

Anti-inflammatory Property of Methanolic Extract of *Zanthoxylum leprieurii* Guill. & Perr. Root Bark

ABSTRACT

Purpose: This study was initiated following the folkloric claim that *Zanthoxylum leprieurii* Guill and Perr. (Family Rutaceae) has an anti-inflammatory property and is used in traditional medicine for treating tonsillitis and throat inflammation. The study is aimed at evaluating the phytochemical and anti-inflammatory properties of the aqueous methanolic extract of *Zanthoxylum leprieurii* root bark.

Methods: Preliminary phytochemical screening of the aqueous-methanol extract of the root bark of *Z. leprieurii* was done to determine the main secondary metabolites. The anti-inflammatory activity of the crude extract of *zanthoxyluml eprieurii* root back was determined using the egg albumin-induced model of inflammation, and the results were analyzed using a one-way ANOVA and student t-test.

Results: The yield after extraction was 5%. The phytochemical screening indicates the presence of the following metabolites: tannins, flavonoids, alkaloids, and saponins. The percentage inhibition of paw edema by 200 mg/kg and 400 mg/kg was 14.8%, 26.5%, and 46.3%, 70.3% at the 3rd and 4th hours, respectively.

Conclusion: The result of the *in vivo* anti-inflammatory evaluation carried out showed statistical significance and therefore justified the folkloric uses of the plant as an anti-inflammatory agent.

Key words: anti-inflammatory, phytochemical screening, methanolic extract, egg albumin-induced model, *Zanthoxylum leprieurii*

INTRODUCTION

A variety of stimuli from both living and non-living agents, such as bacteria, fungi, viruses, parasites, protozoa, immunological injury, trauma, heat, cold, toxins or poison, and irradiation, can cause inflammation, which is the body's response to disturbed normal homeostasis [1] (Guyton, 2006). It is part of the complex biological response of body tissues to harmful stimuli, which can be acute, sub-acute, or chronic [2] (Heasman et al., 2003). The pathological conditions associated with inflammation around the world include gonitis, bursitis, metritis, glossitis, keratitis, stomatitis, duodenitis, blepharitis, salpingitis, sore throat, peritonitis, sialadenitis, encephalitis, oesophagitis, and others [3] (Nickrent, D.L. 2002). Inflammatory bowel disease incidence is on the rise on nearly every continent and often surfaces when patients are in their teens, twenties, or thirties, leading to many years of pain and disability as well as costly monitoring [4] (Charles, 2002).

Non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs, and corticosteroids have been the main drugs used to treat inflammation in the past [5] (Aguwa, 2007). However, for socio-economic reasons prevalent, especially in developing countries, factors such as availability, acceptability, affordability, biodegradability, and cultural acceptance of medicinal plants have contributed to this resort to herbal drugs [6] (Makata, 2014). Plants and their extracts provide significant potential for inflammatory management and treatment due to their affordability and effectiveness, as they are generally safe to use and seldom cause hypersensitivity reactions [7] (Raina et al., 2008). In vivo and in vitro studies have shown a lot of evidence to support the idea that different compounds from plants that have anti-inflammatory properties work by changing the cytokine system [8] (Ruddle, 1997). *Zanthoxylum leprieurii* Guill and Perr. (Family Rutaceae), also known as *Fagara angolensis* Engl. and *Fagara leprieurii* Engl., has wide distribution across tropical Africa. In southeastern Nigeria, particularly among rural inhabitants in the Nsukka district of Enugu State, people chew the root or stem bark and ingest the juice to treat gum and intestinal inflammations [9] (Fabian et al., 2020). Venezuelan traditional medicine commonly uses *Zanthoxylum monophyllum* to treat runny nose or nasal mucosal inflammation, jaundice, ophthalmology, and as an anesthetic [10] (Abubakar et al., 2007).

