label the bottles and store in the refrigerator]. Centrifuge at 3000 rpm till RBCs separated out. Wash RBC with NS (Normal Saline) solution (Place 1 to 3 drops of blood in the tube). Aim the tip of the saline bottle towards the center of the tube and forcibly squirt saline into the tube. Fill the tube 3/4 full of saline (there will be less splattering in the centrifuge). Centrifuge long enough spin to pull most of cells into a button in the bottom of the tube. Decant out the saline completely. Shake the tube to resuspend cell button before washing the cells again. It will depend on the procedure being done as to how many washings are going to be done. Make 10% suspension with normal saline (HRBC suspension). Use this solution for further testing.

- **Preparation of extract**

Take purified plant extract add distilled water to make various concentrations. (50, 100, 200 and 400 µg /ml). These are used as assay concentrations.

- **Phosphate buffered saline (PBS) Reagents**

Take Sodium chloride NaCl 40.0g, Potassium chloride KCl 1.0g, Potassium dihydrogen phosphate anhydrous KH_2PO_4 1.0g, Disodium hydrogen phosphate anhydrous Na_2HPO_4 4.6g dissolve in 1L distilled water. Mix well and made final volume to 5 L with distilled water. Adjust the pH of solution at 7.3 to 7.4. Autoclaved at 121°C for 15 minutes. Use a slow exhaust. Allow to cool and then tighten the lids and label the bottles.

- **Assay Test**

Take Assay concentration add 1 ml of Phosphate Buffer. Add 2 ml of hyposaline (0.36%) solution and add 0.5 ml of HRBC suspension. Incubate the samples for 30 min at 37°

C. Centrifuge for 30 min. and read the absorbance of Hb concentration of supernatant spectrophotometrically at 560nm = Test OD

- **Standard Test**

Take Diclofenac solution add 1 ml of Phosphate Buffer. Add 2 ml of hyposaline (0.36%) solution. Add 0.5 ml of HRBC suspension. Incubate for 30 min at 37ºC. Centrifuge for 30 min and read the absorbance of Hb concentration of supernatant spectrophotometrically at 560 nm = Standard OD

- **Control Solution**

One ml of Phosphate Buffer. Add 2 ml of hyposaline (0.36%) solution. Add 0.5 ml of HRBC suspension. Incubate sample solution for 30 min at 37ºC and centrifuge it for 30 min. and read the absorbance of Hb concentration of supernatant spectrophotometrically at 560 nm = Control OD

Calculations

% of protection =100 – (Test OD / Control OD) X 100

Result and discussion

Anti-inflammatory activity of mangrove bark anti-inflammatory activity of prop root and stem bark of *A. officinalis, R. mucronata* and *S. alba* is shown in Table 1 and Fig. 1. The aqueous and methanolic extracts of root and stem bark of *A. officinalis, R. mucronata* and *S. alba* were studied for in-vitro anti-inflammatory activity by the human red blood cells (HRBC) membrane stabilization method.

