# In Vitro Anti-Inflammatory and Anti-Diabetic Activities of Methanolic Extract of Sour Plum (*Xymenia caffra*) Leaves

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Posted Date: 11 March 2026

doi: 10.20944/preprints202603.0866.v1

Keywords: *Ximenia caffra*; anti-inflammatory; antidiabetic; enzyme inhibition; medicinal plants

 doi:10.20944/preprints202603.0866.v1



*Article*

# In Vitro Anti-Inflammatory and Anti-Diabetic Activities Methanolic Extract of Sour Plum (*Xymenia caffra*) Leaves

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**Abstract**

This study evaluated the in vitro anti-inflammatory and antidiabetic activities of methanolic leaf extracts of *Ximenia caffra* (sour plum), a medicinal plant widely used in traditional healthcare systems across tropical Africa. Medicinal plants remain an important source of bioactive phytochemicals, and growing interest in phytopharmaceuticals has intensified the search for natural compounds with therapeutic potential. The present investigation aimed to scientifically validate the ethnomedicinal use of *X. caffra* leaves by assessing their enzyme inhibitory and anti-inflammatory properties. Fresh leaves of *X. caffra* were collected, authenticated, air-dried, pulverized, and extracted using methanol through maceration. Anti-inflammatory activity was determined using protein denaturation inhibition and membrane stabilization assays, while antidiabetic potential was evaluated through $\alpha$-amylase and $\alpha$-glucosidase enzyme inhibition assays. The extract exhibited concentration-dependent biological activities across all experimental models. Anti-inflammatory evaluation showed significant inhibition of protein denaturation and membrane stabilization, with $IC_{50}$ values of 129.83 µg/mL and 288.11 µg/mL, respectively. Similarly, the extract demonstrated appreciable antidiabetic activity, inhibiting $\alpha$-amylase and $\alpha$-glucosidase enzymes with $IC_{50}$ values of 227.01 µg/mL and 179.35 µg/mL, respectively, indicating stronger inhibition of $\alpha$-glucosidase. These findings suggest that *X. caffra* leaves contain bioactive compounds capable of modulating inflammatory responses and carbohydrate-digesting enzymes, thereby supporting their traditional medicinal use. The study highlights the potential of *X. caffra* as a promising natural source for the development of plant-based anti-inflammatory and antidiabetic therapeutic agents.

**Keywords:** *Ximenia caffra*; anti-inflammatory; antidiabetic; enzyme inhibition; medicinal plants

## 1. Introduction

*Xìmenia caffra*, the large sourplum, is part of the Olacaceae family which is native throughout tropical regions. In particular, the sour plum is native to regions in South East Africa, mainly Botswan, Kenya, Malawi, Mozambique, South Africa, Tanzania, Uganda,Zambia, and Zimbabwe [1,2].The sourplum tree produces several fruits on an annual basis. These are generally sour with a dry aftertaste, however contains significant amounts of potassium [3]. The tree itself is fairly hardy, with frost resistance and drought tolerance. The tree,fruit, seed, leaves, and roots are all used for human consumption, medicinally, or for fuel, and research has shown it to have some pharmaceutical and medicinal importance (Jacob et al., 2021a, 2021b, 2021c, Jacob *et al*., 2023)[4–7].

Natural products are chemical compounds or substances isolated from living organism [8]. The chemistry of the natural product include their biosynthesis, extraction, identification, quantification,

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structural elucidation, physical and chemical properties and reactions They are produced by the pathway of primary or secondary metabolism [9,10].

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. The curative properties of medicinal plantsare attributable to the presence of various bioactive phytochemicals which may explaintheir traditional uses against various ailments (Sajeesha*et al*., 2018)[11–15]. Despite significant development of rural health services, rural dwellers still use herbal folk medicines to a good extent for treatment of common ailments like cough, cold andfever, headache and body-ache, constipation and dysentery, burns, cuts and scalds, boils,ulcers, skin diseases and respiratory troubles and others [16,17]. Nevertheless, a growing world-wide interest in the use of phytopharmaceuticals as complementary or alternative medicine, either for prevention or amelioration of manydiseases has been noted in recent years [18].

In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without anyadverse side effect especially when compared with synthetic drugs. Majority of the humanpopulation are ignorant of importance of sour plum leaves ranging from its nutritional tomedicinal value. It is known that leave part of plants are synthesized into drugs and that they contain phytochemicals which serve as antibiotic principles of plants. The need for a cheap, renewable, easily available and nutritive source of material as active ingredients for medicine, hence, it is pertinent to investigate the antidiabetic and anti-inflammatory properties present in sour plum.

