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ORIGINAL ARTICLE

Restorative Efficacy of *Elaeocarpus gaussenii* Weibel (Elaeocarpaceae) through *In Vitro* Evaluations

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ABSTRACT
Many Indian ethnic groups utilize the well-known genus Elaeocarpus, belonging to the family Elaeocarpaceae for relieving several ailments. The current investigation focuses on Elaeocarpus gaussenii (Weibel), an indigenous plant species that has not been well studied from a phytochemical and pharmacological standpoint. The study assesses the in vitro antioxidant and anti-inflammatory properties of the plant part extracts of E. gaussenii. Current investigation revealed that the ethanolic leaf extract manifested the highest level of DPPH radical scavenging activity (22.4 µg/mL), while its bark extract provided high anti-oxidant activity in terms of ABTS (137013.9 µg TE/g), and ferric reducing power (808.88 mM Fe (II) E/mg extract) activity in the in vitro antioxidant assays. Apparently, the leaf ethanolic extracts of E. gaussenii also demonstrated significant anti-inflammatory effects in heat-induced hemolysis (56.86 µg/mL), hypotonic solution-induced hemolysis (66.93%), and the denaturation of bovine serum albumin (25.94%). Bark extracts of E. gaussenii exhibit noteworthy antioxidant and anti-inflammatory activity, positioning them as a promising natural source for the development of novel therapeutic agents and a valid candidate for the production of pharmaceutical products.
Keywords: antioxidant, anti-inflammatory, bark, ethanolic extract, protein denaturation.

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INTRODUCTION

Medicinal plants play a pivotal role in the lives of rural people, particularly in remote parts of developing countries with limited health facilities. Both developing and developed countries are increasingly using plant-based medicines, food supplements, health products, and cosmetics due to their non-toxic nature, minimal side effects, and ease of availability and accessibility [4]. Nowadays, there is an increasing interest worldwide related to herbal medicine, accompanied by enhanced laboratory investigations on the pharmacological properties of the bioactive ingredients used to treat various diseases. Through the exploration of ethnopharmacology and traditional medicines, numerous drugs have entered the international market, and it is evident that certain herbal remedies have peaked at par with synthetic drugs [3]. Inflammation is defined as a local response of living mammalian tissue to treat injury due to any agent. It provides a protective response that aids in the healing of tissues. At times, inflammation can trigger severe conditions such as rheumatoid arthritis and hay fever, which can potentially lead to life-threatening diseases [27]. Inflammation manifests usually in the form of painful swellings associated with some changes in the skin covering the site [13]. Many factors, including pathogens, ischemia, heat, physical damage, and the interaction between antigens and antibodies, trigger this reaction chain [16]. People widely use non-steroidal anti-inflammatory drugs (NSAIDs) to treat acute and chronic inflammation, pain, and fever. They are effective but associated with the reappearance of symptoms after discontinuation. Their use is associated with adverse effects like severe gastritis, peptic ulcers, nausea, vomiting, salt and water retention, worsening renal function in renal or cardiac and cirrhotic patients,

hypersensitivity, etc. [31]. The risk of death as a result of the use of NSAIDs is high and the risk increases tenfold for those over 75 years old, furthermore, synthetic drugs are very expensive to develop [1]. Contrarily, people have been using many medicines of plant origin for a long time without experiencing any adverse effects. Therefore, it is crucial to develop new plant-based drugs with anti-nociceptive and anti-inflammatory effects that are both economically feasible with fewer side effects. The genus *Elaeocarpus* is commonly known as Rudraksha and is one of the largest genera of the family Elaeocarpaceae. *Elaeocarpus* is endemic to the humid subtropical regions of eastern and southern India, where it thrives at temperatures between 500 and 2000 meters above mean sea level. The fruits of these trees are a favorite food for many animals, including birds, the Nilgiri langur, the Malabar giant squirrel, hornbills, and others [17]. The trees also have religious and medicinal significance in India. Wearing rosaries and bracelets made of seeds is said to be beneficial for the heart and nerves because of their electromagnetic qualities [14]. Medicinal uses for *Elaeocarpus* species include anti-inflammatory, antibacterial, and antidiabetic effects [24]. According to Rahman *et al* [20], green fruits are popular for their culinary uses, such as soup, jelly, marmalade, and chutney; while these fruits have a history of being used for diarrhea. Religious jewelry made of the hard ornamental rocky endocarp (in the shape of beads) is popular in Southeast Asia and India [10,21]. Therefore, the present study was addressed to examine the antioxidant and anti-inflammatory properties of *Elaeocarpus gaussenii* because of its promising medical applications.