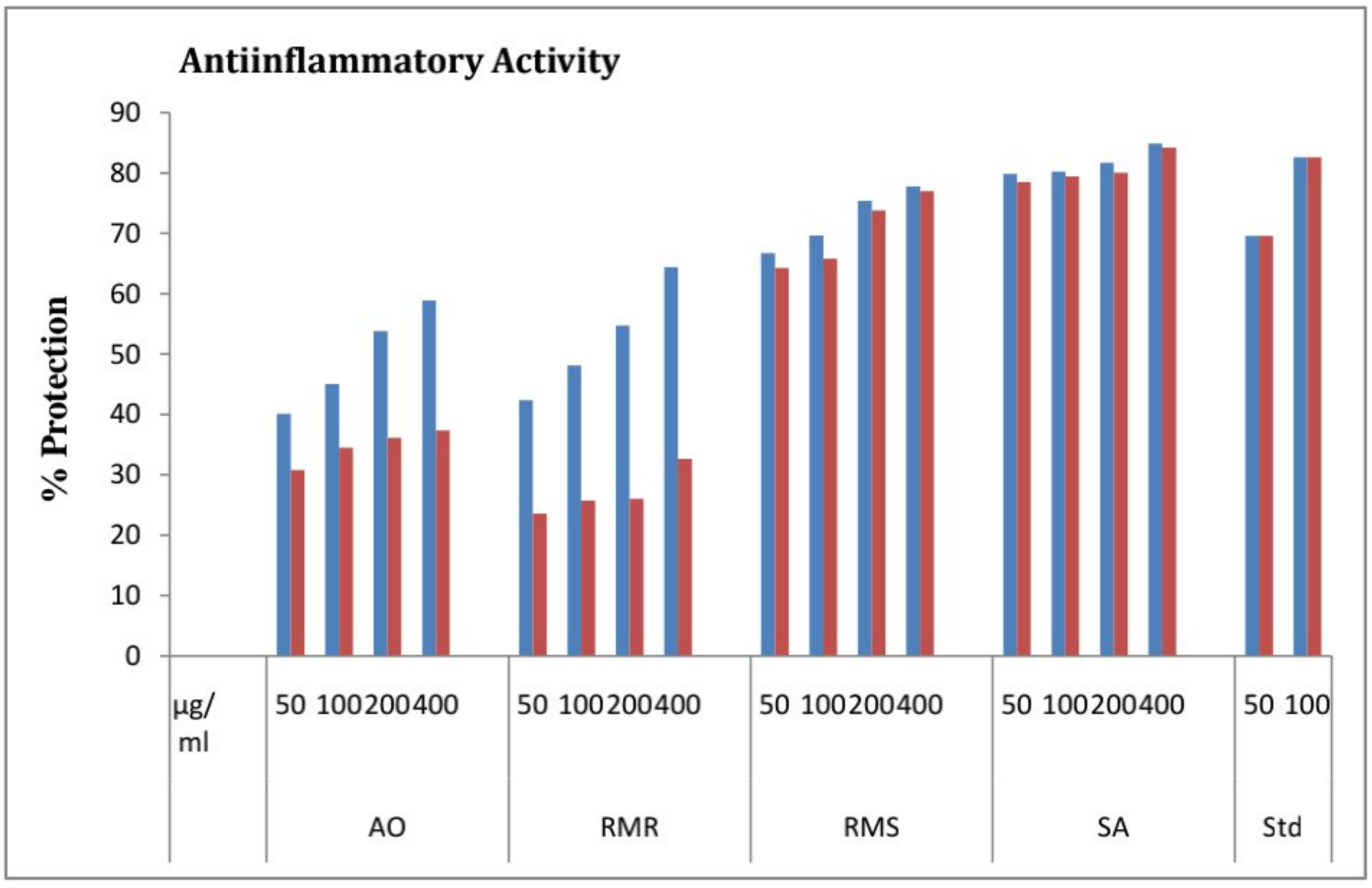
It is observed that in-vitro anti-inflammatory activity of the bark extracts are concentration dependent showing increased activity with increased concentration of extracts. Among both the extracts the aqueous extracts, the *A. officinalis, R. mucronata* and *S. alba* shows 58.8, 64.36, 77.77 and 84.85% protection of HRBC in hypotonic solution while standard diclofenac shows 69.60 and 82.58 % protection. It is also noticed that the percent protection of HRBC is higher in response to the aqueous and methanolic extracts of stem bark of *R. mucronata* and *S. alba*.

Table 1. Anti-inflammatory activity of prop root and stem bark of *A. officinalis, R. mucronata* and *S. alba.*

Sr. No.	Group	Concentration (µg/ml)	% protection mean ± S.D. (N= 6)	
			Aqueous extracts	Methanolic extracts
1	**Control**	-	-	-
1	***A. Officinalis* (stem)**	50	40.10±1.95	30.84±4.44
2		100	45.09±1.17	34.49±5.43
3		200	53.82±2.07	36.14±5.91
4		400	58.88±1.63	37.24±5.91
1	***R. mucronata* (prop root)**	50	42.36±1.81	23.62±1.38
2		100	48.14±1.11	25.75±3.73
3		200	54.73±2.13	26.06±3.64
4		400	64.36±1.14	32.65±4.08
1	***R. mucronata* (stem)**	50	66.74±2.18	64.23±2.68
2		100	69.61±1.06	65.77±4.50
3		200	75.40+1.24	73.79+3.03
4		400	77.77±1.23	76.96±1.9
1	***S. alba* (stem)**	50	79.85±2.56	78.50±3.12
2		100	80.20±2.62	79.41±3.08
3		200	81.68±2.17	80.04±2.16
4		400	84.85±0.54	84.19±1.03
1	**Standard diclofenac**	50	69.60±1.05	69.60±1.05
2		100	82.58±2.69	82.58±2.69

*Data given are mean of three replicates means are with standard deviation (SD) ±

Fig. 1. Anti-inflammatory Activity of bark of *A. officinalis*, *R. mucronata* and *S. alba*



A. Officinalis stem (AO), *R. mucronata* prop root (RMR), *R. mucronata* stem (RMS) *S. alba* stem (SA)
Std – Standard diclofenac

In view of Ahmadiani *et al.* (1998), Ghani (2003) and Gambhire *et al.* (2009), current drugs available such as Opioids and Non-Steroidal Anti-inflammatory Drugs (NSAIDs) drugs and corticosteroids are not useful in all cases of inflammatory disorders, because of their toxic effects with gastrointestinal side effects. As a result of the inherent problems associated with the current non-steroidal as well as steroidal anti-inflammatory agents, there is continuous search especially from natural sources for alternative agents (Akah *et al.*, 2003). Various herbal extract as well as products being employed in the treatment of painful inflammatory disorders (Ghani, 2003).

