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# Anti-inflammatory activity and secondary metabolite profiling of *Eucalyptus camaldulensis* (Myrtaceae)

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## Abstract

**Aim:** This study investigated the anti-inflammatory effect of the extract and fractions of *Eucalyptus camaldulensis* and also profiled the secondary metabolites of its most active fraction.

**Methods:** The leaves of *E. camaldulensis* were collected, authenticated and extracted with methanol. The extract (100, 200, and 400 mg/kg), normal saline (negative control), and aspirin (positive control) were administered orally to egg-induced paw oedema rats of five groups of five rats each. The extract was partitioned, and each of the solvent fractions was assayed for its anti-inflammatory activity. The results obtained were subjected to one-way analysis of variance (ANOVA) followed by Bonferroni post hoc tests, and $p < 0.05$ was considered significant. Also, the most active fraction was subjected to gas chromatography-mass spectrometry (GC-MS) analysis.

**Results:** The extract at 100 mg/kg demonstrated the best anti-inflammatory effect at 29%, while the *n*-hexane (N-HEX) fraction gave the highest inflammatory inhibition at 43%. α-Phellandrene, *o*-cymene, *n*-hexadecanoic acid, and beta-sitosterol were identified as the most abundant compounds in the N-HEX fraction.

**Conclusions:** The study concluded that the methanol extract of *E. camaldulensis* possesses good anti-inflammatory properties, and its non-polar fraction was responsible for the observed activity. Bioassay-guided purification of anti-inflammatory constituents of the N-HEX fraction is recommended for future studies.

## Keywords

inflammation, *Eucalyptus camaldulensis*, extract, GC-MS

## Introduction

Inflammation is a complex biological response of the immune system to harmful stimuli, including pathogens and non-pathogens [1]. It is primarily caused by factors such as toxic chemicals, environmental agents, trauma, overuse, or infection [2, 3]. Inflammation plays a crucial role in wound healing and infection control, but it is also linked to chronic diseases, including cardiovascular disease and cancer [4, 5].

There are two main types of inflammation: acute and chronic. Acute inflammation has a rapid onset, typically resolving within a few days, and is characterized by classic signs and symptoms, including erythema, swelling, and pain [6, 7]. Chronic inflammation, on the other hand, has a slow onset and long duration, often persisting for years, and is characterized by a cellular infiltrate composed of monocytes, macrophages, and lymphocytes [8, 9].

During an inflammatory response, activated immune cells produce and secrete pro-inflammatory mediators, including histamine, serotonin, eicosanoids, and cytokines [10, 11]. These mediators play a crucial role in destroying the effects of inflammation and promoting tissue repair [11]. Inflammation is a complex biological response that plays a crucial role in maintaining tissue homeostasis. Understanding the mechanisms of inflammation and the role of inflammatory mediators can provide insights into the development of new therapeutic strategies for the treatment of inflammatory diseases [12, 13].

Medicinal plants, such as *Eucalyptus camaldulensis*, have been traditionally used to treat various diseases, including inflammatory conditions [14]. *E. camaldulensis* Dehn. (family: Myrtaceae) is a medium-sized to tall (30 m), evergreen and perennial tree [15]. In Nigeria, *E. camaldulensis* is widely used in traditional medicine to manage a variety of ailments [16, 17]. It is primarily employed in the treatment of respiratory conditions such as sore throat, cough, and other lung-related diseases, owing to the expectorant and antibacterial properties of its essential oils [18]. Additionally, the plant is used to treat skin infections, typhoid fever, malaria, as well as gastrointestinal disorders, including diarrhea and dysentery [19]. Various pharmacological activities have been reported for different morphological parts of *E. camaldulensis*, including anti-inflammatory, analgesic, anti-nociceptive, cytotoxic, insecticidal, antimicrobial, and antidiabetic properties [16, 20–26].

Several phytoconstituents have been reported in *E. camaldulensis*, contributing to its pharmacological activities. These include essential oils, sterols, alkaloids, glycosides, flavonoids, tannins, polyphenols, and terpenoids [27–30]. However, previous studies did not identify the specific fraction(s) responsible for the observed bioactivities. Therefore, this study aims to evaluate the anti-inflammatory effects of the methanol extract and its partitioned fractions of *E. camaldulensis* leaves. It also identifies the chemical constituents of its most active fraction.

