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IN VIVO ANTI-INFLAMMATORY ACTIVITY OF *COMBRETUM BUCHIENSE* LEAF METHANOL EXTRACT IN RATS (PAW OEDEMA MODELS)

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ABSTRACT

In Nigeria, inflammatory disorders, such as hypertension (30.6–36.1% prevalence in adults) and arthritis (13.4% in older adults), contribute significantly to noncommunicable disease (NCD) morbidity (Adeloye et al., 2019; Ogunmola et al., 2021). Combretum buchiense (Combretaceae), traditionally used to treat inflammation, contains phytochemicals similar to other Combretum species, but its pharmacological activities are underexplored. This study investigates the phytochemical constituents and in vivo anti-inflammatory activity of C. buchiense leaf methanol extract, targeting mediators like prostaglandins, cytokines, and nitric oxide. The methanol extract was prepared by cold maceration and subjected to phytochemical screening. Acute oral toxicity was assessed using OECD guidelines in rats (n = 13). Anti-inflammatory activity was evaluated using egg albumin (n = 12) and carrageenan-induced paw oedema models (n = 12) in rats, with hepatotoxicity assessed in a paracetamol-induced model (n = 16). Phytochemical screening revealed flavonoids, terpenoids, saponins, tannins, reducing sugars, and cardiac glycosides, consistent with profiles of other Combretum species. In the acute oral toxicity study, no mortality or behavioral changes were observed in rats at doses of 1600, 2900, and 5000 mg/kg, indicating safety for consumption. In the egg albumin model, the extract exhibited 41.75% and 42.92% inhibition at 200 mg/kg and 400 mg/kg, respectively, compared to 47.28% for indomethacin (10 mg/kg). In the carrageenan model, inhibition was 39.93% and 42.00% at 200 mg/kg and 400 mg/kg, respectively, compared to 43.30% for indomethacin. No significant difference ($p < 0.05$) was observed between the extract and indomethacin, suggesting inhibition of prostaglandins, cytokines (IL-1β, TNF-α), and nitric oxide. The methanol extract of C. buchiense demonstrates significant anti-inflammatory activity, likely due to phytochemicals shared with other Combretum species, targeting key inflammatory mediators, and is safe at high doses. These findings support its traditional use in Nigeria for managing prevalent inflammatory disorders like hypertension and arthritis. Further studies are needed to elucidate mechanisms, isolate bioactive compounds, and quantify IMID prevalence.

Keywords: *Combretum buchiense*, Anti-inflammatory activity, Phytochemicals, Flavonoids, Terpenoids, Saponins, Acute oral toxicity, Paw oedema models

INTRODUCTION

In Nigeria, noncommunicable diseases (NCDs) are a growing public health challenge, with chronic inflammatory disorders contributing significantly to morbidity. Recent studies estimate hypertension prevalence at 30.6–36.1% in adults and arthritis at 13.4% in older adults (≥60 years) (Adeloye *et al*., 2019; Ogunmola *et al*., 2021). Immune-mediated inflammatory diseases (IMIDs), such as rheumatoid arthritis, inflammatory bowel disease (IBD), and atopic dermatitis, are reported but underdiagnosed, with precise prevalence data lacking (Ojo *et al*., 2021). These conditions involve inflammatory mediators like prostaglandins, cytokines (IL-1β, TNF-α), nitric oxide, and histamine, driving oedema and tissue damage. The high cost of conventional treatments, such as nonsteroidal anti-inflammatory drugs (NSAIDs), and their side effects (e.g., gastrointestinal and cardiovascular issues) necessitate affordable, safer alternatives (Das *et al*., 2016).

