



## **Antinociceptive and anti-inflammatory activities of the aqueous leaf extract of *Erlangea tomentosa* (Asteraceae) in rats and mice**

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### **Abstract**

*Erlangea tomentosa* (Asteraceae) is used traditionally in the preparation of herbal remedies for management of several diseases including pain and inflammation. However, its efficacy and safety have not been scientifically validated. The aim of this study was to investigate the antinociceptive and anti-inflammatory activities of the aqueous leaf extract of this plant and its acute toxicity profile in animal models. Antinociceptive activity and anti-inflammatory activity were determined using the acetic acid-induced writhing model in mice and carrageenan induced inflammation model in rats respectively. The oral median lethal dose (LD<sub>50</sub>) was determined using the Lorke's Method. The extract inhibited pain due to acetic acid significantly ( $p < 0.05$ ) at doses of 250 mg/kg and 500 mg/kg. The inflammation due to carrageenan was also significantly ( $p < 0.05$ ) reduced at dose of 500 mg/kg body weight but not 250 mg/Kg. The LD<sub>50</sub> value of the extract was greater than 5,000 mg/kg implying that the extract was safe in 24 hours when administered in a single high oral dose. Preliminary phytochemical screening revealed the presence of alkaloids, anthraquinones, coumarins, saponins, tannins and resins. The present study has demonstrated the antinociceptive and anti-inflammatory potential of aqueous leaf extract of *Erlangea tomentosa* in rats and mice; thus validates the folkloric use of the plant.

**Keywords:** *Erlangea tomentosa*; Antinociception; Anti-inflammation; Acetic acid; Carrageenan

### **INTRODUCTION**

Inflammatory diseases and pain are a common cause of morbidity and mortality worldwide [1]. Therapeutic management of these conditions involve the use of nonsteroidal anti-inflammatory drugs, corticosteroids and opioid analgesics [2]. All these drugs are associated with severe adverse drug reactions, expensive and some cause

dependence [2]. This in addition to limited access to primary health care in developing countries has resulted into widespread of herbal medicines due to the availability, accessibility, affordability and cultural acceptance across different ethnic backgrounds [3-5].

*Erlangea tomentosa* (Oliv. & Hiern) S. Moore (Asteraceae) is an erect woody herb that

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grows up to 50 cm high. It is known as *Ekyoganyanja* in Runyankore [4]. The leaves of this plant are widely used by traditional herbal medicine practitioners to prepare herbal remedies for management of various ailments. These include stomachache, colic pains, syphilis, miscarriage fever [5], diarrhoea, skin infections, anaemia, appetite boosting, syphilis [3], conjunctivitis, convulsion in children, and mental confusion [6]. The diverse bioactivity of this plant has been attributed to presence of phytochemicals such as tannins, flavonoids, anthocyanins, saponins, coumarins and steroid glycosides [6,7].

Despite the widespread use of *E. tomentosa* in preparation of herbal medicines for management of pain and inflammation, there was paucity of scientific data regarding its antinociceptive and anti-inflammatory activity. Therefore, the aim of this study was to validate the above claimed bioactivity so as to increase confidence among traditional medicine practitioners and also provide alternative source of molecules for development of better anti-inflammatory and analgesic agents.

## EXPERIMENTAL

**Collection, identification and preparation of plant materials.** The leaves of *Erlangea tomentosa* were collected from *Rukararwe* Eco Centre, Bushenyi district, in September 2018. The plant was identified by Dr. Eunice Olet, a botanist and the Head of the Department of Botany, Mbarara University of Science and Technology, Uganda. A voucher specimen number (50891) was deposited in the national herbarium at Makerere University Botany Department, Uganda for future reference. The leaves were air-dried for 14 days until crumpled and then grounded into fine powder by using a mortar and pestle. About 250 grams of the powder was macerated in 2 liters of distilled water for 24 hours. The mixture was then filtered using Whatman filter paper. The filtered extract was then freeze-dried in 200ml

portions using a Freeze Dryer (Modulyo-Edwards). The freeze-dried powder was then pooled together into airtight containers, weighed and stored at room temperature (23°C approx.) until ready for use.

**Phytochemical screening.** Phytochemical screening was conducted as described by Trease and Evans [8] to identify the different classes of phytochemicals present in the extract.

**Animals and ethical consideration.** Ethical approval was sought and granted from the school of Pharmacy research committee and the institution research and Ethics committee of Kampala International University. Adult Wistar albino rats (120-170g) and Swiss Albino mice (20-30g) of both sexes were obtained from the animal house facility at the Department of Pharmacology and Toxicology, Kampala International University, Western Campus (KIU-WC). The animals were maintained in standard cages under standard environmental conditions of temperature, humidity and illumination cycle. The animals were fed with standard rodent pellet diet and water *ad libitum*. Animal welfare and rights were ensured as described in the *Guide for the Care and Use of Laboratory Animals* by the National Institutes of Health (Publication No. 80-23, revised 1996).

