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FUNCTIONAL POTENTIAL OF *PROSOPIS AFRICANA* WHOLE FRUIT: INSIGHTS INTO ANTIOXIDANT, ANTIDIABETIC, AND IMMUNOMODULATING ACTIVITIES

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ABSTRACT

The global rise in chronic conditions such as diabetes, immune dysfunction, and oxidative stress-related disorders has intensified the search for multifunctional plant-based remedies. This study evaluated the antioxidant, α-glucosidase inhibitory, and immunomodulatory properties of the whole fruit extract and fractions of *Prosopis africana*. Whole fruits were extracted with 70% methanol and fractionated into hexane (PAF-1), dichloromethane (PAF-2), ethyl acetate (PAF-3), and aqueous (PAF-4) components. Antioxidant activity was assessed via DPPH radical scavenging. α-Glucosidase inhibition was analysed through enzyme kinetics using acarbose as a standard. Immunomodulatory effects were evaluated by measuring ROS inhibition in stimulated human peripheral blood mononuclear cells (PBMCs). All extracts exhibited dose-dependent antioxidant activity. PAF-1 and PAF-2 demonstrated strong radical scavenging capacities with IC_{50} values of 0.23±0.02 and 0.259±0.01 µg/mL, respectively, significantly lower than gallic acid (22.1±1.85 µg/mL). PAF-1 showed the most potent α-glucosidase inhibition (IC_{50} = 44.83±1.09 µg/mL), surpassing acarbose (IC_{50} = 377.75±1.34 µg/mL), while PAF-2 showed the highest ROS inhibition (44.7%) in PBMCs, although lower than ibuprofen (73.2%). *P. africana* whole fruit exhibits significant multifunctional bioactivities, validating traditional claims and highlighting its potential as a source of nutraceutical or phytopharmaceutical agents for managing chronic diseases.

Keywords: *Prosopis africana*, Antioxidant, a-glucosidase Inhibition, ROS, Immunomodulation, Plant-based therapy

INTRODUCTION

The escalating global burden of chronic diseases such as diabetes mellitus, immune dysregulation, and oxidative stress-related disorders has intensified the quest for multifunctional, safer, and culturally relevant therapeutic alternatives (Wang & Zhang, 2024). Natural products, particularly those derived from ethnomedicinal plants, offer a diverse repository of bioactive compounds capable of addressing multiple disease pathways simultaneously (Ahirwar et al., 2024). Among these is *Prosopis africana* (Guill. & Perr.) Taub., a leguminous tree endemic to sub-Saharan Africa, has garnered significant ethnopharmacological interest (Ogunniyi et al., 2023). Traditionally, its bark, leaves, and roots are used in the management of infections, fever, and gastrointestinal disorders (Adamu et al., 2024). However, despite the fruit's integral role in local diets and traditional remedies, it remains notably understudied in contemporary biomedical science.

Recent phytochemical investigations have identified key constituents in the fruit, such as flavonoids, tannins, and phenolic acids**,** which are renowned for their ability to scavenge free radicals, chelate metal ions, and modulate enzyme activity. Specifically, flavonoids and tannins are known to inhibit carbohydrate-digesting enzymes like α-glucosidase, thereby improving postprandial hyperglycemia, while phenolic acids contribute significantly to neutralizing reactive oxygen species (ROS) and dampening pro-inflammatory signaling pathways (He et al., 2019; Rudrapal et al., 2022). Interestingly, one of our earlier phytochemical investigations of the whole fruit extract led to the isolation of a new sesquiterpene (Prosoterpene-1) and eleven known compounds (Ali et al., 2023). The presence of these phytochemicals suggests a strong potential for modulating oxidative stress and inflammation, which are central to the pathogenesis of many chronic diseases (Thiruvengadam et al., 2021). Nevertheless, the therapeutic potential of the whole fruit extract, particularly in the context of synergistic interactions among its constituents, has not been rigorously evaluated, representing a critical gap in scientific validation (Chen, Li, Zhang, & Deng, 2022).

