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Anti-inflammatory Activity of Ethanolic Leaf Extracts of *Euphorbia heterophylla* L. and *Tamilnadia uliginosa* (Retz.) Tirveng. & Sastre

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ABSTRACT

The eathanolic leaf extracts of the medicinal plants, *Euphorbia heterophylla* and *Tamilnadia uliginosa* were tested for anti-inflammatory activity using carrageenan induced rat paw oedema. Wistar albino rats weighing about 180-200g were separated into 5 groups of 6 in each. The animals which served as positive control were given Diclofenac sodium (5mg/kg). The extract doses were administered orally one hour before commencing the experiments at a dose of 100, 200 and 400mg/kg body weight. The paw volume was measured at 0, 1, 3 and 5 hours after injection of carrageenan and percentage inhibition was calculated. The volume of paw oedema in carrageenan induced rats treated with ethanolic leaf extracts of *Euphorbia heterophylla* and *Tamilnadia uliginosa* was found to be concentration dependent. The ethanolic extract of *Tamilnadia uliginosa* leaf showed 0.906±0.036, 0.819±0.043 and 0.781 ±0.036 ml respectively in 100, 200 and 400 mg/kg treatment groups after 3rd hour of treatment. The maximum decrease of oedema volume (0.781 ±0.036 ml) was observed in 400 mg/kg treatment group after 3 hrs, next to diclofenac standard. Among the plant extracts tested, the leaf extract of *Tamilnadia uliginosa* showed highest percentage inhibition of 49.013 in 400 mg/kg treatment group 3 hrs after treatment. The study suggests that the ethanolic leaf extracts of *Euphorbia heterophylla* and *Tamilnadia uliginosa* are possessing anti-inflammatory activity.

Introduction

Medicinal plants are playing a vital role in providing various drug sources for curing different ailments. Natural products research, many times is guided by ethnopharmacological knowledge, and has brought substantial assistance to drug innovation by providing novel chemical structures and/or mechanisms of action (Rates, 2001). Medicinal plants are among the most attractive sources of new drugs, and have been shown to produce promising results for the treatment of gastric ulcer (Borrelli and Izzo, 2000).

Plants produce tens of thousands of different natural products also referred to as secondary metabolites. These metabolites were once consideration to be the result of unusual metabolism, or a form of passing storage of consequence and intermediates thereof. Although the true role of such metabolites in plants remains mostly unknown, it is evident that plants invest a great deal of resources in synthesizing, accumulating and sorting such metabolites, often produced through complex and highly regulated biosynthetic pathways operating in multiple cellular and sub-cellular compartments (Lewinsohn and Gijzen, 2009).

Plant natural products can be roughly ascribed to three main classes of compounds, phenyl propanoids, isoprenoids, and alkaloids, widely distributed in plant foods and medicinal herbs (Holstein and Hohl, 2004). Thereby, a great array of molecules, derived from plant secondary metabolism, is of extreme interest in human nutrition and pharmacology, in addition to perfumery and cosmetic industries. Phenolic compounds including gallotannins, condensed tannins and flavonoids as well as saponins and alkaloids have been implicated in pharmacological activities such as anthelmintic, antimicrobial and anti-inflammatory activities (Makkar et al., 2007).

In the present study, ethanolic extracts of two plant species, *Euphorbia heterophylla* L. and *Tamilnadia uliginosa* (Retz.) Tirveng. & Sastre mainly because of their medicinal properties. Various studies clearly showed that the plant *Tamilnadia uliginosa* possesses antimicrobial and antioxidant activities (Hossain et al., 2014; Kalpana and Prakash, 2015). The aqueous leaf extracts of *Euphorbia heterophylla* is reported to possess significant anti-inflammatory activity where the authors used aqueous extracts (Falodun et al., 2006). Hence in the present study, ethanolic leaf extracts of the selected medicinal plants have been chosen to study their anti-inflammatory activity using carrageenan induced rat paw oedema.

Materials and methods

Collection of leaves

The leaves of the medicinal plants selected for the present study were collected from Sirumalai hills (Eastern Ghats), Dindigul, Tamil Nadu and the identification was confirmed using standard local floras (Gamble and Fischer, 1957; Matthews, 1983). The names of the plants identified were *Euphorbia heterophylla* L. (Euphorbiaceae) and *Tamilnadia uliginosa* (Retz.) Tirveng. & Sastre (Family: Rubiaceae). The leaves collected were shade dried and powdered using mortar and pestle. A fine powder obtained was stored in air tight poly bags and used for preparation of extract.

