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Anti-Inflammatory Activity of Ethanol Root Extract and Fractions of *Ficus capensis* Thunb in Rat

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Abstract

Inflammation is a natural defense mechanism against injury and infection; however, chronic inflammation can lead to various diseases. Although non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used, their long-term use is associated with adverse effects, prompting interest in safer alternatives. This study evaluated the anti-inflammatory activity of the ethanol root extract of *Ficus capensis* and its solvent fractions in rat models. The roots of *Ficus capensis* were collected, authenticated, and extracted using 80% ethanol. The crude extract was fractionated into n-hexane, ethyl acetate, butanol, and water. Phytochemical screening was performed according to standard protocols. Acute toxicity was assessed using Lorke's method. Anti-inflammatory activity was evaluated using formalin- and carrageenan-induced paw edema models in albino rats. Phytochemical analysis revealed the presence of flavonoids, alkaloids, tannins, saponins, terpenoids and glycosides. The LD_{50} of the extract was > 5000 mg/kg, indicating low acute toxicity. In both inflammation models, the ethanol extract and its fractions produced significant, dose-dependent anti-inflammatory effects compared to the control ($P < 0.05$). The n-hexane fraction exhibited the highest anti-inflammatory activity, comparable to that of diclofenac sodium. This study confirms the traditional use of *Ficus capensis* as an anti-inflammatory agent. These findings support the potential development o*f Ficus capensis*-based anti-inflammatory therapies as safer alternatives to NSAIDs.

Keywords: *Ficus capensis*, inflammation, phytochemicals, anti-inflammatory activity, rat model, paw edema.

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Introduction

Inflammation is a vital protective response of the body to harmful stimuli, such as pathogens, damaged cells, or irritants, and is characterized by redness, swelling, heat, pain, and loss of function (Amani *et al.* 2022). While acute inflammation is beneficial for tissue repair and infection control, chronic inflammation can contribute to the development and progression of several diseases, including arthritis, cardiovascular disease, diabetes, and cancer (Medzhitov, 2021; Furman *et al.*, 2019). Conventional pharmacological management of inflammation largely relies on non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. Although effective, these agents are associated with a range of side effects, including gastrointestinal irritation, renal impairment, and increased cardiovascular risk, particularly with long-term use (Singh *et al.*, 2021; Amani *et al.*, 2022). These concerns have prompted increased interest in plant-derived compounds that may offer safer alternatives with fewer adverse effects. Medicinal plants have historically played a central role in healthcare, particularly in traditional African medicine, where various plant species are used to treat inflammation and pain. One such plant is *Ficus capensis* Thunb., a member of the Moraceae family, commonly referred to as the bush fig. This species is widely distributed in the tropical and subtropical regions of Africa and is traditionally used to treat several ailments, including gastrointestinal disorders, fever, wounds, and inflammatory conditions (Ekor *et al.*, 2020; Oboh *et al.*, 2017). Phytochemical studies of *F. capensis* have revealed the presence of bioactive compounds, such as flavonoids, alkaloids, tannins, saponins, and terpenoids, many of which are known to possess anti-inflammatory, antioxidant, and analgesic properties (Ajiboye *et al.*, 2022; Oladeji *et al.*, 2021).

Despite its wide ethnomedicinal use, there is a paucity of scientific data validating the anti-inflammatory efficacy of the root extracts and solvent fractions of *Ficus capensis*. Hence, this study aims to investigate the anti-inflammatory potential of ethanol root extract and various fractions of *F. capensis* in rat models of formalin-induced and carrageenan-induced paw edema. The findings from this research could support the development of natural anti-inflammatory agents and provide a scientific basis for the traditional use of *F. capensis* in the management of inflammatory disorders.

Materials and Methods

Animals

Albino rats weighing (120-150g) of either sex was procured from Department of Veterinary Medicine, University of Nigeria Nsukka. They were kept in the animal house, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka, Agulu Campus and were given access to water and pelletized vital grower feed. Animals were handled in conformity with the National Institute of Health Guidelines for the care and use of laboratory animals for research purpose (Pub No. 85-23, revised 1985).

Collection and Authentication of plant materials

Root of *fiscus capensis* were collected between 6:00 and 7:30 am in the month of February, 2019 in Irri community Isoko South L.G.A, Delta State, Nigeria. Plant sample was validated by expert plant taxonomist from the Department of Botany, Faculty of Life Sciences, Nnamdi Azikiwe University, Nigeria. It was deposited in the herbarium of the department with voucher number "PCG/474/M/019" was assigned to it.

