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**Anti-inflammatory Properties of *Psorospermum febrifugum* Stem Bark  
Ethanol Extract**<sup>\*</sup>Asogwa F.C., <sup>2</sup>Asogwa C.J. <sup>3</sup>Okoye C.O.B.<sup>1</sup>\*Department of Pure & Industrial Chemistry, University of Nigeria, Nsukka, Nigeria.<sup>2</sup>Department of Biochemistry/Microbiology, University of Nigeria, Nsukka, Nigeria.<sup>3</sup>Department of Pure & Industrial Chemistry, University of Nigeria, Nsukka, NigeriaEmail; [fredrick.asogwa@gmail.com](mailto:fredrick.asogwa@gmail.com)**ABSTRACT**

Many medicinal plants have shown potent anti-inflammatory effects in the treatment of inflammation using various models. The plant *Psorospermum febrifugum* (Spach) is a specie of flowering plants belonging to the family Hypericaceae. It is a shrub or small tree growing in the tropical regions of South America, Africa and Madagascar. It has been used for a long time in traditional medicine for the treatment of skin diseases like dermatitis, scabies, eczema and subcutaneous wounds. This research was conducted to investigate the anti inflammatory properties of *P. febrifugum* stem bark ethanol extract. Systemic and topical treatment of acute inflammation in rats and mice were respectively achieved by Carrageenan-induced foot paw oedema and Xylene- induced ear oedema. Animals were divided into five groups (n=5). Group I: Served as the negative control and received 5 mL/kg body weight of distilled water. Groups II, III and IV: Received 100, 200 and 400 mg/kg body weight of extract respectively. Group V: Served as the positive control and received 10 mg/kg body weight of indomethacin. After 1h, 0.1 mL of Carrageenan (1% in normal saline) was injected into the subcutaneous tissue of the right hind paw of each animal and the paw volumes were measured at 1, 2, 3, 4 and 5h. For topical treatment using mice, Groups I, II, and III received 100, 200 and 400 mg/kg body weight of the extract respectively. Group IV received 4 mg/kg body weight of dexamethasone and Group V received normal saline as negative control. The results showed that *Psorospermum febrifugum* possess good anti-inflammatory activity. The percent inhibitions of carrageenan-induced paw oedema per hour were 43.54, 48.30 and 50.00 for 1, 2 and 3 hr respectively for 400 mg/kg of extract while Indomethacin had 29.93, 43.54 and 47.86 for the same time range. Apart from 100 mg/kg body weight of extract that had equal percentage inhibition (29.93%) with indomethacin at the first 1hr every other dose had a progressive increase in reduction of paw oedema. The result of the topical treatment of the xylene-induced ear oedema at 400 mg/kg was more effective than other doses and had comparable activity ( $p < 0.05$ ) with dexamethasone. Topical application of the extract reduced ear weight from 0.025g of negative control to 0.009g while the standard drug, dexamethasone reduced it to 0.008g. The result shows that *P. febrifugum* possess good anti-inflammatory activity.

**Key words:** Anti-inflammatory, Carrageenan, Dexamethasone, Indomethacin, *Psorospermum febrifugum*, xylene.

## INTRODUCTION

Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain [1]. Acute inflammation may be an initial response of the body to harmful stimuli but in chronic inflammation, the inflammatory response is out of proportion causing damage to the body [2].

Even though inflammation is the defense mechanism of the body to eliminate or limit the spread of injurious stimuli and heals the wounds, excessive inflammation is associated with onset of diseases like rheumatoid arthritis, asthma, inflammatory bowel disease, atherosclerosis, Alzheimer's disease and even cancer such as gall bladder carcinoma Medzhitov (2008)[3]. Non steroidal anti-inflammatory drugs are widely used in the treatment of acute and chronic inflammation, pain and fever. Their use is associated with adverse effects like severe gastritis, peptic ulcer, nausea, vomiting, salt and water retention, worsening of renal function in renal or cardiac and cirrhotic patients, hypersensitivity etc [4].