## 2. Materials and Methods

The materials and apparatus used are *Ximeniacaffra,*beaker, measuring cylinder, mortar and pestle, weighing scale, filter paper, funnel, masking tape, cotton wool and foil paper, methanol

*Collection and Preparation of Plant Material*

Fresh leaves of *Ximenia caffra* were collected from a verified botanical source and authenticated at the Department of Biological science, Prince AbubakarAudu University Anyigba by a Botanist, Mr AyegbaSule. The plant material was washed thoroughly with distilled water to remove dirt and debris, followed by Air-drying at room temperature for two weeks. The dried samples were then pulverized using morter and pestle to obtain a fine powder, and stored in airtight containers until further use.

*Solvent Maceration Extraction*

The powdered plant material (50 g) was subjected to successive maceration using **methanol** (200 mL) as solvent.

*Methanol Extraction*

50g of the powdered sample was soaked in 200 mL of methanol in a conical flask. The mixture was agitated occasionally and left to stand for **72 hours at room temperature**. After maceration, the extract was filtered, the filtrate was concentrated using a rotary evaporator under reduced pressure at 40 °C to obtain the methanolic extract. All extracts were weighed to determine the yield, stored in amber-colored vials, and kept until further use.

*Determination of Percentage Yield*

The percentage yield of each extract was calculated using the formula:

$$\text{Percentage} = \frac{\text{Mass of extract (g)}}{\text{Mass of sample used (g)}} \times 100$$

- **Mass of extract obtained** was measured using an analytical balance.
- **Mass of plant material used** was recorded before extraction.

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This gave the percentage of extract relative to the initial sample weight.

*In Vitro Anti-Inflammatory Activity Assay*

The anti-inflammatory activity of the extract was evaluated using the **protein denaturation inhibition assay**, as per the modified method of Mizushima and Kobayashi (1968)

Procedure

A reaction mixture consisting of 0.2 mL of egg albumin, 2.8 mL of phosphate-buffered saline (PBS, pH 6.4), and 2 mL of the extract concentration was prepared. The mixture was incubated at **37 °C for 15 minutes**, followed by heating at **70 °C for 5 minutes**. After cooling, the turbidity was measured at **660 nm** using a UV-Vis spectrophotometer.

Calculation

$$\%\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of Control}} \times 100$$

**Diclofenac sodium** (standard anti-inflammatory drug) was used as a reference control.

*In Vitro Anti-Diabetic Activity Assay*

The antidiabetic potential of the *Xymenia caffra* extracts was evaluated in vitro using two standard enzyme inhibition assays:

$\alpha$-Amylase Inhibition Assay

This assay was used to evaluate the ability of the extracts to inhibit the activity of $\alpha$-amylase, a key enzyme involved in carbohydrate digestion.

Preparation of Reagents**:**

**$\alpha$-Amylase enzyme** (porcine pancreatic, 1 U/mL in phosphate buffer, (pH 6.9), **Substrate** (1% soluble starch in phosphate buffer), **DNSA reagent** for color development.

Procedure**:**

Different concentrations (100–1000 µg/mL) of each extract were prepared.0.5 mL of extract was mixed with 0.5 mL of $\alpha$-amylase and incubated for 10 min at 37 °C.Then 0.5 mL of starch solution was added and incubated for 15 min.The reaction was stopped with 1 mL of DNSA reagent, boiled for 5 minutes, cooled, and absorbance was read at 540 nm.

Calculation:

The percentage inhibition was calculated as:

$$\%\ \text{Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

$\alpha$-Glucosidase Inhibition Assay

This assay tested the extracts ability to inhibit $\alpha$-glucosidase, which is also involved in carbohydrate digestion.

Reagents**: $\alpha$-Glucosidase enzyme (**1 U/mL), **p-Nitrophenyl-$\alpha$-D-glucopyranoside (PNPG)** as a substrate, phosphate buffer (pH 6.8).

Procedure**:**

Various concentrations of the extracts were mixed with $\alpha$-glucosidase and incubated.PNPG was added and the mixture was incubated at 37 °C for 15 min.The reaction was stopped with 0.1 M $Na_2CO_3$.Absorbance was measured at 405 nm.

