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Preliminary Evaluation of the Anti–Inflammatory Activity of the Leaves of *Ficus umbellata* (VAHL) in Cotton Pellet Induced Granuloma Inflammation Model

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Abstract

Inflammation is a local response of living tissues to injury. *Ficus umbellata*, an evergreen plant popularly known as house plant has traditional application in the management of menopause physiological disorders, dyspepsia, diarrhoea and cancer. This study aimed to evaluate the anti-inflammatory effect of the leaves of *Ficus umbellata* using cotton pellet induced granuloma model. The methanol, aqueous, n-butanol, ethylacetate and n-hexane extracts of the leaves were administered at the dose of 400 mg/kg body weight respectively after surgical insertion of cotton pellets into groin region of rats. When compared to the negative control, the extracts caused significant ($P < 0.05$) increase in anti-inflammatory activity. The aqueous, n-butanol, n-hexane, ethylacetate, and methanol extracts caused 75%, 64%, 55%, 63%, and 62% reduction in inflammation compared to the negative control. This study provided experimental evidence that extracts of *F. umbellata* present a strong anti-inflammatory response in cotton pellet induced granuloma in albino rats.

Keywords: Anti-Inflammation, Cotton pellet, Ganuloma, *Ficus umbellata.*

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Introduction

Inflammation, a fundamental defense mechanism against foreign pathogens, tissue injury, or chemical irritants, is a complex biological cascade designed to eliminate noxious stimuli and initiate healing, although prolonged inflammation can lead to chronic diseases such as rheumatoid arthritis (Modak *et al*., 2021). This intricate process involves a coordinated interplay of cellular and metabolic events, mediated by a diverse array of signaling molecules including cytokines, interleukins, prostaglandins, and thromboxanes (Majid *et al.*, 2018, Beg *et al*., 2011). While acute inflammation is a protective response, dysregulated or chronic inflammation can have severe consequences, contributing to the pathogenesis of various conditions including arthritis, cardiovascular disorders, and cancer (Kim and Kang, 2023). Sustained activation of immune cells, such as macrophages, in chronic inflammatory states leads to the continuous production of pro-inflammatory cytokines like TNF-α and IL-1β, which further perpetuate tissue damage (Koukoui *et al*., 2024, Sattar *et al*., 2023). This persistent inflammatory response contributes to numerous age-related musculoskeletal, neurodegenerative, cardiovascular diseases, and even certain types of cancer (Akhtar *et al*., 2016, Harikrishnan *et al*., 2018). Given the prevalence and severity of inflammatory diseases, including various rheumatic conditions, the screening of plant-derived compounds for anti-inflammatory and anti-arthritic activity has become a critical area of drug development (Nile and Park, 2012. (Basavarajaiah *et al*., 2023). Chronic inflammation, is implicated in numerous debilitating diseases, including atherosclerosis, diabetes, inflammatory bowel disease, cancer, and rheumatoid arthritis (Sarkar *et al*., 2023). Conventional therapies for chronic inflammation, such as nonsteroidal anti-inflammatory drugs and corticosteroids, often present significant side effects and limited efficacy in sustained management, highlighting the urgent need for novel therapeutic interventions with improved safety profiles (Madhiri and Barik, 2023, Zhang *et al*., 2025). Consequently, the exploration of natural products, particularly medicinal plants with established anti-inflammatory properties, offers a promising avenue for the discovery of safer and more effective anti-inflammatory agents (Tasneem *et al*., 2018).

Ficus umbellata Vahl (Moraceae) has garnered scientific interest due to its traditional use in managing various physiological disorders, including menopause and certain cancers, alongside its documented antioxidant and anti-inflammatory properties (Silihe *et al*., 2022). I Its methanol bark extract has demonstrated significant anti-edematous effects (Silihe *et al*., 2022 This study, therefore, aimed to investigate the anti-inflammatory activity of the methanol, aqueous, n-butanol, ethylacetate and n-hexane extracts of *Ficus umbellata* leaves, thereby contributing to the understanding of its pharmacological profile and potential as an anti-inflammatory phytotherapeutic agent for the management of chronic inflammation (Kalita *et al* 2023).

