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Investigation of *In Vitro* Anti-Inflammatory and *In Vitro* Anti-Oxidant Activity of Bark of *Albizia procera* Benth

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ABSTRACT

Albizia procera belongs to the family mimosaceae, found in the sub-Himalayan tracts from Yamuna eastwards to west Bengal, Gujarat, South India and in the Andamans, Madagascar which is used in the treatment of ulcer, cancer, stomach and intestinal diseases and rheumatism. The qualitative phyto-chemical screening showed the presence of phenols, steroids, alkaloids, flavanoids, tannins, carbohydrates, terpenoids, saponins and proteins. The Petroleum ether, Chloroform, Ethyl acetate, Ethanol and Hydro alcohol extracts of the bark of *Albizia procera* were subjected to *In vitro* Anti- Inflammatory activity by HRBC membrane stabilization method and *In vitro* antioxidant activity by Lipid Per Oxidation method in various concentrations i.e. 10, 50, 100, 200, 400, 800, 1000 µg/ml. In Anti-Inflammatory activity the effect was represented as follows Ethanol> Ethyl acetate > Hydro alcohol > Chloroform>Petroleum ether. In Lipid per oxidation activity, the effect was represented as follows Ethanol > Hydro alcohol> Ethyl acetate > Chloroform >Petroleum ether *Albizia procera* exhibits the above activity which can be accredited by phenolic acids present in it.

Keywords*: Albizia procera*, Lipid Per Oxidation, HRBC Membrane Stabilization.

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INTRODUCTION

In recent times, drug discovery from natural products has provided unique opportunities in the last decade due to mélange of natural product chemistry with modern biology. There is a demand for cost effective medication. Plant based antioxidant compounds play a major role by prevention of free radicals. Natural antioxidants are free from side effects and less expensive[1]. *Albizia procera* (Family: mimosaceae). (Local Name : kondavagai) is one of the medicinal plant scientifically investigated by the medicinal plant researchers. It is a medicinally important plant used for the treatment of stomach and intestinal diseases. A decoction of the bark is given in rheumatism and haemorrhage. The work on the chemical composition of the bark revealed the presence of flavonoids and tannins. Many flavonoids have remarkable anti-inflammatory activity. The present study was carried out to evaluating the anti-oxidant and anti-inflammatory activity of the Bark of *Albizia procera*[2].

PLANT

The Barks of *Albizia procera* were collected from the Sengottai, Tirunelveli, Tamil Nadu, India in the month of November 2009. The plant material was identified and authenticated by Mr. V.Chelladurai, Retired Research officer-botany, C.C.R.A.S. Govt. of India, Tirunelveli. The Collected plant material was free from diseases and also free from contamination of other plants.

REPORTED ACTIVITY

Spasmolytic activity, Cytotoxicity activity, Haemolytic activity and Spermicidal activity

PRIOR ISOLATED CONSTITUENTS

Bi flavonoids, Triterpene glycosides, Saponins, Sapogenins and Fatty acids

USES IN TRADITIONAL MEDICINE [3]

The plant is used for stomach and intestinal diseases. A decoction of the bark is given in rheumatism and haemorrhage. It is used as a fish poison. The pulp is suitable for writing and printing papers. All parts of the plant are used for the treatment of cancer.

METHODOLOGY

Determination of anti-inflammatory activity by HRBC membrane stabilization method [3]

HRBC (human red blood cell) method was used for the estimation of anti-inflammatory activity.10 ml of Blood was collected from healthy volunteer and mixed with equal volume of sterilized Alsevers solution (2% dextrose, 0.8% sodium citrate, 0.08% citric acid and 0.42% of sodium chloride in distilled water). The blood was centrifuged at 3000 rpm the packed cells

were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline. The different concentrations of drug samples were prepared. The assay mixture contains the drug, 1 ml phosphate buffer, 2 ml hyposaline, 0.5 ml HRBC suspension. Diclofenac sodium as the reference drug and 2 ml of distilled water as control. All the assay mixtures was incubated at 37^0C for 30 minutes and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560nm. The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100 %. Percentage of protection was calculated using the following equation.

