



Secondary metabolite profile, antioxidant, and antimicrobial activities of *Spondias mombin* L. (Anacardiaceae) seed methanol extract

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Abstract

Spondias mombin L. (Anacardiaceae) seeds, derived from the Hog plum tree, are rich in polyphenols and exhibit significant antioxidant and antibacterial properties. This study evaluates the chemical profile of the methanol seed extract and its biological activities. Phytochemical screening using colour and precipitation techniques revealed the presence of flavonoids, phenols, alkaloids, tannins, and saponins. Folin-Ciocalteu and aluminium chloride quantitative assays showed high phenolic content (96.54 ± 0.68 mg GAE/g) and moderate flavonoid content (9.02 ± 0.88 mg QE/g), respectively. Antioxidant activity was confirmed through multiple assays, including DPPH ($IC_{50}=20.21$ μ g/mL), FRAP (97.02 ± 0.72 mmol Fe(II)/L), and phosphomolybdate (74.85%). The 200 mg/mL extract exhibited greater antibacterial susceptibility than the 100 mg/mL extract and demonstrated inhibitory effects comparable to those of nitrofurantoin. The Gram-positive strain, *Staphylococcus aureus*, exhibited moderate susceptibility to the extract. But the growth of Gram-negative bacteria (*Proteus vulgaris* and *Pseudomonas aeruginosa*) was suppressed, with MIC and MBC values of 25 mg/mL. These findings highlight the extract's relevance as a plant-derived source of antioxidant activity and antibacterial agent.

Keywords: *Spondias mombin*, secondary metabolites, polyphenols, antibacterial activity, radical scavenging assays

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Introduction

Spondias mombin Linn. (Anacardiaceae), known as yellow mombin or hog plum, is naturalized in southwestern Nigeria (called *Ogheghe* in Esan, *Iyeye* in Yoruba, and *Ngulungwu* in Igbo) and other regions, including the Ivory Coast, India, Bangladesh, Sri Lanka, the Bahamas, Nepal, and Indonesia, with origins in the tropical Americas [1-4]. Ripe fruits are yellow, acidic, and aromatic, with drupes varying from round to ovoid (20-50 mm) [5,6]. Traditional uses include seed infusion for diuresis, bladder issues, and leucorrhea in Brazil. Other parts are used for treating diabetes, inflammation, and infections [7].

Plant extracts contain secondary metabolites like alkaloids, phenols, tannins, flavonoids, saponins, triterpenoids, glycosides, and steroids, which confer on them pharmacological properties including antioxidant, antimalarial, anti-obesity, and anti-aging effects [8-12]. These compounds scavenge free radicals, inhibit oxidation, and reduce reactive oxygen/nitrogen species, mitigating cellular damage and exerting bactericidal activity [13-15].

Previous studies assessed DPPH scavenging by hydro-methanol seed/leaf extracts and antibacterial activity of various *S. mombin* parts in multiple solvents [16-20].

However, quantitative data on DPPH, phosphomolybdate, and FRAP antioxidant capacities, plus antimicrobial effects, remain scarce for methanol seed extracts. But there are extensive reports on the leaves and bark.

Methanol (99%) was selected for its versatility in extracting a wide range of phytochemicals. This study addresses the gap by screening secondary metabolites in *S. mombin* seeds, quantifying the flavonoids and polyphenols, evaluating integrated antioxidant potentials (DPPH, phosphomolybdate, FRAP), and testing antimicrobial activity against clinical isolates, highlighting the untapped pharmaceutical and nutraceutical value of the seeds.

Materials and Methods

Sample Collection, Identification, Processing, and Extraction

Ripe hog plum fruits were plucked from the tree in July 2022 at the Department of Optometry on the Ugbowo Campus of the University of Benin, Benin City, Nigeria, with a latitude of $6^{\circ} 20' N$ and $6^{\circ} 58' N$, and a longitude of $5^{\circ} 35' E$ and $5^{\circ} 41' E$. The plant was identified and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria,



by Prof. H.A. Akinibosun. It was given the voucher number of UBH-S345.

The fruits were crushed with a hand milling machine to separate the seeds from the pulp. Seeds dried with hot air, at 45 °C in an oven, for 4 days. A mechanical grinder was used to pulverise the dry seeds into fine powder. A portion of the seed powder (150 g) was macerated in 1 L of methanol (99 %) for 3 days. The mixture was agitated every 30 min during the first 2 h and thereafter, every 6 h. After filtration (No. 1 filter paper), the mixture was concentrated *in vacuo* at 50 °C, to yield 28.55 g of residue (19.03%). The resulting extract was preserved at 4 °C, awaiting further assay. This extract was utilised for the various assays.