Preparation of extract

The cold extraction procedure was used for extracting leaves with solvents as per the procedure adopted by Prakash and Karmegam (2012) and Vigneshwari et al. (2014). The leaves collected were transported to the laboratory for further processing. The leaves of the plants collected were individually washed with tap water, blotted with filter paper and spread over news paper for air drying under shade. After complete dryness, the leaves of individual plants were powdered using a mixer grinder. A known quantity of leaf powder (100 g) of each plant leaves was taken in a 250 ml conical flask and added with 100-200 ml of ethanol individually. The solvent-leaf powder mixtures were kept at room temperature for 48 hrs and rapidly stirred using glass rod every 8 hrs. After 48 hrs, the extract of each plant was filtered through Whatmann No.1 filter paper to exclude the leaf powder. Then each filtrate was kept in beaker on a water bath at 45ºC until the solvent gets evaporated. A greasy final material (crude extract) obtained for each plant was transferred to screw cap tubes and stored under refrigerated condition till use.

Anti-inflammatory activity

For acute anti-inflammatory study, carrageenan induced rat paw oedema method of Winter et al.

(1962) was followed. Wistar albino rats weighing about 180-200g were separated into 5 groups of 6 in each. The rat paw oedema was provoked by sub-plantar injection of 0.1ml aqueous suspension of 1% carrageenan sodium [in 0.9% NaCl (w/v)] in the left hind-paw. The hind-paw volume was measured by dipping the foot in the mercury bath of the Plethysmograph-apparatus up to the anatomical hairline on lateral malleolus (Goldenberg and Ilse, 1977). The initial paw volume was measured and recorded. The first group of animals was served as negative control and given 0.75% CMC (5ml/kg).

The second group of animals was served as positive control, which were given Diclofenac sodium (5mg/kg). The extracts and salts were administered orally one hour before commencing the experiments at a dose of 100 (Group 3), 200 (Group 4) and 400mg/kg (Group 5) body weight. 1% carrageenan suspension in normal saline was prepared 1hour before use. 0.1ml was injected under the plantar aponeurosis. After 3 hrs of the carrageen injection the hind-paw volumes were recorded. The animals were used with proper institutional ethical approval. The differences between the initial and final paw volume indicated the oedema volume due to inflammation. The percentage inhibitions produced by the standard drug and plant extracts were calculated which are directly indicative of the anti-inflammatory activity exerted. The paw volume was measured at 0, 1, 3 and 5 hours after injection of carrageenan.

$$\text{Inhibition}(\%) = \frac{\text{Oedema Vol. in Control - Oedema Vol. in Treatment}}{\text{Oedema Volume in the Control}} \times 100$$

Statistical analysis

Data are expressed as Mean±SEM. The level of statistical significance was determined by ANOVA and Duncan's multiple range tests using SPSS statistical software.

Results and discussion

The volume of paw oedema in carrageenan induced rats treated with ethanolic leaf extracts of *Euphorbia heterophylla* and *Tamilnadia uliginosa* was found to be concentration dependent (Tables 1 and 2). The increase in volume of paw oedema in standard (diclofenac) was very minimum and was statistically highly significant (p<0.001) when compared with control. The paw oedema volume was 0.997±0.040, 0.965±0.039 and 1.298±0.052 ml respectively at 1st, 3rd and 5th hour of treatment with 100 mg/kg ethanolic leaf extracts of *Euphorbia heterophylla* (Table 1). The maximum decrease of oedema volume was observed in 400 mg/kg treatment group after 3 hrs, next to diclofenac standard.

Table 1. Effect of ethanolic extract of *Euphorbia heterophylla* leaf on carrageenan induced rat paw oedema.