Determination of lipid peroxidation activity [5]

Liver was quickly excised after decapitation, washed several times with ice-cold saline solution (0.15M kcl, pH 7.4) and then homogenized in the same saline solution. A 10% liver homogenate was prepared and test solution contained homogenate with a protein concentration of 500µg/ml. The lipid peroxidation was initiated by addition of 25 µM ferrous sulphate, 100 µm ascorbate and 10 mM potassium hydrogen phosphate. And the homogenates was incubated at 37^0C for 30 minutes with different concentrations of extracts. The lipid peroxidation was measured by estimation of thiobarbituric acid reactive substance (TBARS) and the standard was curcumin. The absorbance was measured at 532nm

$$\text{Percentage inhibition} = \frac{\text{Control - Test}}{\text{Control}} \text{ X } 100$$

RESULTS AND DISCUSSION

The various extracts of the Bark of *Albizia procera were* subjected to *In vitro* anti-inflammatory activity in various concentrations i.e.10, 50, 100, 200, 400, 800 & 1000 µg/ml. In anti-inflammatory activity, the percentage inhibition was found to be 67.32% (Petroleum ether), 68.12% (Chloroform), 76.37% (Ethyl acetate), 84.23% (ethanol)82.66% (Hydro alcohol) and 94.20% (Diclofenac sodium). All the extracts showed positive response. The Chloroform, Ethyl acetate, Ethanol and Hydro alcoholic extracts showed dose dependent response. The Petroleum ether extract showed bi phasic response. This effect may be due to the presence of steroids, alkaloids and flavonoids present in various extracts. The effect was represented as follows

Ethanol > Hydro alcohol > Ethyl acetate > Chloroform > Petroleum ether

The IC_{50} values were found to be 138.9µg/ml for petroleum ether, 336.8 µg/ml for Chloroform, 222.8 µg/ml for Ethyl acetate, 216.4µg/ml for ethanol and 205.4µg/ml for Hydro alcohol.

In Lipid per oxidation activity, the percentage inhibition was found to be 75.81% (Petroleum ether), 77.51 % (Chloroform), 80.42% (Ethyl acetate), 85.93% (ethanol) 83.30% (Hydro alcohol) and 94.20% (Diclofenac sodium). All the extracts showed positive response. The Petroleum Ether, Chloroform, Ethyl acetate and Ethanol extracts showed dose dependent response. The Hydro alcohol extracts showed bi phasic response. This effect may be due to the presence of steroids, alkaloids and flavonoids present in various extracts. The effect was represented as follows

Ethanol > Hydro alcohol > Ethyl acetate > Chloroform > Petroleum ether

In Lipid per oxidation activity, the percentage inhibition was found to be 67.45% (Petroleum ether), 68.13% (Chloroform), 75.53% (Ethyl acetate), 78.39% (ethanol) 76.43% (Hydro alcohol) and 92.32% (Curcumin). All the extracts showed positive response. The Ethyl acetate, Ethanol and Hydro alcoholic extracts showed dose dependent response. The Petroleum Ether and chloroform extracts showed bi phasic response. This effect may be due to the presence of steroids, alkaloids and flavonoids present in various extracts. The effect was represented as follows

Ethanol > Hydro alcohol > Ethyl acetate > Chloroform > Petroleum ether.

The IC_{50} values were found to be 148.7µg/ml for petroleum ether, 162.6 µg/ml for Chloroform, 166.0 µg/ml for Ethyl acetate, 194.5µg/ml for ethanol and 177.0 µg/ml for Hydro alcohol.