Assessment of phytochemical constituents

The screening of *S. mombin* seed powder for phytochemicals followed the protocol described by [21-22]. Phytochemicals screened for include alkaloids, flavonoids, phenolics, tannins, reducing sugar, terpenoids, and saponins.

Estimation of polyphenols

S. mombin seed total phenolic content was evaluated by mixing previously diluted 0.5 mL Folin-Ciocalteu's reagent (1:10 v/v), deionised water (4.5 mL), and 0.5 mL of extract (1 mg/mL) and kept at ambient temperature for 5 min. More deionised water (2 mL) and 5 mL of 7% sodium carbonate solution were added to the reaction mixture. After 90 min of reaction, the mixture was shaken vigorously and its absorbance was determined at 750 nm. The final result was expressed as gallic equivalents per gram of the extract (mg GAE/g extract). Serial dilutions of gallic acid were prepared across a working range (12.5 to 150 mg/mL), selected to encompass low-to-high response levels for calibration [23].

To determine the flavonoid content, a 0.5 mL methanolic solution of the extract (1 mg/mL) was reacted with 0.1 mL of 10% aluminium chloride and 0.1 mL of 0.1 M potassium acetate, followed by dilution with distilled water (2.8 mL) to complete the assay volume. After standing for 30 minutes at room temperature, absorbance was determined at 415 nm. Flavonoid concentration was calculated relative to quercetin and expressed per gram of extract (mg QE/g extract). Three separate runs were done for the experiment to ensure reliability. A standard quercetin curve was prepared from solutions with a concentration range of 12.5 to 150 mg/mL [24].

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Antiradical Activity of *S. mombin* Seed Extract

The *in vitro* experiment involves adding and vortexing 1.0 mL of 0.2 mM DPPH with 3.0 mL of the extract

(concentration range of 1.0 to 300.0 mg/mL). The mixture was left to stand in the dark for 30 min. Absorbance for each mixture was measured at 517 nm with ascorbic acid used as a standard. The DPPH radical scavenging ability was expressed as a percentage calculated from the equation:

$$\text{Percentage radical scavenging (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where A_0 = absorbance of DPPH radical + methanol

A_1 = absorbance of DPPH radical + sample extract/standard.

The 50 % inhibitory concentration (IC₅₀) value was determined from the DPPH radical scavenging versus concentration curve of *S. mombin* seed extract by extrapolating the effective concentration (x-axis) at the 50 % mark on the DPPH radical scavenging (y-axis) [25].

Phosphomolybdate Total Antioxidant Capacity of *S. mombin* extract

Complete antioxidant capacity of *S. mombin* extract was evaluated by mixing 1 mL aliquot extract (from 0.10 µg/mL to 0.60 µg/mL) with 1 mL of ammonium molybdate (4 mM), sodium phosphate (28 mM) and 0.60 M H₂SO₄, (1:1:1). This mixture was maintained at ambient laboratory temperature and measurement of absorbance was carried out at 765 nm against 1 mL of reagent blank while maintaining ascorbic acid as standard. Total antioxidant capacity (TAC) was expressed as milligram equivalent ascorbic acid per gram (mg EAA/g) [26].

TAC of the extract was determined as follows:

$$\text{Total antioxidant capacity (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Abs control = Absorbance of control;

Abs sample = Absorbance of sample

Ferric Reducing Antioxidant Power (FRAP) Assay for *S. mombin* Seed Extract

To obtain the FRAP reagent, 25 mL of 300 mM acetate buffer (pH 3.6) is mixed with 2.5 mL of 2,4,6-tripyridyltriazine in 40 mM hydrochloric acid and 2.5 mL of 20 mM FeCl₃ solution. 3 mL of FRAP solution was mixed with 1 µL of *S. mombin* extract (1 mg/mL), and absorbance was measured at 593 nm after 10 min. 3 mL of FRAP reagent, dissolved in 100 µL of methanol, was used as the blank and ascorbic acid was used as the reference antioxidant. A standard curve within the linear range of 0.1 to 1.0 mM (100-1000 µM) FeSO₄·7H₂O was plotted, and results were expressed in mmol of Fe (II) per Litre [27].