Treatment	Dose (mg/kg)	Paw oedema volume (ml)		
		1st hour	3rd hour	5th hour
Control	-	1.312 ± 0.066	1.571 ± 0.079	1.609 ± 0.080
Standard	5	0.801*** ± 0.048	0.613 *** ± 0.037	0.707 *** ± 0.042
Ethanolic leaf extract of *Euphorbia heterophylla*	100	0.997 ** ± 0.040	0.965 ** ± 0.039	1.298 * ± 0.052
	200	0.952 ** ± 0.052	0.843 *** ± 0.046	1.098 ** ± 0.060
	400	0.898 *** ± 0.046	0.805 *** ± 0.041	1.052 *** ± 0.054

Values expressed are Means ± SEM; n=6; ***p< 0.001, **p< 0.01 and *p< 0.05 in comparison with control. Standard = diclofenac sodium.

The percentage inhibition of paw oedema in experimental animals was higher (60.980%) in diclofenac standard 3 hrs after treatment (Fig. 1). In all the experiments the percentage increase was found to be higher at 3 hrs after treatment. The higher percentage inhibition (48.77%) of oedema volume in experimental animals was found in 400 mg/kg ethanolic extracts of *Euhporbia heterophylla* leaves which showed highly significant than control (p<0.001). However, it was lower than that of standard. In a study by Falodun et al. (2006), the aqueous leaf extracts of *Euphorbia heterophylla* is reported to possess significant anti-inflammatory activity (p<0.001) comparable to the reference drug indomethacin (10 mg/kg). At the different dose range used (50, 100 and 150 mg/kg), there were no significant differences in their anti-inflammatory activity hence they were not dose-dependent (Falodun et al., 2006). However, the present study showed dose-depended anti-inflammatory activity in ethanolic leaf extract of *Euphorbia heterophylla*.

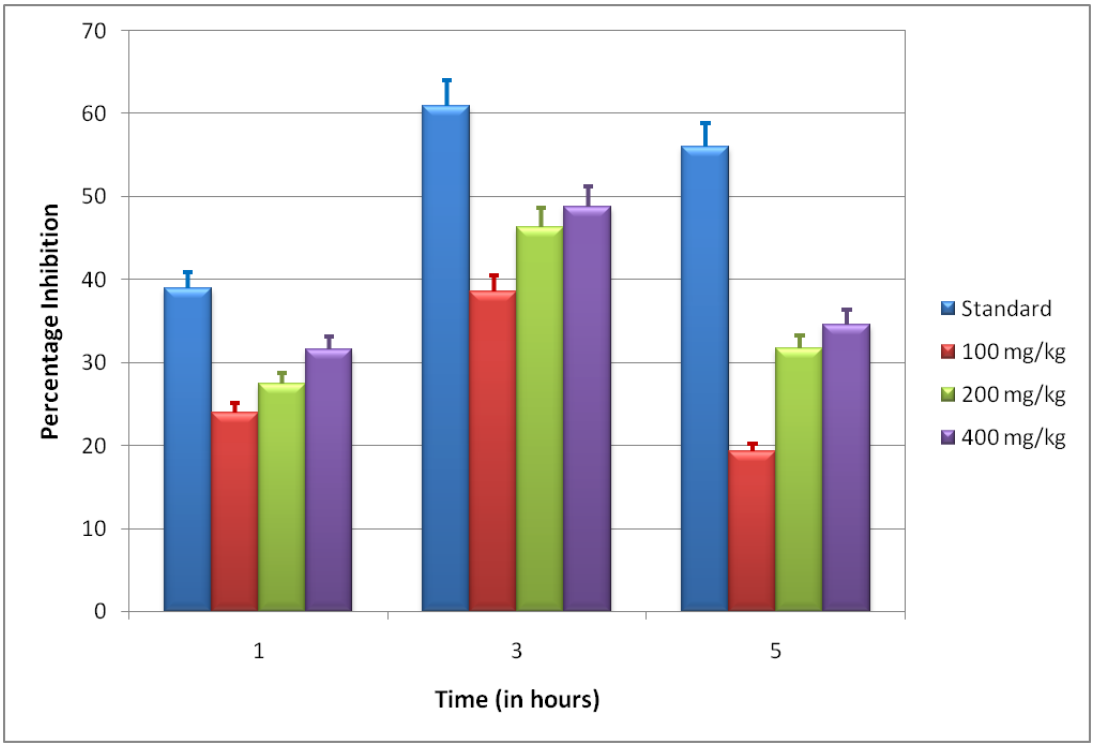

Fig. 1: Percentage inhibition of carrageenan induced rat paw oedema with the application of ethanolic leaf extracts of *Euphorbia heterophylla* in comparison with the standard drug diclofenac sodium (5mg/kg).

