



Anti-Inflammatory Properties of Acetone Extract of Green Coffee Beans: An *In Vitro* Assay

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Abstract: This study evaluated the anti-inflammatory potential of the acetone extract of green coffee beans (*Coffea excelsa*) using *in vitro* assays. The extract was assessed for its ability to inhibit protein denaturation and stabilize biological membranes using heat-induced hemolysis of human erythrocytes as a model. The extract exhibited an overall increase in inhibitory effect across the tested concentrations in both assays. In the protein denaturation assay, percentage inhibition increased from 13.10% at 100 µg/mL to 70.45% at 1000 µg/mL, with an IC₅₀ value of 249.8 µg/mL. Similarly, in the membrane stabilization assay, inhibition ranged from 21.16% to 62.06% across the same concentration range, with an IC₅₀ value of 391.6 µg/mL. Statistical analysis using one-way ANOVA followed by Dunnett's *post hoc* test indicated that all tested concentrations produced significant effects compared to the control ($p < 0.0001$). IC₅₀ values were determined using nonlinear regression analysis ($n = 3$). Although the extract showed lower activity compared with acetylsalicylic acid, it demonstrated moderate anti-inflammatory activity. These effects may be associated with the presence of bioactive compounds such as phenolics and flavonoids; however, this cannot be confirmed since no phytochemical analysis was conducted in this study. Overall, these results indicate that the acetone extract of green coffee beans possesses measurable anti-inflammatory properties *in vitro*, suggesting that it may serve as a potential source of bioactive compounds for further investigation. Further studies, including phytochemical characterization and *in vivo* evaluations, are required to validate these findings.

Introduction

Bioactive plants and their extracts possess tremendous therapeutic potential for the treatment of various diseases and for drug discovery, although synthetic drugs currently dominate and are widely used in the pharmaceutical industry (1, 2). Historically, humans have depended on natural products as a primary source of healthcare (3, 4). In developing countries, the active compounds found in plants, which are both affordable and accessible, have shown effectiveness in treating several diseases with minimal or no significant side effects. This has sparked increasing scientific interest in the use of natural products for the development of new drugs (5, 6).

The human body activates a process called inflammation when it is invaded by harmful substances such as bacteria, viruses, pollutants, chemicals, or even damaged cells (7, 8). While this response is essential for protection and healing, excessive or prolonged inflammation can result in tissue damage (9, 10). Tissue

and cellular damage occur due to changes in the structure and function of key molecules such as proteins, lipids, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA), as well as cellular membranes, caused by chronic inflammation. Protein denaturation can lead to the formation of abnormal structures that promote inflammatory reactions, while membrane destabilization results in the release of intracellular components that aggravate tissue injury (11, 12).

The stabilization of proteins and biological membranes represents an important strategy in the management of inflammation. *In vitro* assays such as inhibition of protein denaturation and membrane stabilization using red blood cell models are widely employed as simple, reliable, and cost-effective methods for evaluating potential anti-inflammatory agents (13, 14). These approaches provide insight into the ability of compounds to preserve structural integrity and modulate inflammatory processes under stress conditions.

Non-steroidal anti-inflammatory drugs (NSAIDs) are

$$\% \text{ inhibition} = \frac{A_c - A_s}{A_c} \times 100 \quad (\text{Eq. 1})$$

widely used for the treatment of inflammation. However, irritation of the stomach lining, which can result in gastric ulcers, is one of the major side effects associated with NSAIDs (15, 16). This has increased interest in identifying safer, plant-derived alternatives with comparable therapeutic potential (13).

Coffee is a dicotyledonous plant belonging to the family Rubiaceae. More than 100 species of the coffee plant have been identified (17). However, *Coffea arabica*, *Coffea canephora* (commonly called Robusta), and *Coffea liberica* are the most common species (18).

