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Anti-Inflammatory Activity Of The Various Solvent Extracts Of Eichhornia crassipes (Mart.) Solms

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Abstract: The various solvent extracts of *Eichhornia crassipes* (Mart.) Solms was studied for its anti-inflammatory activity on formaldehyde induced paw oedema in Male Swiss Albino mice. Due the presence of wide range of phytochemical constituents in the petroleum ether, ethyl acetate and aqueous extracts of *Eichhornia crassipes* as noted from the results of the phytochemical screening, these extracts were assayed for the *in vivo* anti-inflammatory activity. It was found that all the tested extracts of leaves and shoot portion of *Eichhornia crassipes* possess significant anti-inflammatory activity. Percent inhibition obtained from anti-inflammatory activity has shown that the extracts have very strong activity to prevent pains which provides strong scientific evidence to the folkloric use of this plant in the treatment of inflammation in animals.
Keywords: *Eichhornia crassipes*; hyacinth; extract; anti-inflammatory; ketoprofen; formaldehyde; paw oedema.

Introduction

Aquatic plants have economic and environmental uses, depending on the natural characteristics (1). Originally from South America, *Eichhornia crassipes* (Mart.) Solms, a free-floating vascular plant, is one of the world's most pervasive aquatic plants. It is universally called Water hyacinth, but the precise common name is 'Waterhyacinth' and not 'Water hyacinth' (2).This plant is known to cause major ecological and socio-economic changes (3). Although it is a most obnoxious weed, it possesses nutritionally important compounds like phenolics, flavonoids, glutathione and many metabolites (4). Several phenalenones have been isolated from the ethyl acetate extract of the plant (5, 6). Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins. Alkaloids and flavonoids have been used as antiviral, antibacterial and anticancer agents (7). The uses of plant-derived products as disease control agents have been studied, since they tend to have low toxicity to mammals, less environmental effects and wide public acceptance (8).

In Chhattisgarh, *E.crassipes* is being used as styptic. The fresh juice of this weed is used to treat fresh wounds as the tribes believe that it stops further spread of infection. Rice farmers consider this as a best first aid remedy for minor injuries. Along with vinegar, it is being used in treatment of septic wounds (9). Back in folklore medicine, *E.crassipes* has been used to ease swelling, burning, haemorrhage, and goiters. In the animal kingdom, it has been used as a tonic for the skin of horses, for irritation and inflammation (10). Due to lack of modern medical facilities in remote and forest areas, tribal people who have the traditional knowledge, use indigenous plants in treating various ailments. This indigenous art of healing has to be transformed to an exact

science (11). Hence, the present work was aimed to evaluate the anti-inflammatory activity of various extracts of *E.crassipes.*

Experimental

Plant Collection

E.crassipes was collected from Singanallur boat house, Coimbatore, Tamil Nadu in the month of March. The plant was identified by Dr.G.V.S.Murthy, Scientist F & Head of Office, Botanical Survey of India, Sourthern Regional Centre, Coimbatore- 641 002 with the number BSI/SRC/5/23/2011-12/Tech.

Plant Extraction

The roots of *E.crassipes* were removed and the plant was washed under running tap water to remove the trash. The leaves and shoot portion of the fresh plant material was chopped into small pieces. The fresh plant was allowed to dry under shade for about two weeks. A portion of the dried plant was extracted with petroleum ether (PE). Another portion was extracted with ethyl acetate (EA) and aqueous (AQ) by conventional refluxing method. The extracts were concentrated in a rotary evaporator at reduced pressure and stored in refrigerator for further use.

Determination Of In Vivo Anti-Inflammatory Activity

Animals

Male Swiss Albino mice (19-30 g) were used for this study. Animals were housed in polypropylene cages under room temperature and fed with standard pellets and provided with *ad libitum* drinking water.

Anti-inflammatory studies

The study was carried out by adopting the procedure of Nuhu *et al*., 2010 (12) with slight modifications (Ethical committee number KMCHRET/PhD23/2009-10). Swiss Albino mice were divided into 6 groups of 4 mice each. The groups were treated intraperitoneally; thus group 1 received 10 mg of ketoprofen per kg (+ve control), group 2 received 1 ml normal saline per kg (-ve control), groups 3, 4 and 5 received 100 mg of petroleum ether, ethyl acetate and aqueous extracts per kg body weight of mice respectively. Thirty minutes later, mice in all groups were injected with formalin and the difference in diameter of the right hind paw and left hind paw was noted. The increase in paw diameter was measured using Vernier caliper. Measurement was done immediately before and after 1-5 h following formalin injection. The percentage inhibition of the growth of oedema was calculated from the expression:

$$\text{Inhibition (\%)} = \frac{\text{Mean control} - \text{Mean treated}}{\text{Mean control}} * 100$$

Statistical analysis

Percentage inhibition of inflammation by *E.crassipes* extracts on formaldehyde induced paw oedema in mice at 4 h was analysed by one way ANOVA (Analysis Of Variance) using Sigmastat 4.1.