Calculation:

The percentage inhibition was calculated as:

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$$\% \text{ Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

## 3. Results

In vitro Anti-inflammatory Activity Assay

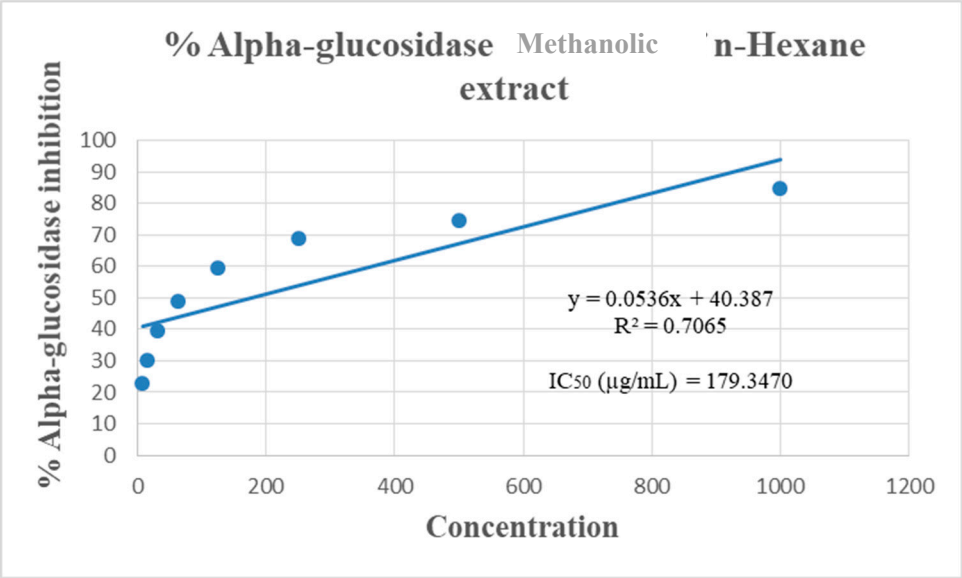


**Figure 1.** Percentage inhibition of $\alpha$-glucosidaseonextract.

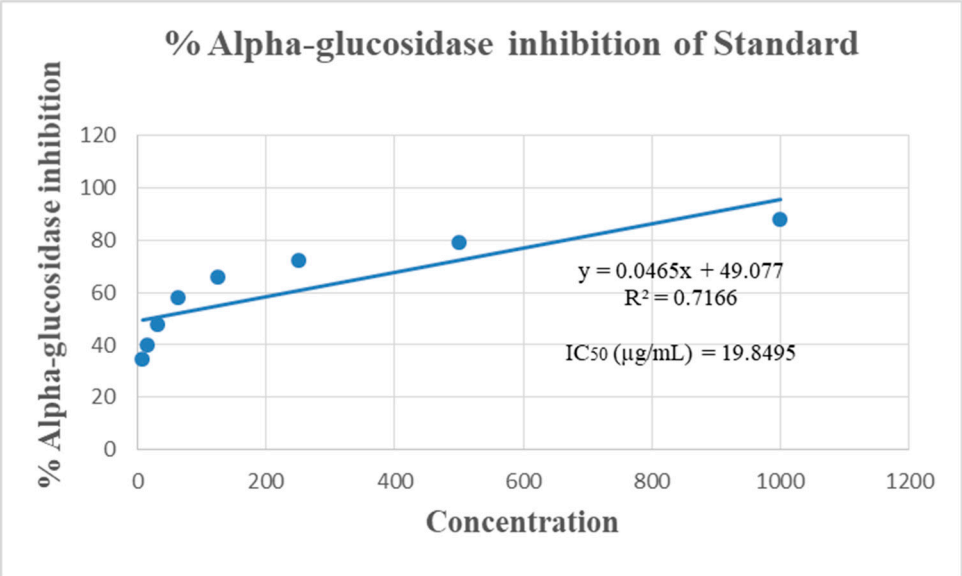


**Figure 2.** Percentage inhibition of $\alpha$-glucosidaseonstandard.

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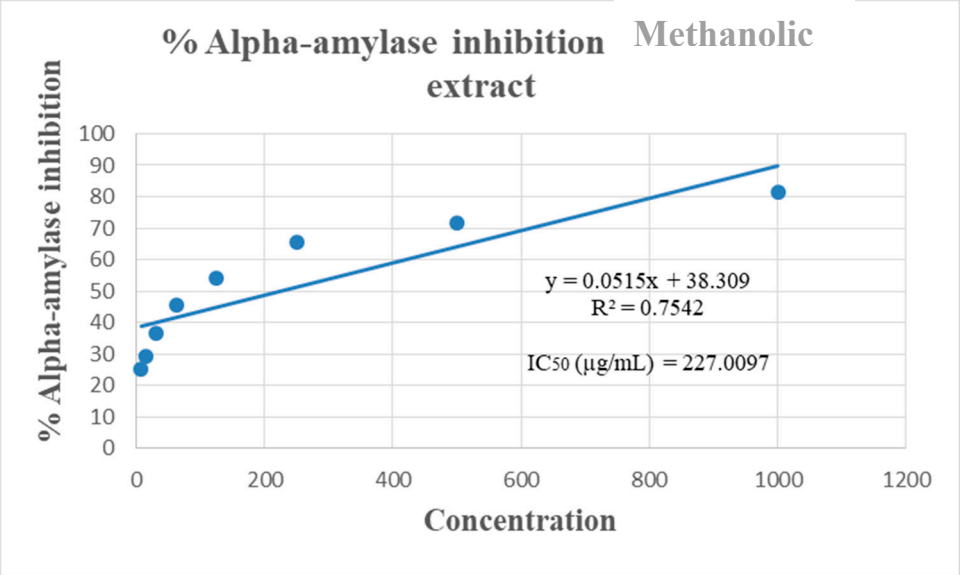


