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IN VITRO ASSESSMENT OF ANTIARTHRITIC ACTIVITY OF *SPHAERANTHUS AMARANTHOIDES* BURM F.

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ABSTRACT

Present study was done to assess the *in vitro* antiarthritic activity of the plant *Sphaeranthus amaranthoides* Burm F (Asteracea). Four different extract of increasing polarity namely petroleum ether, chloroform, ethyl acetate and methanol were prepared. Antiarthritic potential of the plant was assessed by two methods namely, effect on protein denaturating and effect on membrane stabilization. The results indicated that by both the methods ethyl acetate extract was having highest activity and the activity was comparable with that of standard, diclofenac sodium.

Key Words: *Sphaeranthus amaranthoides* Burm F, membrane stabilization, protein denaturation

INTRODUCTION

When the vascular tissue of the body comes in contact with harmful stimuli like irritants, pathogens, pollens etc it releases chemical mediators like prostaglnadins, leukotrienes, prostacyclins etc which results in inflammation. Inflammation is marked by redness, swelling, and pain. Sometimes inflammation may become serious leading to chronic disease like rheumatoid arthritis[1]. Rheumatoid arthritis is an autoimmune disease. During this condition, the immune system, which protects our health by attacking foreign cells, instead attacks the body's own tissues like synovium which is a thin membrane that lines the joints. Therefore fluid builds up in the joints, causing pain and inflammation in the joint. There are number of synthetic drugs used as anti inflammatory agents, but are unsafe. Therefore an attempt has been made to evaluate the in vitro anti inflammatory and anti arthritic activity of *Sphaeranthus amaranthoides* Burm F. This is a small medicinal herb used in siddha medicine as sivakaranthai powder. Phytochemical analysis of the plant revealed the presence of flavanoids, carbohydrates, tannins, phenolics etc in various extracts. Earlier work reported in this plant were protective role of the plant on dermal wounds in Wistar rats[2], anti microbial antidiarrhoeal and phytochemical analysis[3], protective role of the plant on drug metabolizing microsomal enzymes in β-O-Galn induced hepatic rats[4], antibacterial and phytochemical screening against human pathogenic bacteria[5], antioxidant, anti mutagenic, antimicrobial activities[6], analgesic and anti inflammatory activity[7].

MATERIALS AND METHOD

Collection of the plant: The whole plant of *Sphaeranthus amaranthoides* Burm F was collected from Thutukudi district, Tamil nadu. Authentication was done by Mr.V.Chelladurai, Retired research officer, Botany, CCRAS, Government of India.

Drugs and chemicals: All the chemical used were of analytical grade. All the reagents were prepared with double distilled water.

Preparation of extract: Plant material was allowed dry in shade at room temperature. Dried material was grinded to get coarse particles. The powder was extracted with petroleum ether, chloroform, ethyl acetate and methanol in a soxhlet apparatus. The solvents were distilled and extracts were concentrated

to a constant weight using rotary evaporator. The extracts were stored in desiccators.

Preliminary phytochemical analysis: As per the standard procedure[8,9] preliminary phytochemical analysis was done on different extracts and the results were given in table 1.

Inhibition of Protein Denaturation**:** Test solution consist of 0.45 ml of Bovine serum albumin (5 % W/V aqueous solution) and 0 .05 ml of extracts of different concentration varying from 10, 50, 100, 200, 400, 600, 800 and 1000 µg/0.05 ml. Test control contains 0.05 ml of distilled water instead of extracts. Product control consisted of only different concentrations of extract and distilled water. It didn't contain bovine serum albumin. Product test control also lacked bovine serum albumin. pH of the solutions were adjusted to 6.3 using little quantities of 1 N hydrochloric acid and incubated at room temperature for 20 mins followed by 57°C for 3 mins. Cooled and added 2.5 ml of phosphate buffered saline ph 6.3 to all the test tubes. The absorbance of the resulting solution was measured at 416 nm. Diclofenac sodium was used as a standard[10,11]. The results were depicted in table 2 and figure 1.

