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Evaluation of anti-inflammatory activities of ethanolic extract and solvent fractions of *Cleistopholis staudtii* leaves

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Abstract

The ethanol leaf extract of *Cleistopholis staudtii* and its solvent fractions were evaluated for safety and bioactivity. The aim of this study was to investigate acute and chronic anti-inflammatory potential of an ethanolic leaf extract and fractions of *C. staudtii* in animal models. The ethanolic extract and fractions of *C. staudtii* leaf extract was prepared and administered orally to experimental animals used. The anti-inflammatory activity was determined by egg albumin induced paw edema in mice and formaldehyde-induced arthritis in rats. Acute oral toxicity testing revealed an LD_{50} >5000 mg/kg with no mortality, indicating exceptional safety. Pharmacological evaluation demonstrated potent dose-dependent anti-inflammatory effects: the n-butanol fraction (500 mg/kg) showed 97.44% inhibition in acute egg albumin-induced paw edema (4 h) and 41.12% inhibition in chronic formaldehyde-induced arthritis (day 14). LC-QTOF-MS analysis of bioactive sub-fractions identified high-confidence compounds (scores >90%), including Salicin ($C_{13}H_{18}O_7$; precursor to aspirin; glycoside) – analgesic/anti-inflammatory; Eugenol ($C_{10}H_{12}O_2$; phenylpropanoid) – COX-2 inhibition & antioxidant; Kaempferol-3-O-β-diglucoside ($C_{27}H_{30}O_{16}$; antioxidant flavanoid) – NF-κB pathway modulation; 3,4,5-Trimethoxycinnamic acid ($C_{12}H_{14}O_5$; anti-inflammatory phenolic) – TNF-α suppression; and Neobavaisoflavone ($C_{20}H_{18}O_4$; antioxidant flavanoid) – dual COX/LOX inhibition. These results provide a scientific basis for *C. staudtii*'s traditional use and identify its butanol fraction as a rich source of multi-target anti-inflammatory agents with clinical potential.

Keywords: *Cleistopholis staudtii*; Inflammation; n-butanol; N-hexane; Methanol; Fractionation; Extract

1. Introduction

Inflammation is a natural protective and biologically essential response of the body to stimuli that are harmful to the body, such as irritants, pathogens or damaged cells. [1, 2]. It typically involves a complex sequence of physiological changes initiated by the immune system, molecular and cellular mediators and blood vessels [1, 2]. Inflammation is mediated by the activation of immune cells (e.g., macrophages, neutrophils) and the release of inflammatory mediators such as cytokines (e.g., TNF-α, IL-1β), prostaglandins, nitric oxide (NO), and reactive oxygen species (ROS). The nuclear

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factor-kappa B (NF-κB) signaling pathway is a central regulator of inflammatory gene expression [3]. Inhibition of NF-κB activation, suppression of pro-inflammatory cytokines, and reduction of prostaglandin synthesis via cyclooxygenase (COX) inhibition are common mechanisms through which natural and synthetic agents exhibit anti-inflammatory effects [4].

Cleistopholis staudtii is a medium-sized to tall evergreen tree belonging to the family Annonaceae, commonly found in the tropical rainforests of West and Central Africa [5]. The tree typically reaches heights of 20–35 meters, with a straight bole and smooth, greyish bark that releases a fragrant, oily exudate when incised [5]. The leaves are simple, alternate, oblong to elliptical, and glabrous, with a characteristic aromatic scent. Flowers are solitary, large, and greenish-yellow, and the fruits are cylindrical to slightly curved, containing numerous seeds embedded in a yellow pulp [5].

This research was designed to evaluate the anti-inflammatory properties of *Cleistopholis staudtii* leaves extract using adult Swiss albino rats as test animals.

2. Materials and Methods

2.1. Materials

Fresh egg albumin, Diclofenac tablets. Analytical grade ethyl acetate, n-hexane, methanol, and n-butanol were purchased from an accredited vendor.

Fresh leaves of *Cleistopholis staudtii* were collected from a single location at Amawbia, Anambra State, Nigeria on the 4th of May 2021.

Adult Swiss albino rats (180 – 200 g) of both sexes were used for this study.