The ethanolic extract of *Tamilnadia uliginosa* leaf showed 0.906±0.036, 0.819±0.043 and 0.781 ±0.036 ml respectively in 100, 200 and 400 mg/kg treatment groups after 3rd hour of treatment (Table 2). The maximum decrease of oedema volume (0.781 ±0.036 ml) was observed in 400 mg/kg treatment group after 3 hrs, next to diclofenac standard. Among the plant extracts tested, the leaf extract of *Tamilnadia uliginosa* showed highest percentage inhibition of 49.013 in 400 mg/kg treatment group 3 hrs after treatment (Fig. 2) than in other treatments with extracts. Neerugatti et al. (2014) reported that the methanol extract from the roots of *Tamilnadia uliginosa* at dose 400mg/kg produced significant (p<0.001) percentage of inhibition when compared to other doses which was very close to the results of the present study with leaf extracts. Apart from anti-inflammatory activities, recent reports also support the various pharmacological activities of *Tamilnadia uliginosa.* Hossain et al. (2014) reported that in DPPH radical scavenging assay, the IC50 value of the crude

chloroform extract of *Randia uliginosa* (*R. uliginosa* is a synonym of *Tamilnadia uliginosa*) was 399.12 µg/mL, whereas IC50 value for the reference ascorbic acid was 8.77 µg/mL. In case of nitric oxide scavenging assay, the IC50 value of the crude chloroform extract was 58.27 µg/mL, whereas IC50 value for the reference ascorbic acid was 51.07 µg/mL. Moreover, at 200 µg/mL extract concentration, lower grade total antioxidant activity (36.27±1.39 mg/g equivalent to ascorbic acid) was observed (Hossain et al., 2014). The present study results indicate that the ethanolic extracts of leaves of *Euphorbia heterophylla* and *Tamilnadia uliginosa* are possessing anti-inflammatory activity.

Table 2. Effect of ethanolic extract of *Tamilnadia uliginosa* leaf on carrageenan induced rat paw oedema.

Treatment	Dose (mg/kg)	Increase in oedema volume (ml)		
		1st hour	3rd hour	5th hour
Control	-	1.312 ± 0.066	1.571 ± 0.079	1.609 ± 0.080
Standard	5	0.801*** ± 0.048	0.613*** ± 0.037	0.707*** ± 0.042
Ethanolic leaf extract of *Tamilnadia uliginosa*	100	0.928** ± 0.037	0.906** ± 0.036	1.115* ± 0.045
	200	0.855 ** ± 0.044	0.819*** ± 0.043	0.996 ** ± 0.052
	400	0.830** ± 0.037	0.781*** ± 0.036	1.104** ± 0.050

Values expressed are Means ± SEM; n=6; ***p< 0.001, **p< 0.01 and *p< 0.05 in comparison with control. Standard = diclofenac sodium.