Antimicrobial Activity of *Spondias mombin* extract

Preparation of agar

The antibacterial susceptibility study was achieved by the agar well diffusion method. The procedure involves dissolving 38 g of Mueller-Hinton agar in 1000 mL of distilled water, then boiling at 100 °C under a foil cover to ensure complete dissolution. This was autoclaved at 121 °C and 15 lbs for 15 min. After cooling to 45 °C, the mixture was dispensed under a sterile lamp into a Petri dish at 30 mL each. The plates were allowed to solidify and then air-dried with hot air at 50 °C in an oven for 10 min, after which they were stored at 5 °C [28].

Collection and Preparation of Microorganisms

Drug-resistant clinical isolates (*Proteus vulgaris*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) were employed for this investigation. They were supplied by the Microbiology Laboratory of the University of Benin Teaching Hospital. Their identities were confirmed by testing with standard antibiotics following overnight culturing on nutrient and Sabouraud agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines [29].

Bacterial Suspension Preparation

Sterile nutrient broth media was initially prepared and poured (5 mL) into four different sterile test tubes. These were utilised in preparing the individual bacterial cultures: *P. vulgaris*, *S. aureus*, *P. aeruginosa* and *E. coli*, from various sub-cultures. This was accomplished by individual sterile loops and incubating at 37 °C for 24 h. The MacFarland solution (0.5 mL) was used to compare the turbidity of the inoculum suspension.

Microbial Assay

Overnight cultures were used to grow bacteria which were carefully harvested using sterile wire loop and inoculated on nutrient agar plates aseptically. These were allowed to react at a regulated temperature of 37 °C over a 24 h period, before using a sterilized cork borer to cut wells of 8 mm diameter on the agar plate. The base of each well was sealed with molten agar to create a uniform and stable condition for proper diffusion and interaction of the extract against the bacterial isolates [30]. The extract (2 µL of 100 mg/mL and 200 mg/mL) were dissolved in dimethylsulphoxide (1 %) then introduced into the well using a calibrated pipette with a teat. Nitrofurantoin (300 µg) was used as the standard in this study. The plates were allowed to stand for 30 min before 24 h incubation upside down at room temperature. A

ruler and a pair of dividers were used to measure the zones of inhibition.

Determination of growth-inhibitory and bactericidal concentration thresholds

The minimum inhibitory concentration (MIC) of *S. mombin* seed methanol extract was evaluated by a two-fold successive broth microdilution procedure for the test organisms. Sterile (750 µL) Hinton-Mueller Broth (HMB) was carefully poured individually into six (6) sterile labelled vials. *S. mombin* extract (200 mg) was dissolved in 1 mL of dimethylsulphoxide and from this stock solution; series of graded concentrations (200 to 12.5 mg/mL) were made. The sample solution (250 µL) was added to the 750 µL HMB vial to make the final concentration of the vial ranges from 50 mg/mL to 3.125 mg/mL [31].

The Minimum bactericidal concentration (MBC) was determined by sub-culturing the diluted samples from the MIC assay test tubes onto MHA plates, followed by incubation at ambient temperature for 24 h [28].

Statistical Analysis

Results are summarised as average responses, with dispersion described by the standard deviation where necessary. The experiments were carried out in triplicate except otherwise stated. Statistical significance among the zones of inhibition of the extract was evaluated using one-way analysis of variance (ANOVA), followed by Turkey's post-hoc multiple comparison test to evaluate the difference among the treatment groups. Statistical analyses were performed using GraphPad Prism (version 8.0, GraphPad Software, San Diego, CA, USA). Differences were considered statistically significant at $p \leq 0.05$, while highly significant differences were defined at $p < 0.001$.

Results and Discussion

Phytochemical constituents

Table 1. Phytochemical analysis of powdered *Spondias mombin* seed

Phytochemical Constituents	Inference
Saponins	+
Alkaloids	+
Tannins	+
Reducing Sugar	+
Phenolic compounds	+
Terpenoids	-
Flavonoids	+

Key: + = Present - = Absent

The results of the qualitative assessment of the secondary metabolites in the powder of *S. mombin* seed are shown in **Table 1**. Reducing sugars, saponins,

alkaloids, tannins, phenolic compounds, and flavonoids were present, while terpenoids were absent.

