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EVALUATION OF THE INVITRO ANTI-INFLAMMATORY ACTIVITY OF THE PLANT *POLYCARPAEA AUREA*

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ABSTRACT

The species of Polycarpaea are used for many medicinal purposes such as in diabetes, reduction in cholesterol level, anti microbial action, hepatoprotective activity etc. Preliminary phytochemical screening for successive solvent extracts, revealed the presence of alkaloids, tannins, carbohydrates, sterols, gums and mucilage. Anti inflammatory studies using HRBC (membrane stabilization) and protein denaturation methods.

Key words: *Polycarpaea aurea*, anti inflammatory, protein denaturation, HRBC.

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INTRODUCTION

Man has been using herbs and plants products for combating diseases since times immemorial. The traditional system of medicine is so engrained in our culture that, even now 75% of the Indian population depend on this indigenous system for relief. With such a huge section of an ever-increasing population relying on herbal remedies, it is imperative that the plant products which have been in use for such a long time be scientifically supported for their efficacy. The whole plant *Polycarpaea aurea Wight & Arn* is used as a diabetic and diaphoretic. The native practitioners in and around Chittoor District, India, have claimed that the whole plant is being traditionally used in the treatment of diarrhea.

According to folk medicine the plant is used for antioxidant, anti-inflammatory and antimicrobial (1). The inflammatory response represents a generalized response to infection or tissue damage and is designed to remove cellular debris to localize invading organisms and arrest the spread of infection. The inflammatory response is characterized by the following symptoms: Reddening of the localized area, swelling, pain and elevated temperature (2, 3).]

MATERIALS AND METHODS

Plant Collection and Drying

The plant *Polycarpaea aurea* Wight & Arn were collected from Kasaragod. The plant material was taxonomically identified by the botanist, Mr. Biju P, Assistant professor, Department of Botany, Government College, Kasaragod. The plant dried under shade for about 7 days and then powdered with mechanical grinder and stored in an air tight container.

Preliminary Phytochemical Screening

Extraction of the dried powder of the *Polycarpaea aurea* was carried out by successive solvent extraction using solvents of increasing polarity viz. petroleum ether, n-hexane, chloroform, acetone, ethanol and water. Each extract was then filtered, the solvent distilled off and finally the dried extract was obtained. These extracts were used for preliminary phytochemical screening. Preliminary phytochemical screening reveals the presence of alkaloids, flavanoids, sterols, tannins, carbohydrates, aminoacid, saponins, gum and mucilages (4).

Extraction of Total Aqueous and Alcoholic Extracts of Plant

The total alcoholic extract was prepared by using soxhlet apparatus. Total aqueous extract was prepared by Maceration method (4).

Anti-Inflammatory Activity

Inhibition of Protein Denaturation (5-7)

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of plant extracts of 50, 100, 150, 200µg/ml concentration (total alcoholic and aqueous) and pH was adjusted to 6.3 using 1 N HCl. The samples were incubated at 37^0C for 20 min and then heated at 57^0C for 3min. Diclofenac was used as a standard drug (5, 10, 50, 100, µg/ml). After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Absorbance was measured spectrophotometrically at 660 nm. For control tests 0.05 ml distilled water was used instead of extracts while product control lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.

Percentage

$$\text{Inhibition} = (Abs_{Control} - Abs_{Sample/standard})/Abs_{Control} \times 100$$

Calculation of IC_{50} (50% inhibitory concentration)

The concentration (µg/ml) of the drug required to denature 50% protein was calculated from the graph. The IC_{50} value was calculated for inhibitory concentration of both the samples and standard.

Membrane Stabilization Test (6-9)

Preparation of red blood cells (RBCs) suspension

Fresh whole human blood (10ml) was collected and mixed with equal volume of sterilized Alsever Solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm for 10 minutes and the packed cells were washed three times with isosaline (0.85% pH 7.2). Volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline.