**Figure 3.** Percentage inhibition of $\alpha$-amylaseonextract.

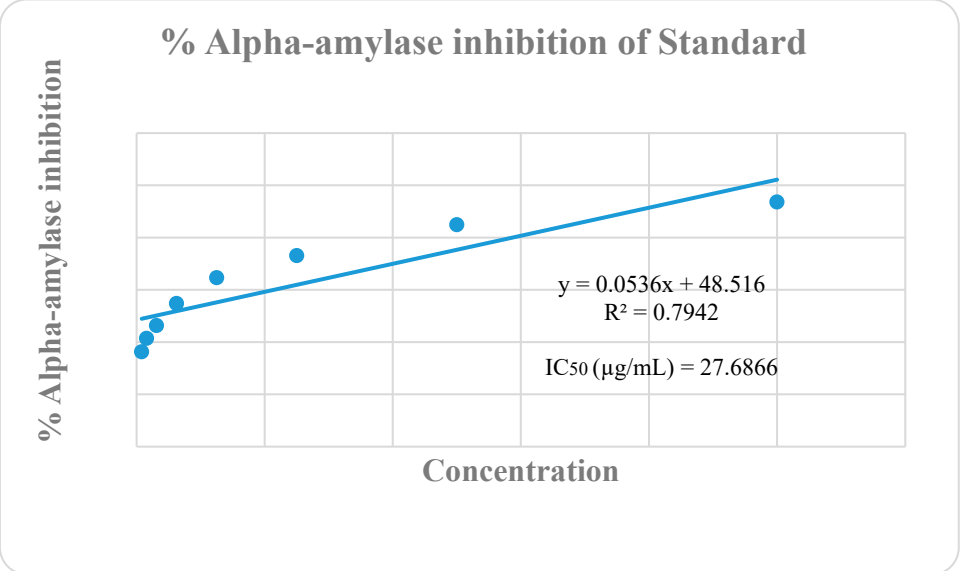


**Figure 4.** Percentage inhibition of $\alpha$-amylaseonstandard.

In vitro Anti-diabetic Activity Assay

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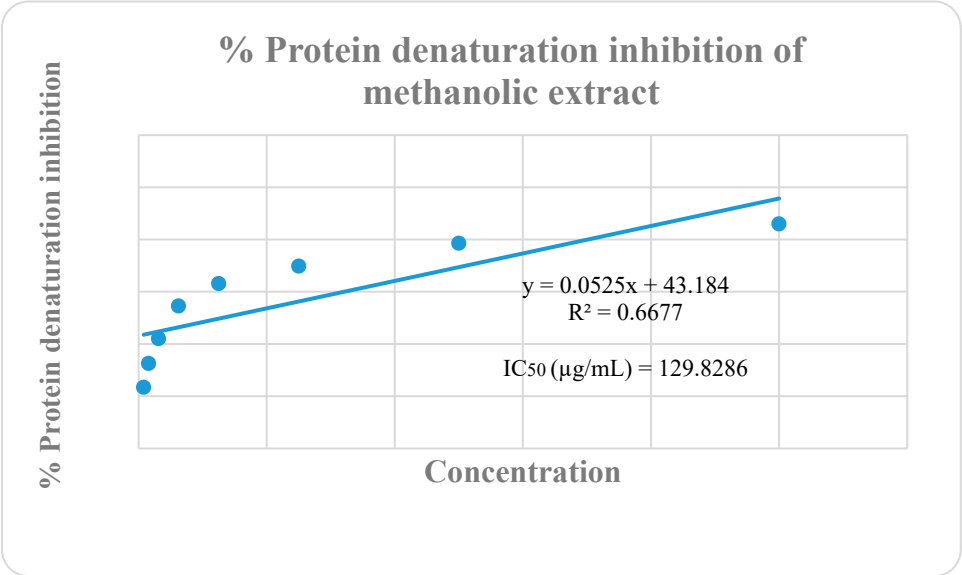


