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IN VITRO COMPARATIVE STUDY OF MEMBRANE STABILIZATION CAPACITY OF DIFFERENT EXTRACTS OF *BLEPHARIS MADERASPATENSIS* (L.) HEYNE EX ROTH. AND *BLEPHARIS MOLLUGINIFOLIA* PERS. GROWN IN THE REGION OF MYSORE, KARNATAKA

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ABSTRACT: *Blepharis* is a genus belonging to family Acanthaceae. It is an Afro-asiatic genus comprising 129 species which occur in arid and semi-arid habitats. They are used for the treatment of a number of ailments. Anti-inflammatory activity was performed based on the folk lore information. The present study is aimed at the evaluation of anti-inflammatory property of the petroleum ether, ethyl acetate and ethanolic extracts of *Blepharis maderaspatensis* (L.) Heyne ex Roth. and *Blepharis molluginifolia* Pers. whole plant by using Human red blood cell (HRBC) membrane stabilization method. Ibuprofen is used as reference drug for comparison. HRBC method was selected for the *in vitro* evaluation of anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. The results revealed that both *Blepharis maderaspatensis* (L.) Heyne ex Roth. and *Blepharis molluginifolia* Pers. have anti-inflammatory activity. Among the test samples tested for membrane stabilization, *Blepharis maderaspatensis* ethyl acetate extract showed better membrane stability with 323.3±15.7 IC_{50} (µg/ml), followed by with same plant alcoholic extract with 377.6±21.3 IC_{50} (µg/ml). The result of the present study authenticates the folk lore information on the anti-inflammatory properties of the above plants.

INTRODUCTION: Inflammation is a reaction of living tissues and it comprises systemic and local response [1]. Inflammation can be classified as either acute or chronic.

Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue.

Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process [2].

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Now a day's much interest has arisen in the search of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agents without too many side effects [3].

The commonly used drugs for management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers [4]. Therefore, naturally originated agents with very little side effects are desirable to substitute chemical therapeutics [5].

Blepharis is a plant genus comprising of many medicinal property species in it. Juice extracted from leaf of *Blepharis maderaspatensis* is heated with gingerly oil and applied topically on affected places to heal wounds [6]. Leaf juice is used in throat troubles and asthma [7]. Whole plant is used to treat urine problems [8].

Plant is used in wound healing [9]. Leaf is ground into a paste and applied or taken orally to treat bone fracture and deep cuts [10]. Plant is used for treatment of a number of ailments like dysuria, headache and diseases of nervous system, diuretic and aphrodisiac [11]. The plant is used to treat brain disorders [12].

Blepharis molluginifolia plant is used traditionally to treat bone fractures, skin diseases, urinary discharges and allergies [13]. Flat branches of the plant is heated and tied in case of joint pains. Leaves are roasted and then extract is obtained, this extract is drunk as a remedy against flatulence. Roots are employed as antidote on snake bite [14].

Survey of literature revealed that there is no scientific validation for the activity of these plants against inflammation. No approach has been made to study the anti-inflammatory activity of different extracts of *Blepharis maderaspatensis* and *Blepharis molluginifolia.* So present study was undertaken to establish scientific evidence for anti-inflammatory activity of plant extracts of *Blepharis maderaspatensis* and *Blepharis molluginifolia.*

MATERIALS AND METHODS:

Plant material: For the present study the two plants *Blepharis maderaspatensis* (L.) Heyne ex Roth. and *Blepharis molluginifolia* Pers. belonging to the family Acanthaceae has been selected. These plants have been collected from the Mysore district, Karnataka, India, in the month of September 2013.

Chemicals: Potassium dihydrogen orthophosphate, sodium monohydrogen orthophosphate, sodium carbonate, trisodium citrate, sodium chloride (SD fine chemicals, Mumbai) all the reagents were of analytical grades. All the solutions, reagents and buffers were prepared with glass distilled water, Ibuprofen tablets (400 mg).