Shirwaikar *et al.* (2011) speculated that anti-inflammatory agent act through the inhibition of lysosomal enzyme, cyclooxygenase enzyme responsible for conversion of Arachidonic acid to Prostaglandins (PG) while NSAIDs acts as an inhibitor of enzyme cyclooxygenase or stabilizes the lysosomal membrane and Chou (1997) noticed that the *Tripterygium wilfordii* plant extracts exhibited membrane stabilization effects by inhibiting hypotonicity induced lyses of erythrocyte membrane. Shirwaikar *et al.* (2011) also reported that the erythrocyte membrane is analogous to lysosomal membrane and its stabilization implies that the *Thespesia populnea* fruit extract may well stabilize lysosomal membranes. They speculated that stabilization of lysosomal membrane is important in limiting the

inflammatory responses by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which further tissue inflammation and damage up on extra cellular release. Berenguer *et al.* (2006) summarized that some of the NSAIDs are exhibits membrane stabilization due to osmotic loss of intracellular electrolyte and fluid components.

Thus, these extracts may exert anti-inflammatory effect by inhibiting the synthesis of Prostaglandin. The presence of methyl salicylate, an anti-inflammatory constituent with Prostaglandin inhibitory activity has been isolated from the roots of *Securidaca longepedunculata* (Costa *et al.*, 1992). The methyl salicylate content accounts for about 90% of the volatile materials in the root bark (Jayasekara *et al.*, 2002). These volatile constituents including methyl salicylate may be chiefly present in the petroleum ether fraction which was shown to contain resins. Resins are often associated with a variety of compounds such as volatile oils, acids, alcohols, phenols etc. (Evans, 1989).

Methyl salicylate is also a common ingredient of most topical anti-inflammatory/ analgesic preparations. In addition to cyclooxygenase enzyme inhibition, methyl salicylate exerts analgesic effect on relevant application (Bowman and Rand, 1980). Yang *et al.* (2001) observed that other phytochemical constituents isolated from the root bark of *Securidaca longepedunculata* such as flavonoids and methyl salicylate are known to have anti-inflammatory activity. Anti-inflammatory compounds like oleanic acid, beta-sitosterol (Yang *et al.*, 2002), salicylic acid and benzoic acid have also been reported in *S. longepedunculata*, contribute to the anti-inflammatory activity.

Kumari *et al.* (2012) revealed GC-MS analysis of *Sarcostemma secamone* entire plant shows the presence of phytol, 9, 12, Octadecadienoic acid (Z, Z)-, phenyl methyl ester and 9-Octadecanoic acid (Z)-, phenyl methyl ester. These compounds may have the role in anti-inflammatory effects (Aparna *et al.*, 2012). *Sarcostemma secamone* have marked anti-inflammatory effect against its carrageenan induced paw edema (Kumari *et al.*, 2012). In the present investigation it is reported that methanolic extract of *R. mucronata* proproot bark extract and *S. alba* stem bark have 9,12-Octadecadionoic acid and 9- Octadecadionoic acid which may exhibit anti -inflammatory activity.

Conclusion

In-vitro anti-inflammatory activity of the bark extract showed concentration dependent with increasing concentration of extracts. Among both the extracts, aqueous extracts of *A. officinalis, R. mucronata* and *S. alba* showed 58.88, 64.36, 77.77 and 84.85% protection of HRBC in hypotonic solution. Standard diclofenac shows 69.60 and 82.58 % protection. The percent protection of HRBC displayed higher in response to the aqueous and methanolic extracts of stem bark of *R. mucronata* and *S. alba.* The prop root and stem bark extracts of *R. mucronata* and *S. alba* inhibits hypotonicity induced lysis of erythrocyte membrane displaying the membrane stabilization effect. It can be concluded that the extract of prop root and stem of *A. officinalis, R. mucronata* and *S. alba* showed significant stabilization property comparable to standard drug diclofenac. The bioactive compounds such as Caryophyllene oxide and Cycloheptasiloxane, tetradeca-methyl of stem bark extracts of *R.*

mucronata and 1,2,3, Benzenetriol of stem bark extracts of *S. alba* might be responsible for anti-inflammatory activity.

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