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A STUDY OF *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY OF COMBINED EXTRACTS OF *AZADIRACHTA INDICA* AND *BASELLA ALBA*

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ABSTRACT

The aim of the present study was to study the *in-vitro* anti-inflammatory activity of *Azadirachta indica* (neem) *and Basella Alba* (Alba) individual extracts and in combination. The powdered plant materials were subjected to maceration processes. The yield was collected and prepared different concentrations (200µg/ml and 400µg/ml). Human red blood cells membrane stabilization method was used as an *in-vitro* anti-inflammatory method by using diclofenac as a standard drug. *Azadirachta indica* showed significant inhibition of haemolysis of 77.03%, 82.96% at the concentrations of 200µg/ml, 400µg/ml respectively. *Basella Alba* showed significant inhibition of haemolysis of 70.3%, 81.4% at 200µg/ml, 400µg/ml respectively. The combined extracts showed significant inhibition of haemolysis of 85.9%, 90.3% at a concentration of 200µg/ml, 400µg/ml respectively.

KEYWORDS

Inflammation, Anti-inflammatory, Human red blood cell, *Azadirachta indica* and *Basella Alba*.

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INTRODUCTON

Plants are the main source of medicine credited with numinous and almost supernatural powers of healing. Medicinal plants play the major role in current medicines. Traditionally medicinal plants are used to treat various diseases[1].

Azadirachta indica and *Basella Alba* are selected for the present study. The genus *Azadirachta* belongs to family *Meliaceae* consists of about 46 generations and the plant list includes a further 3,155 scientific plant names of species rank for the family *Meliaceae* of these 559 are accepted species names[2]. The principal constituents of *Azadirachta*

indica leaves include minerals, protein, carbohydrates, Vitamin-C, calcium, carotene, phosphorus, they also contain aspartic acid, tyrosine, glutamic acid, proline, alanine, cysteine and various fatty acids[3].

The genus *Basella* belongs to family *Basellaceae*. *Basella* contains five known species. Three species are endemic to Madagascar, and one is endemic to Southeastern Africa. The fifth is widespread across the Indian subcontinent, Southeast Asia and New Guinea[4]. *Basella Alba* is excellent source of folic acid, Vitamin-A, Vitamin-C, calcium, iron and magnesium[5].

MATERIAL AND METHODS

Azadirachta indica leaves were collected from Laxmi Nagar Colony, Old Safilguda, Malkajgiri Mandal, Secunderabad. *Basella Alba* leaves were collected from Bahadurpally, Quthbullapur Mandal, Secunderabad, these were authenticated by the Department of Botany Osmania University Hyderabad, Telangana, India. Fresh leaves collected, and then washed, shaded, dried for three weeks and powdered mechanically. The dried powder was sieved through mesh No.44 and stored in an airtight container until use.

EXTRACTION PROCEDURE

Plants extract preparation

Azadirachta indica

The plant extract was prepared by cold maceration method. The procedure involves soaking of the powdered leaves in 99.9% ethanol for 7days and filtered to get the extract. The extract was evaporated to dryness under vacuum to get the desired product. 50gms of dried leaf powder was subjected to cold maceration using ethanol for 7days. The percentage yield was found to be 5%w/w.

Basella Alba

The plant extract was prepared by cold maceration method. The procedure involves soaking of the dry powder in 99.9% ethanol for 5days and filtered to get the extract. The extract was evaporated to dryness under vacuum to get the desired product. 50gms of dried leaf powder was subjected to cold maceration using ethanol for 5days. The percentage yield was found to be 7%w/w.

Phytochemical Analysis of the Extracts

Detailed phyto-chemical examinations were carried out for both the extracts as per the standard methods. The phyto-chemical constituents match with the reported studies[6,7,5,8]. The phyto-chemical constituents shown in Table No.1.

In-Vitro Anti-Inflammatory Activity

The human red blood cell (HRBC) membrane stabilization method

The blood is collected from healthy human volunteer who has not taken any NSAIDS for 2weeks 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 3,000rpm. The packed cells are washed with isosaline and a 10% suspension is made.