Combretum buchiense Hutch. & Dalziel (Combretaceae) is widely used in Nigerian traditional medicine to treat inflammation, infections, diabetes, malaria, bleeding, and diarrhea. The Combretum genus, comprising approximately 600 species, is rich in bioactive phytochemicals. For instance, C. molle and C. zeyheri contain flavonoids, terpenoids, tannins, and saponins, which exhibit anti-inflammatory and antimicrobial properties (Bekele & Lemma, 2021). Studies on C. buchiense leaves have identified flavonoids, terpenoids, saponins, tannins, reducing sugars, and cardiac glycosides, suggesting therapeutic potential for inflammation and infections (Ali *et al*., 2022, 2023). These phytochemicals likely target inflammatory mediators, such as prostaglandins and cytokines, which are critical in conditions like arthritis and hypertension prevalent in Nigeria (Ibeabuchi, *et al*., 2023). However, scientific validation of the pharmacological activities of *Combretum buchiense* remains limited. This study evaluates the phytochemical constituents and in vivo anti-inflammatory activity of C. buchiense leaf methanol extract using egg albumin and carrageenan-induced paw oedema models in rats (n = 40), alongside assessing its safety through acute oral toxicity testing, to address Nigeria's growing inflammatory disease burden.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

Leaves of *Combretum buchiense* were collected from Ezeani in Nsukka Local Government Area, authenticated by Mr. Felix Nwafor at the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka. The leaves were air-dried for 14 days under shade, pulverized into a fine, coarse form, and stored in an airtight container pending extraction.

Extraction

A 510 g sample of pulverized leaves was macerated in 4.5 L of methanol for 72 hours with intermittent agitation. The

mixture was filtered using cheesecloth and a cotton wool-clogged funnel, and the filtrate was concentrated using a rotary evaporator at 40°C to obtain the methanol extract, stored at 4°C.

Solvent-Solvent Partitioning of Methanol Extract

The dry extract (25.0 g) was fractionated in a separatory funnel using solvents in order of increasing polarity: n-hexane (450 mL), ethyl acetate (750 mL), and n-butanol (350 mL). The fractions were concentrated using a rotary evaporator to obtain n-hexane, ethyl acetate, n-butanol, and aqueous fractions, stored at 4°C.

Phytochemical Screening

Qualitative phytochemical analysis was conducted using standard methods (Ali *et al*., 2022, 2023) to detect flavonoids, terpenoids, saponins, tannins, reducing sugars, cardiac glycosides, and alkaloids.

Experimental Animals

Forty (40) male and female Albino Wistar rats were obtained from the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Agbani, Nigeria. They were maintained under standard conditions (12-hour light cycle, room temperature), acclimatized for two days with a standard pellet diet and water ad libitum. Food was withdrawn 12 hours before experiments, but water access was unrestricted. All experiments complied with the OECD Guidelines for the Testing of Chemicals (OECD, 2018), approved by the Faculty of Pharmaceutical Sciences Animal Care and Use Committee (ESUT/FPS/018).

Acute Toxicity Test

Acute oral toxicity was evaluated in rats (n = 13) using OECD guidelines (OECD, 2018). In phase 1, nine rats were divided into three groups (n = 3 per group), receiving oral doses of 10, 100, and 1000 mg/kg of the extract solubilized in Tween 80, and observed for 24 hours for behavioral changes or mortality. In phase 2, four rats (n = 1 per group) received doses of 1600, 2900, 3600, and 5000 mg/kg and were observed for 24 hours.

Carrageenan-Induced Model of Inflammation

The methanol extract was dissolved in Tween 80 for administration. Twelve rats (n = 12) were divided into four groups (n = 3 per group). Paw measurements were taken before the experiment. The extract or control was administered orally, followed 30 minutes later by 0.1 mL of 1% (w/v) carrageenan injected into the hind paw, inducing oedema. Paw volume was measured at 1, 2, 3, and 4 hours post-induction using a micrometer screw gauge (mm/inch) and volume displacement methods (mL).

