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Phytochemical Screening and Anti-Inflammatory Activities of *Eremomastax polysperma* (Benth.) Dandy

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Abstract

In this study, the phytochemical screening and anti-inflammatory activity of *Eremomastax polysperma* was investigated using the carrageenan. Carrageenan-induced rat paw edema is a widely used test to determine anti-inflammatory activity and constitutes a simple and routine animal model for evaluation of pain at the site of inflammation without any injury or damage to the inflamed paw. Qualitative phytochemical screening, Acute toxicity test (LD₅₀) and inflammatory analysis were carried out. Results revealed *Eremomastax polysperma* contained alkaloids, tannins, saponins, terpenes, flavonoids, anthraquinones and cardiac glycosides. The ethanolic leaf extracts produced various degree of toxicity ranging from writhing, decreased respiration to mortality. The intensities of these effects were proportional to the dose administered. The intraperitoneal LD₅₀ of *E. polysperma* was 4242.64 mg/kg. Toxicity analysis revealed that the leaves *Eremomastax polysperma* are non toxic at a particular dose. Administration of different percentages of ethanolic leaf extracts showed marked inhibition at different measured interval and extract of *Eremomastax polysperma* (1272.79 mg/kg) prevented the formation of edema induced by carrageenan, thus showing significant anti-inflammatory activity (p<0.05) and also inhibited the edema by 19.91% after 5h injection of noxious agent as compared to the control group. Also, ethanolic leaf extracts of leaves *Eremomastax polysperma* inhibited inflammation of hind paw edema of albino rats induced by carrageenan. The synergistic effect of saponin and flavonoid present in the plant which has been investigated to possess anti-inflammatory activity is suggested to have caused rapid inhibition of the edema of the hind paw induced by carrageenan. Therefore, the efficacy and efficiency of leaves of *Eremomastax polysperma* is recommended therapeutically, locally, traditionally and pharmaceutically for inhibition and treatment of inflammation.

1. Introduction

The use of plants/herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The World Health Organization [1] estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care. The annual global export value of pharmaceutical plants in 2011 accounted for over US\$2.2 billion [1]. Among the 120 active compounds currently

isolated from the higher plants and widely used in modern medicine today, 80% show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived. More than two thirds of the world's plant species, at least 35,000 of which are estimated to have medicinal value, come from developing countries. Some of these compounds are saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds which have curative properties [2], [3]. These complex chemical substances of different compositions are found as secondary plant metabolites in one or more of these plants.) Plants contain certain other compounds that moderate the effects of the active ingredients [4].

Inflammation (Latin, *inflammatio*) is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants [5]. Although, in ancient times inflammation was recognised as being part of the healing process, up to the end of the 19th century, inflammation was viewed as being an undesirable response that was harmful to the host. However, beginning with the work of Metchnikoff and others in the 19th century, the contribution of inflammation to the body's defensive and healing process was recognised [6]. Furthermore, inflammation is considered the cornerstone of pathology in that the changes observed are indicative of injury and disease [7].

Eremomastax polysperma belongs to the Family Acanthaceae. *Eremomastax polysperma* is an erect or somewhat scrambling perennial herb, 1.3–2 cm tall; stems glabrous to sericeous-puberulent when young. It is widespread from West Africa through Central African Republic and North Congo-Kinshasa to South Sudan and South West Ethiopia, Madagascar, Ghana, Togo, Cameroon, Guinea, Nigeria, Liberia, Ivory Coast with altitude range of 900–1900 m [8]. In Nigeria, the plant is commonly known as a blood tonic plant: Akwa Ibom people (Ibibio) identify it as *edemididuo* meaning purple bark, Yoruba as *Oyun*, Hausa as *Esinyin*, and Igbo as *Akwukwo* [9].



Figure 1. Leaves of *Eremomastax polysperma* (Benth.) Dandy.

Leaf extract of *Eremomastax polysperma* has been suggested to be used as a haematonic and a therapy for anaemic conditions [10]. *Eremomastax polysperma* has shown significant benefit in the use of its extracts in the management of female infertility and its devastating psycho-social effects on couples in the society [10]. Ethanolic extract of *Eremomastax polysperma* has also revealed its effectiveness in inhibiting sickling activity [11]. Thus, this research was carried out to evaluate the phytochemical constituents and toxicity level (LD_{50}) of *Eremomastax polysperma* (leaves) extract on the experimental mice.