Preparation of Plant Extract

The roots *were* washed in a running tap to remove dust and other debris, and air dried for two weeks. The dried roots *were* pulverized with electrical blender and kept in clean air tight amber bottle. About, 750 g of the powdered material was cold macerated in 80% ethanol. The mixture was agitated continually for two days (48 hours). The filtrate was recovered and concentrated to dryness using a water bath at 40^0C. The extract was stored in a refrigerator until use. (Onyegbule *et al.,* 2014).

Fractionation

Fractionation of the crude ethanol root extracts of the plant was carried out as described by Ihekwereme *et al. (*2016). Fractionation was performed using N-hexane, Ethyl Acetate and Butanol. The crude extract (150 g) was dispersed in 500 ml of distilled water and poured into a separating funnel. Then, 500 ml of n-hexane was added to the funnel and shaken thoroughly to mix. The mixture was allowed to separate into two distinct layers at room temperature. The n-hexane portion (upper layer) was separated, and the other portion was subjected to fresh n-hexane until the n-hexane solvent became clear. After the n-hexane phase, the other portion was subjected to ethyl acetate and butanol successively using the same process as described for n-hexane. The various fractions were filtered and concentrated to dryness using a water bath set at 40^0C, then stored.

Phytochemical screening

Phytochemical evaluation for the presence of phytoconstituents was performed following the method described by Harborne (1973).

Acute toxicity test

Acute toxicity, LD_{50} test was carried out using the method described by Lorke (1983). A total of 13 rats, weighing 100-120 g all were used in two phases.
In the first stage, the animals were divided into three groups of three mice each, and the extract was administered at three dose levels (10, 100, and 1000 mg/kg) of body weight. The animals were monitored for 24 h. The absence of deaths in the first phase led to the use of 2000, 3000, 4000, and 5000 mg/kg doses of extract for four groups of one animal each. The animals were examined again for 24 h. The number of deaths (s) were noted for each group, and the LD50 was calculated as follows:
$LD_{50} = \sqrt{(D_0 \times D_{100})}$.
Where: D_0 = Highest dose that gave no mortality
D_{100} = Lowest dose that produced mortality.

Anti-inflammatory studies

Formalin induced inflammation

A formalin-induced inflammation test was performed as described by Hosseinzadeh and Younesi (2002). Thirteen (13) groups of 5 rats per group weighing between 120-150 g were used. Group 1 received normal saline (0.9 g) and served as the normal control group. Group 2 was induced but not treated and served as the negative control group. Diclofenac sodium (100 mg/kg) was administered to group 3 and used as a positive control. The rats in group (4-13) were administered 250 and 500 mg/kg as low and high doses of the crude extract and fractions, respectively. Inflammation was induced by injecting 0.05 ml of 2.5% formalin into the left hind paw of each rat. This was performed 30 min after the administration of individual extracts at the stated doses (for test experiments). Hourly changes in paw size and reduction in paw edema were determined using a Vernier caliper. The paw diameter before and after treatment was recorded, and the percentage change in paw diameter was calculated.

Carrageenan-induced rat paw edema

Anti-inflammatory evaluation was performed using the carrageenan-induced rat paw edema method (Winter et al., 1962, Turner, 1965). Rats weighing (120-150 g) were randomly distributed into 13 groups of five animals each. The first group served as control, second group served as standard (received Diclofenac sodium 100 mg/kg, orally), while the third group was induced but received no treatment. Group 4 to 13 were given their various treatments which were: ethanol n-hexane, ethyl acetate, butanol and water fraction, at dose of 250 and 500 mg/kg. After 1h of, treatment, the animals were injected with 0.1mL of 1% w/v suspension of carrageenan into the sub-plantar region of the right hind paw. The paw volumes were measured at 0, 1, 2, 3, 4 and 5 hours after carrageenan injection using plethysmometer, and the mean increase in paw volume. The edema volumes in control (Vc) and in the treated groups (Vt) was calculated.
The percentage inhibition was calculated using the formula:
% Inhibition = Vc-Vt × 100 Where, Vc = Edema volume of control Vt = Edema volume of test.
The third group was induced but received no treatment. Groups 4 to 13 were administered various treatments, including ethanol, n-hexane, ethyl acetate, butanol, and water fractions, at doses of 250 and 500 mg/kg. After 1h of, treatment, the animals were injected with 0.1mL of 1% w/v suspension of carrageenan into the sub-plantar region of the right hind paw. Paw volumes were measured at 0, 1, 2, 3, 4, and 5 h after carrageenan injection using a plethysmometer, and the mean increase in paw volume was calculated. The edema volumes in the control (Vc) and treated groups (Vt) were calculated. The percentage inhibition was calculated using the following formula:

% Inhibition = Vc-Vt × 100 Where, Vc = Edema volume of control Vt = Edema volume of test

Statistical analyses

Data obtained from the study were analyzed using the Statistical Package for Social Sciences (SPSS-27). Results are presented as mean ± standard error of the mean (SEM) of the sample replicates. Raw data were subjected to one-way analyses of variance (ANOVA) followed by post hoc Turkey's test. Statistical significance was set at $P < 0.05$.

