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Original Research Article

In vitro Cytotoxicity, Antioxidant, and Anti-inflammatory Activities of *Eucalyptus camaldulensis* Leaf Extracts

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ABSTRACT

Chronic inflammation and oxidative stress are key contributors to the onset and progression of several degenerative diseases. Medicinal plants play a vital role in their management due to their rich content of bioactive compounds, which possess antioxidant and anti-inflammatory properties. This study evaluated the cytotoxic, antioxidant, and anti-inflammatory activities of *Eucalyptus camaldulensis* leaf extracts collected in Kano, Nigeria. The powdered leaves were extracted with ethanol and fractionated into n-hexane, chloroform, ethyl acetate, and methanol fractions. Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, anti-inflammatory activity by inhibition of protein denaturation, and cytotoxicity using the brine shrimp lethality test. The methanol extract exhibited the strongest antioxidant effect (IC_{50} = 4.785 µg/mL), which was lower in potency than ascorbic acid (IC_{50} = 0.943 µg/mL). The n-hexane fraction demonstrated the highest anti-inflammatory activity (IC_{50} = 3.215 µg/mL) and cytotoxicity (LC_{50} = 3.810 µg/mL), while the methanol fraction was non-toxic (LC_{50} > 1000 µg/mL). These findings indicate that *Eucalyptus camaldulensis* possesses solvent-dependent biological activities, with the methanol fraction showing strong antioxidant and safe pharmacological properties, and the n-hexane fraction displaying significant anti-inflammatory and cytotoxic effects. The study provides scientific support for the traditional use of *Eucalyptus camaldulensis* and suggests its potential as a source of natural therapeutic agents. Further studies should focus on the isolation, characterization, and *in vivo* evaluation of its bioactive compounds.

Keywords: *Eucalyptus camaldulensis*, antioxidant Activity, anti-Inflammatory Activity, aytotoxicity, DPPH assay, protein denaturation, brine shrimp lethality.

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Introduction

Medicinal plants are recognized as important sources of bioactive compounds with therapeutic potential[1, 2]. Chronic inflammation and oxidative stress are key contributors to the onset and progression of various diseases, including cancer, diabetes, and cardiovascular disorders[3]. Consequently, research on medicinal plants as natural antioxidants and anti-inflammatory agents has intensified, aiming to identify safer and more effective alternatives to synthetic drugs[4]. *Eucalyptus camaldulensis* (river red gum), a member of the M*yrtaceae* family, is widely distributed and frequently used in traditional medicine[3]. Previous studies have documented its antimicrobial[5], anti-diabetic[6], and cytotoxic[7] properties. However, many of these investigations focused on samples from other regions or included limited biological analysis. Therefore, comprehensive validation of the antioxidant, anti-inflammatory, and cytotoxic properties of Nigerian populations of *E. camaldulensis* is lacking, particularly considering possible phytochemical variations due to environmental and climatic differences[8,9]. The present study was therefore designed to evaluate the cytotoxic, antioxidant, and anti-inflammatory activities of different solvent extracts of *Eucalyptus camaldulensis* leaves collected in Kano, Nigeria. This study not only aims to provide scientific justification for its ethnomedicinal use but also to explore its potential as a source of bioactive compounds for pharmaceutical development.

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Figure 1: *Eucalyptus Camaldulensis* Tree (Left) and Leaves (Right)

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Materials and Methods

Plant Collection and Authentication
Fresh leaves of Eucalyptus camaldulensis were collected in December 2024. The plant was identified and authenticated by a taxonomist in the Department of Biological Sciences, Bayero University, Kano. A voucher specimen (No. BUKHAN 0347) was deposited at the Bayero University Herbarium for future reference

Extraction and Fractionation
The powdered leaves (50 g) were macerated in 200 mL of 95% ethanol for seven days with intermittent shaking. Afterward, the mixture was decanted and filtered using Whatman filter paper to separate the liquid extract from the solid residue. The filtrate was left to evaporate at room temperature for three days to obtain the crude extract, which yielded 6.4 g. One gram (1 g) was removed for crude analysis, while the remaining 5.4 g was fractionated successively with solvents of increasing polarity (n-hexane, chloroform, ethyl acetate, and methanol), following the procedure described by Usman et al. (2021)[10].