Effect on Membrane Stabilization**:** Human red blood cell membrane is stabilized by hypo tonicity induced membrane lysis. The assay mixture contains 1 ml of phosphate buffer [pH 7.4, 0.15 M] and 2 ml of hypo saline [0.36 %]. To this 0.5 ml of HRBC suspension [10 % v/v]) and 0.5 ml of plant extracts of various concentrations (10, 50, 100, 200, 400, 600, 800 and 1000 µg/0.5ml) were added. Diclofenac sodium (10, 50, 100, 200, 400, 600, 800 and 1000 µg/0.5ml) was used as a standard drug and control consisted of 2 ml of distilled water instead of hyposalin (so that it produce 100 % hemolysis). All the test tubes were incubated at 37º C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm[12,13]. The results were depicted in table 3 and figure 2.

Statistical analysis: For each sample, three replicates were used for statistical analysis and the values were reported as mean ± SD.

RESULTS AND DISCUSSION

Preliminary phytochemical evaluation of ethylacetate extract indicated the presence of all the tested secondary metabolites except steroids and glycosides. In arthritis auto antigens are produced due to proteinase action, denaturation of protein and membrane lysis. Therefore an agent that prevents denaturation of protein can be considered as anti inflammatory agent. In the present study effect of various extracts of the plant *Sphaeranthus amaranthoides* Burm F were evaluated for their inhibitory effect on bovine serum albumin. The results showed that except petroleum ether extract all other extracts were having more inhibitory effect. Percentage inhibition of chloroform extract, methanol extract and petroleum ether extract were found to be 80.43, 75.17, 64.9 % respectively and ethyl acetate extract was found to exhibit more inhibition of protein denaturation of about 91.48%. In HRBC stabilization method also all the extracts were found to be more active except petroleum ether extract. The stabilizing activity of the extract may be due to shrinkage of the cell or expansion of membrane, and an interaction with membrane proteins there by resulting an increase in surface area/volume ratio of the cells[14, 15]. Percentage protection of petroleum ether was found to be only 69.11 % only, at a concentration of 1000 µg/ml. The IC_{50} value was found to be 125 µg/ml and 200 µg/ml for ethyl acetate and chloroform extract respectively.

CONCLUSION

In the present study inhibition of protein denaturation of different extracts of the plant was studied using bovine serum albumin. The results represented that ethyl acetate extract was more active. In HRBC membrane stabilization method of all the four extract also revealed that ethylacetate extract was having more activity. By both the methods it was found that ethyl acetate extract was having highest activity and the activity was comparable with that of standard, diclofenac sodium. Further it is necessary that the active molecule which is responsible for the above said activities should be identified and isolated.

Table 1 Phytoconstituents present in different extracts

Constituents	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract
Terpenoids	-	+	++	-
Flavonoids	-	++	+	+
Steroid	++	+	-	-
Glycoside	++	+++	-	-
Sugar	-	+++	+	-
Alkaloid	-	++	++	+
Quinone	-	-	+	+++
Phenol	-	++	+	++
Tannin	-	+	+	+

+++ more, ++ moderate, + mild, - Absent

Table 2: Inhibition of protein denaturation

Concentration µg/ml	Percentage protection				
	Standard	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract
100	64.49±0.84	39.66±0.64	48.58±1.18	44.95±0.86	41.89±1.42
200	71.92±1.42	46.29±1.02	50.42±0.96	60.42±1.35	55.73±0.46
400	79.58±1.24	52.73±0.57	61.59±1.44	68.26±0.92	60.36±0.95
600	83.76±0.75	55.19±0.74	72.64±0.60	81.05±0.85	62.42±0.68
800	91.62±1.31	59.32±0.91	77.13±1.49	87.12±1.72	71.44±0.29
1000	96.03±0.73	63.81±1.10	81.43±1.11	92.95±0.58	76.20±1.19