## Materials and methods

### Materials and reagents

The following equipment and reagents were used in this study: rotary evaporator (RE301/601/801 model, Yamato Scientific America, Inc., U.S.A), chiller (Churchill, Instrument Co. Ltd, U.K), vacuum pump (MB 338618 model, Edwards High Vacuum Int., England), oven (Hearson & Co. Ltd, London), Mettler electronic weighing balance (AB 54 model, Mettler Toledo, U.S.A), methanol, *n*-hexane (N-HEX, GFS Chemical Inc), dichloromethane (DCM, GFS Chemical Inc), ethyl acetate (EtOAc, GFS Chemical Inc), Tween 80, normal saline.

### Plant collection and extraction

The leaves of *E. camaldulensis* were collected from Obafemi Awolowo University, Ile-Ife campus. A voucher specimen of the plant was prepared and deposited in the Faculty of Pharmacy herbarium, where the

voucher number FPI 2356 was obtained. The plant material was air-dried, pulverized, and sealed in an air-tight nylon bag. Five hundred grams (500 g) of the plant material was extracted with methanol (1 L) three times for 48 h each to obtain both polar and non-polar chemical constituents. The methanolic solution was filtered and concentrated with a rotary evaporator to obtain the crude extract (76 g). Thereafter, the extract was suspended in distilled water and partitioned successively into N-HEX, DCM, and EtOAc to afford N-HEX (19 g), DCM (16 g), EtOAc (10 g), and aqueous (AQ, 23 g) fractions.

### Experimental animals

The Animal Breeding House, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria, supplied healthy male Wistar rats (120–150 g), which were acclimated for two weeks. Standard rat food (vital feeds) and unlimited water were given to them. The Guide for the Care and Use of Laboratory Animals (The National Academies Press, 2011) was followed in all animal research. Additionally, the Institute of Public Health at Obafemi Awolowo University in Ile-Ife, Osun State, Nigeria, granted ethical clearance for the study (HREC NO: IPHOAU/12/1772).

### Investigation of the anti-inflammatory property of *E. camaldulensis* leaf extract and fractions

The experimental animals were randomly divided into test (extract/fraction), positive control, and negative control groups, with five animals in each group. A 10% Tween 80 solution served as the negative control, while aspirin was used as the reference drug. The test groups received the extract at doses of 100, 200, and 400 mg/kg. Oedema was induced in the rats by injecting 0.1 mL of egg albumin into the subplantar region of the right hind paw in the test, positive, and negative control groups. Thirty minutes after oedema induction, the animals were treated with the extract/fraction, aspirin, and Tween 80 in the respective groups. Paw thickness was measured in millimeters using a digital Vernier caliper before oedema induction and subsequently at 1, 2, 3, and 4 hours post-induction [30]. For the anti-inflammatory activity of the fractions (N-HEX, DCM, EtOAc, and AQ fractions), the most active dose of the extract was adopted for assaying their effect on egg albumin-induced paw oedema, while aspirin and 10% Tween 80 were retained as the positive and negative controls. The percentage inhibition of oedema was calculated using the following formula:

$$\% \text{ Inhibition } = 100\,(1 - \text{Vt} / \text{Vc})$$

Vc represents oedema volume in control; Vt represents oedema volume in the group treated with the extract or fractions.

### Gas chromatography-mass spectrometry (GC-MS) analysis of *E. camaldulensis* N-HEX fraction

The phytochemical constituents of the N-HEX fraction of *E. camaldulensis* were analyzed using GC-MS with a Varian 3800/4000 gas chromatograph coupled to a Varian 4000 mass spectrometer. The system was fitted with an Agilent fused silica capillary CP-Sil 5 CB column (30 m × 0.25 mm i.d.). The mass spectrometer operated in electron ionization mode at 70 eV, scanning a mass range of m/z 30–1,000 amu, with the ion source temperature set at 230°C and the quadrupole temperature maintained at 150°C. The oven temperature was initially set at 150°C and gradually increased to a maximum of 300°C. A 1 µL aliquot of the N-HEX fraction, dissolved in acetone, was injected automatically into the GC inlet maintained at 250°C. Nitrogen served as the carrier gas at a constant flow rate of 0.8 mL/min, and the total analysis time was 40 minutes. Identification of the compounds was performed by comparison with spectra from the National Institute of Standards and Technology mass spectral database (NIST MS Library, 2009). Retention indices were determined relative to a homologous series of *n*-alkanes (C6–C28) analyzed under identical chromatographic conditions on the same column. The relative abundance of each component was estimated based on peak area normalization without applying response factor corrections.

### Data analysis

Data from this study were subjected to GraphPad Prism 5.01 software package using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. Values were considered statistically significant at $p < 0.05$ and expressed as mean ± SEM.

