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SCREENING OF *IN VITRO* ANTI-INFLAMMATORY ACTIVITY OF *FICUS VIRENS* BARK

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ABSTRACT

Ficus virens (Syn: *Ficus infectoria*) is a plant belonging to the genus *Ficus* and family Moraceae is found in India, Malaysia and northern Australia. Its common name is white fig and locally in Hindi language known as pilkhan. Methanolic extract of *Ficus virens* bark was screened for *invitro* antiinflammatory effect. Significant RBC membrane stabilisation effect in heat induced haemolysis method and egg albumin protein inhibition was studied for *invitro* anti-inflammatory action. The phytochemicals like alkaloids, phenolics, flavonoids and tannins present in the extract might be responsible for the desired anti-inflammatory effect of the methanolic extract, which was confirmed by the preliminary phytochemical tests and by estimation.

Keywords: *Ficus virens* bark, Moraceae, alkaloids, phenolics, flavonoids, haemolysis and protein inhibition.

INTRODUCTION

Ficus virens (Syn: *Ficus infectoria*) is a plant belonging to the genus *Ficus* and family Moraceae is found in India, Malaysia and northern Australia[1]. Its common name is white fig and locally in Hindi language known as pilkhan· It is a monoecious large spreading deciduous tree, epiphytic in young stages, with a few aerial roots. leaves coriaceous, glabrous, ablong-ovate, entire or undulate, acuminate, base acute, truncate or subcordate, lateral nerves 8-12 pairs; petiole articulate, glandular at the apex below[2]. Receptacles unisexual, paired, axillary, shortly peduncle, globose, whitish with red dotted. In investigation of *Ficus virens leaf* revealed that phenolics compound form the major photochemical components of the leaves are responsible for the excellent antioxidant capacity of the extracts and the stem bark of the tree is employed in the indigenous systems of medicine for the variety of purpose like astringent, antiseptic[3]. It is also used as a inflammatory swellings, boils and wound healing but none of the earlier works has not yet been documented. Hence in present study, an attempt was made to screen out invitro anti-inflammatory activity on the bark extract of *Ficus virens*.

MATERIALS AND METHODS

Plant material

The plant was obtained from the Tirumala Forest, Tirupathi and the bark was identified by Dr. Madhava Chetty.

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Authentication has done by Dr. P.Jayaraman, Director, Plant Anatomy Research Centre, Chennai (Voucher specimen number.2194).

Preparation of extract

The bark was dried under shade and powdered. The powder was defatted with petroleum ether and subjected to Soxhlet extraction with methanol. The extract was evaporated to dryness. The dark brownish semisolid mass obtained was stored in a well closed airtight resistant container.

Phytochemical screening

The methanolic extract was taken and was proceed for investigating the chemical constituents present in them. The extracts were taken to investigate for screenings of carbohydrate, glycoside, tannins, flavonoids, phenolics compounds, gum, mucilage, resins, volatile oil etc. all the test was performed and the results were recorded[4].

IN VITRO ANTI-INFLAMMATORY ACTIVITY

Heat induced hemolytic method[5]

Preparation of red blood cells (RBCs) suspension

Fresh whole human blood (10ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10 % v/v suspension with normal saline[5]. The reaction mixture (2ml) consisted of 1ml of test sample solution and 1ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was taken as a

standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56° C for 30 min. at the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent membrane stabilization activity was calculated by using the formula (Table No-2). % of inhibition = 100X {Vt / Vc-1}. Where, Vt = Optical density of test, Vc = Optical density of control.

Inhibition of albumin denaturation[6]

The reaction mixture (5 ml) consisted of 0.2 ml egg albumin from fresh hen's egg, 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of extract so that so that final concentrations become 50, 100, 200, 400 and 800 µgm/ml. Similar volume of double distilled water served as control. Then the mixtures were incubated at 37± 2°C in a BOD incubator for 15 mins and then heated at 70°C for 5 mins. After cooling the absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank. Diclofenac sodium at the final concentration of 50, 100, 200, 400 and 800 µgm/ml was used as reference as reference drug and treated similarly for absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula: Percentage inhibition = 100 X [Vt – Vc/1], Where, Vt = absorbance of test sample, Vc = absorbance of control.

Table 1: *Invitro* anti-inflammatory activity of *Ficus virens* bark methanolic extract by Heat induced hemolytic method

S. No	Extract	Dose (µg / ml)	Absorbance (nm)	% inhibition of denaturation
1	Control	-	0.075±0.01	-
2	FVBME	100	0.114±0.12	26.34±0.00
3	FVBME	200	0.133±0.18	50.64±0.01*
4	FVBME	500	0.142±0.23	64.63±0.01*
5	Aspirin	200	0.185±0.60	90.52±0.12*

Table 2: *Invitro* anti-inflammatory activity of *Ficus virens* bark methanolic extract by in Protein denaturation

S. No	Extract	Dose (µg / ml)	Absorbance (nm)	% inhibition of denaturation
1	FVBME	50	0.095±0.006	45.6±0.00
2	FVBME	100	0.128±0.004	50.2±0.33*
3	FVBME	200	0.167±0.003	78.6±0.33*
4	DS	20	0.149±0.005	69.23±0.33*
5	DS	40	0.166±0.003	79.16±0.33*
6	DS	100	0.199±0.006	95.38±0.33*

RESULTS

The inhibition of hypo tonicity induced HRBC membrane lysis i.e) stabilization of HRBC membrane was taken as a measure of the anti inflammatory activity. The percentage of membrane stabilization for methanolic extracts and aspirin were done at 100, 200, 500µg/ml. methanolic extract of *Ficus virens* are effective in inhibiting the heat induced hemolytic of HRBC at different concentration are shown in the Table No 2. It showed the maximum inhibition 64% at 200µg/ml. The inhibitory effect of different concentration FVBME on protein denaturation are shown in the Table No.2. FVBME at different concentration showed significant inhibition of denaturation of egg albumin in a dose dependent manner.

The *in vitro* anti inflammatory activity of the extract was comparable to the diclofenac sodium. A significant difference in the inhibition of thermally induced protein denaturation was observed in core of extract. When compared with standard drug at concentration of 200µg/ml.

DISCUSSION

The enzyme phosholipase A_2 Is known to be responsible for the formation of mediators of inflammatory such as prostaglandins and leukotrienes which by attracting polymorpho nuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals[7]. Phospolipase A_2 comments phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase to prostaglandins, which are major components that induce pain inflammation[8]. Erythrocytes have been used as a model system by a number of scientists to investigate interaction of drugs in the membranes.

The erythrocyte membrane resembles to lysosomal membrane and as seen the erythrocyte could be extrapolated to the stabilization of lysosomal membrane[9]. Hence, the methanolic extract of *Ficus virens* possesses membrane stabilization properties, limiting protein denaturation process and white blood cell anti migration properties. Therefore, the extract leads to effective RBC membrane stabilization and protein inhibition, denaturation both contributing to invitro anti-inflammatory activity. Further, definitive studies were necessary to ascertain the mechanism and constituents behind its anti-inflammatory actions.

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