Traditional use of *zanthoxylum leprieurii* as an anti-inflammatory agent lacks scientific assessment of its anti-inflammatory properties. Therefore, the study aims to conduct additional research to ascertain its influence on treating inflammation in vivo.

MATERIALS AND METHODS

Plant material

The root bark of *Zanthoxylum leprieurii* was collected from a farm in the Nsukka local government area of Enugu State, Nigeria, and authenticated by Mr. A.O. Ozioko of the International Centre for Ethnomedicine and Drug Development, InterCEDD, Nsukka, where a voucher specimen (voucher no.: InterCEDD/16017) was kept. The root bark of *Zanthoxylum leprieurii* was collected, washed in clean water, dried under shade at 28 to 30 °C, and pulverized by a mechanical grinder into powder.

Preparation and Extraction of Plant Material

A total of 500 g was used for successive extractions with 1.5 liters of 95% methanol by the cold maceration method at room temperature. The mixture was stirred intermittently for 48 hours, sieved using a muslin cloth, and then filtered with filter paper. The extract was recovered using a rotary evaporator at 40°C under minimal pressure, yielding 25 g (5%) crude extract of *Zanthoxylum leprieurii*.

The percentage yield was obtained using the formula.

$$(\%) \text{ yield} = (\text{weight (g) of conc. of extract} / \text{weight (g) of grounded plant}) \times 100$$

Experimental Animal

Wistar albino rats (18 rats) were collected from the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Science, University of Nigeria and were kept in stainless steel cages and served with a normal commercial rat diet, given water ad libitum, and maintained under laboratory conditions (temperature of 28 to 30 °C, relative humidity of 60–70%, and normal light-dark cycle). Each animal weighed between 100 and 250 g. The animals were acclimatized for seven days with a standard pellet and water before the experiment. This was done to reduce the stress of experimental handling and conditions. Techniques and methods used in this study were performed following the National Institute of Health Guidelines for the Care and Use of Laboratory Animals [11] (NIH, Department of Health Services Publication No. 83-23, revised 1985). The protocols for the study were approved by the ethics committee of the University of Nigeria, Nsukka, as registered by the National Health Research Ethics Committee of Nigeria (ref no. NHREC/05/01/2008B).

Fractionation of the Plant Extract

The extract weighing 10 g was put in a mortar, and 100 g of silica was introduced into it. It was thoroughly mixed with the aid of a pestle to form a uniform mixture with the plant extract, after

which 100 ml of N-hexane was introduced into the separating funnel. The mixture was mixed for 3 minutes and allowed to stand for 24 hours, after which the N-hexane fraction was tapped off and another 100 ml of N-hexane was introduced into the extract. The same procedure was repeated, as described above. The fractionation continued until the color of N-hexane became very clear, with an indication that there was no N-hexane-soluble component in the extract, after which the N-hexane fraction was evaporated using a rotary evaporator and the fraction was used in conducting the experiment. The same procedure was repeated using ethyl acetate, methanol, and water.

Qualitative phytochemical screening

Preliminary phytochemical screening of the aqueous-methanol extract of the root bark of *Z. leprieurii* was done to determine the main secondary metabolites according to the established method [12,13,14] described by Evans (2009), and the total alkaloid, saponins, tannins, flavonoid, and steroid content of the extract was determined according to the existing method [15] described by Agyare *et al.* (2012).

Acute toxicity test

The established Lorke method (1983) procedure for LD₅₀ determination was used [16]. This method employs 15 albino mice. The experiment involved a preliminary test in which three groups of mice (n = 3) were orally administered 10 mg/kg, 100 mg/kg, and 1000 mg/kg of *Zanthoxylum leprieurii* extract. The animals were constantly observed for 2 hours, intermittently for the next 4 hours, and then overnight. No deaths of animals per group were recorded at the end of the 24 hours. From the results obtained above, the second stage of the acute toxicity test was performed using doses of 1000, 1600, 2900, and 5000 mg/kg, respectively.