Oxidative stress, defined as an imbalance between reactive oxygen species (ROS) and antioxidant defences, is a hallmark of metabolic and degenerative disorders, often aggravated by poor lifestyle and nutritional habits (Jomova et al., 2023). Natural antioxidants are known to mitigate ROS accumulation and also modulate redox-sensitive cellular pathways, thereby exerting systemic protective effects (Rudrapal et al., 2022). Similarly, the global rise in diabetes prevalence has underscored the limitations of synthetic α-glucosidase inhibitors, such as acarbose, which are frequently associated with gastrointestinal side effects (Mushtaq et al., 2023). In contrast, plant-derived inhibitors often exhibit multifaceted mechanisms with improved tolerability and safety profiles, thus gaining increased scientific and clinical attention (Dehelean et al., 2021).

Concurrently, immune dysregulation has emerged as a key feature of several modern health challenges, including autoimmune diseases, infections, and chronic inflammation, necessitating the development of safe and effective immunomodulators (Dehelean et al., 2021). The use of *P. africana* fruit in traditional medicine for gastrointestinal complaints may reflect broader immunological activity, suggesting a potential for modulating inflammatory pathways, although this remains largely unexplored in modern pharmacology (Ndung'u et al., 2024).

Notably, previous studies on *P. africana* have largely focused on isolated plant parts or purified compounds, often neglecting the holistic approach inherent in traditional medicine. This reductionist perspective may hinder the development of whole-plant extracts as functional foods or nutraceuticals, a term for food-derived products that provide medical or health benefits, including the prevention and treatment of disease, where the concept of phytochemical synergy is central to efficacy (Mukherjee et al., 2022). For instance, the co-occurrence of flavonoids and tannins could enhance α-glucosidase inhibition via allosteric mechanisms, while also affecting nutrient absorption and glycemic control (He et al., 2019). Additionally, the phenolic-rich matrix of the fruit may impact immune signalling cascades such as NF-κB activation, as reported in other species (Dejani et al., 2021).

This study aims to bridge these knowledge gaps by evaluating the multifunctional bioactivities of *P. africana* whole fruit extract, including its antioxidant capacity (DPPH scavenging and ROS inhibition assays), α-glucosidase inhibitory effect through kinetic comparison with acarbose, and immunomodulatory potential based on ROS suppression in human immune cells. We hypothesise that the interplay between flavonoids, tannins, and other constituents within the extract will yield enhanced biological activity through synergistic mechanisms.

To our knowledge, this is the first comprehensive investigation into the multifunctional bioactivity of *P. africana* whole fruit extract. By aligning traditional uses with scientific validation, the study offers novel mechanistic insights into its potential as a source of natural antioxidant, antidiabetic, and immunomodulatory agents. Furthermore, this work supports global priorities on plant-based interventions for chronic diseases (Thompson et al., 2023) and contributes to sub-Saharan Africa's bio-prospecting efforts. The findings are expected to inform innovation in functional food development and enhance the therapeutic and socioeconomic value of this underutilised plant species (Knez et al., 2024).

MATERIALS AND METHODS

Plant Collection, Authentication, and Preparation

Whole fruits of *Prosopis africana* were collected from the University of Ilorin campus, Ilorin, Nigeria. The plant material was authenticated at the Forest Herbarium Ibadan (FHI), where a voucher specimen was deposited and assigned the number FHI 111953. The fruits were air-dried at ambient temperature for two weeks and subsequently ground into fine powder using a mechanical grinder. The powdered material was stored in airtight containers under dry conditions until further use, following standard phytochemical preparation protocols (Silpa et al., 2021).

Extraction and Fractionation

Ten kilograms (10 kg) of the powdered fruit were subjected to cold maceration with 70% methanol (25 L) for 72 hours at room temperature with intermittent agitation. The resulting mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to obtain the crude methanol extract, designated as PAF-0. The extraction yield was calculated to be 17% w/w (Muhammed & Mashi, 2025).