2.2. Methods

2.2.1. Plant material authentication and preparation

The plant material was identified by Prof. B.A. Anyinde of the Department of Pharmacognosy, University of Benin and authenticated by Mr. Ozioko of Bioresources Development and Conservation Programme (BDCP). A voucher specimen PCG/473/A/021 was deposited at the Herbarium of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. The plant materials were cleaned, spread on a clean sack and placed under the shade for air drying at room temperature for 7 days. The plant materials were further dried in the oven maintained at 40 °C followed by milling into a coarse powder using modern laboratory electric milling machine. The powdered materials were stored in airtight container before use.

2.2.2. Plant extraction and fractionation

Two and half kilogram of the pulverized leaves *C. staudtii* extracted by cold maceration in 7.5L of ethanol for 72 h with intermittent shaking. The resulting solution was filtered, and the filtrate concentrated to dryness in vacuo using rotary evaporator (RE300 Model, United Kingdom) at 40 °C. Then 200 g of the extract was dissolved in 400 ml of water and subjected to liquid-liquid partition successively and exhaustively with n-hexane, ethyl acetate and n-butanol in increasing order of polarity. Fractions soluble in these solvents were concentrated in vacuo using rotary evaporator to obtain the n-hexane fraction (HF), ethyl acetate fraction (EAF) and n-butanol fraction (BF). The remaining fraction was the water fraction (WF). The plant extract and fractions were stored in refrigerator between 0-4 °C.

2.2.3. Animal Conditioning

Adult swiss albino mice weighing between 25 g – 30 g and adult swiss albino rats weighing 180 g – 200 g were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Nigeria. The animals were maintained on standard laboratory animal conditions and fed with rodent feed (Guinea Feeds Nigeria Ltd). They were allowed free access to water ad libitum. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals and approved by the Nnamdi Azikiwe University Animal Ethical Committee with approval number NAU/AREC/2025/0076.

2.2.4. Acute Inflammation Assay

The systemic acute inflammation of methanol extracts and fractions of *Cleistopholis staudtii* was studied using the method described by Winter *et al*, [6]. A total of 50 rats were used. They were fasted for 5 h and deprived of water only

during the experiment to ensure uniform hydration and to minimize variability in oedematous response. They were grouped into 10 groups of five rats per group. Grouped 1 received 10ml/kg distilled water. Grouped 2 received 50mg/kg diclofenac; group 3 and 4, received 250mg and 500mg of crude extract respectively; group 5 and 6, received 250mg and 500mg of n-hexane fractions respectively; group 7 and 8 received 250mg and 500mg of ethyl acetate fraction respectively; while group 9 and 10, received 250mg and 500mg of butanol fractions respectively. 1-hour post treatment, the animals received sub-plantar injections of 0.1ml of fresh egg albumin in the right hind paw. The paw size was measured before (basal) and at 1, 2, 3, 4, and 5 hours after egg albumin injection using fluid displacement method. Increase in the paw volume was quantified as inflammation.

$$\%\ Inhibition = 100\left[1 - \frac{a - x}{b - y}\right]$$

Where;
a= Mean paw volume of treated rats at various time interval after egg albumin injection.
x= Mean paw volume of treated rats before egg albumin injection.
b= Mean paw volume of control rats at various time interval after egg albumin injection.
y= Mean paw volume of control rats before egg albumin injection.

2.2.5. Formaldehyde-induced arthritis

The effect of the methanol extract and fractions of *Cleistopholis staudtii* on arthritis was studied using formaldehyde-induced arthritis in rats as described by Selye [7]. A total of 55 adult albino rats were used for the study. They were grouped into 11 groups of 5 rats per group. Group 1 received 10ml/kg of distilled water orally. Group 2 received 50ml/kg of diclofenac. Group 3 received 1. Group 4 received 250ml/kg of extract orally. Group 5 received 500ml/kg of extract orally. 1 hour after the administration on day 0, the animals received sub-plantar injection of 0.1ml of 2.5% (v/v) formaldehyde in the right hind paw on day. The paw volume was measured before (basal) and on day 3, 7, 14, 21, and 28. On day 3, the paw was re-inflamed with formaldehyde. The extract was administered once daily for the 28 days. The volume of water displaced by the inflamed paw was used as a measure of edema, increase in paw volume is quantified as inflammation. Percentage inhibition of edema was calculated using the formula:

$$\%\ Inhibition = 100\left[1 - \frac{a - x}{b - y}\right]$$

Where;
a= Mean paw volume of treated rats at various time interval after formaldehyde injection.
x= Mean paw volume of treated rats before formaldehyde injection.
b= Mean paw volume of control rats at various time interval after formaldehyde injection.
y= Mean paw volume of control rats before formaldehyde injection.