Many medicinal plants have shown potent anti-inflammatory effects in the treatment of inflammation using various models [2]. Plant-based drugs used in traditional medicine have attracted great attention because they are easily available, relatively less expensive and have no side effects Cathrine, Prabavothi (2011)[5]. Plants have the ability to synthesize a wide variety of phytochemical compounds as secondary metabolites that have been effectively used to treat various ailments for mankind. Different plant alkaloids and flavonoids have separately been reported to have anti-inflammatory properties [6].

The plant *Psorospermum febrifugum* (Spach) also known with the common name "Christmass berry" is a species of flowering plants belonging to the family *Hypericaceae*. It is a shrub or small tree growing in the tropical regions of South America, Africa and Madagascar. Most of the species of the genus *psorospermum* have been used for a long time in the ethno medical folk traditions of indigenous African population as febrifugal, antidote

against poisons (eg as relief against bites of spiders and scorpions), purgative and as a remedy for the treatment of leprosy, skin disease (Like dermatitis, scabies and eczema) and subcutaneous wounds [7].

## MATERIALS AND METHODS

### ANIMALS

Adult albino rats (150-200g) and mice (25-30g) were used for the experiments. The animals were sourced from the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State, Nigeria. They were housed in groups of 5 animals per cage to acclimatize and were kept at room temperature of 20-25°C on 12h light/dark cycle with food and water administered *ad libitum*. All experiments were performed adhering to the International Guide for the Care and Use of Laboratory Animals[8].

### DRUGS AND CHEMICALS

Acetone and ethanol were ordered from USA and supplied by Bristol Scientific Limited (Sigma Aldrich Rep) Lagos, Nigeria. Goya Olive oil made by Andalucia, Spain was purchased from Vegil Pharmacy, Nsukka while dexamethasone, Indomethacin and xyline were obtained from veterinary clinic, University of Nigeria, Nsukka, Enugu state, Nigeria. The chemicals were of analytical grade[9].

### PLANT MATERIALS

The plant *Psorospermum febrifugum* (Spach) stem bark were collected from a bush in Nsukka, Enugu state, Nigeria in November, 2015. The plant was authenticated by a taxonomist at the International Centre for Ethnomedicine and Drug Development, Nsukka, Enugu State, Nigeria. A voucher specimen (voucher No: INTERCEDD/16024) was deposited at the Centre's Herbarium[10].

### PREPARATION OF PLANT EXTRACT

The stem bark was air dried under a shade for about 14 days and pulverized into a coarse powder using Thomas Willy Laboratory Mill Model 4. About five hundred grams of

the pulverized material were macerated in 95% ethanol and extracted for 7 days with occasional agitation. The mixture was filtered first with chess cloth followed by filter paper and evaporated under reduced pressure using a rotary evaporator. (Buchi; CH-9230 Switzerland)[11].

### ACUTE TOXICITY STUDIES

The acute toxicity studies of the extract was carried out according to the method described by Mahesh *et al*<sup>[10]</sup> (2013) with slight modifications. Mice approximately 20-25g were divided into five groups (n=5). Animals were administered 10mg/kg body weight of extract up to the dose of 5000 mg/kg body weight and monitored for clinical signs and mortality[11].

### EVALUATION OF ANTI-INFLAMMATORY PROPERTY IN ALBINO RATS

#### CARRAGEENAN-INDUCED PAW OEDEMA

Carrageenan-induced paw oedema was one of the models used for evaluation of anti-inflammatory effects of drugs. The method adopted was as described by Winter *et al*<sup>[11]</sup> with some modifications. The baseline values of the paw volume were taken at 0h using a plethysmometer. Animals were divided into five groups (n=5).

Group I: Served as the negative control and received 5 mL/kg body weight of distilled water[12].

Groups II, III and IV: Received 100, 200 and 400 mg/kg body weight of extract respectively.

Group V: Served as the positive control and received 10 mg/kg body weight of indomethacin.

