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Bioactivities of *Prosopis africana* Whole Fruit: Antibacterial and Urease Inhibition Properties

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Abstract

Prosopis africana (Guill. & Perr.) Taub. (Fabaceae) is a West African tree traditionally used for treating infections. However, a systematic evaluation of the bioactivities of its fruit remains limited, and studying the fruit offers a more sustainable and conservation-friendly approach compared to harvesting the bark or roots. This study aimed to investigate the antibacterial and urease inhibitory properties of the whole fruit extract of *P. africana* and its solvent-partitioned fractions. The powdered whole fruit was macerated in 70% methanol to obtain a crude extract (PAF-0). A 1.1 kg portion of PAF-0 was subsequently partitioned using 1 L volumes per cycle into n-hexane (PAF-1), dichloromethane (PAF-2), ethyl acetate (PAF-3), and aqueous (PAF-4) fractions. Antibacterial activity was evaluated against Gram-positive and Gram-negative bacteria using the Microplate Alamar Blue Assay (MABA). Jack bean urease inhibition was assessed spectrophotometrically. All experiments included vehicle controls (DMSO) and were performed with three independent replicates (n=3). The dichloromethane fraction (PAF-2) exhibited the strongest antibacterial activity, showing 84% and 88% inhibition against *Escherichia coli* and *Salmonella typhi*, respectively, which was comparable to the standard drug ofloxacin. In the urease inhibition assay, the n-hexane fraction (PAF-1) and the crude extract (PAF-0) were the most potent, with IC_{50} values of 27.1 µg/mL and 27.6 µg/mL, respectively. The findings indicate that the dichloromethane and n-hexane fractions of *P. africana* fruit possess significant antibacterial and urease inhibitory activities. This provides a scientific basis for its traditional uses against infections and highlights its potential as a rich source of bioactive compounds for further pharmacological development.

Keywords: African mesquite; Antimicrobial resistance; Jack bean urease; Solvent partitioning; Bioactive fractions.

INTRODUCTION

The global health landscape is increasingly challenged by antimicrobial resistance (AMR), which undermines the efficacy of treatments against common bacterial pathogens (Salam et al., 2023). Natural products, with their immense structural diversity, remain a cornerstone in the discovery of new drugs, offering unique mechanisms of action against various diseases (Chopra & Dhingra, 2021; Singh et al., 2025). The systematic investigation of medicinal plants, guided by ethnobotanical knowledge, represents a strategic approach to identifying new bioactive leads (Dean, 2024).

Prosopis africana (Guill. & Perr.) Taub., commonly known as African mesquite, is a leguminous tree native to the savanna regions of West and Central Africa (Malongweni, Mrubata, van Tol, Abd Elbasit, & Harebottle, 2025). Its fruits and seeds are used for food, while various parts of the plant, including the bark and leaves, are employed in traditional medicine to manage ailments such as fever, pain, inflammation, and infections (Alimata et al., 2021; Zhong et al., 2022). Previous phytochemical studies have reported the presence of alkaloids, flavonoids, tannins, and saponins in different parts of *P. africana* (Adamu, Ibrahim, Amin, Mahmoud, & Idris, 2024; Doughari & Saa-Aondo, 2021). These classes of compounds are often associated with a range of pharmacological activities, including

antimicrobial effects (Kebede, Gadisa, & Tufa, 2021; Pikhtirova, Pecka-Kiełb, & Zigo, 2023).

Urease enzyme, produced by pathogens like Helicobacter pylori, is a key virulence factor involved in the pathogenesis of gastric ulcers and urinary stones (Almarmouri et al., 2025). Inhibiting this enzyme is a valid therapeutic strategy, and plant-derived compounds are considered promising alternatives to synthetic inhibitors like acetohydroxamic acid, which has limitations due to its side effects (Al-Rooqi et al., 2023; Almarmouri et al., 2025).

