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A COMPARATIVE STUDY ON THE ANTI-INFLAMMATORY EFFECT OF TWO VARIETIES OF BOUGAINVILLEA FLOWERS

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ABSTRACT

Bougainvillea spectabilis and *Bougainvillea Alba* belongs to the family Nyctaginaceae having white and pink flowers. In traditional medicine, flower extract has been used to treat various diseases such as diarrhoea, stomach acidity, hepatitis, and diabetes. The aim of the study was to compare the anti-inflammatory activity of 50% hydroethanolic extract of two different bougainvillea flowers. *Bougainvillea spectabilis* flower extract exhibit the maximum inhibiting effect on albumin denaturation and proteinase activity. RBC membrane stabilization effect was also more produced by using *Bougainvillea spectabilis* flower extract. The result obtained in the present study indicates that hydroethanolic extract of *Bougainvillea spectabilis* can be used as a potential source of anti-inflammatory agent.

KEYWORDS: Anti inflammatory, Protein denaturation, Membrane stabilization.

INTRODUCTION

Inflammation is derived from ‘inflammare' which is determined by the defense mechanism of the human body towards the pathogenic microorganisms, physical (or) chemical agents and any injuries. It is classified as acute and prolonged inflammation. Acute inflammation is the primary responsibility of the body to harmful stimuli developing redness, pain in the area of infection. Prolonged inflammation which is also known as chronic inflammation participating lymphocyte and macrophage required fibrosis and tissue necrosis resulting in arthritis, coronary heart diseases, asthma etc.[1]

Lysosomal enzymes are released during inflammation and they produce various disorders. The extracellular activities of lysozymes are associated with acute (or) chronic inflammation. Stabilization of lysosomal membrane is important in the control of inflammatory response by the liberation of lysosomal constituents of activated neutrophils such as bacteriocidal enzymes and proteases.[2] Human red blood cell (HRBC) is similar to the lysosomal membrane[3] and the stabilizing effect of drug on erythrocyte membrane may correlate with lysosomal membrane stabilization. The heat induced haemolysis of erythrocytes is used as a simple tool to determine the anti-inflammatory activity of flower extracts.

Medicinal plants are used as herbal medicine to prevent and cure diseases. There are a variety of therapeutically valued plant resources, which are used as drugs and medicines for several diseases. In one of the important studies by the WHO, it was estimated that 80% of the population in many developing countries relies on historically plant oriented herbal medicines for their basic health requirement, because of easy availability, low side effects, low prices and lasting curative property.[4] The management of inflammation is a real problem in the rural community, the population in these areas use many alternative drugs such as substances produced from the medicinal plant.[5]

The genus *Bougainvillea* has 14 species with three are horticulturally important namely *Bougainvillea spectabilis, Bougainvillea glabra,* and *Bougainvillea Peruvian*. *Bougainvillea speactabilis* and *Bougainvillea alba* is a large climber with distinctive thorns and hair on stem and leaves. It is commonly known as the paper flower in English.[6] The plant is used for fertility control by tribal people in many countries.[7] It has been reported to exhibit antibacterial, antidiabetic, amylase inhibition, antifertility, antihyper-lipidemic, radical scavenging activity, antiatherogenic, hematologic effect, thrombolytic activity, analgesic activity and antiulcer activity.[8]

To the best of our knowledge no scientific details concerning the anti- inflammatory activity of *Bougainvillea spectabilis* and *Bougainvillea alba* are available and hence the present study was undertaken to evaluate the anti- inflammatory activity of flower extract using in vitro models.

MATERIALS AND METHODS

Plant material

The flowers of *Bougainvillea spectabilis* and *Bougainvillea alba* were collected in fresh condition from Agricultural University, Coimbatore, Tamil Nadu. The flowers were washed with fresh water, shade dried and then ground into a uniform powder using an electronic blender and stored in polyethylene bags at room temperature.

Preparation of plant extract

10 gm of the dried powder was cold macerated with 50% hydroethanol with occasional stirring for three days. After three days the suspension was filtered through a fine muslin cloth. The filtrates were evaporated under reduced pressure and dried using a rotary evaporator at 55^{o}c.

Assessment of *in vitro* anti-inflammatory activity

Inhibition of albumin denaturation

The anti-inflammatory activity of *Bougainvillea spectabilis* and *Bougainvillea alba* were studied by using inhibition of albumin denaturation according to the method of Mizushima[9] and Kobayashi[10] with minor modifications. The reaction mixture consists 1ml of 50% hydroethanolic extract of *Bougainvillea spectabilis* and *Bougainvillea alba* at (62.5 µg, 125µg, 250µg, 500µg, 1000µg) different concentrations and 1ml of 1% aqueous solution of bovine albumin. The pH of the reaction mixture was adjusted to 6.5 using 1N Hcl. The flower extracts were incubated at 37◦c for 20 minutes. Denaturation was induced by keeping the reaction mixture at 57◦c water bath for 20 minutes. After cooling the turbidity was measured at 660nm. The experiment was done in triplicate and average is taken. Aspirin is used as a standard drug. The percentage inhibition of denaturation was calculated as follows:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

Proteinase inhibition

The test was conducted according to The improved method of Sakat et al [10]. The reaction mixture (2ml) was containing 0.06mg trypsin, 1ml of 20Mm Tris Hcl buffer (Ph 7.4) and 1ml of plant sample of different concentration(62.5 µg, 125µg, 250µg, 500µg, 1000µg). The mixture was incubated at 37◦c for 5 minutes and then 1ml of 0.8% casein was added. The mixture was incubated for additional 20 minutes. 2ml of 70% Perchloric acid was added to terminate the reaction. The reaction mixture was centrifuged and the absorbance of the

supernatant was measured at 210nm against buffer as blank.[11] The experiment was performed in triplicate. Protein inhibitory activity (%) was calculated as follows:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

Membrane stabilizing activity

Preparation of human red blood cells (HRBC) suspension

Fresh whole human blood (10ml) was collected and transferred to the heparinized centrifuged tubes. The tubes were centrifuged at 3000rpm for 10mintues. The tubes were washed three times with normal saline. The volume of the blood was measured and reconstituted as 10% U/V suspension normal saline.