2. Materials and Methods

2.1. Plants Collection and Authentication

The leaves of *Eremomastax polysperma* was collected from plants growing in the Department of Botany and Ecological Studies, University of Uyo Botanical Garden, Nigeria. The plant samples used for this research work were authenticated by a Plant Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Nigeria.

2.2. Preparation of Plant Extracts

The fresh leaves of *E. polysperma* was air dried for 7 days and coarse powdered, 400g each was extracted using 6000ml of 70% ethanol and shaken intermittently for 72 hours. It was filtered and the filtrate concentrated oven dried at 40°C in a water bath. The extract was weighed and stored in 150ml beaker, labeled and covered with foil paper and preserved in the refrigerator at 4°C for use in analysis.

2.3. Qualitative Phytochemical Screening

The methods of [3], [12], [13] were used for qualitative phytochemical screening of the leaf extracts. These included tests for saponins, tannins, flavonoids, anthraquinone, terpenes, phlobatannins, alkaloids and cardiac glycosides.

2.4. Acute Toxicity Test (LD_{50} Determination)

Fifty Swiss Albino Mice (*Mus musculus*) weighing 18 – 35 g were used to determine the LD_{50} of the extract. Five groups of five mice each were kept and handled according to standard guidelines for the use and care of laboratory animals. Food was withdrawn for 18 hours before the onset of the experiment according to methods of [14]. The mice were administered with 5000-3000 mg/kg of *Eremomastax polysperma*. The groups were observed for manifestation of physical signs of toxicity and mortality rate within 24 hours, the median lethal dose (LD_{50}) was calculated for intraperitoneal route (i.p.) of administration according to the methods of [15] with this formula:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where:

D_0 = Maximum dose producing 0% mortality

D_{100} = Minimum dose producing 100% mortality

2.5. Experimental Animals

Fifty (50) either sex albino rats weighing 80 – 120g were randomly assigned to five groups of five rats in each group and were kept at the Laboratory Animal Unit, Faculty of Pharmacy, Department of Pharmacology and Toxicology Laboratory, University of Uyo Nigeria for assessment of inflammatory activities. The 10%, 20% and 30% of LD₅₀ were used as working doses implying low, middle and high dose.

Group I receive 10 ml/kg of normal saline, group II receive 100 mg/kg of standard drug (Aspirin), group III receive 424.26 mg/kg of *Eremomastax polysperma* extract, group IV receive 848.53 mg/kg of *Eremomastax polysperma* extract, group V receive 1272.79 mg/kg of *Eremomastax polysperma* extract.

2.6. Inflammatory Analysis

Inflammation was examined using carrageenan-induced paw edema in rats according to the method of [16] with slight modifications. Five groups of five rats each were treated orally with dose of (424.26, 848.53 and 1272.79 mg/kg p.o.), Aspirin (ASA) (Standard Drug - 100 mg/kg) and normal saline (10 ml/kg) of *Eremomastax polysperma*. One hour after the administration of the various agents, edema was induced by injecting carrageenan (0.1 ml, 1% in saline) into the sub plantar tissue of the right hind paw. Paw edema was measured with a vernier calliper. Measurements were made immediately before injection of the phlogistic agents and

afterwards at 1 h intervals, for 5 h.

The percentage inhibition was calculated thus:

$$\%Inhibition = \frac{V_c - V_t}{V_c} \times 100$$

Where:

V_c = Mean increase in paw volume in control group,

V_t = Mean increase in paw volume in test group

2.7. Statistical Analysis

The statistical analysis was carried out to find the effect of ethanolic leaf extract of *Eremomastax polysperma* on induced inflammation in albino rats. One way analysis of variance was adopted for comparison of the control with the treated groups and the results were expressed as mean \pm standard error of mean (S.E.M.) of five replicates. The significant was determined at (p<0.05) according to the methods of [17].

3. Results and Discussion

3.1. Qualitative Phytochemical Screening

Qualitative phytochemical screening of ethanolic leaf extracts of *Eremomastax polysperma* revealed a number of bioactive constituents, as summarized in Table 1. Ethanolic leaf extracts of *Eremomastax polysperma* revealed alkaloids and saponins were present in abundance. Flavonoids and cardiac glycosides were moderately detected. Tannins, terpenes and anthraquinones in trace amount.