MATERIAL AND METHODS

Collection and preparation of plant materials

Fresh plant of *E. gaussenii* were collected during the month of October 2023 and the taxonomic identity of the plant was authenticated by the Botanical Survey of India (BSI), Southern Regional Center, Coimbatore, Tamil Nadu. The fresh leaves and bark were washed under running tap water to remove the surface pollutants and were air dried under shade. Then it was homogenized into fine powder using a pulverizer. The pulverized plant portions were further successively extracted with petroleum ether, ethyl acetate, and ethanol using the Soxhlet apparatus. Aqueous extract was obtained using cold maceration. All the extracts were dried using a rotary vacuum evaporator and stored in plastic containers for further analysis.

In vitro antioxidant assays

DPPH radical scavenging activity

Antioxidant activity was measured using hydrogen donating or radical scavenging utilizing the stable radical DPPH, according to Braca *et al* [6]. Sample extracts were adjusted to 100 µL with methanol at different concentrations. Shaking the samples and standard (BHT and Rutin) aliquots with 3 mL of 0.004% methanolic DPPH solution was vigorous. A negative control was created by adding 100 µL methanol to 3 mL methanolic DPPH solution. Then after 30 minutes at 27°C, the tubes were rested. Against the methanol blank, samples and control absorbance were read at 517 nm. Sample IC_{50} was considered as the concentration needed to block 50% of DPPH concentration, representing their radical scavenging effect.

ABTS scavenging activity

The total antioxidant activity of the samples was determined by Re *et al* [22] using the ABTS radical cation decolorization test. The reaction of 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12–16 hours at room temperature yielded ABTS. The sample was diluted in ethanol (1:89 v/v) and equilibrated at 25°C to achieve an absorbance of 0.700 ± 0.02 at 734 nm before testing. Diluted ABTS solution (1 mL) was added to 30 µL sample solution and 10 µL Trolox (final concentration 0-15 µM) in ethanol. The negative control was a test tube with 1 mL diluted ABTS solution and 30 µL ethanol. All test tubes were vortexed and incubated right at room temperature for 30 minutes. Following incubation, samples, and standards (BHT and Rutin) were measured at 734 nm against the ethanol blank. The quantity of Trolox with equal antioxidant activity was measured in µg/g sample extracts.

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capabilities of the sample extracts were evaluated using Pulido *et al* [18]. Mix 900 µL of freshly made, 37°C-incubated FRAP reagent with 90 µL of distilled water and 30 µL of test sample or methanol (blank). BHT and rutin were used as standards. In a water bath, all the test tubes were incubated at 37°C for 30 minutes. The final reaction mixture dilution of the test sample was 1/34. Mix 2.5 mL of 20 mM TPTZ in 40 mM HCl, and 2.5 mL of 20 mM FeCl3. 6H2O, and 25 mL of 0.3 M acetate buffer (pH-3.6) to make the FRAP After incubation, the blue color's absorbance was measured at 593 nm against the reagent blank. Methanolic solutions with $FeSO_4.7H_2O$ concentrations from 500 to 4000 µM were

utilized to create the calibration curve. The equivalent concentration was the antioxidant concentration that reduced ferric-TPTZ like 1 mM $FeSO_4.7H_2O$.

***In vitro* anti-inflammatory activity**

Membrane Stabilization Ability

Hypotonic Solution-Induced Hemolysis

To make Alsever's solution, dissolve 2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in distilled water and sterilize [25]. Goat retinal blood was taken and the blood was mixed with an equal volume of sterilized Alsever's solution. Then it was centrifuged at 3000 rpm for 10 minutes, washed three times with isosaline (0.9%, pH 7.2), and the packed cells were prepared as 10% (v/v) suspensions. The 4.5-mL reaction mixture contains 1 mL phosphate buffer (pH-7.4), 2 mL hyposaline (0.45%), 1 mg/mL plant extract, and 0.5 mL of RBC suspension. The reference drug was diclofenac sodium. The control was a plant-free reaction mixture and the blank was a phosphate buffer. The assay solutions were incubated at 37°C for 30 minutes and were then centrifuged again and the absorbance of the supernatant was read at 560 nm.