Results And Discussion

The anti-inflammatory effect of petroleum ether, ethyl acetate, aqueous extracts of the leaves and shoot portion of *E. crassipes* was investigated in this study. In the anti-inflammatory studies, the formaldehyde induced oedema is a multimediated phenomenon liberating diversity of mediators which could be in two phases, the first being the release of serotonin and histamine while the second after the one hour is mediated by prostaglandins. Kinins provide the cyclooxygenase products and the continuity between the phases (13, 14).

The result obtained showed a significant reduction in the growth of oedema in the hind paw of the mice when treated with the extracts. Different solvent extracts showed variable anti- inflammatory activity. This may be due to the presence of flavonoids in the petroleum ether extract, anthroquinone and phenolic compounds in ethyl acetate extract. Alkaloids, flavonoids, sterols, anthroquinone, anthocyanins, proteins and quinones were detected in the aqueous extract (15). The ethyl acetate extract and petroleum ether extract have shown maximum inhibition of the oedema (67.5% and 64.81% respectively) (Table 1) with ethyl acetate extract demonstrating the

highest anti-inflammatory activity. Anthroquinones have been demonstrated to show high anti-inflammatory activity (16). Phenolic compounds have been reported to exert anti-inflammatory activity (17). Alkaloids and flavonoids are known for their ability to inhibit pain perception. Flavonoids inhibit enzymes involved in the production of chemical mediator of inflammation (18).

Table 1-Anti-inflammatory activity studies of the various extracts of shoot and leaves of *Eichhornia crassipes*

Treatment (mg/kg)	Mean paw diameter (cm) in hours				
	T1	T2	T3	T4	T5
N/saline	0.62 ± 0.12^{b}	0.722 ± 0.10^{b}	0.52 ± 0.07^{a}	0.37 ± 0.02^{a}	0.31 ± 0.04^{a}
Ketoprofen (10 mL)	0.44 ± 0.08^{c}	0.52 ± 0.05^{d}	0.34 ± 0.03^{c}	0.25 ± 0.02^{c}	0.17 ± 0.02^{c}
PE	0.61 ± 0.12^{b}	0.71 ± 0.12^{a}	0.51 ± 0.05^{a}	0.13 ± 0.01^{e}	0
EA	0.61 ± 0.13^{a}	0.42 ± 0.07^{c}	0.18 ± 0.01^{d}	0.12 ± 0.01^{e}	0
AQ	0.61 ± 0.15^{a}	0.54 ± 0.06^{c}	0.41 ± 0.05^{b}	0.29 ± 0.02^{b}	0.25 ± 0.05^{b}

Key: PE-Petroleum ether; EA-Ethyl acetate
Values are mean ± SD of six samples in each group
Values in the column not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT)

The mean paw diameter for the standard ketoprofen at fifth hour is 0.13cm whereas it is 0.18cm for ethyl acetate extract at third hour. The standard ketoprofen showed anti-inflammatory activity at third hour whereas ethyl acetate extract showed anti-inflammatory activity starting from second hour which demonstrates the rapid action of the extract in inhibition of inflammation. The mean paw diameter for ethyl acetate extract decreases gradually from the second hour and becomes normal at fifth hour. For the ketoprofen treated mice, the mean paw diameter increases in the second hour and then it decreases from the third hour. Petroleum ether extract treated mice showed the same trend as ketoprofen but the mean paw diameter was 0.13 cm at fourth hour. Although aqueous extract showed maximum phytochemicals compared to other tested extracts, it showed a lesser percentage inhibition (21.62) of anti-inflammatory activity (Table 2). Percent inhibition obtained from anti-inflammatory activity has shown that the leaves and shoot portion have very strong activity to prevent pains.

Table 2- Percentage inhibition of Anti-inflammatory activity of *Eichhornia crassipes* extracts on Formaldehyde induced paw oedema in mice at fourth hour

Group/Treatment	Mean paw oedema + S.E.M At 4 hours	% inhibition
N/saline	0.37 ± 0.02^{a}	……
Ketoprofen (10 ml)	0.25 ± 0.02^{c}	32.43
PE	0.13 ± 0.01^{e}	64.81
Aqueous	0.29 ± 0.02^{b}	21.62
EA	0.12 ± 0.01^{e}	67.5

Values in the column not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT)

Conclusion

The formaldehyde induced anti-inflammatory activity carried out with the petroleum ether, ethyl acetate and aqueous extracts of *E.crassipes* has proved to be a scientific evidence for the use of this plant in the treatment of inflammation. The presence of phytochemicals in the extracts of this plant might be the reason for the activity. Among the studied extracts, the ethyl acetate extract showed better anti- inflammatory activity. The traditional knowledge of tribes can hence be further utilized for a better application of such plants in pharmacology.

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