Results

Qualitative phytochemical analysis extract and fractions

Qualitative phytochemical analysis of the extract and its fractions revealed the presence of several bioactive compounds (Table 1). The crude extract had a high presence (+++) of flavonoids, alkaloids, steroids, phenols, terpenoids, saponins, tannins, and

cardiac glycosides. Similar compounds were identified in the various solvent fractions (n-hexane, ethyl acetate, butanol, and water), although with varying degrees of intensity.

Acute toxicity (Ld_{50}) result

No mortality or observable signs of toxicity were recorded in either phase of the acute toxicity test. The LD_{50} of the ethanol extract was greater than 5000 mg/kg.

Anti-inflammatory activities of the extract and fractions on formalin-induced inflammation in Rats

Rats treated with 500 mg/kg of n-hexane fraction (NHF) demonstrated the most significant ($P < 0.05$) reduction in paw edema across all time points, showing 87.96% inhibition at the 5th hour compared to 70.36% in the diclofenac-treated group.

The ethanol root extract (ERE) at 500 mg/kg also showed 49.79% inhibition at the 5th hour. The ethyl acetate (EAF), butanol (BTF), and water fractions (WF) exhibited dose-dependent activity, with 500 mg/kg EAF 500 mg/kg WF reaching 62.68% inhibition, 60.84% inhibition, and 60.03% inhibition, respectively (Table 2).

Anti-inflammatory activities the extract and fractions on carrageenan-induced inflammation in Rats

The n-hexane fraction at 500 mg/kg produced the highest anti-inflammatory effect, reaching 83.71% inhibition by the 5th hour. Diclofenac sodium (10 mg/kg) exhibited 81.36% of inhibition. The ethanol root extract (ERE) at 500 mg/kg exhibited 54.14% inhibition, whereas the ethyl acetate, butanol, and water fractions at 500 mg/kg yielded 65.8%, 79.95%, and 70.36% inhibition, respectively (Table 3).

Table 1: Qualitative phytochemical results

Phytochemicals	Ethanol Crude extract	N-hexane fraction	Ethyl Acetate fraction	Butanol fraction	Water fraction
Flavonoids Shinoda test Alkaline reagent test	+++	++	+++	+	+
Alkaloids Wagner's test	++	+	++	++	+
Steroids Liebermann-Burchard test	+++	+++	++	++	-
Phenols Ferric chloride test Lead acetate test	+++	+++	+++	++	+++
Terpenoids Salkowski test	++	+++	++	+	++
Anthroquinone Borntrager s test	+	+	++	++	+
Saponin Frothing test	+++	+++	+++	+++	+++
Tannins Gelatin test	+++	+++	+++	++	+
Carbohydrates Iodine test	+	+	++	+	+
Proteins & Amino acids Ninhydrin test Millon's test	+	++	++	+	+
Reducing sugar	++	++	++	+	++
Resins	++	+	+	+	+
Cardiac Glycosides Keller- Killani test Liebermann's test	+++	+++	+++	++	+++

(-) => Not Present, (+) => Faintly Present, (++) => moderately present, (+++) => Highly present

Table 2: Anti-inflammatory activities the extract and fractions on formalin-induced inflammation in Rats