Heat-Induced Hemolysis

The test was performed according to the modified method described by Henneh *et al* [9]. Each tube was loaded with 1.0 mL of 10% GRBC and 1.0 mL of various solvent-based plant extracts (1 mg/mL) in a two-tube reaction mixture, followed by gentle mixing. Positive control consisted of 1.0 ml of GRBC and 1.0 mL of diclofenac sodium (10, 30, 100 µg/mL). Negative controls were 1.0 ml of 10% erythrocyte suspension and normal saline. The solution was heated at 56°C for 30 minutes, cooled to room temperature, and then centrifuged at 2500 rpm for 10 minutes. As a measure of hemolysis, the supernatant was collected and quantified spectrophotometrically (UV mini-1240, Shimadzu) at 560 nm.

Protein Denaturation Abilities

Egg Albumin Denaturation Assay

The modified method of Mizushima and Kobayashi [15] was utilized. 0.2 mL of fresh egg albumin, 2.8 mL of buffered phosphate saline (PBS, pH 6.4), and 1 mL of various solvent plant extracts (1 mg/mL) made up the reaction mixture (5). The positive control includes 0.2 mL fresh egg albumin, 2.8 mL PBS (pH 6.4), and 2.0 mL diclofenac sodium at various doses (10, 30, and 100 µg/mL). Negative controls included the same egg albumin, PBS, and 2.0 mL distilled water. To produce denaturation, the mixture was incubated at 37 ± 2°C for 15 minutes and heated at 70°C for 5 minutes. After cooling, UV-mini 1240 (Shimadzu) absorbance was measured at 660 nm using an empty mixture as a blank. The experiment was done in three replicates.

Bovine Serum Albumin Denaturation Assay

The test was performed according to the modified method of Purnomo *et al* [19]. A 2 mL reaction mixture was prepared using 0.06 mL trypsin, 1 mL 20 mM Tris-HCl buffer (pH 7.4), and 1 mL plant extract solvents (1 mg/mL). The reaction mixture was incubated at 37°C for 10 minutes. Then, 1mL 0.65% (W/V) casein was added. The mixture was re-incubated for 20 minutes. Add 2 mL of 2M $HClO_4$ to stop the process after incubation. The hazy suspension was centrifuged for 15 minutes at 7830 rpm. The supernatant absorbance was read at 280 nm. Tris- HCl buffer was taken as blank.

RESULTS AND DISCUSSION

***In vitro* Antioxidant Activity**

DPPH radical scavenging activity of *E. gaussenii* extracts

The DPPH radical scavenging activities of the leaf and bark extracts of *E. gaussenii* are presented in Figure 1. Among the tested extracts, the ethanolic leaf extract (IC_{50} = 22.4 µg/mL) and bark extract (IC_{50} = 22.96 µg/mL) exhibited superior scavenging activity and the IC_{50} values for the standard natural antioxidant rutin and the synthetic antioxidant BHT were 5.56 µg/mL and 4.44 µg/mL, respectively. The DPPH radical assay is a widely accepted method for assessing the free radical scavenging potential or hydrogen-donating ability of the compounds, used to evaluate the antioxidant properties of plant extracts and food [8]. Balamurugan *et al* [5] analyzed the scavenging activity of *E. variabilis* fruit, reporting their IC_{50} values ranging between 20 to 83.57 µg/mL. Similarly, Joshi *et al* [11] conducted a comparative antioxidant assay on different *Elaeocarpus* species, demonstrating high antioxidant capacity with IC_{50} values of 68.98 µg/mL for *E. sphaericus* (Gaertn.) Heer and 20 µg/mL for *E. tectorius* (Lour.) Poir. When compared to prior reports on DPPH radical scavenging activities of various Elaeocarpaceae members and related genera, the selected plant exhibits promising antioxidant potential, likely attributable to its significant phenolic compound content.