Various concentrations of extracts of *A. indica* and *B. alba* were prepared (200µg/ml and 400µg/ml) using distilled water, and to each concentration 1ml of phosphate buffer, 2ml hyposaline and 0.5ml of HRBC suspension was added, incubated at 37ºC for 30min, then centrifuged at 3000rpm for 20min. The content of the supernatant solution was estimated on UV spectrophotometer at 560nm. Diclofenac (100µg/ml and 200µg/ml) was used as the reference standard and control was prepared by omitting the extracts[9,10]. The percentage inhibition of haemolysis shown in Table No.2.

% Inhibition of haemolysis = {(OD control-OD test samples)/OD control} x 100.

RESULTS AND DISCUSSION

Phyto-chemical Screening

Both the plant extracts screened for phytochemical constituents, the extract of *Azadirachta indica* consists of glycosides, terpenoids and flavonoids. The extract of *Besella Alba* consists of saponins, flavonoids and charbohydrates[11,12].

In the present study, the anti-inflammatory activity was assessed by *in-vitro* HRBC method. During inflammation, lysosomal hydrolytic enzymes are released causes damages the surrounding organelles and tissues with a variety of disorders[13]. The

erythrocyte membrane is analogous to the lysosomal membrane, 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 bactericidal enzymes and *proteases* which cause further tissue inflammation and damage upon extracellular release[14]. In this study, it was reported that the percentage inhibition of haemolysis by ethanolic extract of *A. indica* showed 77.03%, 82.96% and *B. alba* showed 70.3%, 81.4% at 200µg/ml, 400µg/ml respectively. The combined extracts showed, 85.9%, 90.3% inhibition of haemolysis at a concentration of 200µg/ml, 400µg/ml respectively.

Table No.1: Preliminary Phyto-chemical constituents of *Azadirachta indica* and *Basella Alba*

S.No	Contents	*Azadirachta indica* extract	*Basella* extract
1	Alkaloids	-	-
2	Glycosides	+	-
3	Saponins	-	+
4	Terpenoids	+	-
5	Flavonoids	+	+
6	Steroids	+	-
7	Carbohydrates	-	+

+ = *Present,* **-** = *Absent*

Table No.2: Percentage haemolysis of ethanolic extracts and standard drug

S.No	Treatment	Concentration (µg/ml)	Absorbance (Mean ± SD)	% Inhibition of haemolysis
1	Control	-	0.135±0.035	-
2	*Azadirachta indica*	200	0.031±0.003	77.03%
		400	0.023±0.002	82.96%
3	*Basella alba*	200	0.040±0.004	70.3%
		400	0.025±0.015	81.4%
4	Combined	200	0.019±0.010**	85.9%
		400	0.013±0.003**	90.3%
5	Standard (Diclofenac)	100	0.003±0.001	97.7%
		200	0.006±0.004	97.9%