Studies have shown that green coffee beans (the raw, unroasted seeds of the coffee plant) contain a wide variety of polyphenols, known for their health benefits and their contribution to flavor and aroma. Among these compounds, chlorogenic acid is the most abundant, making up about 12% of the phenolic compounds in green coffee beans. Other polyphenols, such as tannins, lignins, and anthocyanins, are also present but in significantly smaller concentrations (19).

The pharmacological properties of coffee, including its potential antioxidant, anti-inflammatory, antimicrobial, and neuroprotective effects, have been reported in previous studies, supporting its potential as a therapeutic agent (20–22). However, many of these investigations have focused on isolated compounds or advanced molecular and cell-based models rather than crude coffee bean extracts evaluated using simple *in vitro* assays.

Hence, there is limited information on the anti-inflammatory activity of crude green coffee bean extracts assessed using models such as protein denaturation and membrane stabilization. Furthermore, the use of less-studied coffee varieties, such as *Coffea excelsa*, and the influence of extraction solvents remain underexplored. Addressing these gaps is important for expanding the understanding of plant-based anti-inflammatory agents using accessible experimental approaches.

Therefore, this study was undertaken to evaluate the anti-inflammatory potential of the acetone extract of green coffee beans by assessing its ability to inhibit protein denaturation and prevent heat-induced hemolysis *in vitro*. The concentration-dependent effects and the IC_{50} values of the extract in these assays were determined, and its activity was compared with that of a standard non-steroidal anti-inflammatory drug (acetylsalicylic acid). It is hypothesized that the acetone extract of green coffee beans may exhibit anti-inflammatory activity by preventing protein denaturation and enhancing membrane stability.

Materials and Methods

Sourcing and Identification

Green coffee beans were procured from Bokkos Market, a major agricultural trading center in Bokkos Local Government Area, Plateau State, Nigeria, known for its supply of locally produced agricultural commodities. The botanical identity of the beans was confirmed by Professor I. I. Ibeawuchi of the Department of Crop Science Technology, Federal University of Technology Owerri, using detailed morphological assessment. A voucher specimen of the plant material was deposited in the department

herbarium for future reference (Voucher No: FUTO/SAAT/CST/001/2025), ensuring traceability of the study.

Chemicals and Reagents

All chemicals used were of analytical grade. Acetone, hydrogen peroxide, sodium carbonate, trichloroacetic acid, phosphoric acid, sodium hydroxide, and ascorbic acid were obtained from a certified local supplier.

Equipment

The study employed standard laboratory equipment, including a UV–Vis spectrophotometer, water bath, incubator, centrifuge, analytical balance, and standard glassware such as beakers, conical flasks, test tubes, and filter paper.

Plant Extraction

The maceration method was used for extraction. Pulverized green coffee beans (290 g) were soaked in 1200 mL of acetone in a conical flask and left at room temperature for 48 h with occasional stirring. The mixture was then filtered using Whatman No. 1 filter paper, and the filtrate was concentrated in a water bath at 40–45 °C to remove the solvent, yielding 29.8 g of semi-solid extract. The % yield of the extract was calculated as 10.3% based on the dry weight of the plant material. The resulting extract was stored in an airtight container at 4 °C until use.

Preparation of Extract Solution (Stock Solution)

A stock solution of the acetone extract was prepared by dissolving 0.5 g of the crude extract in 10 mL dimethyl sulfoxide (DMSO), yielding a concentration of 50 mg/mL. For each assay, an intermediate solution (5 mg/mL) was prepared by diluting 1 mL of the stock solution with 9 mL of distilled water. Working concentrations of 100, 200, 300, 500, and 1000 µg/mL were then prepared from the intermediate solution immediately before use. The final concentration of DMSO in all assay mixtures did not exceed 1%, ensuring that the solvent did not interfere with protein denaturation or red blood cell membrane stability.

Ethical Considerations

The study utilized anonymized discarded human blood samples obtained from Federal Medical Center, Owerri, and complied with institutional guidelines for minimal-risk *in vitro* studies. No identifiable donor information was used.