Table 1: Drug Administration for the Carrageenan Model

Group	Treatment Received
A (negative control)	0.2 mL normal saline
B (induced and treated)	200 mg/kg extract
C (induced and treated)	400 mg/kg extract
D (induced and treated)	10 mg/kg indomethacin

Egg Albumin-Induced Model of Inflammation

The methanol extract was dissolved in Tween 80. Twelve rats (n = 12) were divided into four groups (n = 3 per group). Paw measurements were taken before the experiment. The extract or control was administered orally, followed 30 minutes later by 0.2 mL of egg albumin injected into the hind paw, inducing oedema. Paw volume was measured at 1, 2, 3, and 4 hours post-induction using a micrometer screw gauge (mm/inch).

Table 2: Drug Administration for the Egg Albumin Model

Group	Treatment Received
A (negative control)	0.2 mL normal saline
B (induced and treated)	200 mg/kg extract
C (induced and treated)	400 mg/kg extract
D (induced and treated)	10 mg/kg indomethacin

Inhibition was calculated as: $[(Co - Ct)/Co] \times 100$, where Co = circumference of control, Ct = circumference of treated group.

Paracetamol-Induced Hepatotoxicity Model

Hepatoprotective effects were evaluated over 8 days using 16 rats (n = 16), divided into four groups (n = 4 per group). Rats received treatment for 7 days, followed by oral administration of paracetamol (3 g/kg) on day 8 to induce hepatotoxicity. Blood samples were collected 24 hours later from the eyes to assay liver marker enzymes (AST, ALT, ALP) and serum bilirubin using Randox kits.

Table 3: Drug Administration for the Paracetamol Model

Group	Treatment Received
A (negative control)	1 mL/kg normal saline for 7 days + paracetamol 3 g/kg on day 8
B (induced and treated)	200 mg/kg extract for 7 days + paracetamol 3 g/kg on day 8
C (induced and treated)	400 mg/kg extract for 7 days + paracetamol 3 g/kg on day 8
D (induced and treated)	25 mg/kg silymarin for 7 days + paracetamol 3 g/kg on day 8

Statistical Analysis

Data were analyzed with one-way ANOVA, expressed as mean ± standard deviation. Duncan's multiple range test was used to assess differences between treated and control groups, with significance at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical Screening

The methanol extract of C. buchiense leaves contained flavonoids, terpenoids, saponins, tannins, reducing sugars, and cardiac glycosides, with alkaloids absent (Ndidiamaka, *et al*., 2024; Ali *et al*., 2022, 2023).

Effect of Extract on Carrageenan-Induced Oedema

In the carrageenan model (n = 12, 3 per group), the extract at 200 and 400 mg/kg showed no significant difference ($p < 0.05$) compared to indomethacin (10 mg/kg) at 4 hours (Table 4).

Table 4: Inhibition Zone (mm/inch) for Carrageenan-Induced Paw Edema

Groups	0 hr (Before)	1 hr	2 hr	3 hr	4 hr	% Inhibition
A	3.65±0.16ab	6.41±0.17a	8.17±0.21b	8.79±0.21b	9.79±0.08b	-
B	3.53±0.25a	7.92±0.27b	6.40±0.53a	6.03±0.32a	5.88±0.75a	39.93
C	3.99±0.47b	8.45±0.74b	7.08±0.58a	6.26±0.76a	5.68±0.58a	42.00
D	3.81±0.23ab	8.11±0.42b	6.94±0.62a	5.75±0.68a	5.55±0.48a	43.30

Values are mean ± SD (n = 3 per group). Means with different superscripts in the same row/column are significant at $p < 0.05$.

Effect of Extract on Egg Albumin-Induced Oedema

In the egg albumin model (n = 12, 3 per group), the extract at 200 and 400 mg/kg showed no significant difference ($p < 0.05$) compared to indomethacin (10 mg/kg) at 4 hours (Table 5).