Extraction of plant material: The whole part of the plant have been collected, cleaned, chopped into small pieces and dried under shade at room temperature. The dried whole plant was pulverized using a stainless steel mixer grinder. After pulverization, the powder has been sieved using a commercial sieve (mesh size approx. 1mm) to make the particle size uniform. Powdered and stored in air tight containers. This powder has been subjected to solvent extraction in a Soxhlet apparatus using various solvents viz., petroleum ether, ethyl acetate and ethanol.

Preparation of Test Solutions: Standard drugs (Ibuprofen, 2.5 mg/ml) and extract were prepared in isosaline (0.85% w/v NaCl) to final concentrations of 62.5-1000 µg/ml.

Preparation of human red blood cell: Fresh human volunteer blood samples were collected into an anticoagulant (containing dextrose (2%), sodium citrate (0.8%), citric acid (0.05%) and sodium chloride (0.42%). Blood samples were centrifuged at 3000 rpm on a centrifuge for 10 min at room temperature. The supernatants (plasma and leucocytes) were carefully removed while the packed red blood cell was washed in fresh normal saline (0.85% w/v NaCl). The process of washing and Centrifugation was repeated five times until the supernatants were clear. Then, bovine erythrocytes (2% v/v) were prepared as reported previously.

Assay of membrane stabilizing activity: The membrane stabilizing activity assay was carried out using 2% (v/v) erythrocyte suspension while Ibuprofen was used as standard drug. The assay mixtures consisted of 2 ml of hyposaline (0.25% w/v) sodium chloride, 1.0 ml of 0.15 M sodium

phosphate buffer, pH 7.4, 0.5 ml of 2% (v/v) erythrocyte suspension, 0.0 - 1.0 ml of drugs (standard, extract) and final reaction mixtures were made up to 4.5 ml with isosaline. Drugs were omitted in the blood control, while the drug control did not contain the erythrocyte suspension. The reaction mixtures were incubated at 56°C for 30 min on a water bath, followed by centrifugation at 5000 rpm on Centrifuge for 10 min at room temperature [15]. The absorbance of the released haemoglobin was read at 560 nm. The percentage membrane stability was estimated using the expression:

$$\% \text{ of membrane stability} = \frac{100 - \{\text{Abs of test drug-Abs of drug control}\}}{\{\text{Abs of blood control}\}} \times 100$$

The results are tabulated in **table 1 and 2** and the graphical representations of the results are also made with respect to the results tabulated in the tables 1 and 2.

RESULTS AND DISCUSSIONS: The membrane stabilising action of different extracts of *Blepharis maderaspatensis* (L.) Heyne ex Roth. and *Blepharis molluginifolia* Pers. whole plants were performed on the human erythrocyte membrane.

Among the test samples tested for membrane stabilization, *Blepharis maderaspatensis* ethyl acetate extract showed better membrane stability with 323.3±15.7 IC_{50} (µg/ml), followed by with same *Blepharis maderaspatensis* alcoholic extract with 377.6±21.3 IC_{50} (µg/ml) (Table 1) in comparison with the standard Ibuprofen which has 479.0±17.4 IC_{50} (µg/ml) (**Table 3**).

Blepharis molluginifolia also has membrane stabilising activity of 578.2±22.9 IC_{50} (µg/ml) in ethanolic extract and 662.6±27.1 IC_{50} (µg/ml) in ethyl acetate extract (Table 2). Petroleum ether extracts of both the plants showed very less activity.

TABLE 1: HRBC STABILIZING ACTIVITY OF *BLEPHARIS MADERASPATENSIS*

Test conc.	Petroleum ether		Ethyl acetate		Ethanol	
	%stabilizn.	IC_{50} (µg/ml)	%stabilizn.	IC_{50}(µg/ml)	%stabilizn.	IC_{50}(µg/ml)
1000	37.2±6.5		73.4±3.6		70.6±1.3	
500	18.0±10.0		67.8±1.3		55.5±2.3	
250	0.0	>1000	50.4±0.7	**323.3±15.7**	42.0±3.3	377.6±21.3
125	0.0		15.5±3.3		34.9±5.5	
62.5	0.0		0.0		0.0	