2.2.6. Statistical analysis

Results were presented as mean ± Standard error of mean (SEM). The analysis of variance in the outcome of the treatment (one-way ANOVA) was done using Statistical Package for Social Science (SPSS, version 20). Multiple comparisons for post hoc analysis was done using Turkey's test. The differences between the treatment groups were determined by multiple comparisons of mean ranks for all groups. In all cases, a probability error of less than 0.05 was selected as the criterion for statistical significance. Calculation of fifty percent inhibitory concentration (IC50) of the extracts and fractions was carried out using regression equation in Microsoft Excel, 2007.

3. Results

3.1. Percentage yield of extracts and fractions

After extracting the dried leaves of *C. staudtii* with ethanol, the yield of the ethanol crude extract was about one-quarter of the powdered leaves (19.1%) as is shown in Table 1 below. The bulk of the extract distributed into the n-hexane and ethyl acetate fractions, while the n-butanol fraction gave the least yield when compared to other fractions.

Table 1 Yield of methanol extract and fractions of *C. staudtii*

Extract/Fractions	Yield (g)	Yield (%w/w)
Ethanol extract	764.0	19.1[a]
n-hexane fraction	68	27.2[b]
Ethyl acetate fraction	76	30.4[b]
n-Butanol fraction	56	22.4[b]

[a]Yield calculated from 4000 g of powdered leaves; [b]Yield calculated from 250 g of ethanol extract

3.2. Acute Inflammatory Study

The paw edema response observed in the control group (10 mL/kg distilled water) peaked at 1 hour (0.92 mL) and gradually declined to 0.87 mL at 4 hours, indicating a sustained inflammatory response. In contrast, treatment with 50 mg/kg diclofenac, a standard non-steroidal anti-inflammatory drug (NSAID), significantly suppressed edema formation across all time points, decreasing from 0.75 mL at 1 hour to 0.52 mL at 4 hours, thereby validating the experimental model.

Among the test groups, a dose-dependent inhibition of paw edema was noted for the crude extract of *Cleistopholis staudtii*. At 100 mg/kg, the extract reduced paw volume from 0.77 mL (1 hour) to 0.53 mL (4 hours), while 250 mg/kg and 500 mg/kg further decreased edema to 0.48 mL and 0.45 mL at 4 hours, respectively. These results are comparable to diclofenac, especially at the highest dose, suggesting potent anti-inflammatory activity. The butanol fraction at 500 mg/kg showed a particularly robust reduction (from 0.63 mL at 1 hour to 0.46 mL at 4 hours), supporting the presence of bioactive compounds with anti-inflammatory properties in this fraction.

Ethyl acetate and N-hexane fractions showed moderate effects. The ethyl acetate fractions (250 mg/kg and 500 mg/kg) led to edema reductions to 0.62 mL and 0.59 mL at 4 hours, respectively. N-hexane fractions demonstrated lesser activity, with values remaining above 0.67 mL at 4 hours, indicating weaker anti-inflammatory effects compared to other fractions.

The anti-inflammatory effects observed across the treatment groups, especially in the crude extract and butanol fractions, may be attributed to the presence of phytochemicals such as alkaloids, flavonoids, and tannins, which are known to modulate pro-inflammatory mediators such as prostaglandins, histamine, and bradykinin [8, 9]. Flavonoids, in particular, have demonstrated the ability to inhibit enzymes like cyclooxygenase and lipoxygenase, which are central to the inflammatory cascade [10].

This anti-inflammatory potential has hepatoprotective implications. Chronic liver injury often involves oxidative stress and inflammation as key pathological features [11]. Compounds with demonstrated anti-inflammatory activity may attenuate hepatic inflammation and fibrosis by modulating cytokine production, reducing neutrophil infiltration, and stabilizing hepatic cell membranes [12]. The ability of the extract and its fractions to suppress acute inflammation in the paw edema model suggests that similar pathways may be modulated in hepatic tissues, thereby conferring hepatoprotection.

This hypothesis aligns with previous studies where anti-inflammatory agents showed hepatoprotective effects in chemically-induced liver damage models [13, 14]. Thus, the observed efficacy of *Cleistopholis staudtii* in reducing paw edema lends pharmacological credence to its ethnomedicinal use for liver-related disorders and supports its inclusion in drug development programs targeting hepatic inflammation.