Inhibition of Protein Denaturation

The ability of the extract to inhibit protein denaturation was evaluated using a modified procedure described by Mizushima and Kobayashi (23) and Sakat *et al.* (24). Briefly, 500 µL of 1% bovine serum albumin (BSA) solution was mixed with 100 µL of the extract at various concentrations. The mixture was incubated at room temperature for 10 min, followed by heating at 51 °C for 20 min to induce protein denaturation. After cooling, absorbance was measured at 660 nm. Acetylsalicylic acid was used as positive control. The % inhibition of protein denaturation was calculated using Eq. 1.

Where A_c is the absorbance of the control, and A_s is the absorbance of the sample, IC_{50} values were determined by nonlinear regression analysis using GraphPad Prism.

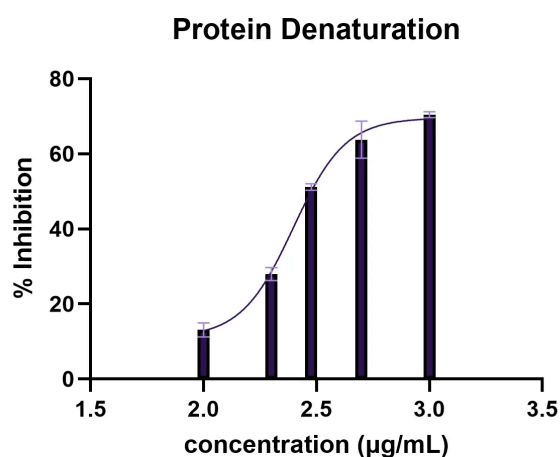


Figure 1. Illustrates the inhibitory effect of the extract on protein denaturation, showing an overall increase with a nonlinear dose–response pattern. Concentrations are presented on a log₁₀ scale (µg/mL).

Stabilization of Membrane

Preparation of Red Blood Cell (RBC)

Anticoagulated human blood was centrifuged at 3000 rpm for 10 min to separate its components. Following three saline washes of the resultant sample, the red blood cell fraction was separated. The RBC fraction was then diluted with 1x phosphate-buffered saline (PBS) to obtain a final concentration of 10% (v/v), following the method of Sadique *et al.* (25) and Sakat *et al.* (24).

Heat-Induced Hemolysis

A 100 µL volume of 10% red blood cell (RBC) suspension was mixed with 100 µL of the plant extract. The mixture was then incubated at 56 °C for 30 min to induce hemolysis, followed by centrifugation at 2500 rpm for 10 min at room temperature. The supernatant was collected, and its absorbance was measured at 560 nm to determine the extent of hemolysis. Acetylsalicylic acid (aspirin) was used as a positive control. The % of membrane stabilization was calculated according to the method described by Shinde *et al.* (26) and Sakat *et al.* (24) using Eq. 1. This assay effectively evaluates the potential of the

extract to provide protection against thermal-induced protein denaturation and cellular damage.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) from three independent biological replicates, each measured in triplicate. Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test. Differences were considered statistically significant at $p < 0.0001$. All analyses were carried out using GraphPad Prism version 11.0. Concentration values were log₁₀-transformed prior to statistical analysis and graphical representation to improve the linearity of the dose–response relationship.

Results and Discussion

Inhibition of Protein Denaturation

Protein denaturation involves the disruption of secondary, tertiary, and quaternary structures, leading to loss of biological function and contributing to inflammatory processes (14, 27). In this study, the acetone extract of green coffee beans (*C. excelsa*) exhibited inhibitory activity against protein denaturation across all tested concentrations (Table 1).

These findings align with the objective of evaluating the anti-inflammatory potential of the extract using *in vitro* models.

The extract exhibited an overall increase in inhibitory activity with increasing concentration, although minor variations were observed at intermediate concentrations, with % inhibition ranging from 13.10% at 100 µg/mL to 70.45% at 1000 µg/mL. Nonlinear regression analysis yielded an IC₅₀ value of 249.8 µg/mL, indicating moderate anti-inflammatory potential.