The average value of three calculations are presented as ±SD

TABLE 2: HRBC STABILIZING ACTIVITY OF *BLEPHARIS MOLLUGINIFOLIA*

Test conc.	Petroleum ether		Ethyl acetate		ethanol	
	%stabilizn.	IC_{50}(µg/ml)	%stabilizn.	IC_{50}(µg/ml)	%stabilizn.	IC_{50}(µg/ml)
1000	39.8±5.4		75.4±0.6		66.5±1.2	
500	28.1±7.8		33.1±5.0		45.5±0.8	
250	18.1±9.0	>1000	0.0	662.6±27.1	26.7±1.4	578.2±22.9
125	0.0		0.0		9.0±4.2	
62.5	0.0		0.0		6.9±5.8	

The average value of three calculations are presented as ±SD

TABLE 3: HRBC STABILIZING ACTIVITY OF IBUPROFEN (STANDARD)

Test Conc. (µg/ml)	Ibuprofen	
	% stabilizn.	IC_{50}(µg/ml)
1000	86.0±6.2	
500	44.3±1.7	
250	27.8±0.8	479.0±17.4
125	10.0±5.5	
62.5	0.0	

The average value of three calculations are presented as ±SD

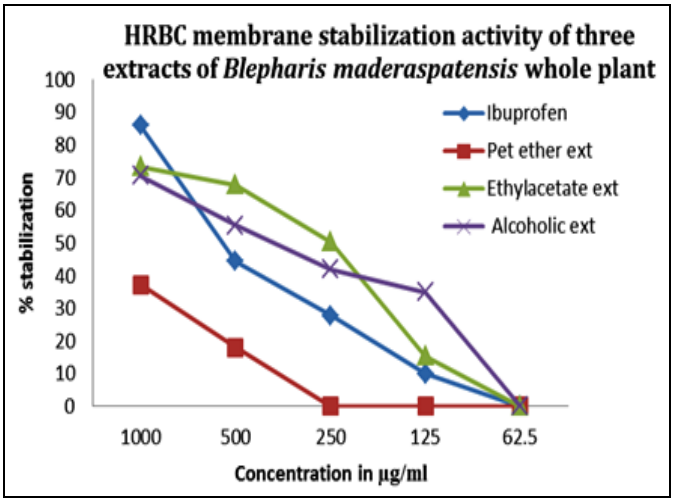

FIGURE 1: COMPARATIVE GRAPH OF % STABILIZATION OF DIFFERENT EXTRACTS OF *BLEPHARIS MADERASPATENSIS*

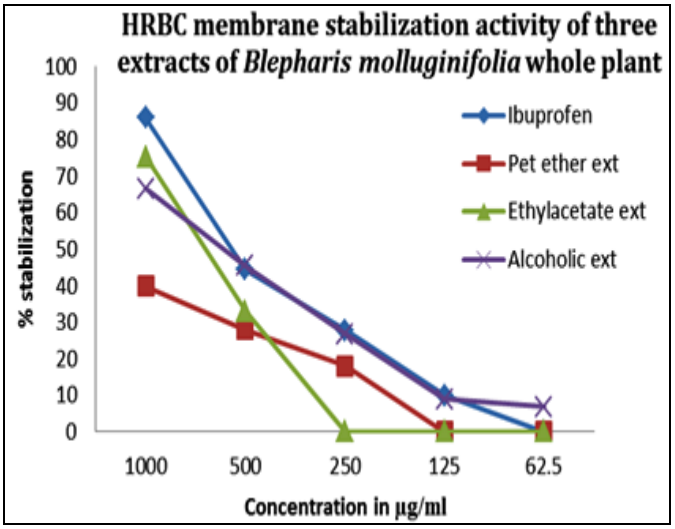

FIGURE 2: COMPARATIVE GRAPH OF % STABILIZATION OF DIFFERENT EXTRACTS OF *BLEPHARIS MOLLUGINIFOLIA*

From the above studies, it could be concluded that *Blepharis maderaspatensis* have maximum anti-inflammatory activity and it could be natural anti-inflammatory source and thus could be useful as therapeutic agents in preventing the diseases. Further studies are needed for their active principle to elucidate.

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