**Figure 5.** Percentage inhibition of protein denaturation onextract.

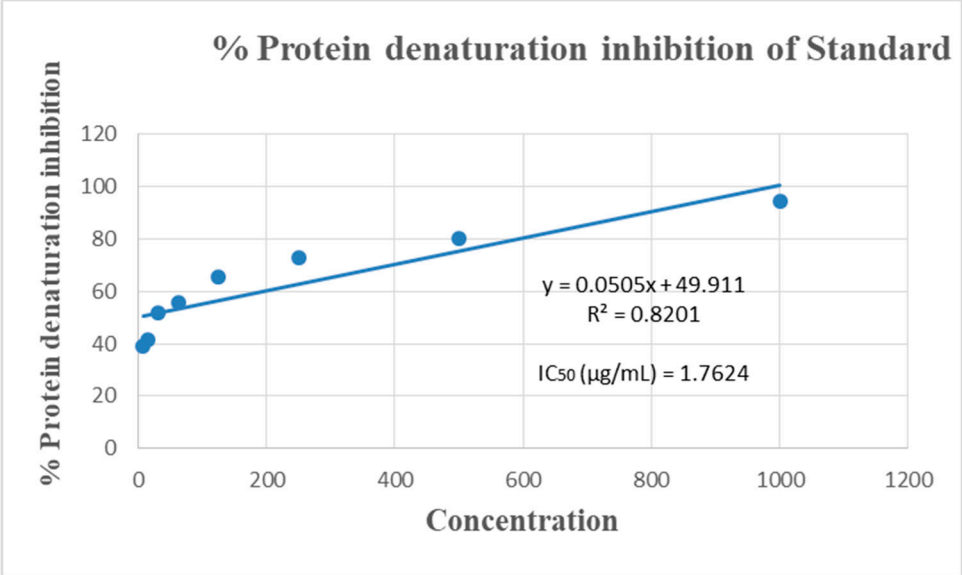


**Figure 6.** Percentage inhibition of protein denaturation onstandard.

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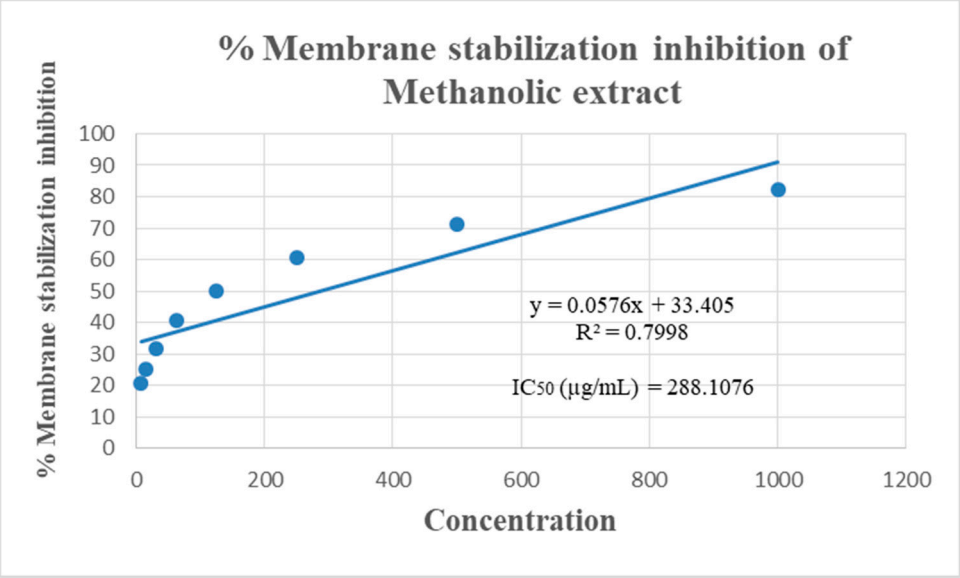