# Results

## Anti-inflammatory effect of *E. camaldulensis* methanol leaf extract

The results of the anti-inflammatory activity of the methanol extract of *E. camaldulensis* compared to the negative control and standard drug (aspirin) are presented in Table 1.

**Table 1. Anti-inflammatory effect of the methanol extract of *E. camaldulensis*.**

| Treatment group | Baseline paw size (mm) | Paw oedema size (mm) per time (h) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| Negative control | 3.56 ± 0.02$^{b}$ | 7.20 ± 0.26$^{a}$ | 6.78 ± 0.21$^{a}$ | 5.96 ± 0.26$^{a}$ | 5.88 ± 0.50$^{a}$ |
| ECE (100 mg/kg) | 3.91 ± 0.03$^{a}$ | 5.80 ± 0.24$^{b}$ | 5.56 ± 0.34$^{b}$ | 5.32 ± 0.30$^{a}$ | 4.20 ± 0.17$^{b}$ |
| | | (19%) | (18%) | (11%) | (29%) |
| ECE (200 mg/kg) | 3.80 ± 0.02$^{a}$ | 5.74 ± 0.25$^{b}$ | 5.64 ± 0.14$^{b}$ | 5.12 ± 0.02$^{a}$ | 4.70 ± 0.21$^{a}$ |
| | | (20%) | (17%) | (14%) | (20%) |
| ECE (400 mg/kg) | 3.79 ± 0.03$^{a}$ | 5.80 ± 0.34$^{b}$ | 5.16 ± 0.24$^{b}$ | 5.38 ± 0.32$^{a}$ | 4.92 ± 0.25$^{a}$ |
| | | (19%) | (24%) | (10%) | (16%) |
| Aspirin | 4.03 ± 0.17$^{a}$ | 6.72 ± 0.21$^{a}$ | 6.58 ± 0.22$^{a}$ | 5.50 ± 0.28$^{a}$ | 5.05 ± 0.15$^{a}$ |
| | | (7%) | (3%) | (8%) | (14%) |

ECE: methanol crude extract. Values are expressed as mean ± SEM ($n$ = 5). Values with different superscripts (a and b) within columns are significantly different ($p < 0.05$).

The results showed that the paw of the rats in the negative control group maintained consistent swelling up to 4 h. The extract at 100 mg/kg demonstrated a significant anti-inflammatory effect after 4 h with 4.20 ± 0.17 (29%) paw size reduction compared to the negative and positive control groups. The 100 mg/kg dose also gave a good anti-inflammatory effect as compared to the 200 and 400 mg/kg doses. Also, the results obtained for the anti-inflammatory effect showed that the activity elicited by the extract is non-time and non-dose dependent.

## Anti-inflammatory activity of fractions of *E. camaldulensis*

The active methanol extract of *E. camaldulensis* was partitioned in order to identify the fraction that may be responsible for the anti-inflammatory activity elicited by the extract, and the results are presented in Table 2.

**Table 2. Anti-inflammatory effects of *E. camaldulensis* fractions on egg albumin-induced rat paw oedema.**

| Treatment group | Baseline paw size (mm) | Paw oedema size (mm) per time (h) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| NS | 3.56 ± 0.02$^{c}$ | 7.2 ± 0.26$^{c}$ | 6.78 ± 0.21$^{a}$ | 5.96 ± 0.26$^{a}$ | 5.88 ± 0.50$^{a}$ |
| N-HEX | 3.21 ± 0.01$^{c}$ | 5.30 ± 0.23$^{a}$ | 4.80 ± 0.03$^{b}$ | 4.24 ± 0.16$^{b}$ | 3.38 ± 0.09$^{c}$ |
| (100 mg/kg) | | (26%) | (29%) | (29%) | (43%) |
| DCM | 3.51 ± 0.03$^{c}$ | 5.55 ± 0.32$^{a}$ | 4.58 ± 0.22$^{b}$ | 4.32 ± 0.14$^{b}$ | 3.86 ± 0.16$^{b}$ |
| (100 mg/kg) | | (23%) | (32%) | (28%) | (34%) |
| EtOAc | 3.09 ± 0.02$^{b}$ | 4.98 ± 0.21$^{b}$ | 4.88 ± 0.13$^{b}$ | 4.14 ± 0.19$^{b}$ | 4.02 ± 0.13$^{b}$ |
| (100 mg/kg) | | (31%) | (28%) | (31%) | (32%) |
| AQ | 3.12 ± 0.05$^{b}$ | 5.52 ± 0.20$^{a}$ | 4.40 ± 0.20$^{b}$ | 4.50 ± 0.23$^{b}$ | 3.76 ± 0.15$^{b}$ |
| (100 mg/kg) | | (23%) | (35%) | (24%) | (36%) |
| Aspirin | 4.03 ± 0.17$^{a}$ | 6.72 ± 0.21$^{a}$ | 6.58 ± 0.22$^{a}$ | 5.50 ± 0.28$^{a}$ | 5.05 ± 0.15$^{a}$ |
| | | (7%) | (3%) | (8%) | (14%) |