Materials and Methods

Chemical and Reagent

Methanol, n-hexane, n-butanol, ethyl acetate, hydrochloric acid, sulphuric acid and ferric chloride (Analytical grades) from BDH Chemicals LTD, Poole England. Chloroform, sodium hydroxide, molisch reagent, fehling solution A and fehling solution B (GHTECH Guandong Guanghua Sci-Tech., Co., Ltd China). Indomethacin 25 mg by Juhel Nigeria Ltd, Enugu Nigeria, Distilled water

Experimental Animals

Albino rats (70 -130 kg) of either sex were obtained from the Animal House of the Department of Pharmacology, Enugu State University of Science and Technology.

Methods

Collection and identification of F. umbellata leaves

The fresh leaves of *F. umbellata* were collected from Agbani in Nkanu West Local Government Area, Enugu State Nigeria, and authenticated by a botanist, Patrick Obi of the Department of Pharmacognosy, Enugu State University of Science and Technology. A voucher specimen number: (PCG/121/B/023) was deposited at the Department of Pharmacognosy Enugu State University of Science and Technology.

Preparation, extraction and fractionation procedures

The leaves were washed and shade dried four weeks. The dried leaves were pulverized into a coarse powder, and were well stored at room temperature. Cold maceration was applied to extract 300 g of the powdered material for 72 hours using 1L of analytical grade methanol with intermittent agitation. After 72 hours, filtration was carried out using Whatman (no.1) filter paper. The crude extract was concentrated in a rotary evaporator at 40 ^{o}C. Solvent-solvent fractionation was carried out on the crude methanol extract using n-hexane, ethyl acetate, and n-butanol and water. The fractions obtained were concentrated in a rotary evaporator at 40 ^{o}C.

Phytochemical screening of the methanol extract of F. umbellata leaves

The crude methanol extract was qualitatively assessed for the major photochemical constituents such as Alkaloids,

tannins, glycosides, steroids, terpenoids, saponins, flavonoids, carbohydrates using standard qualitative phytochemical screening procedures as described by (Kim and Kang 2023)

Acute toxicity (LD_{50}) test

Acute toxicity of the extracts was evaluated using Larke's (1983) method. Thirteen rats were used in two (2) phases. In the first phase, nine of rats divided into 3 groups of 3 animals each and were administered 10,100 and 1000 mg/kg body weight of the methanol extract. The animals were observed for 24 hours for obvious adverse effects and number of deaths in each group. In the second phase, four doses of the extract were selected based on the result of phase 1 and were administered to 4 groups of one animal each. After twenty-four hours, the number of deaths were recorded and the LD_{50} was calculated as the geometric mean of the highest non-lethal dose and the least lethal dose.

Animal Studies

Cotton Pellet Induced Granuloma Inflammation Model

Albino rats (40) were used for the experiment; they were grouped into eight group containing five animals each. Adsorbent cotton weighing 0.1g was cut into pieces and made into pellets. The pellets were then sterilized in a hot air oven at 1200^0C for 2 hours. The abdomen was well shaved, swabbed with 70 % ethanol, and two sterilized cotton pellets were implanted subcutaneously, one on each side of the abdomen of the animal under light ether anesthesia. Test drug was administered once daily throughout the experimental period of 7 days. On the 8th day after implantation, rats were anaesthetized with pentobarbital sodium. The pellets were dissected and dried at 600^0C for 18 hours and weighed after cooling. The mean weight of the cotton pellets of the control groups as well as the test groups was calculated. The transudative weight, granuloma formation and percent granuloma inhibition by the extract and fractions were calculated. The animals were grouped and treated as follows after implantation;
Group 1: no treatment (Negative control)
Group 2: methanol crude extract 400 mg/kg
Group 3: aqueous fraction 400 mg/kg.
Group 4: n-butanol fraction 400 mg/kg.
Group 5: n-hexane fraction 400 mg/kg
Group 6: ethyl acetate fraction 400 mg/kg
Group 7: indomethacin 300 mg/kg (positive control)

Statistical Analysis

The results are expressed as mean ± standard deviation. Results obtained were analyzed using One-way ANOVA. Data was computed for statistical analysis. Differences between the data were considered significant at $P < 0.05$.