Antioxidant Assay (DPPH Radical Scavenging Activity)
The antioxidant activity of the extracts was evaluated using the DPPH radical scavenging method as described by Gülcin (2009)[11] and Falodun et al. (2025)[12] with slight modifications. Briefly, different concentrations of the extracts (7.81-1000 µg/mL) were mixed with DPPH solution in methanol and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV-visible spectrophotometer, with ascorbic acid serving as the positive control. IC_{50} values were determined from dose–response data using SPSS software.

Anti-Inflammatory Assay (Protein Denaturation Method)
The anti-inflammatory activity of the extracts was assessed using the *in vitro* protein denaturation assay. 20 µL of each extract (7.81-1000 µg/mL) was mixed with 0.2 mL of fresh egg albumin and 180 µL of phosphate-buffered saline (PBS) in a 96-well microplate. The mixture was incubated in an oven at 70 °C for 15 minutes, allowed to cool, and the turbidity was measured at 660 nm. IC_{50} values were calculated from dose-response data using SPSS software.

Cytotoxicity Assay (Brine Shrimp Lethality Test)
The cytotoxic activity of the extracts was evaluated using the brine shrimp lethality test as described by Meyer et al. (1982)[13] and further applied in similar studies by Amuamuta (2023)[14] with slight modifications. *Artemia salina* (brine shrimp larvae) was used as the test organism. The eggs were hatched in a 1000 mL beaker containing seawater and kept under constant illumination and aeration for 24–72 h until the nauplii (larvae) emerged. Stock solutions of the extracts (10,000 µg/mL) were prepared by dissolving 30 mg of each extract in 3 mL of ethanol. Working concentrations of 1000, 100, and 10 µg/mL were prepared in test tubes in triplicate. After adding 10 active nauplii to each tube, the total volume was adjusted to 5 ml of seawater. After 24 h of exposure, the number of surviving nauplii was counted, and the percentage mortality was calculated. LC_{50} values were determined from the mortality data using probit regression analysis in SPSS software.

Results and Discussion

Extraction Yield
The crude ethanol extracts of *Eucalyptus camaldulensis* leaves yielded 6.4 g of residue. Fractionation produced n-hexane (0.23 g), chloroform (0.86 g), ethyl acetate (1.02 g), and methanol (1.56 g) fractions. The highest yield was obtained from methanol, consistent with its ability to extract polar phytochemicals such as flavonoids and phenolics[5]. This agrees with previous studies that reported higher polar extract yields in *Eucalyptus* species[15].

Antioxidant Activity
The DPPH radical scavenging activity showed that the methanol fraction had the strongest activity (IC_{50} = 4.785 µg/mL), followed by the crude ethanol extract (IC_{50} = 15.854 µg/mL), while ascorbic acid, used as a positive control, had IC_{50} = 0.943 µg/mL (Table 2, Figure 2).

Table 1: Extraction yield of fractions

Fraction	Weight (g)	Characteristics
n-Hexane	0.23	Oily, dark green
Chloroform	0.86	Viscous, dark green
Ethyl acetate	1.02	Clumpy, brownish green
Methanol	1.56	Powdery, yellowish brown

This indicates that the methanol fraction is rich in polyphenolic compounds capable of donating hydrogen atoms to neutralize free radicals. Previous studies have shown similar antioxidant properties of *Eucalyptus camaldulensis* attributed to flavonoids and tannins[9, 15, 16]. The results justify its ethnomedicinal use as an antioxidant agent.

Anti-Inflammatory Activity
The protein denaturation assay revealed that the n-hexane fraction demonstrated the strongest activity (caused the least protein denaturation, indicating higher membrane stabilization potential, IC_{50} = 3.215 µg/mL), followed by the methanol fraction (IC_{50} = 6.713 µg/mL) (Table 3, Figure 3). The high activity of the nonpolar fraction suggests that lipophilic compounds, possibly terpenoids and fatty acid derivatives, play a significant role in membrane stabilization and inhibition of protein denaturation. These findings are consistent with earlier reports that hydrophobic phytochemicals from *Eucalyptus* species exhibit significant anti-inflammatory effects[6, 8, 9, 17].