Egg albumin-induced rat paw edema

The egg albumin-induced rat paw edema method was used [17] (Adeyemi *et al.*, 2007). The crude extract of *Zanthoxylum leprieurii* was administered orally to animal groups of 5 per dose, and 4 dose levels (50 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg) were used. Thirty minutes after administration of the extract fraction, inflammation was induced by a sub-plantar injection of 0.1 ml of fresh, undiluted egg albumin. Edema was assessed in terms of the volume of distilled water displaced before and after 0, 1, 2, 3, and 4 hours after the induction of inflammation using the LETICA Digital Plethysmometer (LE 7500). The average edema level at each interval was determined in terms of the difference in volume displacement after injecting the egg albumin and at zero time volume displacement of the injected paw ($V_t - V_0$).

The level of inhibition of edema was calculated for each dose of the plant extract.

Statistical analysis

The results obtained from the above experiment were analyzed with a one-way ANOVA expressed as SEM, and a student T-test was used to test the differences between the mean of the treated and control groups, which were considered significant at $P = .05$.

Result

Phytochemical composition

The root bark of *Zanthoxylum leprieurii* was extracted by maceration with 95% methanol, filtered, and the solvent recovered using a rotary evaporator. The extract gave a percentage yield of 5%; it was fractionated, and the compositions of the different fractions are listed below in Table 1.

Table 1: Phytochemical compositions

Phytoconstituents	Results			
	N-hexane	Ethyl acetate	Methanol	Water
Alkaloids	+	+	++	++
Flavonoids	++	+	+++	+
Saponins	+	-	+++	++
Tannins	-	-	++	+

Key

- = absent

+ = Present in small concentration

++ = present in moderate concentration

+++ = present in High concentration

Acute toxicity test (LD₅₀)

The acute toxicity study showed no mortality rate in the animal for up to 10,000 mg/kg. This indicates that the LD₅₀ of *Zanthoxylum leprieurii* is greater than 8,000 mg/kg. It shows that *Z. leprieurii* is safe at high doses.