**Figure 7.** Percentage inhibition of membrane stabilization onextract.

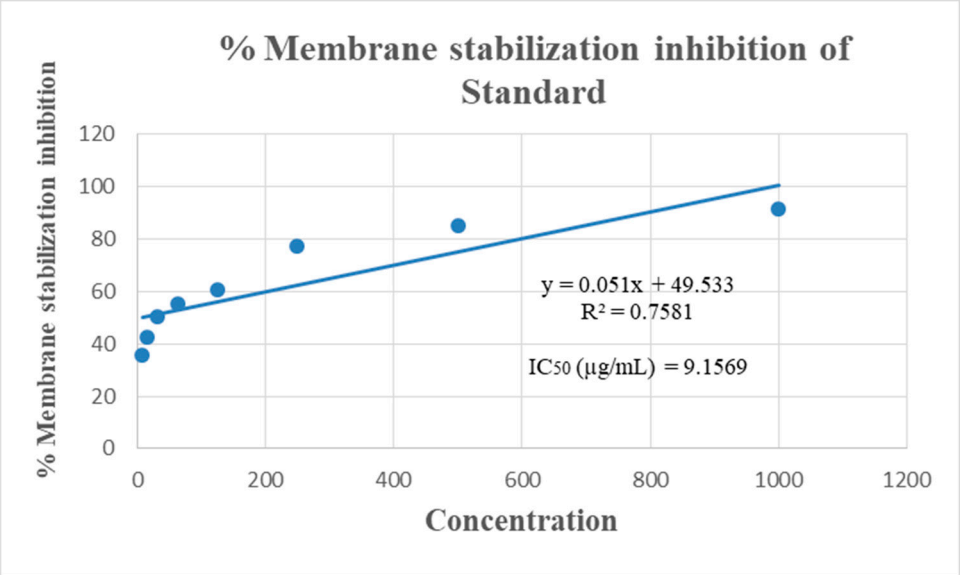


**Figure 8.** Percentage inhibition of membrane stabilization onstandard.

## 4. Discussion

The present study evaluated the in vitro anti-inflammatory and antidiabetic activities of the methanolic leaf extract, with the findings demonstrating pronounced concentration-dependent biological responses across all assays. These results provide strong evidence that the extract contains bioactive phytochemicals capable of modulating inflammatory processes and carbohydrate-metabolizing enzymes, thereby supporting its ethnomedicinal applications.

*Anti-Inflammatory Activity*

Inflammation is frequently associated with protein denaturation, membrane destabilization, and the release of inflammatory mediators. Therefore, inhibition of protein denaturation and protection of cellular membranes are widely recognized indicators of anti-inflammatory potential. In this study, the methanolic extract showed progressive inhibition of heat-induced protein denaturation with increasing extract concentration, suggesting effective stabilization of protein structure. The $IC_{50}$ value

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obtained (129.83 µg/mL) indicates relatively strong activity, implying that the extract may contain polyphenols, flavonoids, or other secondary metabolites capable of interacting with protein functional groups and preventing conformational alterations. Stabilization of proteins under stress conditions is particularly important because denatured proteins can trigger inflammatory cascades and tissue damage.

Similarly, the membrane stabilization assay demonstrated that the extract protected erythrocyte membranes against hypotonic-induced lysis in a concentration-dependent manner, with an $IC_{50}$ value of 288.11 µg/mL. Membrane stabilization is considered analogous to stabilization of lysosomal membranes in vivo; therefore, compounds capable of maintaining membrane integrity can reduce the release of inflammatory enzymes and mediators. The comparatively higher $IC_{50}$ value observed for membrane stabilization suggests that although the extract possesses significant protective activity, protein denaturation inhibition represents a stronger anti-inflammatory mechanism in this case. The observed effects collectively indicate that the extract may exert anti-inflammatory action through multiple pathways, including stabilization of structural proteins and cellular membranes.