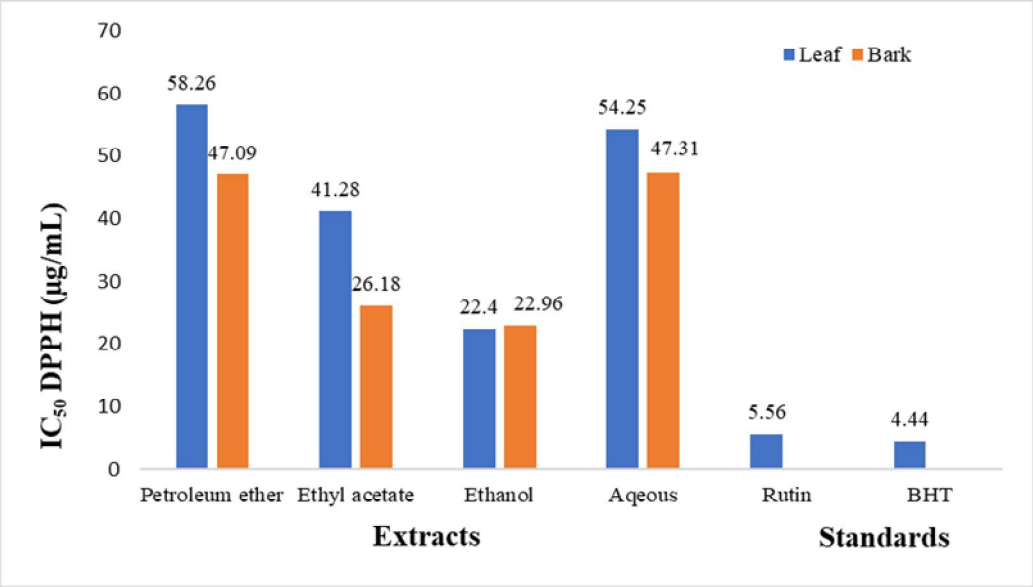


Figure 1: DPPH radical scavenging activity of *E. gaussenii* leaf and bark extracts

ABTS cation radical scavenging activity of *E. gaussenii* extracts

The TEAC (Trolox Equivalents Antioxidant Capacity) was assessed using the improved ABTS radical decolorization assay, a widely used method for measuring antioxidant capacity. This assay evaluates a compound's ability to scavenge the ABTS cation radical, with results expressed as µM Trolox Equivalents per gram of extract. The ABTS cation radical scavenging activities of the leaf and bark extracts of *E. gaussenii* are shown in Figure 2. The ethyl acetate bark extract (137,013.9 µM TE/g) and the ethanol leaf extract (131,527.8 µM TE/g) exhibited the highest scavenging activities. In comparison, the standard natural antioxidant rutin (144,166.7 µM TE/g) and the synthetic antioxidant BHT (145,347.2 µM TE/g) demonstrated slightly higher activities. Balamurugan *et al* [5] reported the radical scavenging activity of *E. variabilis*, with the ethanolic extract of its fruit showing 78.76% activity, while the ethyl acetate extract of *E. tectorius* fruit showed 3203.92 µM TE/g. When compared with other *Elaeocarpus* species, the present study holds that the selected *E. gaussenii* extracts possess higher ABTS cation radical scavenging activity.

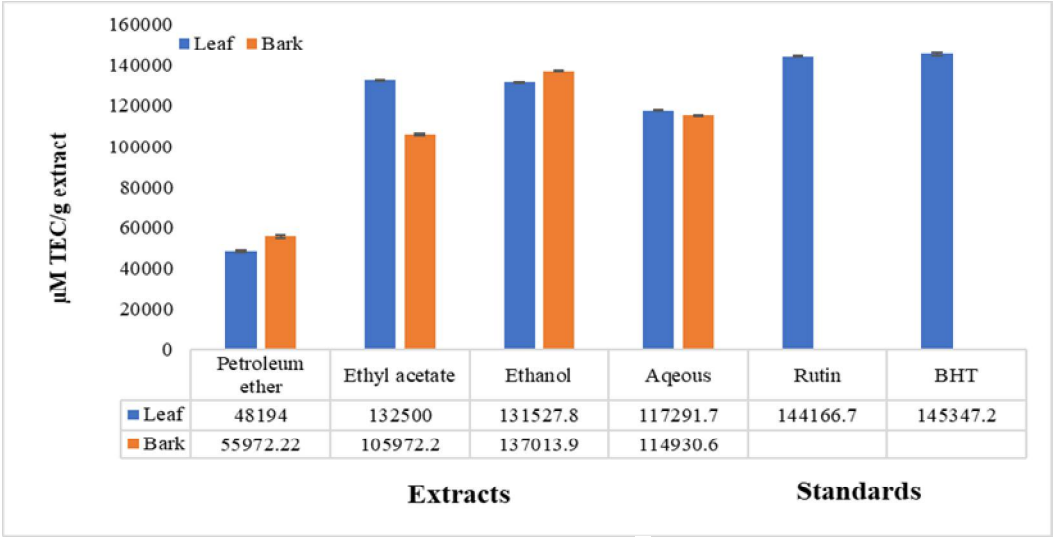


Figure 2: ABTS cation radical scavenging activity of *E. gaussenii* leaf and bark extracts

Values are the mean of triplicate determination (n=3) ± standard deviation; Abbreviations-TE – Trolox equivalent.