Results

The extract yield was 28.4 g (7.9 %)

Results of phytochemical screening of the leaves of F. Umbellata

The results revealed that the methanol extract of the leaves of *F. umbellata* contains phenols, tannins, saponins, alkaloid, steroids, terpenes and flavonoids (Table 1).

Table 1: Photochemical constituents of methanol extract of *F. umbellata*

Phytochemical	Results
Tannins	+
Saponin	+
Alkaloids	+
Steroids	+
Terpenes	+
Phenols	+
Anthraquinone	-
Flavonoids	+

+ = present - = absent

Acute toxicity studies

The preliminary acute toxicity studies demonstrated the extract's safety, with no mortality observed at doses up to 5000 mg/kg

Effects of the extract and fractions on cotton pellet-induced granuloma

The results revealed that the extract at the dose of 400 mg/ kg of aqueous, n-butanol, n-hexane, methanol, ethyl acetate fractions and indomethacin had 71%, 60%, 52%, 59%, 58% and 67% inhibition respectively in weight of cotton pellet when compared to that in the control group as indicated in table 2 below. The results of the different extracts when compared to the negative control showed P-Value < 0.05. The methanol, aqueous, n-butanol, ethylacetate and n-hexane extracts of *F. umbellata* at a dose of 400 mg/kg produced significant inhibition of granulomatous tissue formation, thereby, indicating that the extract can inhibit sub chronic inflammation in which various types of cellular migration are involved (Tazeze *et al.,* 2021).

Table 2: Effects of leaves of *F. umbellata* methanol extract and fractions on cotton pellet induced granuloma in rats.

Treatment groups/ doses (mg/kg)	Weight	% inhibition
Negative control	1.06±0.46	----
Methanol extract 400 mg/kg	0.43±0.10	59%
Hexane fraction 400 mg/kg	0.51±0.10	52%
Ethyl acetate fraction 400 mg/kg	0.44±0.05	58%
Butanol fraction 400 mg/kg	0.42±0.07	60%
Indomethacin 300 mg/kg	0.35±0.09	67%
Aqueous fraction 400 mg/kg	0.31±0.03	71%