Despite its traditional uses, a comprehensive bioactivity profile of *P. africana* fruit, particularly concerning its antibacterial and urease inhibitory potential, has not been fully established. This study represents a novel, systematic investigation of these two distinct bioactivities in the fruit, a more sustainable plant part compared to the commonly studied bark and roots. Therefore, this study was designed to evaluate these activities in the crude 70% methanol extract of *P. africana* whole fruit and its subsequent solvent-partitioned fractions, thereby identifying the most active fractions for future phytochemical investigation.

MATERIALS AND METHODS

Plant Material and Extraction

Whole fruits of *Prosopis africana* were collected in Ilorin, Kwara State, Nigeria, in November 2019. The plant was identified and authenticated at the Forest Herbarium Ibadan (FHI), where a voucher specimen (FHI 111953) was deposited. The fruits were air-dried, pulverized, and extracted by maceration with 70% aqueous methanol (3 × 25 L) at room temperature for 72 hours. The combined filtrate was concentrated under reduced pressure to yield the crude extract (PAF-0). A 1.1 kg portion of this extract was suspended in dilute methanol and sequentially partitioned with n-hexane (3 × 1 L), dichloromethane (DCM) (3 × 1 L), and ethyl acetate (3 × 1 L) to yield the corresponding fractions: PAF-1 (n-hexane), PAF-2 (DCM), PAF-3 (ethyl acetate), and PAF-4 (aqueous).

Antibacterial Assay

The antibacterial activity was evaluated using the Microplate Alamar Blue Assay (MABA) (Salawu, Wang, Maharjan, & Ajaiyeoba, 2020) against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella typhi* ATCC 14028, *Bacillus subtilis* ATCC 23857, and methicillin-resistant *Staphylococcus aureus* (MRSA) NCTC 13277. Bacterial suspensions were standardized to 0.5 McFarland turbidity. Test samples were dissolved in DMSO and screened at a concentration of 3 mg/mL. Ofloxacin (25 µg/mL) was used as the positive control. A vehicle control containing an equivalent concentration of DMSO was included. After 24 hours of incubation at 37°C, Alamar Blue reagent was added, and the plates were incubated for a further 2 hours. The reduction of the dye was measured spectrophotometrically, and the percentage inhibition was calculated.

Urease Inhibition Assay

Jack bean urease (Sigma-Aldrich) inhibition was assessed according to a previously described method (Lu et al., 2021). Briefly, the enzyme was pre-incubated with various concentrations of the test samples for 15 minutes at 30°C. The reaction was initiated by adding urea solution. After 30 minutes of incubation, the amount of ammonia produced was determined using the indophenol method by measuring the absorbance at 630 nm. Acetohydroxamic acid (AHA) was used as the standard inhibitor. The percentage inhibition and IC_{50} values were calculated using non-linear regression in GraphPad Prism.

Statistical Analysis

All experiments were performed in triplicate (n=3), and the results are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test in GraphPad Prism version 9.0. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Extraction Yields

The extraction of 20 kg of powdered *P. africana* fruit with 70% methanol yielded 1.1 kg (5.55% w/w) of crude extract (PAF-0). The sequential solvent partitioning of 1.1 kg of the crude extract yielded 116 g (10.6%) of the n-hexane fraction (PAF-1), 149 g (13.6%) of the DCM fraction (PAF-2), 404 g (36.7%) of the ethyl acetate fraction (PAF-3), and 304 g (27.6%) of the aqueous fraction (PAF-4).

Antibacterial Activity

The results of the antibacterial screening are presented in Table 1. The DCM fraction (PAF-2) demonstrated the most potent activity, inhibiting the growth of *E. coli* by 84% and *S. typhi* by 88%, an effect comparable to the standard drug ofloxacin. The crude extract (PAF-0) and the ethyl acetate fraction (PAF-3) showed moderate activity against *B. subtilis* and *S. aureus*. The n-hexane (PAF-1) and aqueous (PAF-4) fractions exhibited weak or negligible activity against the tested strains. In Table 1, "-" indicates ≤ 3% inhibition.