Ferric-reducing antioxidant power of *E. gaussenii* extracts

The antioxidant potential of *E. gaussenii* leaf and bark extracts was evaluated based on their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II), as shown in Figure 3. The ethanol extracts of both the bark (808.88 mM Fe (II)E/mg) and leaf (721.23 mM Fe (II)E/mg) demonstrated higher ferric-reducing power. Balamurugan *et al* [5] and Keerthana *et al* [12] studied the ethanolic extracts of *E. variabilis* and *E. tectorius*, reporting that the reducing power of bioactive compounds, particularly the presence of low and high molecular weight phenolics, is linked to its addressed antioxidant activity, specifically in scavenging free radicals. Similar studies have also shown that antioxidants, assessed through *in vitro* ferric-reducing antioxidant power assay, enhance the total antioxidant capacity of blood plasma [11].

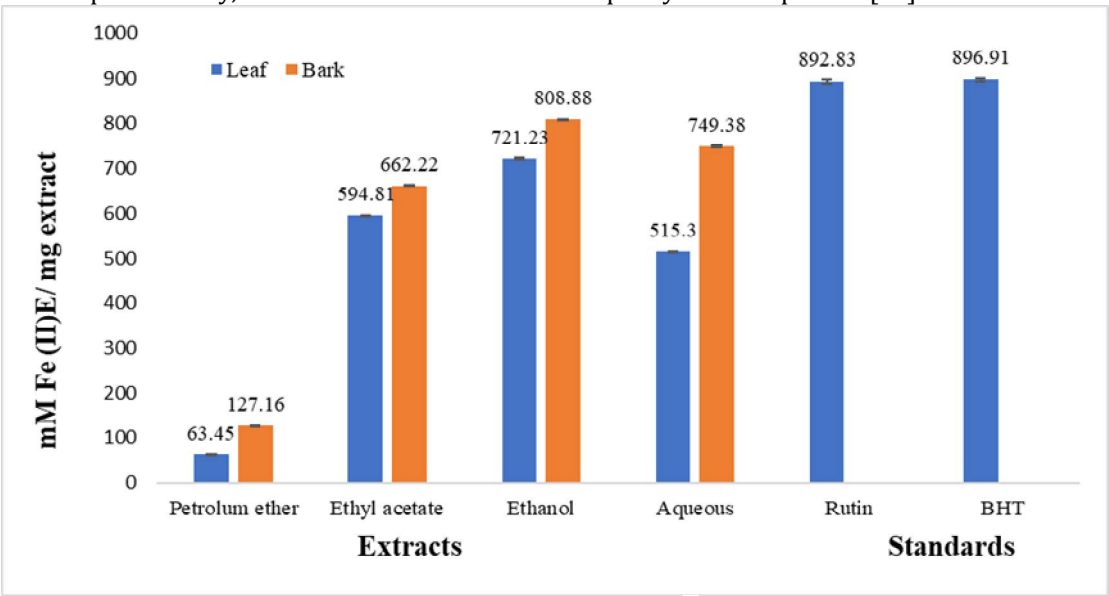


Figure 3: Ferric reducing antioxidant power activity of *E. gaussenii* leaf and bark extracts

Values are the mean of triplicate determination (n=3) ± standard deviation.

***In vitro* Anti-inflammatory Activity**

Hemolysis-based anti-inflammatory activity of *E. gaussenii* extracts

The results of anti-inflammatory activities, assessed through the membrane stabilization assay for the ethanolic leaf and bark extracts of *E. gaussenii*, are shown in Figure 4. The results were expressed as the percentage inhibition of the extracts. In this study, the ethanolic bark extract exhibited the highest inhibition (74.64%), followed by the ethanolic leaf extract with 67.98%. The standard drug, diclofenac, showed an inhibition of 79.14%. Leakage of extracellular lysosomal components is a major contributor to tissue inflammation and injury. Free radicals, generated from lipid peroxidation, make cells more susceptible to further damage [26]. Therefore, stabilization of the lysosomal membrane plays a key role in regulating the inflammatory response [30]. In a similar study, Yesmin *et al* [28] used a membrane stabilization assay through heat-induced hemolysis with crude ethanolic root extract of *P. chaba* in HRBC, observing membrane stabilization by inhibiting membrane lysis. Aidoo *et al* [2] also conducted an *in vitro* study to evaluate the ability of bergapten, a furocoumarin found in various medicinal plants, to prevent hemolysis of human erythrocytes and protein denaturation using hypotonic solutions and heat.