For fractionation, 1,000 g of the crude extract was sequentially partitioned with solvents of increasing polarity: *n*-hexane (PAF-1), dichloromethane (PAF-2), ethyl acetate (PAF-3), and water (PAF-4). Each fraction was individually concentrated under a vacuum and stored at4°C for less than one week until further use. The percentage yields for the fractions were as follows: PAF-1 (12.5% w/w), PAF-2 (8.3% w/w), PAF-3 (15.1% w/w), and PAF-4 (48.2% w/w). These extraction and fractionation procedures were adapted from established phytochemical methodologies (Moomin et al., 2023).

DPPH Radical Scavenging Assay

The antioxidant capacity of the crude extract (PAF-0) and fractions (PAF-1 to PAF-4) was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, with modifications based on the method described in one of our earlier studies (Salawu et al., 2020). Briefly, 2 mL of DPPH solution (0.04 mg/mL in methanol) was mixed with varying concentrations of each sample (6.25–200 µg/mL) and incubated in the dark for 30 minutes at room temperature. Absorbance was measured at 517 nm. IC_{50} values were calculated from the dose-response curves using nonlinear regression analysis. Gallic acid was used as the positive control. Percentage inhibition was calculated, and IC_{50} values were determined from dose-response curves.

α-Glucosidase Inhibitory Assay

The α-glucosidase inhibitory activity of the extracts and fractions was evaluated following the method described in one of our earlier studies (Salawu et al., 2024). Test samples (5.2–330 µg/mL) were incubated with 0.15 U/mL α-glucosidase enzyme in phosphate buffer (pH 6.8) at 37°C for 30 minutes. Subsequently, 5 mM p-nitrophenyl-α-D-glucopyranoside was added as the substrate. The reaction was terminated with 100 µL of 200 mM sodium carbonate, and the absorbance of released p-nitrophenol was measured at 400 nm. Acarbose was used as the standard inhibitor.

In Vitro Immunomodulatory and Cellular Antioxidant Assay

The immunomodulatory potential of the extracts was assessed using isolated human immune cells. The use of human blood samples was approved by the Institutional Ethical Committee (IEC) of the International Center for Chemical and Biological Sciences (ICCBS), University of Karachi (Ref. No. ICCBS/IEC-008-BC-2015). Written informed consent was obtained from all healthy volunteers prior to blood collection. Peripheral blood mononuclear cells (PBMCs) and polymorphonuclear neutrophils (PMNCs) were isolated from freshly collected human whole blood via density gradient centrifugation using Histopaque® (Sigma-Aldrich). For the reported ROS inhibition results, PBMCs were used. Cells were treated with various concentrations (6.25–100 µg/mL) of the extracts for 2 hours, followed by stimulation with zymosan-A or phorbol 12-myristate 13-acetate (PMA) in the presence of luminol or lucigenin probes. Reactive oxygen species (ROS) production was monitored via chemiluminescence, recorded every 30 seconds using a luminometer, and expressed as relative light units (RLU). The procedure was adapted from earlier work reported by Liu and co-workers (Liu et al., 2024).

Statistical Analysis

All data are presented as mean ± standard error of the mean (SEM) from three independent replicates. Statistical analyses were conducted using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test for comparisons with the control group, using GraphPad Prism version 6.0 (GraphPad Software, USA). Differences were considered statistically significant at $p < 0.05$.