TABLE-1: *IN VITRO* ANTI- INFLAMMATORY ACTIVITY OF VARIOUS EXTRACTS OF BARK *OF ALBIZIA PROCERA* BY HRBC MEMBRANE STABILIZATION METHOD

Concentration (µg/ml)	% inhibition					
	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Hydro alcohol	Diclofenac sodium
10	30.53±0.532	31.18±0.359	32.76±1.123	35.60±0.845	33.85±0.639	94.20±0.124
50	37.56±0.756	37.93±1.276	39.33±0.721	42.12±0.612	40.37±0.236	
100	43.29±0.712	44.22±0.601	47.23±0.653	49.74±0.741	46.70±0.589	
200	50.37±0.341	51.67±0.667	55.21±0.512	55.07±0.362	53.53±0.312	
400	58.23±0.612	57.29±1.180	62.32±0.645	63.33±0.526	65.98±0.624	
800	67.32±0.641	62.51±0.422	69.13±0.912	78.37±0.525	74.96±0.612	
1000	60.88±0.576	68.12±0.211	76.37±0.932	84.23±1.034	82.66±0.427	
IC_{50}	138.9	336.8	222.8	216.4	205.4	

Each values represents mean ±S.D of 3 observations

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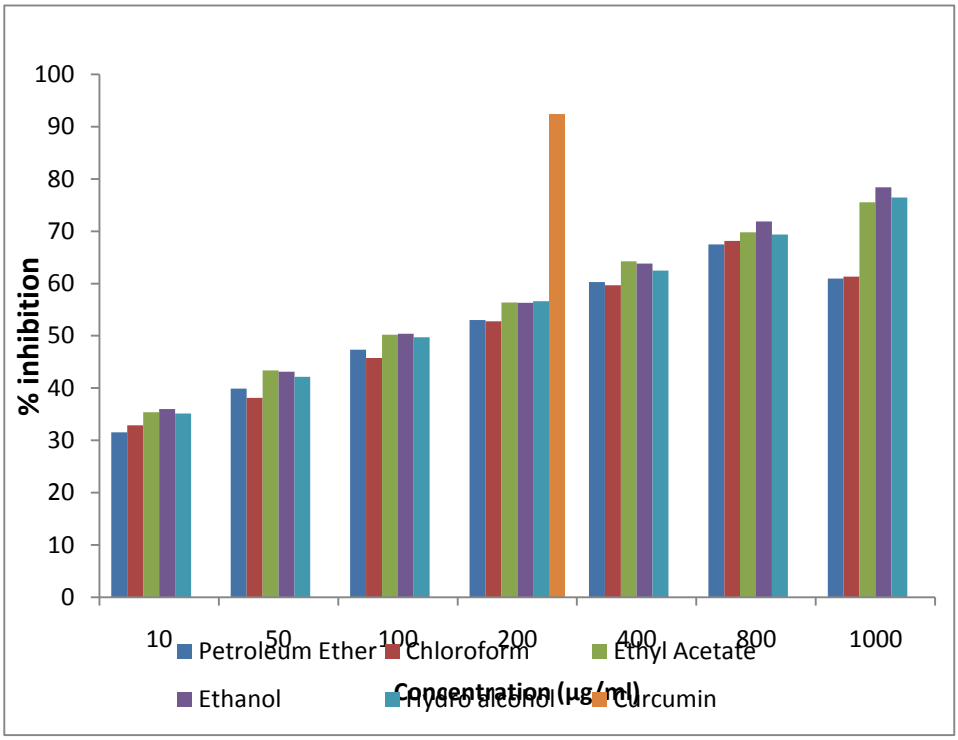
TABLE-2: THE PERCENTAGE INHIBITION OF DIFFERENT EXTRACTS OF BARK OF *ALBIZIA PROCERA* BY LIPID PER OXIDATION METHOD