After 1h, 0.1 mL of Carrageenan (1% in normal saline) was injected into the subcutaneous tissue of the right hind paw of each animal and the paw volumes were measured at 1, 2, 3, 4 and 5h. Oedema was expressed as the mean increase in paw volume relative to control animals. The percentage inhibition of oedema was calculated using the following equation:

$$\% \text{ inhibition} = \frac{[(V_t - V_0)_{\text{control}} - (V_t - V_0)_{\text{treated}}]}{(V_t - V_0)_{\text{control}}} \times 100$$

Where  $V_0$  = paw volume at 0h,

$V_t$  = paw volume at 1, 2, 3, 4 and 5h

#### **XYLENE-INDUCED EAR OEDEMA**

Five groups (n=5) of Mice were topically treated as follows:

Groups I, II, III received 100, 200 and 400 mg/kg body weight of the extract.

Group IV received 4 mg/kg body weight of dexamethasone.

Group V received normal saline as negative control.

The drug was topically applied on the anterior surfaces of the right ear. The left ear was considered as normal control and left without induction or treatment. 60 min after the administration of the drugs, each animal received 20 $\mu$ L of Xylene on the posterior surface of the right ear. After another 60 min, the animals were sacrificed and both ears were sampled with a punch (5 mm diameter) and weighed. The extent of oedema and drug efficacy was evaluated by weight difference between the right and left ear biopsies of the same animal[13].

#### **STATISTICAL ANALYSIS**

The results were expressed as mean  $\pm$  SD. Difference between control and treatment groups were analyzed using one-way analysis of Variance (ANOVA) with SPSS version 16. *p* values  $\leq$  0.05 were considered significantly different.

### **RESULTS AND DISCUSSION**

#### **ACUTE ORAL TOXICITY STUDIES**

There was no death or clinical signs recorded during the period of experiment. Hence, the extract is safe and has a wide range of effective dose.

#### **EFFECTS OF EXTRACT ON CARRAGEENAN-INDUCED PAW OEDEMA**

The various doses of the extract reduced the paw size at the time points in a dose-dependent manner. However, there is no significant difference ( $p < 0.05$ ) between the effects of 200 mg/kg and 400 mg/kg body weight of the extract. At the 3, 4 and 5h, the two doses showed the same activity (Table 1).

Table 1: Effects of varying doses of *Psorospermum febrifugum* stem bark ethanol extract and indomethacin on Carrageenan-induced paw size of rats with time

Treatment/Dose	0h	1	2	3	4	5
Distilled water 5mL/kg	0.33±0.66	1.80±0.10	1.80±0.10	1.73±0.00	1.67±0.06	1.60±0.10**
Extract 100 mg/kg	0.50±0.10	1.53±0.06	1.43±0.06	1.33±0.06	1.23±0.06	1.16±0.06
Extract 200 mg/kg	0.33±0.06	1.33±0.06	1.23±0.06	1.20±0.00	1.10±0.00	1.03±0.06*
Extract 400 mg/kg	0.47±0.15	1.30±0.10	1.23±0.06	1.17±0.06	1.10±0.00	1.03±0.06*
Indomethacin 10 mg/kg	0.47±0.11	1.50±0.00	1.30±0.00	1.20±0.00	1.00±0.00	0.83±0.06

Data presented as mean ± SD (n = 5). \*\* Significantly different (p<0.05) compared to treated groups. \* No significant different (p<0.05) compared to positive control.

Fig 1: Effects of varying doses of *Psorospermum febrifugum* stem bark ethanol extract and dexamethasone topically applied on Xylene-induced ear oedema in mice