RESULT AND DISCUSSION

Antioxidant Activity (DPPH Radical Scavenging Assay)

The DPPH radical scavenging activities of *Prosopis africana* extract and fractions are presented in Table 1. All the tested samples demonstrated concentration-dependent radical scavenging activity. The crude extract (PAF-0) showed moderate activity with 57.1% inhibition at 0.5 µg/mL and an IC_{50} of 0.172±0.01 µg/mL. Among the fractions, PAF-1 and PAF-2 exhibited the highest inhibition (86% and 86.3%, respectively) with IC_{50} values of 0.23±0.02 and 0.259±0.01 µg/mL, respectively. The least activity was observed in PAF-4 (67.7%, IC_{50} = 0.395±0.001 µg/mL). Standard antioxidant, gallic acid, exhibited potent activity with inhibition values of 97.8% and IC_{50} values of 22.1±1.85 µg/mL, respectively (Table 1).

Table 1: DPPH Radical Scavenging Activity of *P. Africana* Extract and Fractions

Sample	% Inhibition	IC_{50}±SEM (µg/mL)
PAF-0	57.1	0.172±0.01***
PAF-1	86	0.23±0.02***
PAF-2	86.3	0.259±0.01***
PAF-3	80.3	0.259±0.01***
PAF-4	67.7	0.395±0.001***
Gallic acid*	97.8	22.1 ±1.85

Keys: *= Positive Control, Statistical significance: ***p < 0.001 vs. Gallic Acid (positive control) using one-way ANOVA followed by Dunnett's post hoc test. Abbreviations: PAF: *Prosopis africana* fraction, DCM: Dichloromethane, EtOAc: Ethyl acetate

α-Glucosidase Inhibitory Activity

Results from the α-glucosidase inhibition assay are shown in Table 2. The hexane fraction (PAF-1) demonstrated the most potent inhibition (98.93%) with an IC_{50} of 44.83±1.09 µg, followed by the crude extract (PAF-0) with a percentage inhibition and IC_{50} of 95.6%, IC_{50} = 230.73±2.3 µg, respectively. The ethyl acetate fraction (PAF-3) with percentage inhibition (83.2%) and IC_{50} (348.62±0.83 µg/mL) showed similar activities to the aqueous fraction (PAF-4) with percentage inhibition of 83.2% and an IC_{50} of 240.45±0.73 µg/mL. Notably, PAF-2 showed no inhibitory effect. Acarbose, the reference drug, had 59.02% inhibition and an IC_{50} of 377.75±1.34 µg/mL.

Table 2: α-Glucosidase Inhibitory Activity of *P. Africana* Extract and Fractions

Sample	% inhibition	IC_{50}±SEM (µg)
PAF-0	95.60	230.73±2.3***
PAF-1	98.93	44.83±1.09***
PAF-2	0.00	NT
PAF-3	83.20	348.62±0.83**
PAF-4	83.20	240.45±0.73***
Acarbose*	59.02	377.75±1.34

Keys: * = Positive control, Statistical significance: ***p < 0.001, **p < 0.01, ns = not significant (p > 0.05) vs. Acarbose (positive control) using one-way ANOVA followed by Dunnett's post hoc test. *Abbreviations: PAF: Prosopis africana fraction,* DCM: Dichloromethane, EtOAc: Ethyl acetate, NT: Not Tested

Immunomodulatory Activity (ROS Inhibition Assay)

In the reactive oxygen species (ROS) immunomodulatory assay, the extract and fractions exhibited varying levels of inhibition (Table 3). PAF-2 showed the highest inhibition among the test samples (44.7% at 25 µg/mL), followed by PAF-4 (32.3%) and PAF-1 (21.6%). The crude extract (PAF-0) and PAF-3 recorded 10.5% and 15.5% inhibition, respectively. Ibuprofen used as a positive control, produced 73.2% inhibition at 25 µg/mL with an IC_{50} of 11.2±1.9 µg/mL.