Heat Induced Hemolysis

The principle involved is stabilization of human RBC membrane by hypotonicity induced membrane lysis. The assay mixture (4ml) consisted of 1 ml 0.15M phosphate buffer (pH 7.4), 2 ml hyposaline (0.36%), 0.5 ml HRBC suspension (10% v/v) with 0.5 ml of test sample of each concentrations (50, 100, 150, 200µg/ml) of total aqueous and alcoholic extracts. Diclofenac was used as a standard drug of concentrations (5, 10, 50, 100µg/ml). All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56^0C for 30min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm 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. Percentage membrane stabilization activity was calculated by the formula as shown below.

$$\% \text{ Hemolysis} = (Abs_{test}/ Abs_{control}) \times 100$$

$$\%\text{Protection} = 100 - [(Abs_{test}/ Abs_{control}) \times 100]$$

Calculation of IC_{50} (50% inhibitory concentration)

The concentration (µg/ml) of the drug required to 50% protection was calculated from the graph. The IC_{50} value was calculated for protection concentration of both the samples and standard.

RESULTS AND DISCUSSION

Protein denaturation method (Bovine serum albumin)

In this method, the total aqueous and alcoholic extracts of *Polycarpaea aurea* (50, 100, 150 and 200µg/ml) displayed significant activity. The extracts at a concentration of 200µg/ml showed maximum activity. The results are shown in table-1 and figure-1.

Table-1 Results of *in vitro* anti inflammatory activity of total aqueous and alcoholic extracts of *Polcarpaea aurea* by Protein denaturation method

S. No	Sample	Concentration (µg/ml)	Absorbance at 660nm	% inhibition
1.	Control	-	1.142	-
2.	Standard (Diclofenac)	5	0.79	30.29
		10	0.661	42.12
		50	0.477	58.23
		100	0.318	72.15
3.	Alcoholic extract	50	0.659	42.29
		100	0.543	52.45
		150	0.428	62.52
		200	0.385	66.28
4..	Aqueous extract	50	0.776	32.05
		100	0.632	44.65
		150	0.498	56.39
		200	0.414	63.74

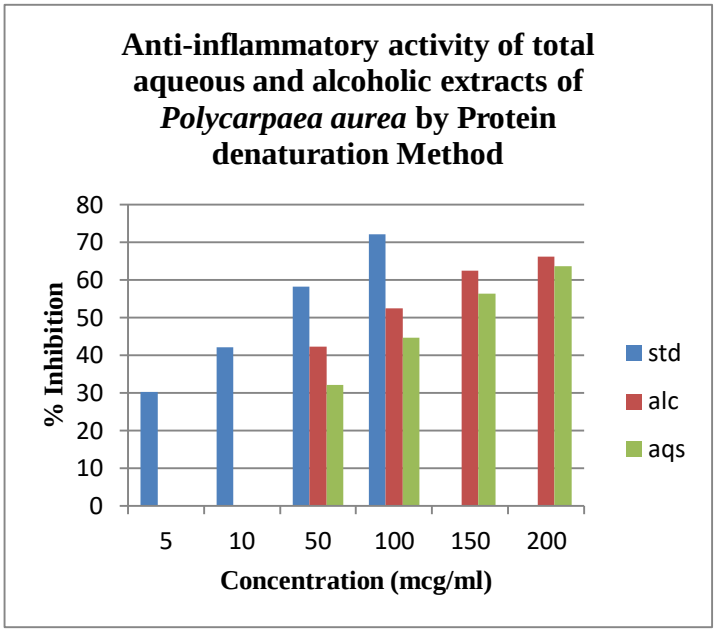

Fig-1 Anti inflammatory activity of total aqueous and alcoholic extracts of *Polcarpaea aurea* by Protein denaturation method

IC_{50} value was calculated for aqueous and alcoholic extracts of *Polycarpaea aurea* and standard from the graph. The values are tabulated in table-2.