Concentration (µg/ml)	% inhibition					
	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Hydro alcohol	Curcumin
10	31.53±0.143	32.89±073	35.39±0.712	36.02±0.56	35.12±0.763	92.32±0.145
50	36.91±1.230	38.16±0.62	43.36±0.684	43.16±0.90	41.19±0.856	
100	45.33±1.357	45.76±0.68	49.21±0.432	51.42±0.83	49.72±0.812	
200	53.04±1.180	52.75±0.42	57.37±0.546	57.30±0.85	56.59±0.527	
400	60.26±0.896	59.66±0.53	62.24±0.512	63.80±0.52	62.50±0.593	
800	67.45±0.732	68.13±0.63	69.81±0.623	71.87±0.57	69.37±0.236	
1000	61.98±0.612	60.34±1.00	75.53±0.662	78.39±0.20	76.43±0.329	
IC_{50}(µg/ml)	148.7	162.6	166.0	194.5	177.0	

Each values represents mean ±S.D of 3 observations

GRAPH-1: *IN VITRO* ANTI- INFLAMMATORY ACTIVITY OF VARIOUS EXTRACTS OF BARK *OF ALBIZIA PROCERA* BY HRBC MEMBRANE STABILIZATION METHOD



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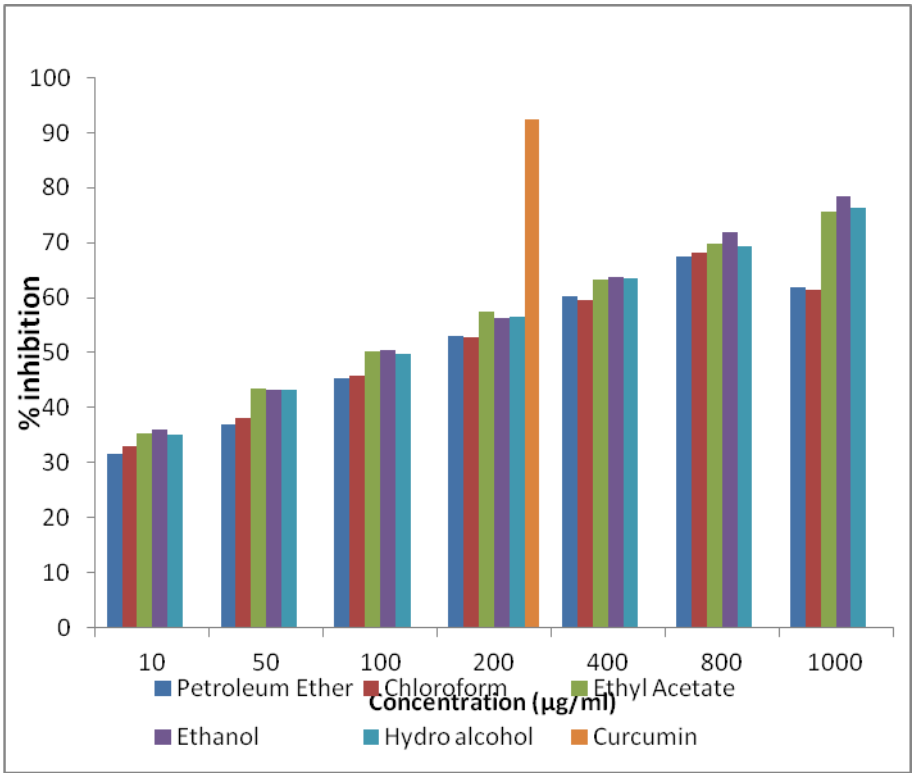
GRAPH-2: THE PERCENTAGE INHIBITION OF DIFFERENT EXTRACTS OF BARK OF *ALBIZIA PROCERA* BY LIPID PER OXIDATION METHOD



CONCLUSION

The *In vitro* studies on bark of *Albizia procera* showed the presence of significant anti-oxidant and anti- arthritic activities. The Ethanol extract shows more anti-oxidant and anti-arthritic activities. All these activities may be due to the presence of terpenoids, steroids, alkaloids, flavonoids and tannins. Our future aim is to isolate the chemical constituents responsible for the above activities and also to carry out the *in vivo* investigation.

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