Table 3: ROS Anti-inflammatory/Immunomodulatory Activity

Sample	Conc. (µg/mL)	% inhibition	IC_{50}±SEM (µg/mL)
PAF-0	25	10.5	NT***
PAF-1		21.6	NT***
PAF-2		44.7	NT***
PAF-3		15.5	NT***
PAF-4		32.3	NT***
Ibuprofen*	25	73.2	11.2±1.9

Keys: * = Positive control, ***p < 0.001 vs. Ibuprofen (positive control) using one-way ANOVA followed by Dunnett's post hoc test. *Abbreviations: PAF: Prosopis africana fraction, DCM: Dichloromethane, EtOAc: Ethyl acetate, ROS: Reactive Oxygen Species, PBMCs: Peripheral Blood Mononuclear Cells.* NT: Not Tested

Discussion

The present study highlights the significant antioxidant, α-glucosidase inhibitory, and immunomodulatory activities of *Prosopis africana* fruit extracts and their solvent fractions. These findings align with recent literature and further validate the therapeutic potential of this underutilised plant.

The DPPH radical scavenging assay demonstrated that the crude extract and fractions exhibited concentration-dependent antioxidant activity. Notably, the dichloromethane (PAF-2) and n-hexane (PAF-1) fractions showed high inhibition rates (86.3% and 86%, respectively), with IC_{50} values of 0.259±0.01 µg/mL and 0.23±0.02 µg/mL. These values are significantly lower (i.e., more potent) than those of gallic acid (IC_{50} = 22.1 ± 1.85 µg/mL), which is widely regarded as a benchmark antioxidant in natural product research. This remarkable potency, while unusual, may be attributed to an exceptionally high concentration of powerful, synergistic antioxidants, such as specific flavonoids or condensed tannins, in these non-polar fractions. The significantly lower IC_{50} suggests that even minimal amounts of these fractions are required to achieve a 50% radical scavenging effect, highlighting their potential efficacy. Comparable or superior antioxidant performance has been reported in related *Prosopis* species, such as *P. cineraria*, but few studies have documented such potency in the fruit of *P. africana*, highlighting the novelty of this result. These results are consistent with previous studies indicating that *P. africana* extracts possess significant antioxidant properties. For instance, a study by Orisakwe et al., 2024 reported that *P. africana* extracts reduced oxidative stress markers and enhanced antioxidant enzyme activities in the cerebral cortex and cerebellum of rats exposed to heavy metal mixtures. Additionally, the presence of phenolic compounds, such as gallic acid and ellagic acid, identified in *P. africana* oil, contributes to its antioxidant capacity. Similarly, in an earlier study, the ethyl acetate fraction and myricetin-3-O-rhamnoside isolated from the leaves demonstrated the highest antioxidant activities (Yanda et al., 2022).

Prosopis africana has been previously reported to exhibit significant *in vivo* antidiabetic activity in streptozotocin-induced diabetic Wistar rats (Bamisaye, Ayodele, Ajuwon, Oluwajobi, & Ajiboye, 2023). For α-glucosidase inhibition, the n-hexane fraction (PAF-1) demonstrated the most potent inhibition (98.93%) with an IC_{50} of 44.83 ± 1.09 µg/mL, significantly surpassing the activity of acarbose (IC_{50} = 377.75 ± 1.34 µg/mL). The markedly lower IC_{50} value of PAF-1 suggests the presence of bioactive compounds that might act via potent competitive or non-competitive inhibition mechanisms, potentially through synergistic interactions between its phytoconstituents. While the ethyl acetate (PAF-3) and aqueous (PAF-4) fractions showed similar activities (83.2%), the lack of activity in PAF-2 is notable and may be due to the absence of, or lower concentration of, the specific α-glucosidase inhibitory compounds present in PAF-1, possibly reflecting a distinct phytochemical profile skewed towards other bioactivities like ROS inhibition. This demonstrates that PAF-1 possesses significantly greater inhibitory capacity than acarbose, a widely used antidiabetic agent (Mahindrakar, 2021). These findings are consistent with earlier reports on other members of the *Prosopis* genus. For example, *Prosopis cineraria* stem bark extract has demonstrated significant blood glucose-lowering effects in hyperglycemic mice. The observed bioactivity may be attributed to the presence of phenolic acids, flavonoids, and other phytoconstituents commonly reported in *Prosopis* species. Taken together, the α-glucosidase inhibitory properties of *P. africana* may represent a key mechanism underlying its antihyperglycemic activity, thereby supporting its traditional use in the management of diabetes mellitus.