Table 1. Antibacterial activity of *P. Africana* whole fruit extract and fractions.

Sample	Percent (%) Inhibition of Extract				
	E. coli	***B. subtilis***	***S. aureus***	***P. aeruginosa***	***S. typhi***
PAF-0	-	25±0.97	18±0.95	4±0.00	-
PAF-1	9±0.30	8±0.73	-	4±0.00	9±0.12
PAF-2	84±1.08	-	-	-	88±1.05
PAF-3	-	18±1.02	5±0.03	-	-
PAF-4	-	-	-	4±0.00	-
Standard Drug*	83±1.21	92±0.56	90±1.04	91±0.96	89±0.92

*Ofloxacin at 25 µg/mL. Note: "–" indicates ≤ 3% inhibition at the screen concentration.

Urease Inhibition Activity

The urease inhibitory potential of the fractions is summarized in Table 2. At a concentration of 0.5 mg/mL, all fractions showed significant inhibition (>79%). The non-polar fraction (PAF-1) and the crude extract (PAF-0) were the most potent, with IC_{50} values of 27.1 µg/mL and 27.6 µg/mL, respectively. The aqueous fraction (PAF-4) also showed high potency (IC_{50} = 25.4 µg/mL). The DCM fraction (PAF-2), while showing high inhibition at the fixed dose, had a moderate IC_{50} of 76.7 µg/mL. The ethyl acetate fraction was the least potent.

Table 2. Urease inhibition by *P. africana* fruit fractions.

Sample	% Inhibition (at 0.5 mg/mL)	IC_{50}±SEM (µg/mL)
PAF-0	79.3	27.6±1.12
PAF-1	95.2	27.1±1.4
PAF-2	96.3	76.7±2.16
PAF-3	90.3	114.1±2.34
PAF-4	81.4	25.4±3.02
Acetohydroxamic acid*	94.4	20.3±0.43

DISCUSSION

This study provides a comprehensive bioactivity profile of *P. africana* whole fruit, revealing that the biological properties are differentially concentrated in specific solvent fractions. The potent antibacterial activity of the DCM fraction (PAF-2) against Gram-negative bacteria is particularly noteworthy, as these pathogens are often more resistant due to their outer membrane (Zhou et al., 2023). The observed antibacterial activity may be attributed to secondary metabolites such as flavonoids (e.g., quercetin derivatives) and alkaloids previously identified in the Prosopis genus, which are known to exert their effects through bacterial membrane disruption and inhibition of vital enzymatic processes (Nava-Solis et al., 2022; Zhong et al., 2022).

The significant urease inhibition exhibited by the n-hexane fraction (PAF-1) and the crude extract (PAF-0) suggests the presence of compounds capable of chelating nickel ions at the enzyme's active site or otherwise interfering with its catalytic function (Akhlaq et al., 2025; Oliyaei et al., 2024). This activity may be attributed to tannins or specific phenolic acids previously reported in *P. africana*. This finding supports the potential use of *P. africana* in managing conditions associated with urease-producing pathogens like H. pylori (Almarmouri et al., 2025).

The differential activity profiles across fractions highlight the importance of solvent partitioning in concentrating specific bioactive constituents. The strong antibacterial effects in the DCM fraction versus the potent urease inhibition in the n-hexane fraction suggest that different classes of compounds may be responsible for these distinct activities.

CONCLUSION

The results of this study scientifically validate the traditional use of *P. africana* fruit for treating infections. The dichloromethane and n-hexane fractions were identified as the most promising sources of antibacterial and urease inhibitory compounds, respectively. The significant bioactivity of these non-polar fractions warrants their selection for future bioassay-guided isolation of the active principles. Further *in-vivo* studies and detailed mechanistic investigations are recommended to fully exploit the therapeutic potential of this plant.

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