Table 1. Qualitative phytochemical screening of *Eremomastax polysperma* leaves.

Constituent	Test	Observation	<i>E. polysperma</i>
Alkaloids	Dragendoff's	formation of red precipitate indicated the presence of alkaloids.	+++
Tannins	Ferric Chloride	a blue-green precipitation	+
Saponins	Frothing	formation of 1 cm layer of foam was observed.	+++
Terpenes	Liebermanns Burchards	formation of brown ring at the junction indicated the presence of phytosterols	+
Flavonoids	Shinoda	a red colour indicated the presence of flavonoids	++
Phlobatannins		no visible colour	-
Free Anthraquinone	Borntrager's	the presence of a pink in the ammoniacal (lower) phase	+
Combined Anthraquinone	Sulphuric acid	violet coloration in the ammonia phase (lower layer)	+
Cardiac Glycosides	Salkowski	a reddish-brown colour at the interface	++
	Keller-kellani	brown ring obtained at the interface	++

+ Tracely detected, ++ moderately detected, +++ abundantly detected, - Not detected

3.2. Acute Toxicity of *Eremomastax polysperma* Ethanolic Leaves Extract

The mice were treated intraperitoneally with a single dose of 0.57 – 1.90 mg/kg of *Eremomastax polysperma* ethanolic leaves extract after being left without food for 18 hours. Intraperitoneal route was chosen because of its sensitivity and rapid results. These ethanolic leaf extracts produced various degree of toxicity ranging from writhing, decreased respiration to mortality. The intensities of these effects were proportional to the dose administered. The intraperitoneal LD₅₀ of *E. polysperma* was 4242.64mg/kg (Table 2).

Table 2. Acute Toxicity of *Eremomastax polysperma* leaves extract on Swiss Albino Mice (*Musmu sculus*).

Groups	Dose (mg/kg)	Wt. of mice (g)	No. of Mice per group	% Mortality
I	5000	27	5	100
II	4500	28	5	100
III	4000	24	5	0
IV	3500	25	5	0
V	3000	24	5	0

Where:

100=Maximum dose producing 100% mortality

0=Minimum dose producing 0% mortality

W_i=Weight

3.3. Anti-Inflammatory Activity of Ethanolic Leave Extracts of *Eremomastax polysperma*

The effects of oral administration of ethanolic leave extracts of *Eremomastax polysperma* (424.26, 848.53 & 1272.79 mg/kg p.o.) in carrageenan induced paw edema in albino rats as the paw size was reduced from 6.60 ± 0.02 mm to 5.39 ± 0.08 mm is shown in Table 3. Administration of different percentages of ethanolic leaf extracts showed marked inhibition at different measured interval and extract

of *Eremomastax polysperma* (1272.79 mg/kg) prevented the formation of edema induced by carrageenan, thus showed significant anti-inflammatory activity ($p < 0.05$) and also inhibited the edema by 19.91% after 5h injection of noxious agent as compared to the control group as in Table 4. On carrageenan induced acute inflammation model the dose of ethanolic leaves extract of *Eremomastax polysperma* (1272.79 mg/kg) produced better inhibition of paw edema than lower doses Table 4.

Table 3. Effect of ethanolic leaf extract of *Eremomastax polysperma* on carrageenan induced paw edema in rat.

Group	Treatment (mg/kg)	Initial	0.5 Hr	1 Hr	2 Hr	3 Hr	4 Hr	5 Hr
A	-ve control (Saline 10ml/kg)	3.72±0.07	6.60±0.02	6.47±0.08	6.56±0.09	6.62±0.08	6.68±0.09	6.73±0.09
B	+ve control (ASA 100)	3.66±0.10	5.79±0.04	5.68±0.05	5.56±0.03	5.23±0.12*	4.99±0.18*	4.85±0.19*
III	Plant extract (424.26)	3.87±0.11	6.51±0.14	6.42±0.04	6.31±0.04*	6.18±0.04	6.04±0.05*	5.82±0.05*
IV	Plant extract (848.53)	3.79±0.06	6.45±0.03	6.32±0.03	6.21±0.03*	6.09±0.05*	5.89±0.07	5.64±0.06*
V	Plant extract (1272.79)	3.86±0.09	6.36±0.05	6.24±0.05	6.17±0.07*	6.04±0.08*	5.63±0.13*	5.39±0.08

Results are expressed as mean values \pm standard error of the mean * $p < 0.05$ when compared to control group.

