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IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF LEAF EXTRACTS OF *BASELLA ALBA* LINN. VAR. ALBA

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Abstract

The leaf extracts of *Basella alba* Linn.var. alba were investigated for *In-vitro* anti-inflammatory activity by human red blood cell membrane stabilization method (HRBC). The increased use of natural product in the pharmaceutical industry has led to an increase in demand for screening for cost effective, nontoxic bioactive compounds in medicinal plants. Now a day's many researchers interest is to search medicinal plants with potent therapeutic activity which may lead to the discovery of new therapeutic agent. In this work the methanolic extract of *Basella alba* (M.E.B.A.) and aqueous extract of *Basella alba* (A.E.B.A.) were studied for its *in-vitro* anti-inflammatory activities. The potency of the extracts was compared with standard Diclofenac sodium (50 and 100 µg/ml). The aqueous extract showed the most significant membrane stabilizing action on human red blood cell membrane.

Key words:

Basella alba, anti-inflammatory, Human red blood cell membrane stabilization method

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Introduction: -*Basella alba L.,* (Basellaceae) commonly has known as "Poi (Hindi), Potaki (Sanskrit) and Pasalakkirai (Tamil) [1]. In present study Diclofenac sodium, a nonsteroidal anti-inflammatory drug (NSAID) was is used as reference standard drug and leaf extracts *of Basella alba* as a test drug. Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation,

mediator release, fluid extravasations, cell migration, tissue breakdown and repair[2]. which are aimed at host defense and usually activated in most disease condition. The critical role of inappropriate inflammation is becoming accepted in many diseases that affect man, including cardiovascular diseases, inflammatory and autoimmune disorders, neurodegenerative conditions, infection and cancer [3].

In appreciating the inflammatory process, it is important to understand the role of chemical mediators. These are the substances that tend to direct the inflammatory response. These inflammatory mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes/macrophages. They are triggered by bacterial products or host proteins. Chemical mediators bind to specific receptors on target cells and can increase vascular permeability and neutrophil chemotaxis, stimulate smooth muscle contraction, have direct enzymatic activity, induce pain or mediate oxidative damage. Most mediators are short-lived but cause harmful effects. Examples of chemical mediators include vasoactive amines (histamine, serotonin), arachadonic acids (prostaglandins, leukotrienes) and cytokines (tumor necrosis factor and interleukin–1) [4].

*Basella alba L.*also known Indian spinach. The aerial part (leaves, stem) of the *plant* serve as edible plant (vegetable) in many parts of world [5]. These contain different components which have extensively used in constipation, as diuretic, in urticaria, as demulcent, antiulcers, and as cooling application for burn[6]. India, due to its geographical and environmental positioning has traditionally been a good source for such products among the Asian countries.

Material and Methods:-

Plant Material

Basella alba Linn var. alba leaves for the proposed study were collected from vegetable market of Tripura (India) and were authenticated (Regd.-PARC/2009/258) by Prof. P. Jayaraman, Director, PARC, National Institute of Herbal Sciences, W.Tambaram, Chennai (Tamilnadu).The fresh collected leaves of *Basella alba* Linn. Were shade dried and used for this study. The coarse powder was subjected to extracted separately using ethanol and water as solvents. The solvents were distilled under reduced pressure using rotary vacuum evaporator. The extracts were dissolved in distilled water.

Phytochemical screening

The Phytochemical examination of the both extracts was performed by the standard methods [11] and shows the presence of various phytochemical constituents tabulated in Table-2.

Method:-

In-vitro anti-inflammatory activity

The human red blood cell (HRBC) **membrane stabilization method:** The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution(2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared (200 and 400 µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. It was incubated at 37^{o}C for 30 min and centrifuged at 3,000 rpm for 20 min. and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 and 200 µg/ml) was used as reference standard and a control was prepared by omitting the extracts. [7].

The percentage of HRBC membrane stabilization or protection was calculated by using the following Formula,

$$\% \text{ Protection} = 100 - \frac{\text{Optical density of drug treated sample}}{\text{Optical density of control}} \times 100$$

Results and Discussion- The results are reported in table 1. The petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of the leaves of *Basella alba L.*were studied for *in vitro* anti-inflammatory activity by HRBC membrane stabilization method. The *in vitro* anti inflammatory activity of the extracts were concentration dependent, with the increasing concentration, the activity is also increased. Among both the extracts, aqueous extract at a concentration of 400 µg/ml showed 71.89 % protection of HRBC in hypotonic solution. All the results were compared with standard Diclofenac which showed 83.54 % protection.

Basella alba L. leaves extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane [8] and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing 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 [9] Some of the NSAIDs are known to posses membrane stabilization properties which may contribute to the potency of their anti-inflammatory effect. Though the exact mechanism of the membrane stabilization by the extract is not known yet; hypotonicity-induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components. [10] Aqueous extract showed significant *in-vitro* anti-inflammatory activity as compared to standard. The aqueous extract showed significant anti-inflammatory activity (71.89 %) at the dose of 400 µg/ml. On the basis of the above results it can be concluded that the *Basella alba* L. have an anti-inflammatory activity.

Table 1: Anti inflammatory activity of leaves extracts of *Basella alba L.* var. alba.

S. No.	Groups	Concentration (ug/ml)	(% Protection) Mean ± S.E.M [n=6]
1	Control	-	-
2	M.E.B.A.	200 400	57.10 ±2.18** 68.38±1.18**
3	A.E.B.A.	200 400	58.46 ±1.61** 71.89 ±1.22**
4	Standard	50 100	70.17 ±0.22*** 83.54 ±0.61***

The results were expressed as mean˙ ± S.E.M¨ [n=6].

Table. 2: Preliminary Phytochemical screening of M.E. and A.E. of leaves of ***Basella alba*** (L.) var. ***alba.***

S. No.	Test	Reagents/method adopted	MEBA	AEBA
1.	Alkaloids	Picric acid Dragendroffs reagents Mayer's reagents	+ + +	- - -
2.	Carbohydrates	Molish test Fehling's test Benedicts test	+ + +	+ + +
3.	Tannins	Ferric chloride test	-	+
4.	Flavonoids	Shinoda test	+	+
5.	Mucilage	Ruthenium red	-	+
6.	Fixed oil	Spot test	+	-
7.	Saponin	Heamolysis test	+	+

* + = Present , – =Absent

CONCLUSION:

Basella leaves extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogues to the lysosomal membrane [8] and its stabilization implies that the extract may as well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bacterial enzymes and proteases which cause

further tissue inflammation and damage[9] From the above study it was concluded that the aqueous and methanolic extracts of leaves of *Basella alba L.* has significant membrane stabilization property comparable to the standard drug diclofenac.

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