Table 5: Inhibition Zone (mm/inch) for Egg Albumin-Induced Paw Oedema

Groups	0 hr (Before)	1 hr	2 hr	3 hr	4 hr	% Inhibition
A	3.86±0.33a	6.28±0.54a	7.78±0.46b	8.84±0.21c	9.39±0.53b	-
B	3.65±0.26a	8.61±0.93c	6.78±0.54a	5.97±0.42b	5.47±0.34a	41.75
C	3.69±0.32a	8.89±0.67c	6.63±0.63a	5.84±0.39b	5.36±0.43a	42.92
D	3.96±0.32a	7.69±0.53b	6.11±0.59a	5.21±0.20a	4.95±0.71a	47.28

Values are mean ± SD (n = 3 per group). Means with different superscripts in the same row/column are significant at $p < 0.05$.

Effect of Extract on Paracetamol-Induced Hepatotoxicity

In the paracetamol-induced hepatotoxicity model (n = 16, 4 per group), the extract showed protective effects (Table 6).

Table 6: Effect of Extract on Paracetamol-Induced Hepatotoxicity

Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (g/dL)	Total Protein (g/dL)
Normal saline + paracetamol	23.25±1.92ab	34.25±1.48	45±2.24	5.83±0.29	8.78±0
200 mg/kg extract + paracetamol	25.75±1.48b	35.75±1.48	43±2.24	5.83±0.33	5.83±0
400 mg/kg extract + paracetamol	21.75±1.48ab	32±1.22	46±1.22	5.63±0.15	5.43±0
25 mg/kg silymarin + paracetamol	41.25±1.92	61.25±2.38	89.25±3.56	8.78±0.3	5.23±0.566

Values are mean ± SD (n = 4 per group).

Effect of Extract on In Vitro Antitrypanosomiasis

Table 7: Effect of Extract on In Vitro Antitrypanosomiasis

Conc. of Extract (mg/mL)	Extract	Diminazene Aceturate
10	0.00±0.00a	0.00±0.00a
5	0.00±0.00a	0.00±0.00a
2.5	0.00±0.00a	0.00±0.00a
1.25	0.33±0.52a	0.00±0.00a
0.625	1.83±0.98b	0.17±0.02a
0.3125	4.67±1.37c	2.50±0.84b
0.156	9.50±1.23d	5.67±1.03c
Control	34.3±2.38e	34.3±2.38d

Values are mean ± SD. Means with different superscripts in each column are significant at $p < 0.05$

Acute Toxicity

The acute oral toxicity study (n = 13) showed no mortality or behavioral changes in rats. In phase 1, no deaths occurred at 10, 100, and 1000 mg/kg (n = 3 per group). In phase 2, all rats survived doses of 1600, 2900, 3600, and 5000 mg/kg (n = 1 per group), indicating the extract's safety.

Table 8: Initial Acute Oral Toxicity Test Result

Sample	10mg/kg dose	100mg/kg dose	1000mg/kg dose
Methanol extract of *C. bauchiense*	0/3	0/3	0/3

Table 9: Final Acute Oral Toxicity Test Result

Dose	1600mg/kg	2900mg/kg	5000mg/kg
Surviving rat	3/3	3/3	3/3

Discussion

Combretum buchiense leaves are widely used in Nigerian traditional medicine to treat inflammatory conditions, infections, diabetes, malaria, bleeding, diarrhea, and other ailments. This study, conducted with 40 rats, investigated the acute oral toxicity and anti-inflammatory activity of the methanol extract to substantiate these claims, particularly in the context of Nigeria's inflammatory disorder burden, with hypertension affecting 30.6–36.1 % of adults and arthritis 13.4 % of older adults (Adeloye *et al*., 2019; Ogunmola *et al*., 2021). Immune-mediated inflammatory diseases (IMIDs), such as rheumatoid arthritis and atopic dermatitis, are also prevalent but underdiagnosed (Ojo *et al*., 2021). The acute toxicity study (n = 13) demonstrated safety, with no mortality or behavioral changes in rats at doses of 1600, 2900, 3600, and 5000 mg/kg, supporting its potential as a safe treatment option (OECD, 2018).