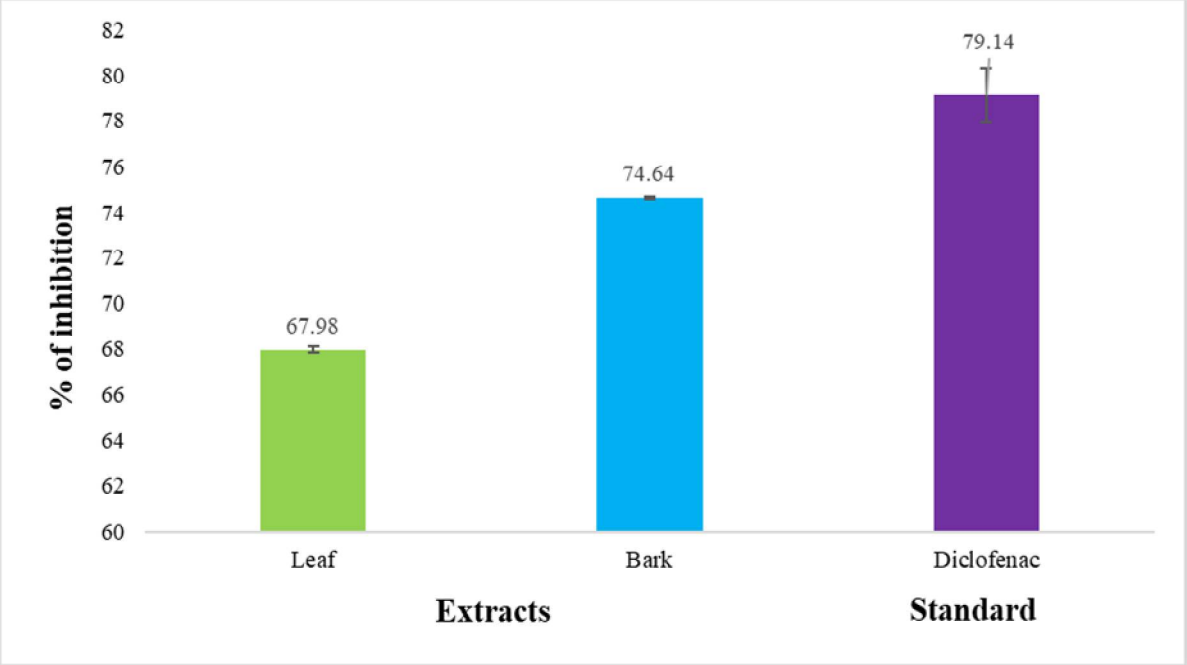


Figure 4: Hypotonic Solution-Induced Hemolysis of leaf and bark ethanolic extract of *E. gaussenii*

Values are the mean of triplicate determination (n=3) ± standard deviation.

Heat-induced hemolysis-based anti-inflammatory activity of *E. gaussenii* extracts

The membrane stabilization ability of the ethanolic leaf and bark extracts of *E. gaussenii* was assessed and is presented in Figure 5. The data are shown as IC_{50} values, with lower IC_{50} values indicating better inhibition of heat-induced hemolysis. The highest heat-induced hemolysis radical scavenging activity was observed in the ethanol bark extract (56.86 µg/mL), followed by the leaf extract (73.88 µg/mL). When lysosomal enzymes in RBCs are ruptured, either by a hypotonic solution or by heat, excessive fluid accumulates within these cells. This leakage of extracellular lysosomal components contributes to tissue inflammation and injury, making cells more susceptible to further damage from free radicals generated by lipid peroxidation. Thus, stabilization of the lysosomal membrane plays a key role in regulating the inflammatory response [7].