Phytochemical analysis identifies various secondary metabolites groups and is essential for elucidating the medicinal properties of plants. Esua and co-workers [32] had reported that the seed of *S. mombin* contains tannins, while flavonoids and saponins were lacking. Asomie and co-worker [33] had also reported the presence of tannins, flavonoids, alkaloids, saponins, and phenolics, similar to the findings in this study. The former results by Esua and co-workers could be due to differences in locality and environmental conditions where the seeds were collected. The presence of tannin in the seed could be responsible for confining the astringent and bitter taste of the seed; these were also observed with the fruit and bark [34]. Phytochemicals such as phenolics and flavonoids are likely indicators that *S. mombin* seeds contain compounds that could scavenge for free radicals.

Total phenolic content

Determining the total phenolic content of *S. mombin* seed extract involves a calibration plot (Figure 1) using gallic acid as the reference compound. Gallic acid concentrations ranged from 12.5 to 150 µg/mL, yielding absorbance values between 0.0626 and 0.4016. The correlation between absorbance and concentration yielded a linear equation of $y = 0.0026x + 0.0403$, with a correlation coefficient of $R^2 = 0.9825$ and total phenolic content of 96.54 ± 0.68 mg GAE/g (Table 2).

Table 2. Total phenolic and flavonoid contents of *Spondias mombin* methanol seed extract

Sample	Mean \pm SD
Total phenolic content of seed extract	96.54 ± 0.68 mg GAE/g
Total flavonoid content of seed extract	9.02 ± 0.88 mg QE/g

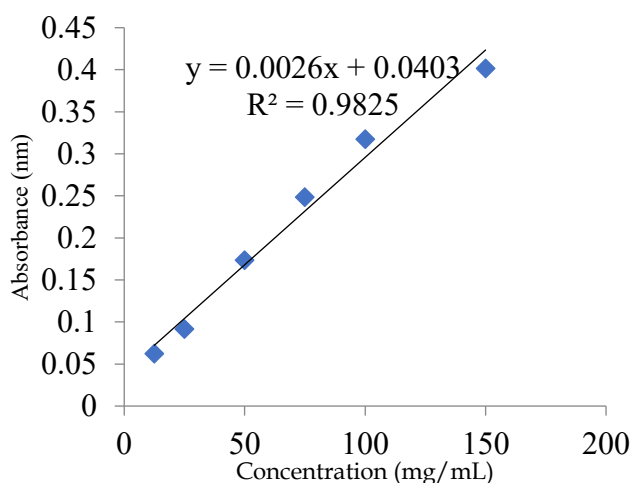


Figure 1: Standard curve of gallic acid for the determination of total phenolics

Total Flavonoid Content

Similarly, total flavonoid content of *S. mombin* seed extract was determined with a calibration plot using a quercetin calibration curve with concentrations ranging from 12.5 to 100 µg/mL, yielding absorbance values between 0.2786 and 1.089 (Figure 2).

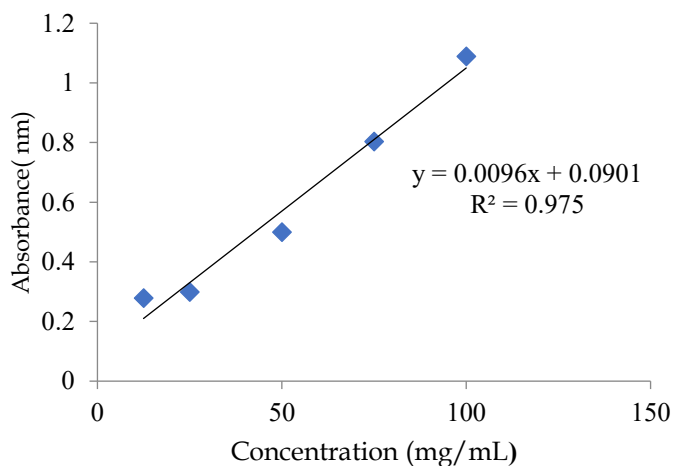


Figure 2. Calibration curve of Quercetin for the determination of total flavonoids

Relationship between absorbance and concentration was expressed in the linear equation of $y = 0.0096x + 0.0901$, while $R^2 = 0.975$ was recorded and total flavonoid content of 9.02 ± 0.88 mg QE/g were recorded (Table 2).