Anti-inflammatory activity

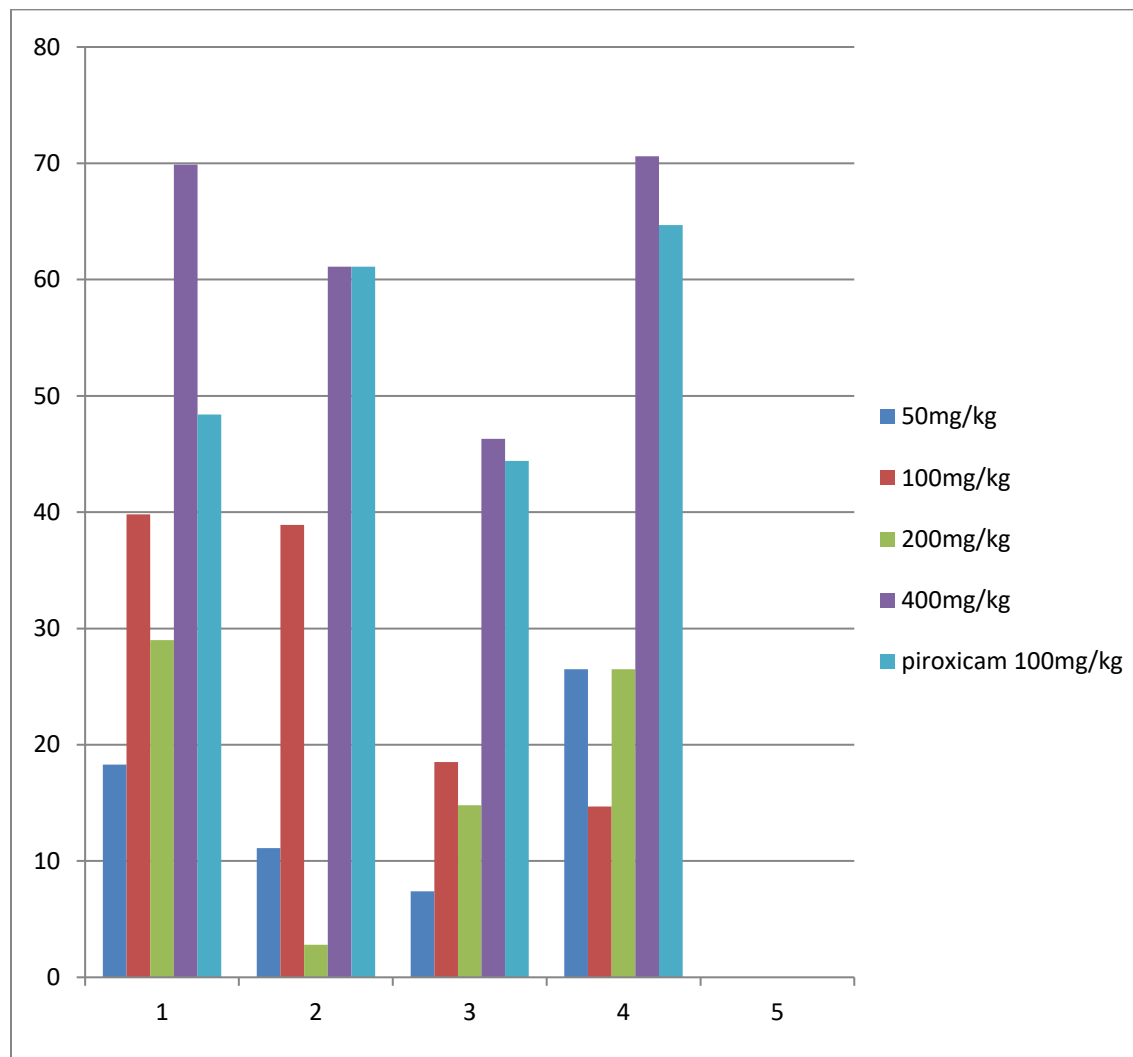
The result in Table 2 shows the doses of 50 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg at 0, 1st, 2nd, 3rd, and 4th hours, respectively. The effect of the crude extract of *Z. leprieurii* root bark was significant ($P = .05$) with the maximum effect at the 4th hour when compared with the standard.

Table 2: Anti-inflammatory activity studies

GROUP	DOSE (mg/kg)	MEAN EDEMA VOLUME (ml) AND PERCENTAGE INHIBITION AT DIFFERENT HOURS				
		0h	1h	2h	3h	4h
ZL ₁	50	0.38±0.033	0.78±0.026 (18.3%)	0.73±0.032 (11.1%)	0.68±0.041 (7.4%)	0.68±0.015 (26.5%)
ZL ₂	100	0.38±0.017	0.70±0.015 (39.8%)	0.65±0.020 (38.9%)	0.66±0.040 (18.5%)	0.68±0.020 (14.7%)
ZL ₃	200	0.4±0.029	0.75±0.039 (2.8%)	0.76±0.028 (29.0%)	0.75±0.057 (14.8%)	0.71±0.008 (26.5%)
ZL ₄	400	0.42±0.044	0.63*±0.008 (69.9%)	0.64*±0.020 (61.1%)	0.64*±0.011 (46.3%)	0.61*±0.029 (70.6%)
Piroxicam	100	0.50*±0.029	0.76±0.024 (48.4%)	0.67±0.038 (61.1%)	0.64*±0.045 (44.4%)	0.65±0.025 (64.7%)

Values are Mean ±SEM, n=3, * values are significantly different from the control group at $P = .05$.

Figure 1: Egg albumin induced paw edema in rat



DISCUSSION

The yield of the crude extract of *Zanthoxylum leprieurii* stem bark was expressed in percentage as 5%. The extractive yield was low when compared with the amount of *Z. leprieurii* root bark powder (500 g) used for the extraction.