***p<0.05*

Figure No.1: *Azadirachta indica*

Figure No.2: *Basella Alba*

Figure No.3: Ethanolic extract of *Azadirachta indica*

Figure No.4: Ethanolic extract of *Basella Alba*

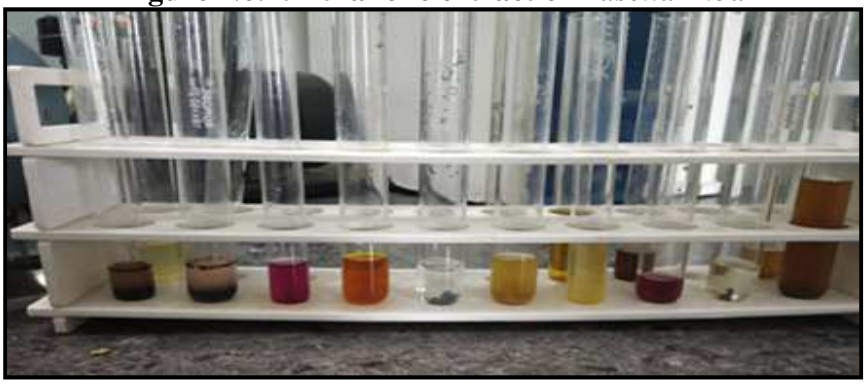

Figure No.5: Phyto-chemical analysis of the extracts

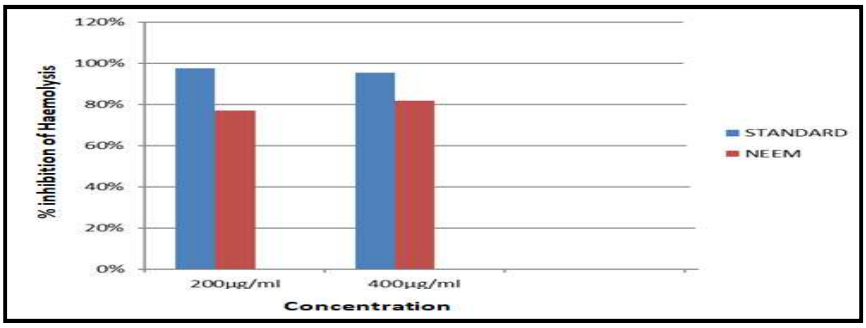

Figure No.6: Membrane stabilizing activity of *A. indica*

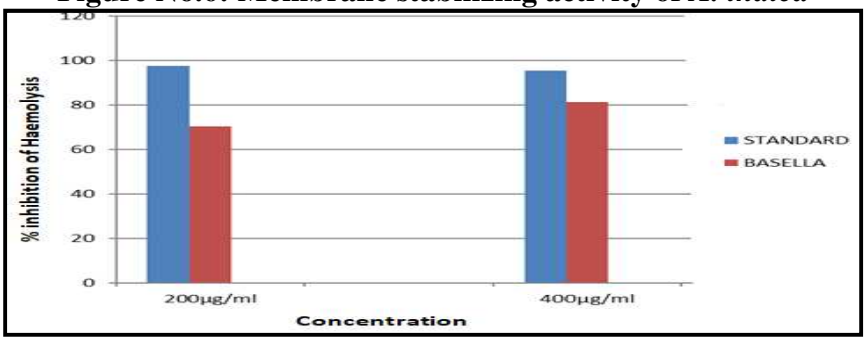

Figure No.7: Membrane stabilizing activity of *B.alba*

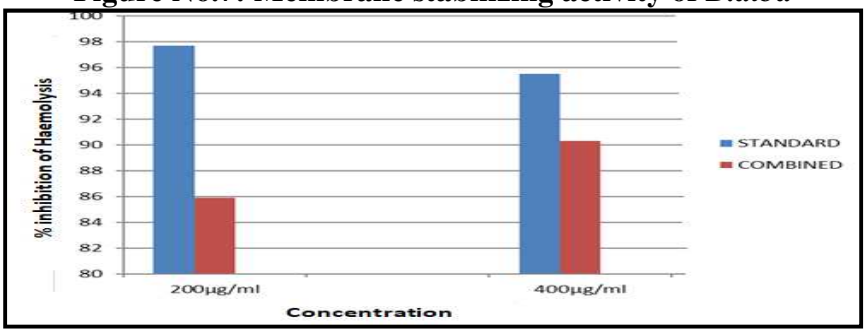

Figure No.8: Membrane stabilizing the activity of combined extracts

CONCLUSION

In the present investigation, the results indicate that the ethanolic leaf extracts of *Azadirachta Indica* and *Basella Alba* possess anti-inflammatory properties. The protective effect of HRBC membrane stabilization is known to be a good index of anti-inflammatory activity; the present study says that the combination of *Azadirachta Indica* and *Basella Alba* possesses greater anti-inflammatory activity when compared with the individual extracts.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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