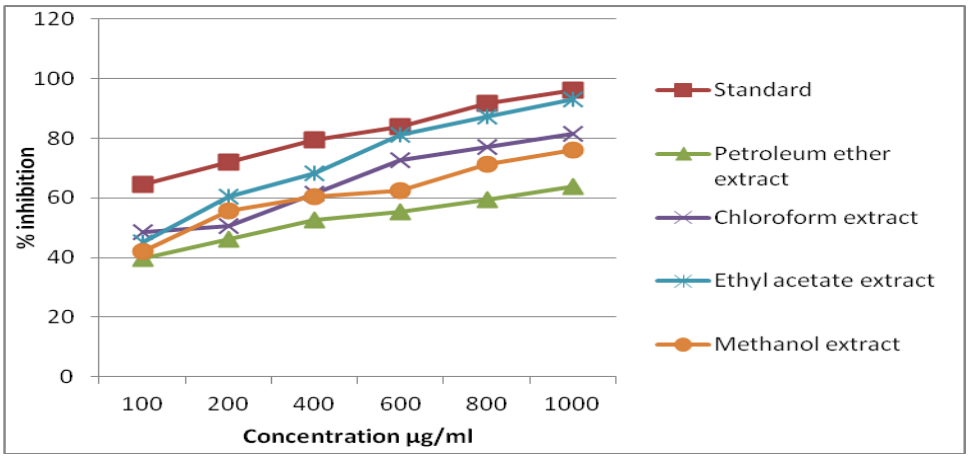

Figure 1: Percentage inhibition of protein denaturation of different extracts

Table 3: Effect on Membrane Stabilization

Concentration µg/ml	Percentage protection				
	Standard	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract
100	70.84±0.69	40.84±1.50	41.92±1.39	44.95±0.86	41.89±1.43
200	75.88±1.67	47.81±1.23	48.42±1.06	59.09±1.50	56.70±1.37
400	81.24±1.40	53.69±1.12	65.93±1.65	67.26±1.65	60.33±0.90
600	84.72±1.17	56.04±1.18	71.31±1.32	80.72±1.07	63.58±1.20
800	91.50±1.36	60.67±0.81	75.53±1.46	85.12±1.37	71.58±0.45
1000	94.35±1.40	64.90±1.04	80.43±1.23	91.48±1.08	75.17±0.87

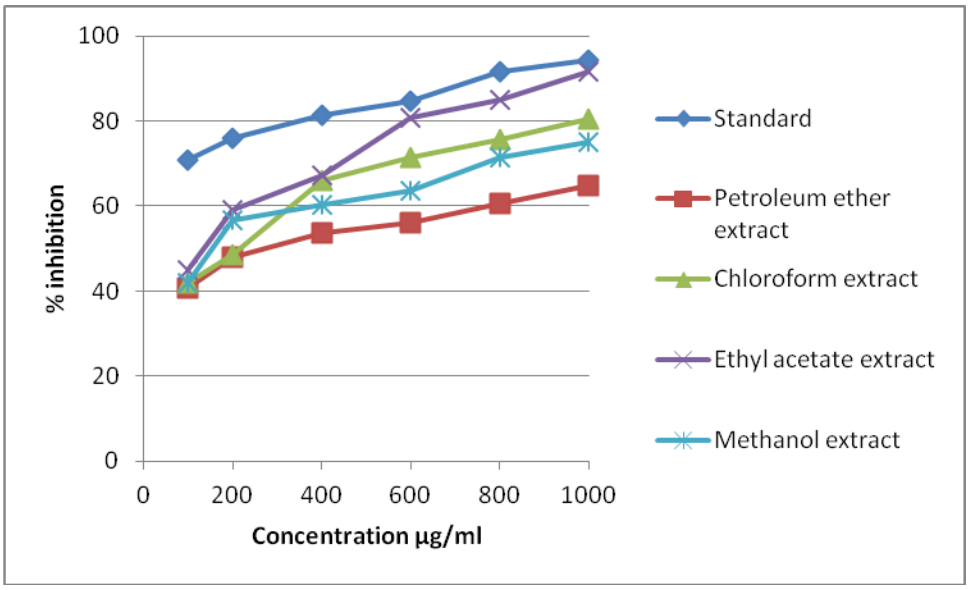

Figure 2 Percentage inhibition of membrane stabilization of different extracts

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