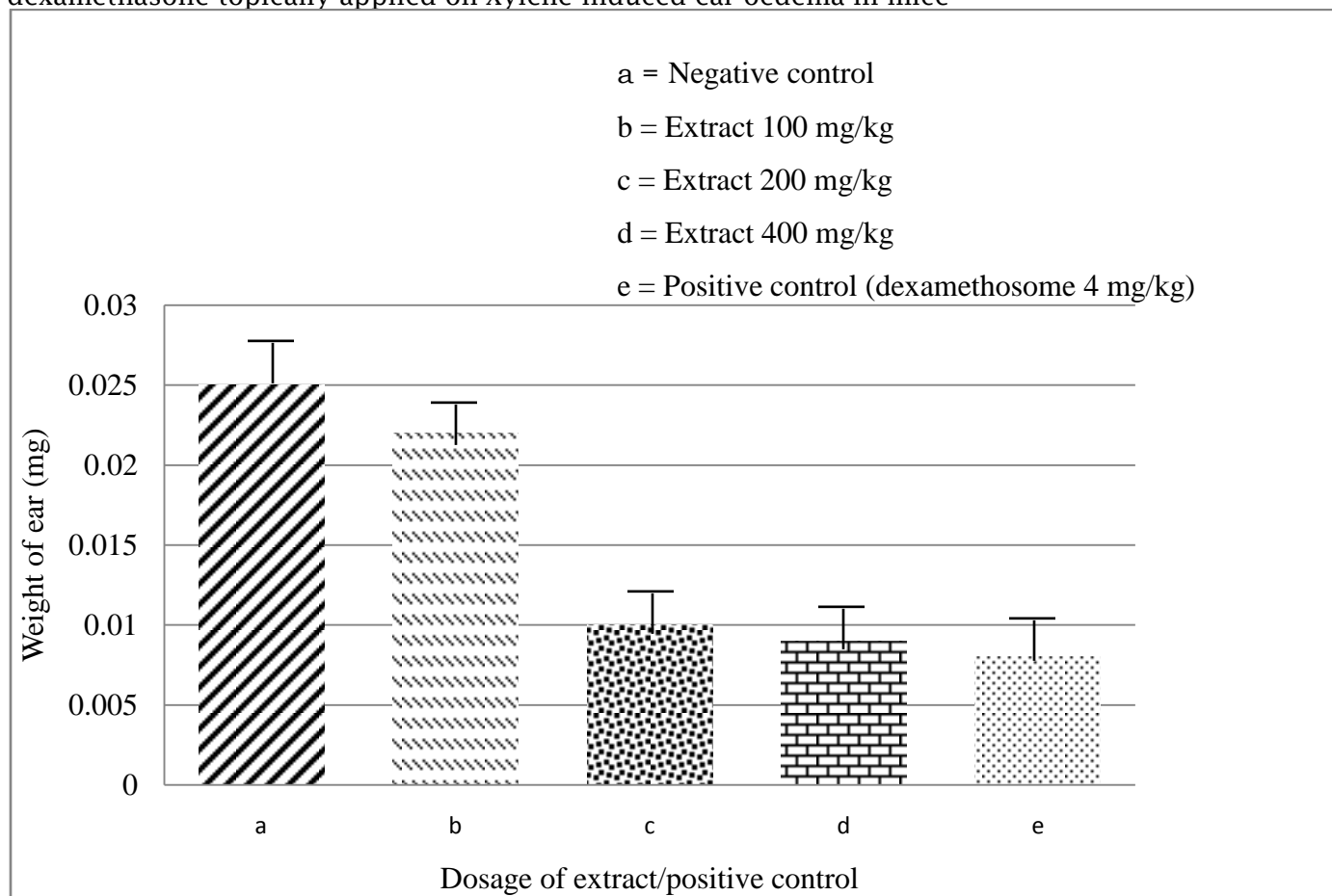


Table 2: Percent (%) inhibitions of Carrageenan-induced paw oedema by *P. febrifugum* stem bark extract and Indomethacin with time

Time (hr)	1	2	3	4	5
<b>Treatment</b>					
<b>Extract 100 mg/kg</b>	29.93	36.73	40.71	45.52	48.03
<b>Extract 200 mg/kg</b>	31.97	38.76	37.78	42.54	44.88
<b>Extract 400 mg/kg</b>	43.54	48.30	50.00	52.99	55.91
<b>Indomethacin 10 mg/kg</b>	29.93	43.54	47.86	60.45	71.65

Skin is well known for its functional role as a protective physical barrier and actively participates in immunological regulatory processes and inflammatory responses<sup>[12]</sup>. Carrageenan-induced paw oedema is a good, reliable and reproducible *in vivo* experimental model employed to investigate anti-inflammatory agents. Following carrageenan injection, the development of oedema is believed to be biphasic Habib, Waheed (2013)[14] and leads to signs of inflammation such as oedema, hyperalgesia and erythema. The early phase is associated with the release of inflammatory mediators such as histamine, serotonin and bradykinins while the late phase involves the release of prostaglandins Lumber-BNarod *et al.*,(1997)[15]. The resultant effect is increased vascular permeability, which enhances the infiltration of neutrophils and the accumulation of plasma fluid into the interstitial space which leads to oedema[16].