N-HEX: *n*-hexane; DCM: dichloromethane; EtOAc: ethyl acetate; AQ: aqueous. Values are expressed as mean ± SEM ($n$ = 5). Values with different superscripts (a, b and c) within columns are significantly different ($p < 0.05$).

The results obtained showed that the N-HEX fraction elicited the highest percentage paw oedema reduction of 3.38 ± 0.09 mm (43%) compared to the positive control. The N-HEX fraction also demonstrated good anti-inflammatory activity compared to the DCM, EtOAc, and AQ fractions. Also, both the N-HEX and DCM fractions gave a time-dependent anti-inflammatory activity. Therefore, the activity of the fractions is in the order of N-HEX > AQ > DCM > EtOAc.

### GC-MS analysis of the N-HEX fraction

The chemical constituents of the N-HEX, which emerged as the most active fraction, were identified, and the results are presented in Table 3. In this study, α-phellandrene, *o*-cymene, *n*-hexadecanoic acid, and beta-sitosterol were identified as the major chemical compounds in the non-polar fraction.

**Table 3. GC-MS analysis of the *n*-hexane fraction.**

| S/N | R.T. (min) | Compound detected | MF | MW | Peak |
|---|---|---|---|---|---|
| 1 | 7.811 | α-Phellandrene | $C_{10}H_{16}$ | 136.24 | 8.45 |
| 2 | 8.036 | Cyclohexene, 1-methyl-4-(1-methylethylidene)- | $C_{10}H_{16}$ | 136.24 | 3.14 |
| 3 | 8.146 | *o*-Cymene | $C_{10}H_{14}$ | 134.22 | 7.53 |
| 4 | 11.704 | Phenol, 2-methyl-5-(1-methyl ethyl) | $C_{10}H_{14}O$ | 150.22 | 1.27 |
| 5 | 16.913 | Neophytadiene | $C_{20}H_{38}$ | 278.52 | 2.55 |
| 6 | 17.635 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 270.45 | 1.24 |
| 7 | 18.114 | *n*-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256.42 | 4.51 |
| 8 | 19.183 | Phytol | $C_{20}H_{40}O$ | 296.54 | 1.36 |
| 9 | 23.122 | 1-Docosene | $C_{22}H_{44}$ | 308.58 | 1.12 |
| 10 | 26.558 | Octacosyl acetate | $C_{30}H_{60}O_2$ | 452.80 | 1.67 |
| 11 | 27.153 | Stigmastan-3,5-diene | $C_{29}H_{48}$ | 396.69 | 1.17 |
| 12 | 30.497 | Beta-sitosterol | $C_{29}H_{50}O$ | 414.71 | 2.98 |
| 13 | 31.270 | Oleana-11,13(18)-diene | $C_{30}H_{48}$ | 408.70 | 1.51 |
| **Total** | | | | | 38.5 |

MF: molecular formula; MW: molecular weight; GC-MS: gas chromatography-mass spectrometry.

## Discussion

Inflammation has been implicated in the pathophysiology of various life-threatening diseases. The egg albumin-induced rat paw edema model is a widely recognized method for screening anti-inflammatory agents due to its simplicity, sensitivity, and reproducibility [30–32]. This model primarily targets the early phase of inflammation, characterized by neutrophil infiltration, free radical release, and the induction of cyclooxygenase (COX)-2 expression, leading to prostaglandin formation. Egg albumin is known to induce an immune response that activates macrophages, neutrophils, and T cells. These immune cells release cytokines and chemokines that further stimulate inflammation by attracting additional immune cells and releasing inflammatory mediators such as prostaglandins, leukotrienes, and histamine [30, 33, 34]. Consequently, these mediators contribute to the hallmark symptoms of inflammation, including swelling, redness, and pain. Given these characteristics, the egg albumin-induced rat paw edema model was selected for this study. Table 1 illustrates the impact of various doses (100, 200, and 400 mg/kg) of the methanol leaf extract on egg-albumin-induced rat paw edema. The reduction of the rat paw edema suggests the anti-inflammatory potential of *E. camaldulensis* methanolic leaf extract.