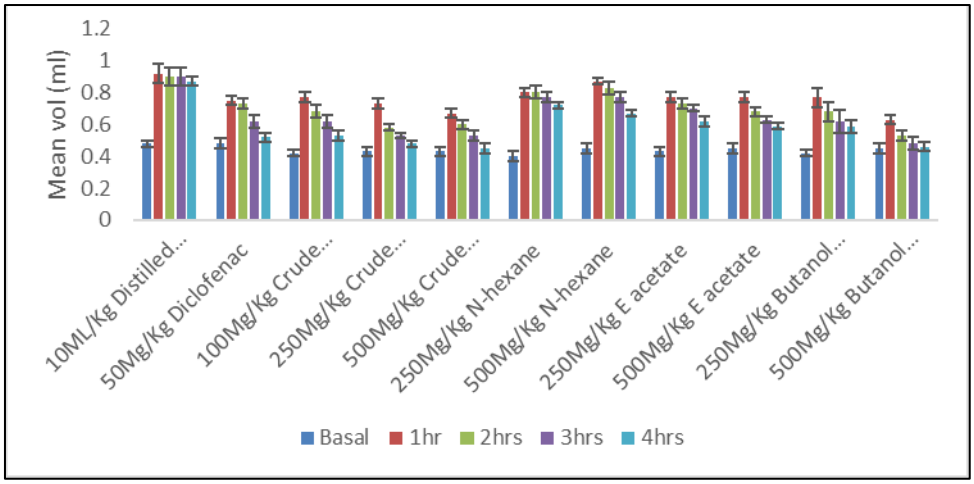

Figure 1 A bar chart of mean paw volume against doses for acute inflammation

3.3. Anti-inflammatory potentials of *Cleistopholis staudtii* extracts and fractions

The figure 2 below presents the percentage inhibition of egg albumin-induced paw edema at various time points in rats treated with crude extract and solvent fractions of *Cleistopholis staudtii*, compared to the standard anti-inflammatory drug, diclofenac (50 mg/kg). The model employed—acute paw edema induction—is widely validated for assessing the anti-inflammatory efficacy of test substances via suppression of fluid accumulation and inflammatory cell infiltration [6].

Diclofenac, as expected, showed high inhibitory activity across all time points, reaching 89.74% by the 4th hour, which confirms the reliability of the model. The crude extract of *C. staudtii* demonstrated a dose-dependent inhibition, with values rising from 20.45% (100 mg/kg at 1 hr) to 94.87% (500 mg/kg at 4 hrs). Notably, both 250 mg/kg and 500 mg/kg of the crude extract surpassed the efficacy of diclofenac from the third hour onward, indicating a strong and sustained anti-inflammatory effect likely attributed to synergistic phytoconstituents in the full-spectrum extract.

The solvent-partitioned fractions showed varying degrees of anti-inflammatory activity. The butanol fraction at 500 mg/kg exhibited the highest percentage inhibition across all time points among the fractions, peaking at 97.44% by the 4th hour, even exceeding diclofenac. This suggests that highly polar compounds—typically flavonoids, tannins, and saponins—may be responsible for the anti-inflammatory action [15]. The ethyl acetate fractions displayed moderate activity, with 500 mg/kg reaching 64.10% inhibition at the 4th hour. Conversely, the N-hexane fractions showed weak anti-inflammatory effects, barely exceeding 43.59% even at the highest dose, suggesting that non-polar constituents (e.g., fatty acids, sterols) contribute less to the extract's bioactivity.