Table 2: Percentage DPPH radical scavenging activity of *Eucalyptus camaldulensis* extracts compared with the standard (Ascorbic acid)

Conc. (µg/mL)	**n-Hexane** (% inhibition)	**Chloroform** (% inhibition)	**Ethyl acetate** (% inhibition)	**Methanol** (% inhibition)	**Crude EtOH** (% inhibition)	**Ascorbic acid** (% inhibition)
1000	93.55	94.80	97.97	96.67	111.33	91.18
500	95.55	95.46	95.02	94.65	100.16	85.21
250	85.79	93.30	93.54	93.39	95.19	78.64
125	47.47	90.31	71.06	92.27	91.75	77.58
62.5	23.81	63.89	42.83	92.09	91.47	73.71
31.25	10.02	27.91	9.81	90.19	73.98	71.73
15.625	3.59	15.04	6.25	71.65	48.96	68.13
7.8125	3.35	12.56	3.97	42.64	25.10	66.34
IC_{50} (µg/mL)	**113.71**	**45.48**	**76.57**	**4.79**	**15.85**	**0.94**

Cytotoxicity
Brine shrimp (*Artemia salina*) lethality results indicated that the n-hexane fraction was the most cytotoxic (LC_{50} = 3.810 µg/mL), while the chloroform and ethyl acetate fractions showed moderate toxicity. The methanol fraction was non-toxic (LC_{50} > 1000 µg/mL) (Table 4, Figure 4). The brine shrimp lethality test was employed as a preliminary bioassay to assess general cytotoxicity and to predict potential

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anticancer activity of the plant extracts. The marked toxicity observed in the n-hexane fraction suggests the presence of bioactive lipophilic compounds with possible anticancer or antiproliferative properties. This observation is in agreement with the findings of Mohammed (2021)[7] and Kumar (2014)[18], who reported cytotoxic effects of *Eucalyptus* extracts against cancer cell lines. Conversely, the non-toxic nature of the methanol fraction indicates its safety and supports its potential use as a natural antioxidant in nutraceutical applications[19, 20, 21].

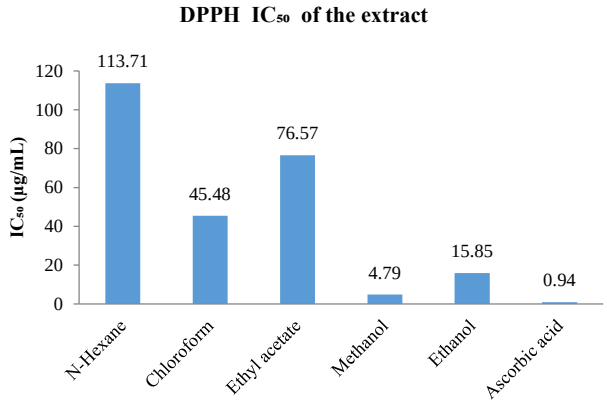

Figure 2: Antioxidant activity of *Eucalyptus camaldulensis* extracts compared with ascorbic acid