Heat induced haemolysis

The test was performed according to the modified method of Shinde et al [12]. The reaction mixture 2ml consist of 1ml of different species of flower extracts (0.1gm/ml) at different concentration(125µg,250µg,500µg,1000µg) and 1ml of 10% red blood cells suspension. 1ml of saline was added to the control test tube. Aspirin is used as a standard drug. The centrifuge tubes containing reaction mixture were incubated in a water bath at 56◦c for 30minutes. After incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500rpm for 5minutes and the absorbance of the supernatant was measured at 560nm. The experiment was performed in triplicate. Membrane stabilizing activity in (%) was calculated as follows:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

Statistical analysis

The results are presented as mean ± SD for three replicates.

RESULT

In our study, the anti-inflammatory activity of *Bougainvillea spectabilis* and *Bougainvillea alba* flowers on protein denaturation, anti-proteinase activity, and membrane stabilization are established in table 1. The hydroethanolic extract of two flowers exhibited a varying degree of anti-inflammatory activity. The maximum albumin denaturation of 71% was observed for *Bougainvillea spectabilis* at 1000µg/ml concentration. Another variety *Bougainvillea alba* showed 59% of albumin denaturation. The standard drug aspirin showed an inhibition of 75% at 0.1gm/ml. The different varieties of *Bougainvillea* also exhibited anti-proteinase activity

which is shown in table1. High activity for anti-proteinase was observed with *Bougainvillea spectabilis (*69%) than *Bougainvillea alba* (52%) at 0.1gm/ml concentration.

Table 1: Effect of hydroethanolic extract of *Bougainvillea spectabilis* and *Bougainvillea alba* on albumin denaturation and proteinase inhibitory activity.

Concentration (µg/ml)	Albumin denaturation (%)			Proteinase inhibitory activity (%)		
	Bougainvillea spectabilis	*Bougainvillea alba*	Aspirin	*Bougainvillea spectabilis*	*Bougainvillea alba*	Aspirin
62.5	10.11±0.5	12.22±0.6	12.33±0.77	8.03±0.4	15.45±7.05	15.23±0.43
125	27.45±1.35	25.13±1.25	15.03±0.63	13.78±0.65	29.87±1.45	18.09±0.80
250	42.15±2.1	35.77±1.75	31.20±1.25	31.92±1.55	36.98±1.8	38.61±1.41
500	59.50±2.95	45.05±2.25	37.63±1.4	35.11±1.75	47.37±2.85	45.83±2.10
1000	71.05±3.55	59.57±2.95	75.69±3.75	69.74±3.45	52.53±3.2	72.29±3.6

The values are expressed as Mean ± SD. (n=3)

The stabilization of RBC membrane by different varieties of flower extract is presented in table 2. Both extracts were effective in preventing the heat induced haemolysis of RBC membrane at different concentrations ranging from 62.5 to 1000 µg/ml. Maximum inhibition of 56% is observed for *Bougainvillea spectabilis* when compared to *Bougainvillea alba* (44%). The standard drug aspirin showed 62% of inhibition.

Table 2: Effect of hydroethanolic extract of *Bougainvillea spectabilis* and *Bougainvillea alba* on Membrane stabilization activity.

Concentration (µg/ml)	% inhibition on heat induced haemolysis		
	Bougainvillea spectabilis	*Bougainvillea alba*	Aspirin
62.5	11.69±5.5	13.48±6.5	22.35±0.98
125	20.84±1.0	16.66±8.0	33.94±2.20
250	27.90±1.35	29.00±1.45	40.3±1.7
500	39.43±1.9	31.01±1.55	55.34±0.56
1000	56.69±2.3	44.23±1.21	62.56±3.6

The values are expressed as Mean ± SD. (n=3)

DISCUSSION

Inflammation is a normal body protective function against injury and infection. The denaturation of protein is a well-documented cause of inflammation. Several authors have reported that protein denaturation is one of the main reason of rheumatoid arthritis caused by generation of auto antigen.[13] The denaturation of protein is due to modification of electrostatic force, hydrogen, hydrophobic and disulfide bonds.[14]

Neutrophils are rich sources of serine proteinase and are seen in lysosomes. It was reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reaction and protection was provided by proteinase inhibitors.[15] The result confirmed that *Bougainvillea spectabilis* flower extract has maximum capacity to inhibit the proteinase.

Stabilization of RBC's membrane was studied to determine the mechanism of anti-inflammatory action of *Bougainvillea spectabilis* flower extract by inhibiting heat-induced hemolysis. The erythrocyte membrane is analogous to the lysosomal membrane and during acute (or) chronic inflammation the membrane is destabilized by the release of membrane enzymes. The flower extract of *Bougainvillea spectabilis* and *Bougainvillea alba* stabilize the lysosomal membrane. These finding clearly indicate that the *Bougainvillea spectabilis* extract has a very good anti-inflammatory activity.[16]

CONCLUSION

In conclusion, the present study showed the in vitro anti-inflammatory activity of 50% hydroethanolic extract of different varieties of Bougainvillea flowers. *Bougainvillea spectabilis* showed a considerable anti-inflammatory activity than *Bougainvillea alba* which might be due to the presence of phytochemicals in it.

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CONFLICT OF INTEREST

There is no conflict of interests.

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