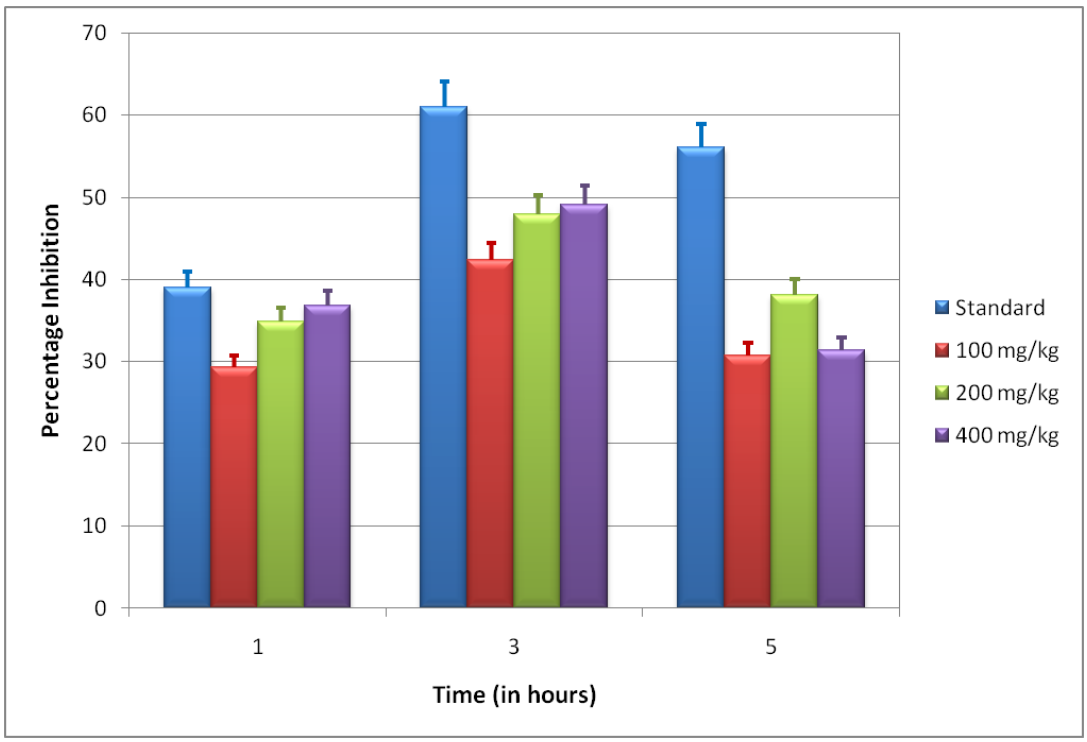

Fig. 2: Percentage inhibition of carrageenan induced rat paw oedema with the application of ethanolic leaf extracts of *Tamilnadia uliginosa* in comparison with the standard drug diclofenac sodium (5mg/kg).

Conflict of interest statement

Authors declare that they have no conflict of interest.

References

Borrelli, F., Izzo, A.A., 2000. The plant kingdom as a source of anti-ulcer remedies. Phytother. Res. 14, 581-591.

Falodun, A., Okunrobo, L.O., Uzoamaka, N., 2006. Phytochemical screening and anti-inflammatory evaluation of methanolic and aqueous extracts of *Euphorbia heterophylla* Linn. (Euphorbiaceae). Afr. J. Biotechnol. 5(6), 529-531.

Gamble, J.S., Fischer, C.E.C., 1957. Flora of the Presidency of Madras (Vols. IIII). Authority of Secretary of State for India in Council, London. 1389p.

Goldenberg, M.M., Ilse, A.C., 1977. Anti-inflammatory activity of EU-2972. Arch. Int. Pharmacodyn. 22(8), 153-161.

Holstein, SA., Hohl, R.J., 2004. Isoprenoids: Remarkable diversity of form and function. Lipids. 34, 293-309.

Kalpana, B., Prakash, M., 2015. Anti-candidal activity of leaf extracts of certain medicinal plants from Tamil Nadu, India. Int. J. Curr. Res. Acad. Rev. 3(7), 438-442.

Lewinsohn, E., Gijzen, M., 2009. Phytochemical diversity: The sounds of silent metabolism. Plant Sci. 176, 161-169.

Makkar, H.P.S., Sidhuraju, P., Becker, K., 2007. Plant Secondary Metabolites. Humana Press Inc., New Jersey, USA.

Matthews, K.M., 1983. The Flora of Tamil Nadu Carnatic, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. 1034 p.

Neerugatti, D., Battu, G. R., Jangiti, R. K., 2014. Anti-inflammatory effect of methanolic extract of *Tamilnadia uliginosa* (Retz.) roots. Int. J. Phytopharmacol. 5(1), 34-37.

Prakash, M., Karmegam, N., 2012. *In-vitro* antibacterial activity of certain plant extracts against plant disease causing bacteria isolated from citrus plant. Int. J. Curr. Microbiol. App. Sci. 1(1), 1-11.

Rates, S.M.K., 2001. Plants as source of drugs. Toxicon. 29, 603.

Vigneshwari, C., Nagaraj, R., Karmegam, N., 2014. Synergistic anti-*Staphylococcus aureus* (methicillin resistant) activity of ethnomedicinal plants from Shevaroy hills (Eastern Ghats), south India. Int. J. Curr. Res. Biosci. Plant Biol. 1(2), 51-59.

Winter, C.A., Risely, E.A., Nuss, G.W., 1962. Carrageenan induced oedema in hind–paw oedema of the rats-an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. 3, 544-547.

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