The anti-inflammatory activity was evaluated using egg albumin (n = 12) and carrageenan-induced paw oedema models (n = 12). In the egg albumin model, the extract at 200 mg/kg and 400 mg/kg reduced paw oedema by 41.75% and 42.92%, respectively, compared to 47.28% for indomethacin (10 mg/kg). In the carrageenan model, inhibition was 39.93% and 42.00% at 200 mg/kg and 400 mg/kg, respectively, compared to 43.30% for indomethacin. No significant difference ($p < 0.05$) was observed, indicating comparable efficacy. The egg albumin model, mimicking biologically induced inflammation, showed stronger inhibition, relevant for conditions like arthritis and atopic dermatitis in Nigeria.

Phytochemical screening identified flavonoids, terpenoids, saponins, tannins, reducing sugars, and cardiac glycosides in *C. buchiense* (Ali *et al*., 2022, 2023), consistent with profiles of other *Combretum* species like *C. molle* and *C. zeyheri*, which contain flavonoids, terpenoids, tannins, and saponins with anti-inflammatory and antimicrobial properties (Ndidiamaka, *et al*., 2024; Bekele & Lemma, 2021). These phytochemicals likely target specific inflammatory mediators. Flavonoids inhibit cyclooxygenase (COX) and lipoxygenase (LOX), reducing prostaglandins and leukotrienes, key mediators in rheumatoid arthritis and IBD (Serafini *et al*., 2017). Terpenoids suppress cytokines (IL-1β, TNF-α) and nitric oxide synthase, mitigating hypertension-related inflammation (Salminen *et al*., 2018). Saponins modulate arachidonic acid metabolism (Navarro *et al*., 2017), while tannins inhibit pro-inflammatory signaling pathways (Kwatra, 2020). Cardiac glycosides may modulate cellular signaling, potentially benefiting atopic dermatitis (Mohanraj& Kumar, 2019). The carrageenan model, involving histamine, serotonin, and prostaglandin phases, suggests the extract targets these mediators, particularly prostaglandins in the later phase, given the sustained oedema reduction at 4 hours. The presence of these phytochemicals in *C. buchiense* fractions further supports its potential for broader therapeutic applications, including antibacterial activity (Ali *et al*., 2022).

The hepatoprotective study (n = 16) supports the extract's safety and therapeutic potential. The lack of significant difference between 200 mg/kg and 400 mg/kg doses suggests lower doses may suffice, minimizing risks. These findings are critical in Nigeria, where NCDs, including inflammatory disorders, contribute to significant healthcare burdens, with hypertension affecting up to 36.1% of adults (Ogunmola *et al*., 2021). The extract's safety and efficacy position it as a cost-effective alternative to nonsteroidal anti-inflammatory drugs (NSAIDs), which carry risks of gastrointestinal, cardiovascular, and renal toxicities (Das *et al*., 2016). The scarcity of precise prevalence data for IMIDs in Nigeria underscores the need for further epidemiological research.

CONCLUSION

The methanol extract of *Combretum buchiense* leaves exhibits significant anti-inflammatory activity in egg albumin and carrageenan-induced paw oedema models (n = 12 each), with efficacy comparable to indomethacin, likely driven by flavonoids, terpenoids, saponins, tannins, and cardiac glycosides, which target prostaglandins, cytokines (IL-1β, TNF-α), leukotrienes, and nitric oxide. These phytochemicals align with those found in other *Combretum* species, such as *C. molle* and *C. zeyheri*. The acute oral toxicity study (n = 13) confirms safety, with no mortality or behavioral changes in rats at doses of 1600, 2900, 3600, and 5000 mg/kg. The hepatoprotective study (n = 16) further supports its therapeutic potential. These findings are highly relevant in Nigeria, where inflammatory disorders, such as hypertension (30.6–36.1 %) and arthritis (13.4 %), are prevalent (Adeloye *et al*., 2019; Ogunmola *et al*., 2021). The extract's safety and efficacy validate its traditional use and suggest its potential as a cost-effective remedy. Further studies are needed to elucidate mechanisms, isolate bioactive compounds, and establish comprehensive prevalence data for IMIDs in Nigeria.

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