This trend is further illustrated in Figure 1, confirming the nonlinear dose–response pattern observed in the inhibition of protein denaturation. The curve shows a gradual increase in inhibitory activity with concentration, followed by a plateau at higher concentrations, suggesting possible saturation of protein-binding interactions.

Statistical analysis using one-way ANOVA revealed a significant difference among treatment groups ($F = 528.3$, $p < 0.0001$). Dunnett's *post hoc* test showed that all concentrations produced significant inhibition compared to the control ($p < 0.0001$).

The observed increase in inhibitory activity with

Table 1. Anti-inflammatory activity of acetone extract of green coffee beans (*C. excelsa*).

Concentrations (µg/mL)	Log ₁₀ (Concentration)	Inhibition of protein denaturation (%)	Inhibition of heat-induced hemolysis (%)
100	2.00	13.10 ± 1.85	21.16 ± 1.21
200	2.30	28.00 ± 1.75	29.11 ± 1.22
300	2.48	51.19 ± 0.89	35.05 ± 1.89
500	2.70	63.74 ± 4.99	50.47 ± 0.75
1000	3.00	70.45 ± 0.84	62.06 ± 1.97
Standard	—	75.79 ± 1.51	67.39 ± 2.51

Note: Values are expressed as mean ± standard deviation (n = 3). Statistical significance was set at $p < 0.0001$. Log₁₀ values were calculated from concentration (µg/mL) and used for statistical analysis and graphical representation.

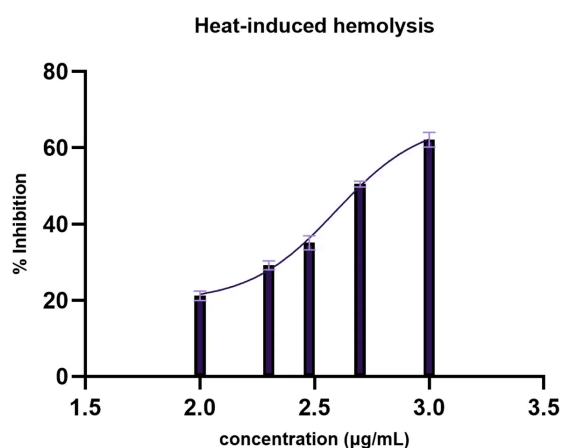


Figure 2. Presents the effect of the extract on heat-induced hemolysis, showing an overall increase in membrane stabilization across the tested range. Concentrations are presented on a \log_{10} scale ($\mu\text{g/mL}$).

concentration, together with slight deviations at the intermediate concentrations, suggests a nonlinear dose-response relationship, which may reflect complex interactions between extract constituents and protein molecules.

Compared with previously reported plant extracts, the IC_{50} value obtained in this study suggests moderate activity, as some plant-derived extracts exhibit lower IC_{50} values and thus higher potency. For instance, an anti-inflammatory study using *Solanum obtusifolium* Dunal fruit reported an IC_{50} value of $197.31 \pm 6.89 \mu\text{g/mL}$ (28), indicating slightly higher inhibitory activity than the present extract. In contrast, extracts of *Ephedra alata* Decne used in a protein denaturation study exhibited higher IC_{50} values ranging from 309.45 to 504 $\mu\text{g/mL}$ based on the extraction solvents used (29), suggesting lower activity compared to the current findings. Similarly, extracts of *Ajuga integrifolia* Buch. -Ham. leaves used in a protein denaturation study reported an IC_{50} value of 532 $\mu\text{g/mL}$ (30). These comparisons place the activity of the present extract within the moderate range relative to other plant-derived anti-inflammatory agents.

Furthermore, the extract showed lower inhibitory activity than the standard drug acetylsalicylic acid (aspirin), indicating comparatively reduced efficacy. At 1000 $\mu\text{g/mL}$, the extract showed 70.45% inhibition compared with 75.79% for acetylsalicylic acid, indicating lower but comparable activity.

The observed activity may be attributed to bioactive compounds such as phenolics and flavonoids, which are known to possess anti-inflammatory properties. However, no phytochemical analysis was conducted in this study; therefore, this attribution remains speculative.