Phenolics and flavonoids share structural similarities, featuring a benzene ring with at least one attached hydroxyl group that confers their antioxidant properties [34]. Quantifying these compounds enhances understanding of the potential antioxidant capacity in *S. mombin* seeds. A comparable study [16] using 70% methanol extraction reported higher phenolic and flavonoid levels, which differed significantly from the present findings, though both observed lower flavonoid than phenolic content. This pattern is attributable to phenolics encompassing a broader class of phytochemicals, including stilbenes, coumarins, flavonoids, phenolic acids, tannins, and lignans [36-37]. Flavonoids represent a substantial subset of phenolics and the largest group of naturally occurring phenolic compounds, existing in free or combined form [38]. They comprise an extensive polyphenol class with a C₆-C₃-C₆ carbon skeleton, linking two benzene rings via a three-carbon chain [39]. Total phenolics and flavonoids assays provide incomplete insights into overall antioxidant capacity due to the non-specificity of detected phenols and flavonoid, their interactions with other compounds, and the influence of additional antioxidants in the reaction medium [40].

Antioxidant activity

DPPH radical sequestering capacity of *S. mombin* seed extract compared well with ascorbic acid (Figure 3), with IC₅₀ of 20.21 µg/mL (extract) and 1.243 µg/mL (ascorbic acid) (Table 3), indicating that the seed extract of *S. mombin* is one-tenth as potent as ascorbic acid in scavenging for free radicals.

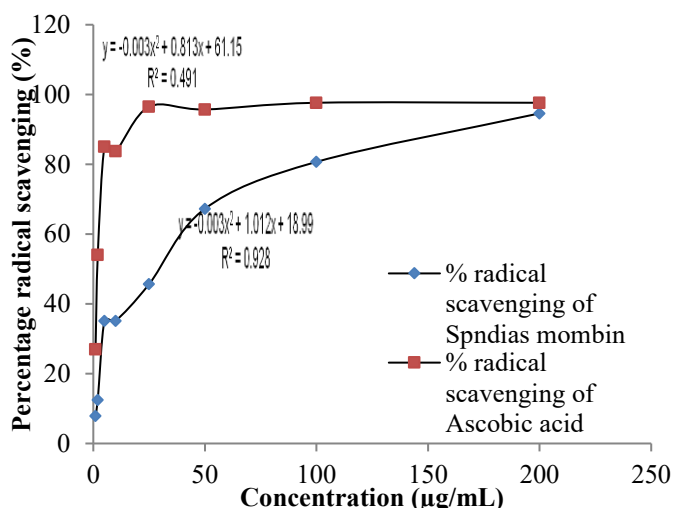


Figure 3. Percentage Radical Scavenging Capacity of Ascorbic Acid and *Spondias mombin*

Table 3. Inhibitory concentration of Ascorbic acid and *S. mombin* seed extract

Samples	IC ₅₀ (µg/mL)
Ascorbic acid	1.243
Seed extract	20.21

The total antioxidant capacity (TAC) of *S. mombin* seed extract showed a gradual reduction in absorbance from 2.702±0.003 to 0.755±0.002 with a gradual rise in the extract’s concentration from 10 to 60 µg/mL (Table 4) and the percentage inhibition varied from 9.99 % to 74.85 %, while 100 µg/mL of ascorbic acid gave 83.84 % inhibition.

Table 4: TAC of *Spondias mombin* methanol seed extract

Treatment	Concentration (µg/mL)	Absorbance at 765 nm	% Inhibition
Control	-	3.002 ± 0.001	-
<i>S. mombin</i>	10	2.702 ± 0.003	9.99
<i>S. mombin</i>	20	1.695 ± 0.005	43.54
<i>S. mombin</i>	30	1.223 ± 0.006	59.26
<i>S. mombin</i>	40	0.937 ± 0.002	68.78
<i>S. mombin</i>	60	0.755 ± 0.002	74.85
Ascorbic acid	100	0.485 ± 0.007	83.84

The FRAP assay for methanol seed extract of *S. mombin* showed value of 97.02 ± 0.72 mmol ferrous sulphate/g (Table 5) from the calibration plot that ranges from 10 to 100 mmol ferrous sulphate/g (Figure 5).