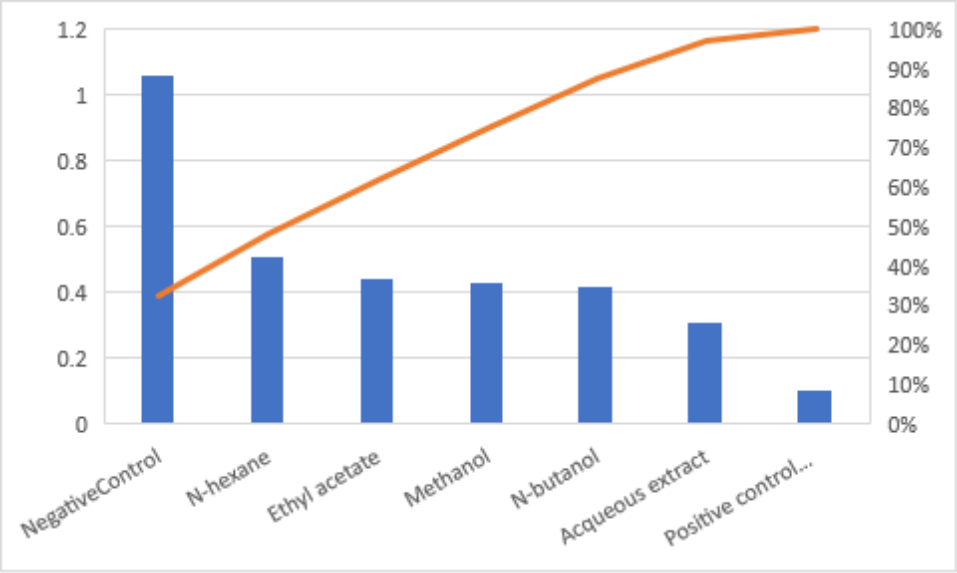

Figure 1: Inhibition of granuloma by the extract and fractions

Discussion

The phytochemical analysis of the methanol extract of *F. umbellata* leaves revealed the presence of compounds which are well-known for their diverse pharmacological activities, including anti-inflammatory effects (Sarkar *et al.,* 2023, Ahoton *et al.,* 2023). Flavonoids and tannins, have been reported to inhibit cyclooxygenase and lipoxygenase pathways, crucial enzymes in the inflammatory cascade, and exert antioxidant effects that can mitigate inflammation (Mradu *et al.,* 2013, Tazeze *et al.,* 2021). Terpenoids and alkaloids also contribute to analgesic and anti-inflammatory actions by interfering with prostaglandin synthesis and modulating neurotransmitter activity (Tamrat *et al.,* 2017). Phenols have the capacity to modulate inflammatory processes by suppressing pro-inflammatory mediators (Chukwuma *et al.,* 2024). Steroids, are recognized for their potent anti-inflammatory properties to suppress immune responses and reduce swelling (Fatema *et al.,* 2024). Saponins, can interfere with inflammation by stabilizing mast cell membranes and inhibit histamine release (Tazeze *et al.,* 2021, Bello *et al.,* 2022). The presence of steroids further supports the potential to reduce inflammation by inhibiting relevant physiological pathways (Azmi *et al*., 2022). This collective phytochemical profile strongly underscores the therapeutic potential of *F. umbellata* in mitigating inflammatory responses, thereby justifying its traditional medicinal use.

The preliminary acute toxicity studies demonstrated the extract's safety, with no mortality observed at doses up to 5000 mg/kg, indicating a high therapeutic index and suitability for further pharmacological investigation (Zingue *et al.,* 2017). In the present study, the anti-inflammatory activity of the extracts of *F. umbellata* has been established using cotton pellet granuloma method. This model is particularly effective for evaluating antiproliferative and exudative phases of inflammation, allowing for a comprehensive assessment of the therapeutic efficacy (Galvão *et al.,* 2018). In this model, the chronic inflammatory response is induced by implanting sterile cotton pellets subcutaneously, leading to granuloma formation characterized by fluid exudation, proliferation of fibroblasts, and infiltration of inflammatory cells (Kalita *et al*., 2023). The dry weight of these pellets serves as a reliable indicator of granulomatous tissue formation, while the moist weight reflects the transudative component of inflammation (Basavarajaiah *et al*., 2023). The reduction in dry pellet weights following treatments indicate inhibitory effects on both the early transudative and later proliferative phases of chronic inflammation in line with previous study (Kalita *et al.,* 2023). Additionally, this model is recognized for its utility in assessing the capacity of agents to diminish leukocyte infiltration and mitigate granuloma formation, indicating a strong potential for reducing leukocyte migration during inflammation (Tazeze *et al*., 2021). Comparatively, the cotton pellet model provides a robust platform for evaluating anti-inflammatory agents against both exudative and proliferative phases, enabling direct comparisons with standard drugs like Indomethacin, which significantly reduces exudate and granuloma formation (Kalita *et al.,* 2023). Therefore, the decrease in granuloma weight revealed suppression of the proliferative phases, which was effectively inhibited by *F. umbellata* in line with previous studies (Zhang *et al.,* 2020, Kalita *et al*., 2023).

Conclusion

This study provides experimental evidence that methanol crude extract, and fractions of F. *umbellate* present anti-inflammatory potentials in cotton pellet granuloma in albino rats. The results support the folkloric use of the leaves of *F. umbellata* for the treatment of chronic inflammatory conditions.

Conflict of Interest

Authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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