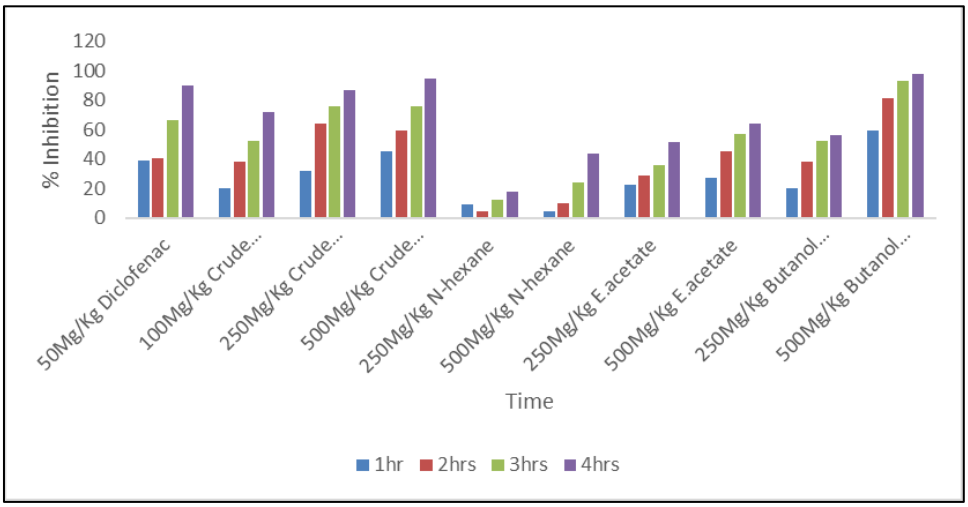

Figure 2 A bar chart of percentage inflammation inhibition against time

4. Discussion

Inflammation is a key pathological feature in acute and chronic liver injury, with inflammatory mediators playing a pivotal role in hepatocyte damage and fibrogenesis [16, 17]. The anti-inflammatory potential of *Cleistopholis staudtii* was assessed using both acute (egg albumin-induced paw edema) and chronic (formaldehyde-induced arthritis) inflammation models, which offer valuable insights into the extract's immunomodulatory and hepatoprotective potential.

In the acute inflammation model, the negative control group exhibited a sustained paw edema response (0.92 mL at 1 hour to 0.87 mL at 4 hours), reflective of a typical inflammatory cascade. In contrast, 50 mg/kg diclofenac sodium significantly suppressed edema formation, validating the assay's sensitivity. Remarkably, the crude extract of *C. staudtii* showed dose-dependent inhibition, with 500 mg/kg reducing paw volume to 0.45 mL by 4 hours, achieving 94.87% inhibition, even surpassing diclofenac (89.74%). This robust and sustained suppression of inflammation suggests that the extract contains bioactives capable of modulating early and late phases of acute inflammation. Among all fractions, the butanol fraction at 500 mg/kg was the most effective, reducing paw volume to 0.46 mL (97.44% inhibition at 4 hours). This significant effect is likely due to the high concentration of polar phytoconstituents—particularly flavonoids, tannins, and saponins—which are known to inhibit cyclooxygenase (COX), lipoxygenase (LOX), and other pro-inflammatory pathways [10, 18]. The pronounced anti-inflammatory activity observed for the butanol fraction aligns with its superior antioxidant and hepatoprotective properties, as shown in previous assays.

The chronic inflammation model further reinforces these findings. While the control group (distilled water) demonstrated a persistent inflammatory response over 28 days, treatment with 500 mg/kg crude extract led to a peak inhibition of 37.38% at Day 7, which remained high (31.63%) at Day 28, indicating prolonged anti-inflammatory activity. This extended effect is pharmacologically relevant, as chronic liver diseases such as hepatitis and steatohepatitis involve sustained inflammatory insults [12, 19].

Similarly, the 500 mg/kg butanol fraction exhibited strong activity in the chronic model, peaking at 41.12% inhibition on Day 7 and maintaining significant suppression throughout the study. The rapid onset and sustained action suggest that the butanol fraction contains water-soluble, rapidly bioavailable compounds capable of modulating inflammatory signaling over time. Such agents may downregulate nuclear factor-kappa B (NF-κB) and pro-inflammatory cytokines like TNF-α and IL-1β, which are implicated in liver inflammation and fibrosis [20].

These anti-inflammatory effects have direct hepatoprotective implications. Inflammation contributes to liver damage by promoting oxidative stress, neutrophil infiltration, and hepatocyte necrosis [16, 21]. The demonstrated ability of *C. staudtii*—particularly its butanol fraction—to suppress both acute and chronic inflammation suggests its potential to attenuate hepatic inflammation, stabilize hepatocyte membranes, and protect against fibrogenic progression.

5. Conclusion

The present study evaluated the anti-inflammatory properties of the crude extract and solvent fractions of *C. staudtii.* The resulting data from the study positively supports the ethnomedicinal use of the plant in managing inflammatory disorders.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare the absence of any conflicts of interest

Statement of ethical approval

Ethical approval for the use of Animals for the study was issued by Nnamdi Azikiwe University Animal Ethical Committee with approval number NAU/AREC/2025/0076.

Contributions of the Authors

All the authors were involved in the conceptualization, planning and the execution of the research study.

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