In the reactive oxygen species (ROS) inhibition assay, the dichloromethane fraction (PAF-2) exhibited the highest inhibitory activity (44.7% at 25 µg/mL), indicating a notable immunomodulatory potential. Although this is lower than the inhibition by ibuprofen (73.2%, IC_{50} = 11.2 ± 1.9 µg/mL), the difference in mechanism is notable; ibuprofen acts primarily via COX inhibition, while plant-based ROS suppression likely involves modulation of NADPH oxidase and upregulation of antioxidant enzymes (González-Ponce, 2018). Furthermore, plant extracts often modulate broader pathways like NF-κB, offering a multi-targeted approach that may be advantageous in chronic inflammatory conditions. Thus, PAF-2 may offer a milder but safer alternative in chronic inflammatory conditions.

This observation aligns with previous reports demonstrating that *P. africana* extracts attenuated oxidative stress and modulated inflammatory responses in heavy metal-exposed rats. While the phytochemical profile of ***P. Africana*** includes bioactive compounds such as caryophyllene and β-sitosterol, recognised for their anti-inflammatory properties, future studies involving GC-MS or HPLC analysis of the active fractions (PAF-1 and PAF-2) are needed to confirm the presence and contribution of these specific compounds to the observed activities. Beyond these activities, *P. africana* has been explored as a promising alternative to conventional antibiotics, with extracts derived from its stem bark, leaves, roots, and essential oils exhibiting a broad spectrum of pharmacological properties. These include antioxidant, antibacterial, antifungal, anti-helminthic, antiviral, hepatoprotective, immunostimulatory, and general antimicrobial activities (John, 2024). Additionally, *P. africana* preparations are rich in essential minerals and amino acids that support enzymatic functions and enhance the body's defence mechanisms against oxidative stress (Sharifi-Rad et al., 2023). Collectively, these findings suggest that *P. africana* whole fruit possesses multifunctional therapeutic potential comparable to, and in some cases exceeding, standard agents. This makes it a viable candidate for development into functional foods or phytopharmaceuticals, particularly in the management of diabetes and immune-related conditions. A limitation of this study is its in vitro nature; future work should include in vivo validation and cytotoxicity assessments to fully establish safety and efficacy.

CONCLUSION

The present study demonstrated that the crude extract and solvent-partitioned fractions of *Prosopis africana* whole fruit possess significant antioxidant, α-glucosidase inhibitory, and ROS-inhibitory activities. Among the fractions, the n-hexane (PAF-1) and dichloromethane (PAF-2) fractions showed particularly remarkable bioactivity, with antioxidant and enzyme-inhibitory potencies surpassing that of standard drugs such as gallic acid and acarbose. This underscores the rich phytochemical content and therapeutic potential of *P. africana*, especially in the context of oxidative stress and metabolic disorders.

Although the ROS inhibition exhibited by the fractions was lower than that of ibuprofen, the observed immunomodulatory effect may still be valuable in chronic or subacute inflammation, where gentler, multi-targeted mechanisms are desirable. The findings justify the traditional use of *P. africana* in managing diabetes and inflammatory conditions and provide scientific support for its further development as a functional food or nutraceutical agent.

This study has limitations, including its *in vitro* design and the lack of toxicity data. Future studies should focus on the isolation and structural elucidation of the bioactive compounds, in-depth exploration of mechanisms of action, and preclinical validation of safety and efficacy in relevant *in vivo* models. The therapeutic versatility of *P.*

africana positions it as a promising candidate in the search for safer, plant-based alternatives for chronic disease management.

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