Sample size (n=5).

ASA = Aspirin

Table 4. Percentage inhibition of paw edema volume in *Eremomastax polysperma*.

Group	Treatment (mg/kg)	Percentage inhibition at various times intervals					
		0.5 hr	1 hr	2 hr	3 hr	4 hr	5 hr
III	Plant Extract (424.26)	1.36	0.77	3.81	6.65	9.58	13.52
IV	Plant extracts (848.53)	2.27	2.32	5.34	8.01	11.83	16.20
V	Plant extracts (1272.79)	3.64	3.55	5.95	8.76	15.72	19.91

The phytochemical screening estimation of the crude yields of chemical constituents of the plant revealed that the leaf of *Eremomastax polysperma* is rich in alkaloids, flavonoids, tannin, saponins, cardiac glycoside and anthraquinone. Alkaloids showed prevalence in *Eremomastax polysperma*. The presence of phytochemicals in these plant species infers a possibility of medicinal efficiency of this plant. Phytochemicals are non-nutritive chemicals that contain protective, disease preventive compound. They are naturally occurring compounds in fruits, vegetables, legumes and grains. These compounds are associated with prevention and treatment of diseases such as cancer, cardiovascular diseases and hypertension [18], [19]. The results of qualitative analyses clearly indicated pronounced presence of tannins, alkaloids, saponins, cardiac glycosides and flavonoids. Tannins have been reported to provide protection against microbial degradation of dietary proteins [20].

Acute toxicity of ethanolic leaves extract of *Eremomastax polysperma* implies that the leaves are non toxic and could be eaten and used as blood supplement due to high magnesium content, an element in chlorophyll of plant which is known to be easily displaced by iron in the haemoglobin of blood system. [21] earlier reported that oral administration of 2,000 mg/kg of aqueous extract of some selected herbs was non-toxic to birds.

The search for new anti-inflammatory agents from the huge array of medicinal plant resources is on the increase this is because medicinal plants may hold assurance for the discovery of novel therapeutic agents capable of suppressing

or reducing inflammation with limited adverse effect. In this present study, the anti-inflammatory activity of *Eremomastax polysperma* was investigated using the carrageenan. Carrageenan-induced rat paw edema is a widely used test to determine anti-inflammatory activity and constitutes a simple and routine animal model for evaluation of pain at the site of inflammation without any injury or damage to the inflamed paw [22], [23]. It is believed to be triphasic. The first phase (0– 2h) of the carrageenan model is mainly mediated by histamine and serotonin; biochemically, the metabolic balance was sharply disturbed in favour of catabolism, this raised the osmotic pressure, attracting extra fluid into the tissue, edema; and also free heat liberated from lytic and other exothermic reactions such as decarboxylation, desamination or glucose fermentation significantly elevate tissue temperature on its way out of the body at the second phase [24]. The second phase (2–3 h) mediated by bradykinin, leukotrienes, polymorphonuclear cells and the last phase (3–5h) which begins after the bradykinin phase and is consecutive to the liberation of prostaglandins produced by tissue macrophages [26].

The result from this study indicates that ethanolic leaf extract of *Eremomastax polysperma* showed significant inhibitory effect on rat paw edema development in the second phase at 2 hours and this suggests that the extracts possibly act by the inhibition of the action of histamine and serotonin and also suggests a possible inhibition of cyclooxygenase synthesis by the extract, because the carrageenan inflammatory model basically reflects the actions of

prostaglandins. This effect is similar to that produced by non steroidal anti-inflammatory drugs such as aspirin, ibuprofen and indomethacin, whose mechanism of action is inhibition of the cyclooxygenase enzyme. The synergistic effect of saponin and flavonoid present in the plants which have been investigated to possess anti inflammatory activity is suggested to have caused rapid inhibition of the edema of the hind paw induced by carrageenan [26], [27].

4. Conclusion

The results from this study revealed that the leaves *Eremomastax polysperma* are non toxic at a particular dose. In this study, the ethanolic leaf extracts of leaves *Eremomastax polysperma* obviously inhibit inflammation of hind paw edema of albino rats induced by carrageenan. Therefore, the efficacy and efficiency of leaves of *Eremomastax polysperma* is recommended therapeutically, locally, traditionally and pharmaceutically for inhibition and treatment of inflammation.

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