Treatment	0 hr	1hr	2hr	3hr	4hr	5hr
Distilled water 10ml/kg	100.00±0.00 (0.00%)	99.92±0.08 (0.08%)	99.92±0.08 (0.08%)	99.84±0.16 (0.09%)	99.92±0.08 (0.08%)	100±0.00 (0.00%)
Formalin	100.00±0.00 (0.00%)	102.40±0.4 (-2.4%)	103.46±0.42 (-3.46%)	104.34±0.34 (-4.35%)	104.73±0.32 (-4.74%)	102.40±0.11 (-2.4%)
Diclofenac 10mg/kg	100.00±0.00 (0.00%)	74.79±0.09 (25.21%)	68.43±0.24 (31.57%)	51.98±0.30 (48.02%)	40.76±0.12 (59.24%)	29.64±0.35 (70.36%)
ERE 250 mg/kg	100.00±0.00 (0.00%)	91.55±1.12 (8.45%)	83.43±0.54 (16.27%)	79.12±0.30 (20.88%)	77.88±0.12 (22.12%)	75.11±1.52 (24.89%)
ERE 500 mg/kg	100.00±0.00 (0.00%)	84.54±0.65 (15.46%)	77.93±0.11 (22.07%)	75.77±0.28 (24.23%)	72.27±0.30 (27.73%)	50.21±0.38 (49.79%)
NHF 250 mg/kg	100.00±0.00 (0.00%)	78.12±0.75 (21.88%)	64.96±0.22 (35.04%)	53.41±0.23 (46.59%)	43.98±0.36 (56.02%)	39.70±0.22 (60.03%)
NHF 500 mg/kg	100.00±0.00 (0.00%)	68.70±0.14 (31.30%)	57.24±0.29 (47.76%)	45.35±0.21 (54.65%)	39.17±0.18 (60.83%)	12.04±0.18 (87.96%)
EAF 250 mg/kg	100.00±0.00 (0.00%)	93.56±1.12 (6.74%)	80.37±0.16 (19.63%)	78.10±0.53 (21.90%)	74.67±0.87 (25.33%)	54.13±0.35 (45.87%)
EAF 500 mg/kg	100.00±0.00 (0.00%)	78.30±0.66 (21.70%)	65.28±0.20 (34.72%)	54.17±0.98 (45.83%)	45.14±1.69 (54.86%)	37.32±1.11 (62.68%)
BTF 250 mg/kg	100.00±0.00 (0.00%)	95.27±0.68 (4.73%)	85.16±0.53 (14.84%)	78.87±0.23 (21.13%)	76.32±0.36 (23.68%)	56.44±0.22 (44.56%)
BTF 500 mg /kg	100.00±0.00 (0.00%)	78. 78±0.60 (21.12%)	66.55±0.18 (33.45%)	56.21±0.37 (43.79%)	47.11±0.12 (52.89%)	39.16±0.25 (60.84%)
WF 250 mg/kg	100.00±0.00 (0.00%)	95.89±0.76 (4.11%)	87.34±1.23 (12.66%)	80.56±0.16 (19.44%)	79.63±0.38 (20.37%)	60.13±0.59 (39.87%)
WF 500 mg/kg	100.00±0.00 (0.00%)	78.12±0.05 (21.88%)	64.96±0.22 (35.04%)	53.41±0.23 (46.59%)	43.98±0.36 (56.02%)	39.70±0.22 (60.03%)

Values presented as Mean ± SEM, * $P < 0.05$versus control (repeated measures ANOVA followed by post hoc turkey's test). Where: BTF= Butanol fraction, WF= water fraction, EAF= Ethyl acetate fraction, ERE= ethanol root extract, NHF= n-hexane fraction

Table 3: Anti-inflammatory activities of the extract and fraction son carrageenan-induced inflammation in rats

Treatment	0 hr	1hr	2hr	3hr	4hr	5hr
Distilled water 10ml/kg	100.00±0.00 (0.00%)	99.92±0.08 (0.08%)	99.92±0.08 (0.08%)	99.84±0.16 (0.09%)	99.92±0.08 (0.08%)	100±0.00 (0.00%)
Carrageenan (5%)	100.00±0.00 (0.00%)	102.40±0.24 (-2.4%)	103.46±0.42 (-3.46%)	104.34±0.34 (-4.35%)	104.73±0.32 (-4.74%)	102.40±0.11 (-2.4%)
Diclofenac 10mg/kg	100.00±0.00 (0.00%)	74.79±0.09 (25.21%)	68.43±0.24 (31.57%)	51.98±0.30(48.02%)	40.76±0.12 (59.24%) *	18.64±0.35 (81.36%) *
ERE 250mg/kg	100.00±0.00 (0.00%)	93.21±0.65 (6.79%)	86.13±0.32 (13.87%)	71.22±0.64 (28.78%)	62.17±0.98 (37.83%)	53.23±1.12 (46.77%)
ERE500 mg/kg	100.00±0.00 (0.00%)	87.33±0.29 (12.67%)	76.66±0.92 (23.34%)	68.98±0.34 (31.11%)	57.36±0.39 (42.64%)	45.86±0.11 (54.14%)
NHF 250 mg/kg	100.00±0.00 (0.00%)	81.66±0.98 (18.34%)	74.98±0.28 (25.02%)	66.21±0.30 (33.79%)	40.09±0.12 (59.91%) *	27.81±0.35 (72.19%) *
NHF 500 mg/kg	100.00±0.00 (0.00%)	72.14±0.13 (27.86%)	59.23±1.44 (40.67%)	48.57±0.16 (51.43%)	35.77±0.08 (64.23%)	16.29±0.54 (83.71%) *
EAF 250 mg/kg	100.00±0.00 (0.00%)	87.29±0.29 (12.71%)	80.11±0.11 (19.89%)	70.23±0.10 (29.77%)	63.22±0.85 (36.78%)	57.49±0.28 (42.51%) *
EAF 500 mg/kg	100.00±0.00 (0.00%)	75.34±0.12 (24.66%)	60.18±01.21 (39.82%)	51.37±0.11 (48.63%)	42.15±0.19 (57.85%) *	34.20±0.76 (65.8%) *
BTF 250 mg/kg	100.00±0.00 (0.00%)	78.12±0.05 (21.88%)	64.96±0.22 (35.04%)	53.41±0.23 (46.59%)	43.98±0.36 (56.02%) *	39.70±0.22 (60.03%) *
BTF 500 mg /kg	100.00±0.00 (0.00%)	57.74±0.32 (42.3%)	50.61±0.39 (49.39%)	40.32±0.44 (59.68%) *	31.09±0.38 (68.91%) *	20.08±0.98 (79.95%) *
WF 250 mg/kg	100.00±0.00 (0.00%)	90.12±0.18 (9.88%)	79.41±0.28 (20.59%)	75.39±0.56 (24.61%)	71.58±0.12 (28.42%)	67.16±0.6 (32.84%)
WF 500 mg/kg	100.00±0.00 (0.00%)	74.79±0.09 (25.21%)	68.43±0.24 (31.57%)	51.98±0.30 (48.02%)	40.76±0.12 (59.24%) *	29.64±0.35 (70.36%) *