**Acute toxicity studies.** The oral median lethal dose (LD<sub>50</sub>) of the extract was estimated using a biphasic method as previously described by Lorke [9]. In the first phase, three groups of three mice each were administered with the extract 10, 100 and 1,000 mg/kg. The animals were observed for signs of toxicity and death for the first 2 hours and intermittently for 24 hours. In the second phase, three mice were each administered with the extract at 1600, 2,900 and 5,000 mg/kg. They were also observed for signs of toxicity and death for the first 2 hours and intermittently for 24 hours. The LD<sub>50</sub> value was estimated by calculating the geometric mean of the lowest dose that

caused death and the highest dose for which the animals survived.

**Evaluation of antinociceptive activity in mice.** Four (4) randomly selected groups of mice (n=5) were orally administered with distilled water (10 ml/kg), acetylsalicylic acid (ASA, 300 mg/kg) and the plant extract (250 and 500 mg/kg). Sixty minutes after oral administration, acetic acid 0.6% v/v (10 ml/kg) was administered intraperitoneally to each mouse and was placed in observation cage. Five minutes post acetic acid injection, the number of writhes was counted for each mouse for a period of 10 minutes. A reduction in the number of writhes as compared to the distilled water treated animals was considered as evidence for the presence of antinociception and expressed as percent inhibition of writhes [10].

% Inhibition =

$$\frac{\text{Mean No. of writhes (D/Water)} - \text{Mean No. of writhes (Test)}}{\text{Mean No. of writhes (D/Water)}} \times 100$$

**Evaluation of anti-inflammatory activity in rats.** Four (4) randomly selected groups of rats (n=5) were orally administered distilled water (1 ml/kg), Acetylsalicylic acid (300 mg/kg), and extract (250 and 500 mg/kg). Sixty minutes post treatment, each rat was injected with 0.1 ml of 1% carrageenan into plantar surface of rat right hind paw. The hind paw oedema was measured and recorded at times 0, 1, 2, 3 and 4 hours using Vernier caliper to determine the diameter of the oedema. The increase in paw diameter (oedema index) for each rat was calculated as the difference in paw diameter before carrageenan injection and after carrageenan injection at each time interval [11]. The percent inhibition of oedema was calculated for each group with respect to its distilled water treated control group using the following relationship:

$$\frac{\text{Mean paw oedema (D/Water)} - \text{Mean paw oedema(Test)}}{\text{Mean paw oedema (D/Water)}} \times 100$$

**Data management and analysis.** All numerical values were expressed as Mean  $\pm$  Standard Error of the Mean (SEM). The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's Post hoc test for Multiple Comparison, using the Graph Pad Prism (statistical) software. The differences between means were considered significant when  $p < 0.05$ .

## RESULTS AND DISCUSSION

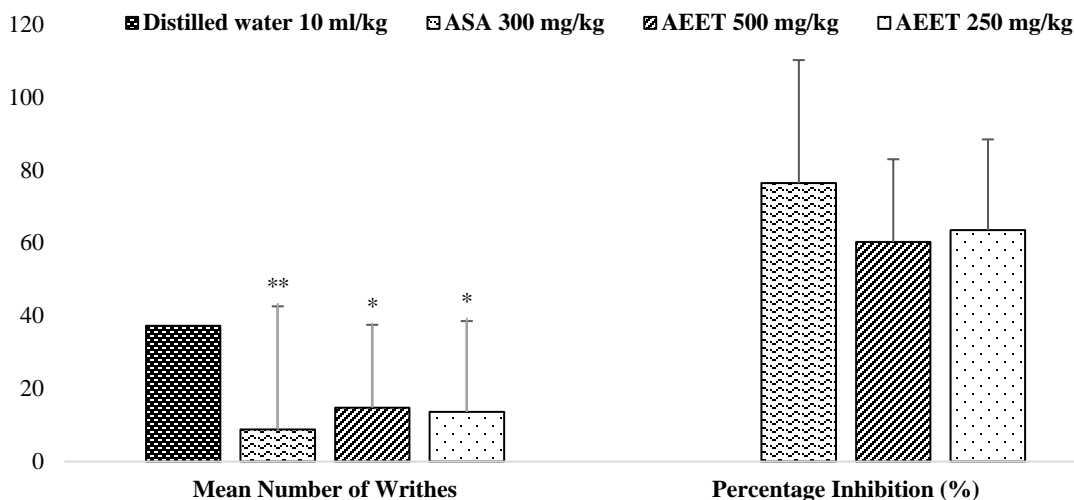
This study was designed to investigate the antinociceptive and anti-inflammatory activities of the aqueous leaf extract of *Erlangea tomentosa*. The oral median lethal dose of the plant extract was greater than 5,000 mg/kg. Administration of doses of the aqueous extract of doses up to 5,000mg/kg caused no death and no observable signs of toxicity. This suggests that the aqueous leaf extract of *E. tomentosa* was relatively safe [12].