The methanol extract of *Zanthoxylum leprieurii* root bark was phytochemically analyzed and found to have a high concentration of flavonoids and saponins and a moderate concentration of alkaloids and tannins. This is in line with what other research has said about the phytochemical composition of *Zanthoxylum leprieurii* [18] (Adesina et al., 2005). The ethyl acetate fraction showed low concentrations of flavonoids and alkaloids, while the N-hexane fraction showed a moderate concentration of flavonoids and a low concentration of alkaloids and saponins. The water fraction showed a moderate concentration of alkaloids and saponins, as well as a low concentration of tannins and flavonoids. Plant alkaloids may promote inflammation by increasing cell proliferation [19] (Barbakadze et al., 2009). Researchers have proven that tannins and flavonoids in the plant act as free radical scavengers, making their presence particularly significant [20] (Marja et al., 1999). Flavonoids and general phenolic compounds act as potent antioxidants that possess significant anti-inflammatory properties by inhibiting the effects of enzymes responsible for the production of inflammatory chemical mediators [21] (Owoyele et al., 2008). Plant flavonoids may improve collagen fibril viability by increasing fiber strength, which promotes DNA synthesis and reduces cell damage and inflammation [22] (Panda and Tripathy, 2009). Tannins found in plants can promote inflammation and epithelialization due to their astringent and antibacterial properties [22] (Panda and Tripathy, 2009).

The lethal dose (LD₅₀) is the dose that produces death in 50% of animals. Lethal dose (LD₅₀) determination is essential because it gives room for safe dosing in subsequent whole animal experiments that will be carried out in the course of the study. Substances more toxic than 1 mg/kg are of no practical interest; LD₅₀ values greater than 5000 mg/kg are of no practical interest; an appropriate figure for the LD₅₀ is usually adequate to estimate the risk of acute intoxication [16] (Lorke, 1983). *The experimental animal tolerated high doses of Zanthoxylum leprieurii.*

The anti-inflammatory effect of crude *Zanthoxylum leprieurii* root bark was studied using egg-albumin-induced rat hind paw edema. The characteristic swelling of the paw is due to edema formation and inhibition of increased vascular permeability; hence, the attendant edema modulates the extent and magnitude of the inflammatory reaction. The injection of egg albumin induced paw edema, which peaked between 30 minutes and 1 hour and gradually decreased over time, while the negative control remained almost constant throughout the experiment.

According to the results of the egg-albumin-induced paw edema model, the crude extract showed dose-dependent inhibition of edema, particularly at 3 to 4 hours. The percentage inhibition of paw edema by 50 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg was 7.4% and 26.5%, 18.5% and 14.7%, 14.8%, and 26.5%, 46.3%, and 70.6% at the 3rd and 4th hours, respectively, while the standard drug (Piroxicam at 100 mg/kg) was 44.4% and 64.7% at the 3rd and 4th hours, as shown

in Table 2. This demonstrates that the standard medicine at 100 mg/kg has greater anti-inflammatory action than the extract at 50, 100, and 200 mg/kg; however, at 400 mg/kg, the extract produces a stronger anti-inflammatory response than the standard drug at 100 mg/kg.

CONCLUSION

The results of this investigation revealed that the crude extract of *Zanthoxylum leprieurii* root bark is safe at large doses and possesses anti-inflammatory properties. The sample contains phytochemical elements such as alkaloids, saponins, tannins, and flavonoids. The non-polar essential oils in the plant may be responsible for its anti-inflammatory effect. The present study provided evidence supporting the use of the plant *Z. leprieurii* in the management of inflammatory disorders. Thus, it serves as a beneficial alternative to orthodox medicine.

We recommend further research to isolate and characterize the phytochemical constituents responsible for the obtained anti-inflammatory activities.

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