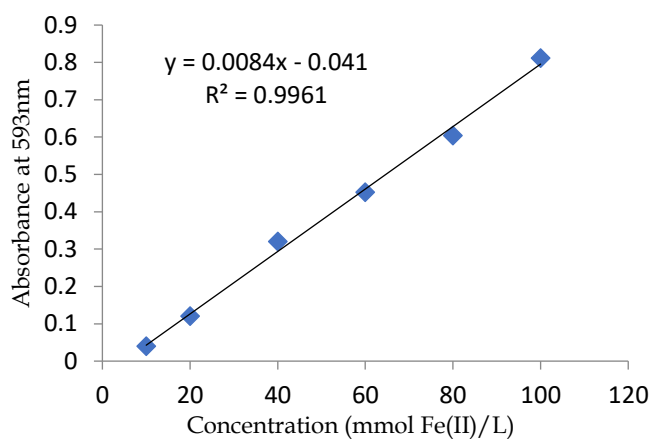


Figure 5. FRAP assay standard curve of absorbance versus concentration

Table 5. FRAP results for methanol seed extract of *Spondias mombin* and ascorbic acid

Sample	Absorbance	Mean concentration ± SD (mmol Fe(II)/L)
<i>S. mombin</i>	0.774	97.02 ± 0.72
Ascorbic acid	0.789	98.81 ± 0.45

Antioxidants prevent cellular damage caused by free radicals, which form as metabolic by-products or from external sources like pollution and radiation. Free radicals are highly reactive molecules with unpaired electrons that serve some bodily functions; however, excess levels can induce oxidative stress and contribute to diseases such as Parkinson’s and Alzheimer’s diseases [41]. In-vitro antioxidant experiment utilises free radical donors such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), which is deeply purple in methanol solution and becomes pale yellow in the presence of an antioxidant. This change in colour is measured spectrophotometrically at 515 nm and can be related to the donation of hydrogen atom or electron to the DPPH [42]. Since the exact content or composition of the extract was unknown during the assay, comparison with standards such as rutin and ascorbic acid was necessary. In this study, ascorbic acid showed better antioxidant potential than the methanol extract of *S. mombin* seed, with a higher IC₅₀ (20.21 µg/mL). In a similar study where the seed extract was macerated with 70 % methanol, a comparable outcome was reported [16]. Measuring the total antioxidant capacity of methanol seed extract of *S. mombin*, using the green phosphomolybdenum complex formation at a wavelength of 695 nm yielded 74.85 %, while ascorbic acid (standard) yielded 83.84 %. Though ascorbic acid exhibited superior activity at 100 µg/mL compared to the

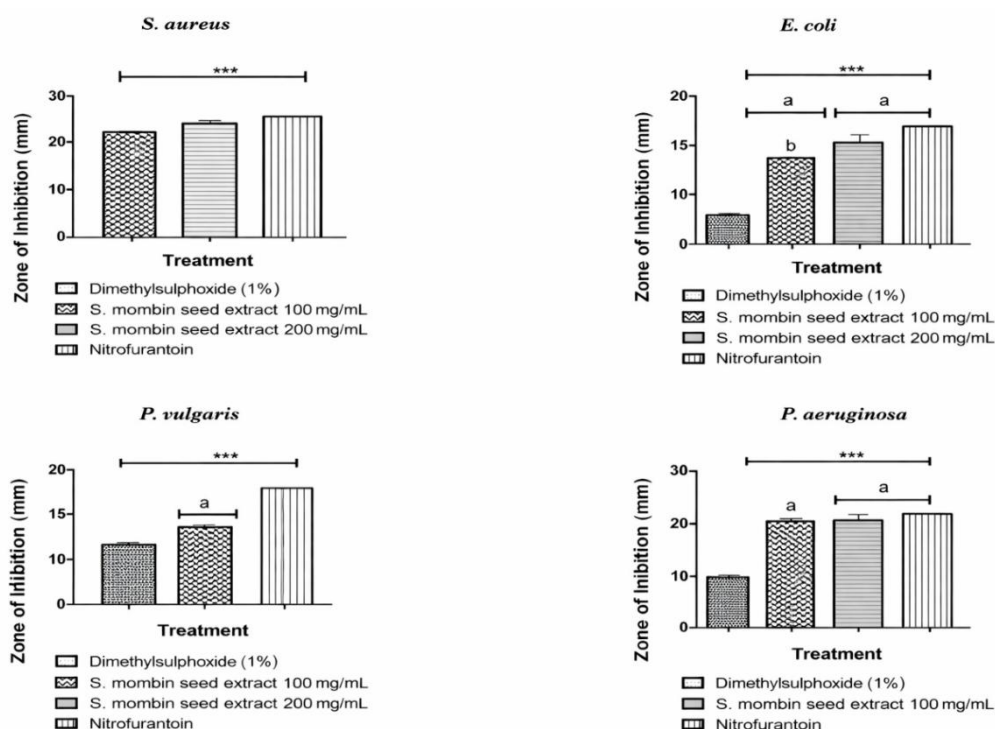


Figure 6. Susceptibility test of *Spondias mombin* seed methanol extract on selected bacterial isolates

seed extract at 60 µg/mL. Speculatively, it is likely that further purification of the crude may yield improved activity.