The intraperitoneal injection of acetic acid elicited the writhing syndrome in control mice with  $37.2 \pm 12.60$  writhes counted in 10 min. The extract produced a significant ( $p < 0.05$ ) reduction in the number of writhes with peak effect of 63.44% inhibition at the dose 250 mg/kg (Figure 1). These results are similar to other previous studies on evaluation of antinociceptive activities of medicinal plant extracts in laboratory animals [13]. There was no significant difference in the percentage inhibition of writhes between 250 and 500 mg/kg. This implies that antinociceptive activity was not dose dependent. The oedema ensued secondary to the carrageenan injection. The dose of 500 mg/kg body weight showed a significant anti-inflammatory activity ( $p < 0.05$ ) at the 1st and 2nd hours after injection while the lower dose of 250 mg/kg was not as effective. The percentage inhibitions of 46.43%, 44.71%, 30.99 and 35.56% were observed at the 1st, 2nd, 3rd and 4th hour respectively with the dose of 500 mg/kg (Figure 2). The anti-inflammatory activity of the extract against carrageenan-induced

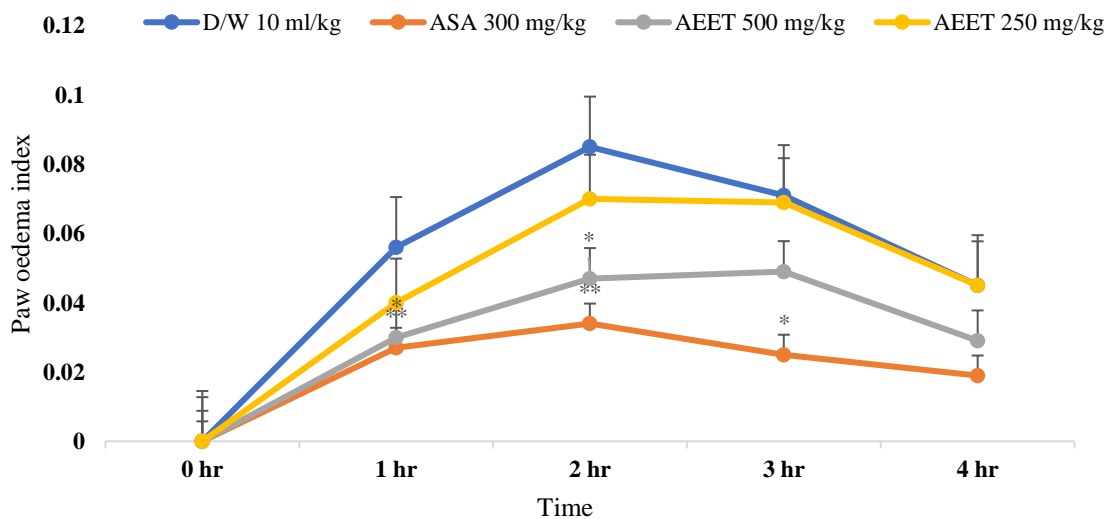
inflammation was therefore dose dependent. The ineffectiveness of the 250mg/kg dose could be attributed to insufficient concentration of the active secondary metabolites [14].

The preliminary phytochemical screening revealed the presence of alkaloids, anthraquinones, coumarins, saponins, tannins and resins. The antinociceptive and anti-

inflammatory effects of extract could be attributed to one or more of these observed phytoconstituents. Odoma et al. [12] reported that tannins, alkaloids and saponins possess antinociceptive and anti-inflammatory activities. However, the study could not identify the real phytochemicals responsible for the observed activity.



**Figure 1:** Effect of aqueous leaf extract of *E. tomentosa* on acetic acid-induced writhes in mice. \*\* =  $p < 0.01$ , \* =  $p < 0.05$  compared to distilled water treated group. ASA= Acetylsalicylic acid, AEET= Aqueous extract of *Erlangea tomentosa*, n=5



**Figure 2:** Effect of aqueous leaf extract of *E. tomentosa* on carrageenan induced oedema in rats. \*\* =  $p < 0.01$ , \* =  $p < 0.05$  compared to distilled water treated group. ASA= Acetylsalicylic acid, AEET= Aqueous extract of *Erlangea tomentosa*, n=5.

**Conclusion.** The data suggest that the aqueous leaf extract of *Erlangea tomentosa* possesses antinociceptive and anti-inflammatory activities and is relatively safe when orally administered in a single dose within 24 hours. This therefore further support the ethno-medical use of the plant in the management of pain and inflammatory conditions. We recommend more studies to isolate and characterize the pure chemical compounds in this plant responsible for this confirmed bioactivity and establish the exact mechanism through which the extract exerts its antinociceptive and anti-inflammatory activity.

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