It was observed that the extract's anti-inflammatory activity was not dose-dependent. The highest percentage inhibition was observed at 100 mg/kg and the lowest at 400 mg/kg (4 h after treatment) (Table 1). Also, there was a statistical difference between the activity of the extract at 100 mg/kg and the control drug at 4 h post-treatment. However, *E. camaldulensis* methanolic leaf extract showed higher oedema inhibition at 100–400 mg/kg than aspirin, suggesting the anti-inflammatory property of the extract.

The anti-inflammatory properties of the N-HEX, DCM, EtOAc, and AQ fractions of *E. camaldulensis* were assayed to understand the class of compounds that may be responsible for the anti-inflammatory activity of the extract (Table 2). All the fractions demonstrated higher anti-inflammatory activity than aspirin at 100 mg/kg. Notably, the N-HEX fraction displayed the highest activity, reducing paw edema by 43% after 4 hours of examination. This indicates that the anti-inflammatory constituents of the extract are predominantly non-polar. This observation corroborates previous research showing that the crude N-HEX extract of *E. camaldulensis* stem bark possesses significant anti-inflammatory properties [35, 36]. This effect may be attributed to the inhibition of prostaglandin synthesis, cell degranulation, serotonin and bradykinin release, and decreased leukocyte mobilization [36]. Additionally, studies have linked the biological and pharmacological activities of plant extracts to the presence of secondary metabolites [30].

The GC-MS analysis revealed α-phellandrene, *o*-cymene, *n*-hexadecanoic acid, and beta-sitosterol. α-Phellandrene has previously been identified as one of the major compounds in *Eucalyptus phellandra*, and the phytochemical has been proven to demonstrate anti-inflammatory, immunomodulatory, and antimicrobial activities [37]. Also, Purushothaman et al. [38] reported that *n*-hexadecanoic acid isolated from *Excoecaria agallocha* significantly exerted anti-inflammatory responses against hypertonicity-induced haemolysis, protein denaturation and paw oedema. Also, α-phellandrene was reported to be responsible for suppressing NF-κB activity, increasing fibroblast proliferation and suppressing cytokines like TNF-α and IL-6 as its dose increased [39, 40]. Furthermore, beta-sitosterol was reported to elicit excellent anti-inflammatory activity, alleviating perianal inflammation and restoring gut microbiota composition in experimental rats [41]. Therefore, the abundance of these phytochemicals in the N-HEX fraction may be attributed to its anti-inflammatory activity.

In conclusion, the extract demonstrated good anti-inflammatory activity at the lowest tested dose. Also, the N-HEX fraction demonstrated the highest anti-inflammatory activity, indicating that the non-polar compounds may be responsible for the observed activity. This is the first report on the anti-inflammatory effect of *E. camaldulensis* methanol leaf extract and its partitioned fraction on egg albumin-induced paw oedema. The major compounds may be responsible for the observed anti-inflammatory activity. Phytochemical isolation from the N-HEX fraction is recommended for further studies.

In terms of its limitations, the study only evaluated the anti-inflammatory activity of the extract and fractions of *E. camaldulensis* and identified the chemical constituents of its active fraction. A bioassay-guided phytochemical purification of anti-inflammatory compounds can be performed. Also, anti-inflammatory activity of the isolated compounds can be evaluated, followed by in silico studies to elucidate their mechanism of action.

## Abbreviations

AQ: aqueous

COX: cyclooxygenase

DCM: dichloromethane

EtOAc: ethyl acetate

GC-MS: gas chromatography-mass spectrometry

N-HEX: *n*-hexane

## Declarations

### Author contributions

KOF: Conceptualization, Methodology, Supervision. OEE: Investigation, Methodology, Data curation. SAO: Methodology, Investigation, Formal analysis, Writing—original draft. AUN: Investigation, Methodology, Data curation. SAA: Investigation, Writing—original draft, Writing—review & editing. EAO: Investigation, Project administration, Writing—review & editing. BOM: Data curation, Formal analysis, Writing—original

draft. GA: Investigation, Supervision. MOB: Data curation, Formal analysis, Writing—original draft. SOF: Conceptualization, Supervision, Writing—review & editing. All authors read and approved the submitted version.

### Conflicts of interest

The authors declare that there are no conflicts of interest.

### Ethical approval

The study was performed according to the ethical clearance of the Institute of Public Health, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria (HREC NO: IPHOAU/12/1772).

### Consent to participate

Not applicable.

### Consent to publication

Not applicable.

### Availability of data and materials

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.


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