Values presented as Mean ± SEM, * $P < 0.05$ versus control (repeated measures ANOVA followed by post hoc turkey's test) n=5, ERE=Ethanol root extract, NHF=N-hexane fraction, EAF= ethyl acetate fraction, BTF= Butanol fraction and WF= water fraction.

Discussion

The findings from this study clearly demonstrate that the ethanol root extract and solvent fractions of *Ficus capensis* exhibit significant anti-inflammatory activity in both formalin- and carrageenan-induced rat paw edema models. These models are well-established and widely used for evaluating acute and subacute inflammation, which mimic the biphasic nature of inflammatory responses—the initial phase (mediated by histamine and serotonin) and the delayed phase (mediated by prostaglandins and cytokines) (Sadeghi *et al.*, 2021; Niaz *et al.*, 2023).

In both models, the n-hexane fraction (NHF) showed the most potent anti-inflammatory effects, even surpassing the activity of the reference drug, diclofenac sodium, particularly at the 5th hour post-induction. This suggests that NHF may contain lipophilic bioactive compounds capable of modulating both early and late inflammatory mediators. Lipophilic phytochemicals such as terpenoids and certain flavonoids have been reported to disrupt membrane signaling, inhibit cyclooxygenase (COX), and suppress the nuclear factor kappa B (NF-κB) pathway, thereby exerting broad anti-inflammatory effects (Yuan *et al.*, 2021; Reuter *et al.*, 2023).

Phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, saponins, terpenoids, and glycosides—all of which have been associated with anti-inflammatory mechanisms (Luo *et al.*, 2022). Notably, flavonoids and tannins, which are abundant in *Ficus capensis*, are known to inhibit the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and prostaglandins by downregulating COX-2 and inducible nitric oxide synthase (iNOS) (Ali *et al.*, 2022; Akinmoladun *et al.*, 2023).

In the carrageenan-induced paw edema model, the anti-inflammatory effect of the fractions was sustained, indicating potential involvement in prostaglandin and leukotriene pathways (Tan *et al.*, 2021). The butanol and ethyl acetate fractions, though moderately active compared to NHF, were still significantly more effective than the control. This suggests that their polar constituents—likely polyphenols and saponins—contributed to the observed activity by stabilizing lysosomal membranes and scavenging reactive oxygen species (ROS) (Rahman *et al.*, 2022).

The high LD_{50} value (>5000 mg/kg) aligns with previous studies suggesting that *Ficus capensis* is relatively safe at therapeutic doses (Ekor *et al.*, 2020). This reinforces its potential for safe application in the development of plant-derived anti-inflammatory agents.

In conclusion, the anti-inflammatory activity of *Ficus capensis* root extract and its fractions can be attributed to its rich phytochemical profile, particularly the lipophilic compounds found in the n-hexane fraction. These results not only validate its traditional medicinal uses but also underscore its promise as a candidate for further pharmaceutical development

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