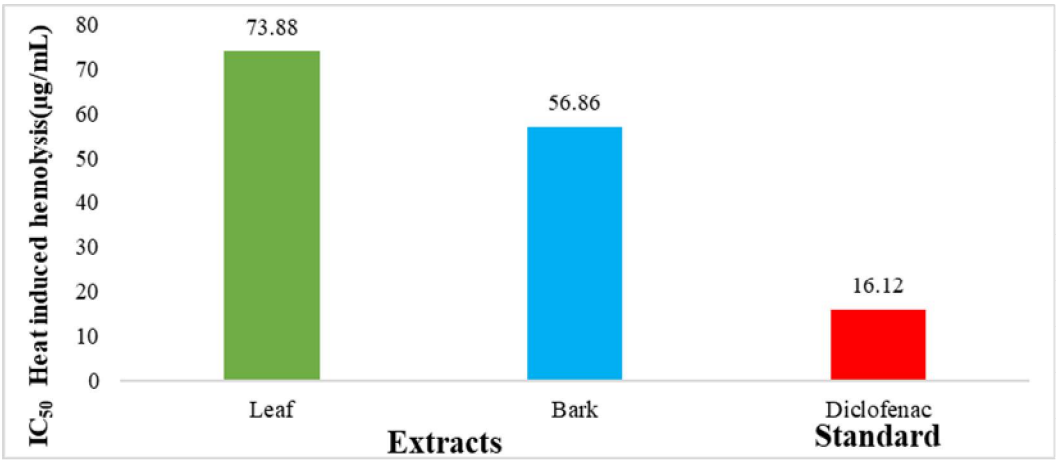
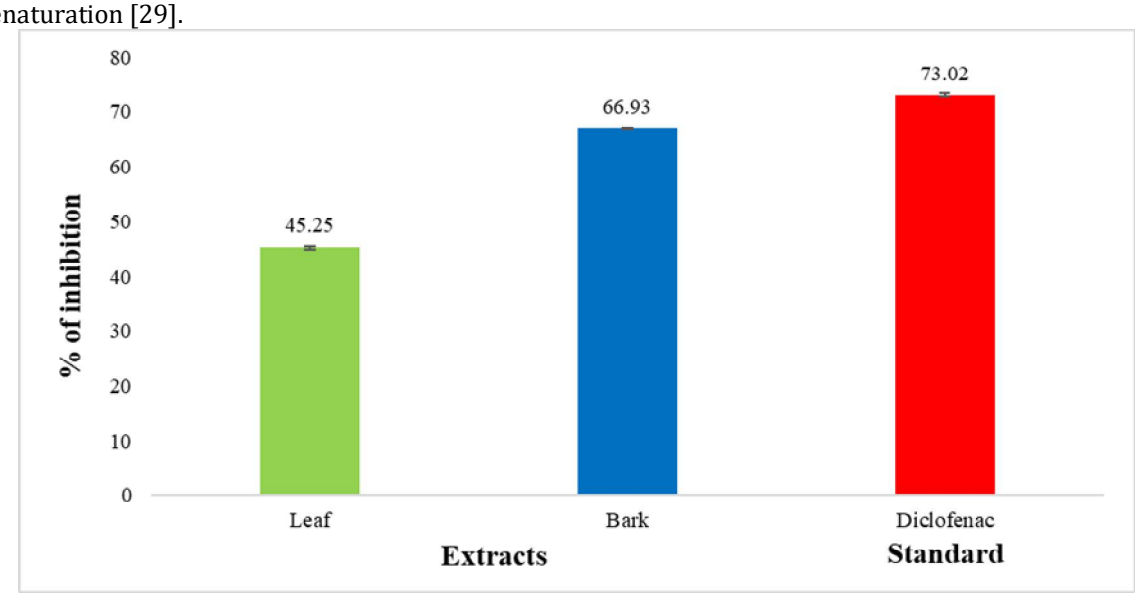


Figure 5: Heat-induced hemolysis of leaf and bark ethanolic extract of *E. gaussenii*

Protein denaturation effect through egg albumin denaturation assay

In this study, the protein denaturation assay for the ethanolic leaf and bark extracts of *E. gaussenii* is shown in Figure 6. The results demonstrate the albumin denaturation inhibition abilities of the plant extracts. The highest protein denaturation inhibition was observed for the ethanolic bark extract (66.93%), followed by the leaf extract (45.25%), compared to the standard drug, diclofenac (73.02%).