Heat-Induced Hemolysis

Membrane stabilization is an important mechanism in the management of inflammation, as destabilization of lysosomal membranes leads to the release of enzymes that exacerbate tissue damage (31, 32). In this study, the acetone extract of green coffee beans demonstrated protective effects against heat-induced hemolysis of erythrocytes (Table 1).

The extract exhibited an overall increase in membrane stabilization across the tested range, with % inhibition ranging from 21.16% at 100 $\mu\text{g/mL}$ to 62.06% at 1000 $\mu\text{g/mL}$. The IC_{50} value was determined to be 391.6 $\mu\text{g/mL}$, indicating moderate membrane-stabilizing activity.

This trend is further illustrated in Figure 2, confirming the nonlinear dose-response pattern observed in membrane stabilization. The graphical profile indicates progressive membrane stabilization with increasing concentration, with slight deviations at intermediate concentrations, reflecting complex interactions between extract constituents and erythrocyte membranes.

Statistical analysis using one-way ANOVA showed a highly significant effect of treatment ($F = 692.2$, $p < 0.0001$; $R^2 = 0.9966$). Dunnett's multiple comparisons test revealed that all concentrations significantly reduced hemolysis compared to the control ($p < 0.0001$).

In comparison with acetylsalicylic acid (aspirin), the extract exhibited lower inhibitory activity, indicating reduced potency relative to conventional anti-inflammatory agents. At 1000 $\mu\text{g/mL}$, the extract showed 62.06% inhibition compared with 67.39% for acetylsalicylic acid, indicating lower activity relative to the standard. However, the IC_{50} value obtained in this study is comparable to those reported for some plant extracts in similar assays. For instance, *Solanum obtusifolium* Dunal fruit used in a hemolysis study reported a lower IC_{50} value of $196.81 \pm 4.59 \mu\text{g/mL}$ (28), indicating higher potency than the present extract, whereas *Ficus religiosa* seeds extract used in a hemolysis study reported substantially higher IC_{50} values ranging from 790.30 to 1094.65 $\mu\text{g/mL}$ depending on the solvent fraction (33), suggesting lower activity compared to the current findings. These comparisons further support the classification of the acetone extract of *C. excelsa* as having moderate membrane-stabilizing activity.

While erythrocyte membranes share structural similarities with lysosomal membranes, extrapolation of these findings to *in vivo* lysosomal stabilization remains theoretical. Therefore, further *in vivo* studies are required to confirm this mechanism.

Conclusion

This study demonstrated that the acetone extract of green coffee beans (*C. excelsa*) exhibited measurable anti-inflammatory activity *in vitro*, as evidenced by its ability to inhibit protein denaturation and stabilize erythrocyte membranes under heat-induced conditions. Although the extract was less potent than acetylsalicylic acid (standard drug), the observed concentration-dependent effects suggest the presence of bioactive compounds that may contribute to its activity, possibly through mechanisms related to the modulation of inflammatory mediators and protection of cellular structures under stress conditions. These findings indicate that crude extracts, despite their lower potency compared to standard drugs, may still offer valuable pharmacological potential as alternative or complementary therapeutic agents.

However, the absence of phytochemical characterization and *in vivo* studies limits the ability to attribute the activity to specific compounds or predict therapeutic relevance. In addition, pharmacokinetic properties and toxicity profiles of the extract remain unknown, which are critical factors for its potential clinical

application. Further research is needed to identify the active constituents, explore their mechanism of action, and evaluate their efficacy and safety in animal models to better understand the potential of green coffee beans as an anti-inflammatory agent, as well as to support the development of standardized formulations and optimized extraction methods for future therapeutic use.

Declaration

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

All data generated or analyzed during this study are included in this published article.

Ethics Statement

The study utilized anonymized discarded human blood samples obtained from Federal Medical Center, Owerri, and complied with institutional guidelines for minimal-risk in vitro studies. No identifiable donor information was used.

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