*Antidiabetic Activity*

The management of diabetes mellitus often involves controlling postprandial hyperglycemia through inhibition of key digestive enzymes such as α-amylase and α-glucosidase, which catalyze the breakdown of complex carbohydrates into absorbable glucose. The methanolic extract exhibited concentration-dependent inhibitory activity against both enzymes, confirming its potential role in regulating carbohydrate metabolism.

The $IC_{50}$ value for α-amylase inhibition was 227.01 µg/mL, indicating moderate enzyme suppression. Excessive inhibition of α-amylase is generally undesirable because it may lead to gastrointestinal disturbances; therefore, moderate inhibition is considered beneficial for therapeutic applications. The extract's activity profile suggests that it may slow the initial stages of starch digestion without completely preventing carbohydrate utilization.

In contrast, the extract showed comparatively stronger inhibition of α-glucosidase, with an $IC_{50}$ value of 179.35 µg/mL. Stronger inhibition of α-glucosidase relative to α-amylase is considered advantageous in diabetes management because it delays the conversion of disaccharides and oligosaccharides into glucose at the intestinal brush border, thereby reducing postprandial blood glucose spikes. The observed enzyme inhibition may be attributed to the presence of phenolic compounds and other phytoconstituents known to interact with enzyme active sites through hydrogen bonding, hydrophobic interactions, or metal ion chelation.

*Comparative Interpretation of Activities*

The concentration-dependent responses observed across anti-inflammatory and antidiabetic assays indicate that the biological effects are dose-related and likely mediated by multiple phytochemical constituents acting synergistically. The stronger inhibition of protein denaturation compared to membrane stabilization suggests that the extract's anti-inflammatory activity may primarily involve protein-protective mechanisms, while its antidiabetic activity appears more pronounced at the level of α-glucosidase inhibition. Such dual pharmacological action is particularly significant because chronic inflammation is often associated with metabolic disorders, including diabetes mellitus; therefore, agents capable of targeting both inflammatory pathways and glucose metabolism may provide enhanced therapeutic benefits.

*Pharmacological Implications*

The results obtained in this study provide scientific validation for the traditional medicinal use of the plant leaves in managing inflammatory conditions and metabolic disorders. The $IC_{50}$ values observed fall within ranges reported for many plant-derived extracts possessing therapeutic potential, indicating that the extract could serve as a promising source of lead compounds for drug

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development. Furthermore, the use of methanol as an extraction solvent likely facilitated the recovery of a wide range of polar bioactive compounds, including flavonoids, tannins, alkaloids, and phenolic acids, which are known contributors to antioxidant, anti-inflammatory, and antidiabetic activities.

## 5. Conclusions

Overall, the findings demonstrate that the methanolic leaf extract exhibits significant anti-inflammatory and antidiabetic properties, as evidenced by effective inhibition of protein denaturation, membrane stabilization, and suppression of carbohydrate-digesting enzymes. The concentration-dependent activities observed across all assays strongly suggest the presence of pharmacologically active phytochemicals capable of modulating inflammatory responses and glucose metabolism. These results highlight the therapeutic potential of the plant as a natural source of bioactive compounds and provide a basis for further investigations involving phytochemical characterization, in vivo studies, toxicity evaluation, and formulation development aimed at producing plant-based therapeutic agents.

**Author Contributions:** For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, and ; methodology, Alifa Jacob; software, Alifa Jacob and Abiodun Dauda; validation, and ; formal analysis, Alifa Jacob, Abiodun Dauda; investigation, Vivian Okonkwo and Nkechi Orji; resources, Alifa Jacob, Abiodun Dauda, Vivian Okonkwo and Nkechi Orji; writing—original draft preparation, Alifa Jacob; writing—review and editing, Abiodun Dauda; visualization, Alifa Jacob, Abiodun Dauda, Vivian Okonkwo and Nkechi Orji; supervision, Alifa Jacob; project administration, Alifa Jacob, Abiodun Dauda, Vivian Okonkwo and Nkechi Orji; funding acquisition, Alifa Jacob, Abiodun Dauda, Vivian Okonkwo and Nkechi Orji. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding".

**Data Availability Statement:** No new data were created.

**Acknowledgments:** The authors have reviewed and edited the output and take full responsibility for the content of this publication.

**Conflicts of Interest:** "The authors declare no conflicts of interest.".

## Abbreviations

In vitro Anti-inflammatory Activity Assay

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