When egg albumin is denatured by heat, antigens are produced from the denatured proteins, which are associated with hypersensitive reactions (type-III), linked to diseases such as glomerulonephritis and serum sickness [4]. These denatured proteins may exacerbate delayed hypersensitivity, functioning similarly to native proteins
[23]. It is well established that conventional NSAIDs, such as phenylbutazone and indomethacin, not only inhibit the production of endogenous prostaglandins by blocking COX enzymes but also prevent protein denaturation [29].



Values are the mean of triplicate determination (n=3) ± standard deviation.

Figure 6: Egg albumin denaturation assay for the ethanolic leaf and bark extract of *E. gaussenii*

Bovine serum albumin denaturation effect of *E. gaussenii*

Protein inhibition potential for the ethanolic leaf and bark extracts of *E. gaussenii*, were analyzed and are presented in Fig 7. The highest inhibition was observed in the ethanolic bark extract (25.94%), followed by

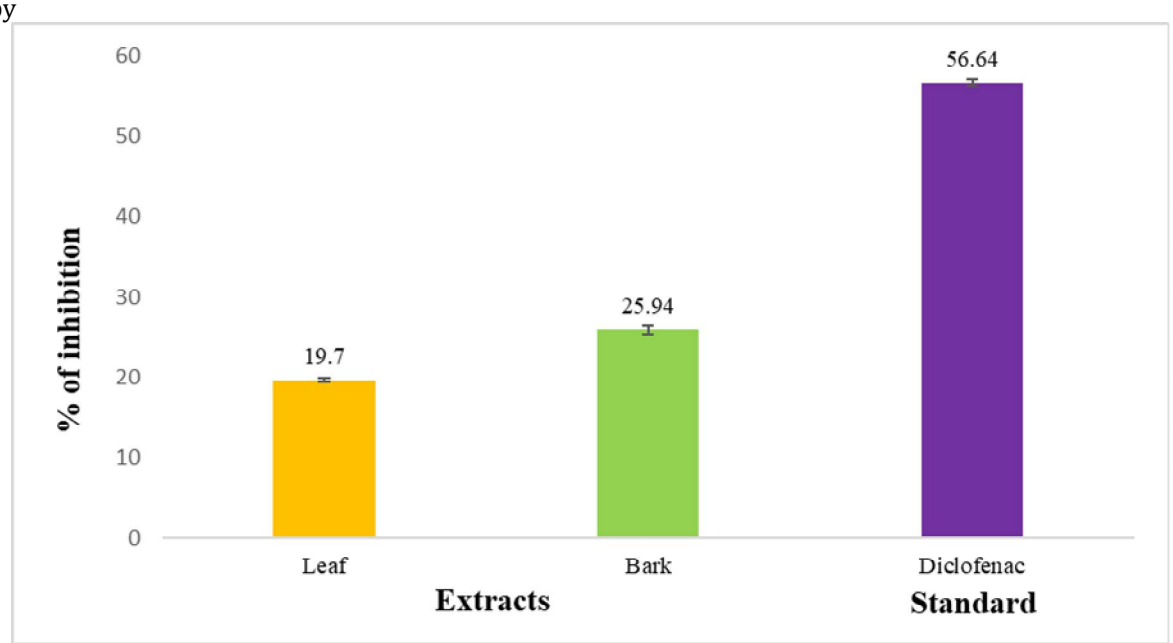


Figure 7: Bovine serum albumin denaturation assay for the leaf and bark ethanolic extract of *E. gaussenii*

Values are the mean of triplicate determination (n=3) ± standard deviation.

the leaf extract (19.7%), in comparison to the standard drug, diclofenac (56.64%). Denatured egg albumin generates antigens that induce type III hypersensitivity, associated with conditions like

glomerulonephritis and serum sickness. These altered proteins can also intensify delayed immune responses. NSAIDs such as phenylbutazone and indomethacin suppress prostaglandin synthesis and hinder protein denaturation [23].

CONCLUSION

Therefore, the present study underscores the exceptional antioxidant and anti-inflammatory properties of *Elaeocarpus gaussenii*, a versatile plant known for its wide range of compounds with diverse chemical structures. With the global shift toward the use of non-toxic herbal products, there is an urgent need to develop safer drugs from *E. gaussenii* for the treatment of inflammation-related diseases. This study aims to inspire further scientific exploration into the medicinal potential of this species to meet the growing demand for effective and sustainable treatments for mankind.

COMPETING INTERESTS

The authors have declared that no competing interest exists.

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