The results of this study showed that *Psorospermum febrifugum* possess good anti-inflammatory activity. Table 1 shows the effects of different doses of *P febrifugum* stem bark extract and indomethacin on carrageenan-induced paw oedema. The effect of dose was only significant ( $p \leq 0.05$ ) for 100 mg/kg body weight of extract compared to the standard indomethacin. 200 and 400mg/kg body weight did not show any significant different ( $p \leq 0.05$ ) between the doses and compared with the positive control. However, in Table 2, the percent inhibitions of carrageenan-induced paw oedema by various doses of *P. febrifugum* stem bark extract and indomethacin with time were shown. It shows that between one to three hours after induction with carrageenan, the extract at 400mg/kg body

weight was more effective than indomethacin. The percent inhibitions per hour were 43.54, 48.30 and 50.00 for 1, 2 and 3 hr respectively for 400 mg/kg of extract while Indomethacin had 29.93, 43.54 and 47.86 for the same time range. Apart from 100 mg/kg body weight of extract that had equal inhibitory effect (29.93%) with indomethacin at the first 1hr every other dose had a progressive increase in reduction of paw oedema. Four hundred milligram per kilogram body weight of the extract reduced foot paw oedema by 55.91 percent to be more effective than other doses but below that of indomethacin which showed 71.65 percent inhibition after 5hours[17].

The result of the topical treatment of the xylene-induced ear oedema was shown in fig 1. A dose dependent activity was observed. The extract at 400 mg/kg was more effective than other doses and had comparable activity ( $p < 0.05$ ) with the positive control. Topical application of the extract reduced ear weight from 0.025g of negative control to 0.009g while the standard drug, dexamethasone reduced it to 0.008g (fig 1). Xylene, a well known inflammatory agent, provokes acute inflammatory response in the ear of the mouse leading to serious oedematous changes in the skin when applied to the ear. The acute inflammatory response, severe vasodilation and oedematous changes of the skin is partially associated with phospholipase A2 as reported by [18]

Data is limited or not available on the anti-inflammatory properties of *P. febrifugum* (Spach). The present work therefore presents a first line report on the anti-inflammatory properties of the stem bark ethanol extract of *P. febrifugum*. However, previous review<sup>[18]</sup> on the plants belonging to the genus *psorospermum* shows that they contain alkaloids, flavonoids steroids, tannins, terpenes, simple and O- and C- prenylatedxanthenes. This reported anti-inflammatory activity of *P. febrifugum* could be related to its composition. Flavonoids were reported to have anti-oxidative and free radical scavenging activities and could regulate cellular activities of inflammation-related cells, thus, mast cells, macrophages, lymphocytes and neutrophils<sup>[19]</sup>. Certain flavonoids modulate the enzyme activities of arachidonic acid, metabolizing enzymes like phospholipase A2,



cyclooxygenase, lipoxygenase and the NO producing enzyme, NO synthase. Inhibition of these enzymes by flavonoids leads to reduced production of arachidonic acid, prostaglandins, leukotrienes and NO, which play very important roles in control of inflammation<sup>[6]</sup>. Alkaloids as well were shown to possess good anti-inflammatory activity. Indole alkaloids from the roots of *I. indogotica* has been found to have inhibitory effects of Nitric oxide production<sup>[7]</sup> and those from *Alstonica scholaris* were found to inhibit both NO, and prostaglandin. Hence, alkaloids exhibit their activities through different mechanisms [19].

### CONCLUSION

This paper presents the result of both the systemic and topical treatment of acute inflammation in rats and mice respectively induced by Carrageenan foot paw oedema and xylene induced ear oedema. The result shows that *P. febrifugum* possess good anti-inflammatory property and we therefore recommend studies on the phytochemistry and further pharmacological assays to ascertain the constituent responsible for the reported activity.

### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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