Table-2 Results showing IC_{50} value of total aqueous and alcoholic extracts of *Polycarpaea aurea* by Protein denaturation method

S. No	Sample	IC_{50} (mcg/ml)
1.	Standard	49.92
2.	Alcoholic extract	117.54
3.	Aqueous extract	135.30

Membrane stabilization test

The total aqueous and alcoholic extracts of the *Polycarpaea aurea* at different concentrations showed significant stabilization towards human RBC membrane. The results are shown in table- 3 and fig-2.

Table-3 Results showing *in vitro* anti-inflammatory activity of total aqueous and alcoholic extracts of *Polycarpaea aurea* by HRBC method

S. No	Sample	Conc (µg/ml)	Absorbance at 560nm	% Haemolysis	% Protection
1.	Control	-	0.471	-	-
2.	Standard (Diclofenac)	5	0.246	52.22	47.78
		10	0.213	45.22	54.78
		50	0.159	33.76	66.24
		100	0.096	20.38	79.61
3.	Alcoholic Extract	50	0.311	66.02	33.98
		100	0.238	50.53	49.47
		150	0.165	35.03	64.96
		200	0.110	23.35	76.64
4..	Aqueous extract	50	0.342	72.61	27.38
		100	0.286	60.72	39.28
		150	0.217	46.07	53.93
		200	0.148	31.42	68.58

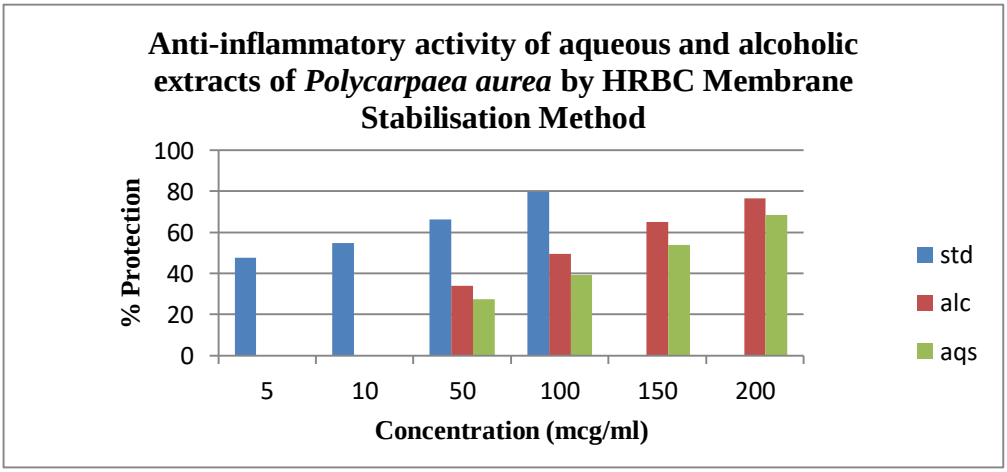

Fig-2 anti-inflammatory activity of total aqueous and alcoholic extracts of *Polycarpaea aurea* by HRBC method

IC_{50} value was calculated for total aqueous and alcoholic extracts of *Polycarpaea aurea* and standard from the graph. The values are tabulated in table-4.

Table-4 Results showing IC_{50} value of aqueous and alcoholic extracts of *Polycarpaea aurea* by HRBC membrane stabilisation method

S. No	Sample	IC_{50} (µg/ml)
1	Standard	33.64
2	Alcoholic extract	113.70
3	Aqueous extract	137.33

CONCLUSION

The air dried powder of *Polycarpaea aurea* subjected to successive solvent extraction using petroleum ether, n-hexane, chloroform, acetone, ethanol and water as per standard procedure. The various extracts obtained from successive solvent extraction were used for preliminary phytochemical analysis it reveals the presence of alkaloids, flavanoids, sterols, tannins, carbohydrates, aminoacid, saponins, gum and mucilages. The *In vitro* Anti-inflammatory activity also performed by Human Red Blood Corpuscle (HRBC) membrane stabilization and protein denaturation methods. The total alcoholic extract of plant showed more significant activity in both methods when compared with standard diclofenac sodium than that of total aqueous extract.

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