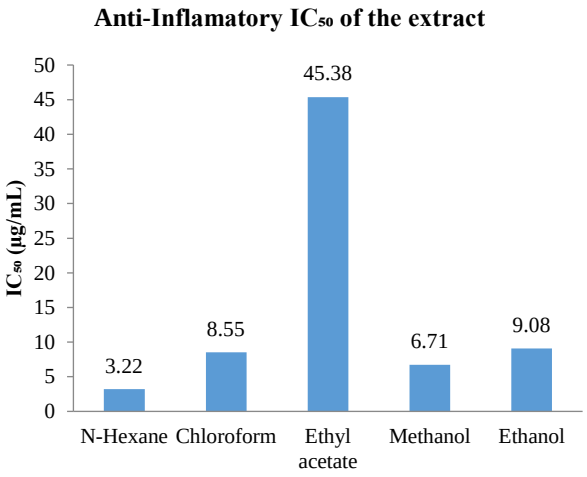

Figure 3: Anti-inflammatory activity of *Eucalyptus camaldulensis* extracts

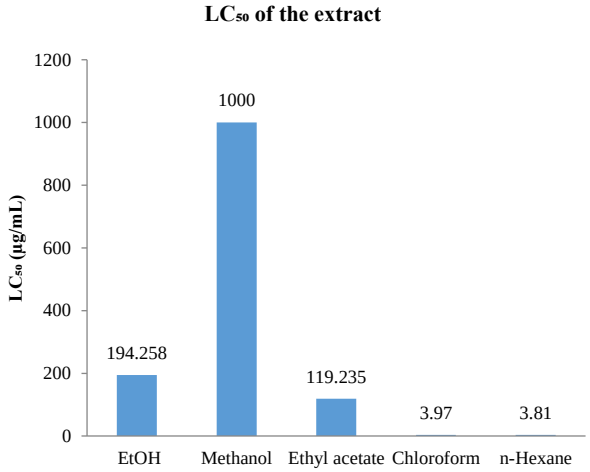

Figure 4: Cytotoxicity of *Eucalyptus camaldulensis* extracts (Brine shrimp assay)

Table 3: Percentage inhibition of protein denaturation (anti-Inflammatory activity) of *Eucalyptus camaldulensis* leaf extracts.

Conc. (µg/mL)	**n-Hexane** (% inhibition)	**Chloroform** (% inhibition)	**Ethyl acetate** (% inhibition)	**Methanol** (% inhibition)	**Crude EtOH** (% inhibition)
1000	94.52	91.31	152.19	96.51	92.56
500	91.98	88.42	78.14	94.82	90.80
250	84.37	84.00	74.97	94.27	92.09
125	82.11	79.67	66.38	91.48	83.39
62.5	79.00	75.65	64.95	88.87	90.05
31.25	74.15	50.38	51.71	79.82	68.42
15.625	79.85	73.62	29.00	61.46	48.34
7.8125	48.59	42.39	17.49	46.63	46.73
IC₅₀ (µg/mL)	**3.22**	**8.55**	**45.38**	**6.71**	**9.08**

The study highlights the solvent-dependent biological activities of *Eucalyptus camaldulensis*. While methanol extracts were safer and richer in antioxidants, nonpolar fractions like n-hexane exhibited strong anti-inflammatory and cytotoxic effects. This suggests selective use of extracts depending on therapeutic goals. Further investigations, including isolation and structural elucidation of bioactive compounds and *in vivo* testing, are necessary to validate these *in vitro* findings

Table 4: Brine shrimp lethality test of *Eucalyptus camaldulensis* extracts

S/N	**Sample**	**Conc. (µg/mL)**	**No. Of Brine shrimp**	**Replica**	**Mortality (24h)**	**% Mortality**	**LC₅₀ (µg/mL)**
1	Crude EtOH	1000	10	3	18	60	194.258
		100	10	3	13	43	
		10	10	3	11	37	
2	Methanol	1000	10	3	12	40	>1000
		100	10	3	10	33	
		10	10	3	9	30	
3	Ethyl acetate	1000	10	3	19	63	119.235
		100	10	3	15	50	
		10	10	3	10	33	
4	Chloroform	1000	10	3	26	87	3.970
		100	10	3	23	77	
		10	10	3	17	57	
5	n-Hexane	1000	10	3	29	97	3.810
		100	10	3	24	80	
		10	10	3	19	63	

Conclusion

This study demonstrated that *Eucalyptus camaldulensis* leaf extracts possess solvent-dependent bioactivities. The methanol fraction showed the strongest antioxidant activity with no detectable cytotoxicity, indicating its safety and potential as a nutraceutical agent. Although the reduced cytotoxicity of the methanol fraction may imply a lower anticancer potential compared to the non-polar fractions, it also emphasizes its suitability for applications where safety and antioxidant efficacy are prioritized. In contrast, the n-hexane fraction exhibited potent anti-inflammatory and cytotoxic effects, suggesting the presence of bioactive lipophilic compounds with possible anticancer properties. These findings provide scientific justification for the ethnomedicinal use of *Eucalyptus camaldulensis* and highlight its potential as a source of natural therapeutic agents and for drug discovery. Further work should focus on isolation, structural characterization of active compounds, and *in vivo* validation.

Conflict of interest

The authors declare no conflict of interest

Authors' Declaration

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

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