Ferric reducing antioxidant power (FRAP) was also used to estimate the antioxidant capacity of the methanol extract of *S. mombin* seed, based on the ability of Fe³⁺ to undergo reduction to Fe²⁺ (intense blue colour complex), which can be measured spectrophotometrically at 593 nm. *S. mombin* seed extract exhibited a FRAP value of 97.02 ± 0.72 mmol Fe(II)/L compared to ascorbic acid. This indicates that the extract demonstrates superior potential for reducing ferric ions to ferrous compared to other antioxidant assays.

Antimicrobial Activity Susceptibility Test

Both concentrations (100 and 200 mg/mL) of *S. mombin* seed methanol extract showed varied inhibition of the growth of the isolates selected for this study. At 100 mg/mL, zones of inhibition range from 10 mm (*S. aureus*) to 23 mm for *P. vulgaris* (Figure 6). At 200 mg/mL, a similar trend was observed. The extract and nitrofurantoin produced significantly larger zones of inhibition compared with the negative control (dimeyhylsulphoxide) (***) $P < 0.001$; one-way ANOVA). Turkey’s post-hoc analysis indicated that treatments sharing the same superscript letter (a or b) were not significantly different, whereas those with different letters differed significantly ($p < 0.05$). Overall, the 200 mg/mL extract concentration showed greater greater antibacterial susceptibility than the 100 mg/mL and

demonstrated inhibitory effects comparable to nitrofurantoin.

Minimum Inhibitory Concentration (MIC)

The minimum concentration of extract preventing growth of the bacterial isolate (MIC) was determined across a range of 50 to 3.125 mg/mL. No growth (NG) was observed at 50 mg/mL and 25 mg/mL for both *P. vulgaris* and *P. aeruginosa*, while *S. aureus* recorded no growth at 50 mg/mL to 6.25 mg/mL concentrations, respectively. *E. Coli* was resistant at all concentrations (Table 7).

Table 7: Minimum Inhibitory Concentration (MIC) of *Spondias mombin* seed extract

Bacteria Isolate	Dosage (mg/mL)				
	50	25	12.5	6.25	3.125
<i>Proteus vulgaris</i>	NGR	NGR	GR	GR	GR
<i>Staphylococcus aureus</i>	NGR	NGR	NGR	NGR	GR
<i>Pseudomonas aeruginosa</i>	NGR	NGR	GR	GR	GR
<i>Escherichia coli</i>	GR	GR	GR	GR	GR

NGR = No Growth; GR = Growth

Minimum Bactericidal Concentration (MBC)

The potency of the seed extract of *S. mombin* against selected bacteria was determined by measuring the extract concentration (in mg/mL) causing ≥99 % death

of viable bacteria. It was observed that the extract was able to inhibit growth (No growth) at 50 mg/mL and 25 mg/mL for *P. vulgaris* and *S. aureus*, while *P. aeruginosa* was inhibited at a 50 mg/mL concentration (Table 8).

Table 8: Minimum Bactericidal Concentration (MBC) of the seed extracts of *S. mombin*

Bacteria Isolate	Dosage (mg/mL)				
	50	25	12.5	6.25	3.125
<i>Proteus vulgaris</i>	NGR	NGR	GR	GR	GR
<i>Staphylococcus aureus</i>	NGR	NGR	GR	GR	GR
<i>Pseudomonas aeruginosa</i>	NGR	GR	GR	GR	GR
<i>Escherichia coli</i>	GR	GR	GR	GR	GR

NGR = No Growth; GR = Growth

Susceptibility test indicated greater vulnerability in Gram-negative bacteria than in Gram-positive bacteria. This may be due to the inability of the compounds in the extract to effectively cross the lipopolysaccharide (endotoxin) layer external to the cell wall in the Gram-negative bacteria, which is absent in the Gram-positive bacteria [43-44]. The presence of this membrane has impacted resistance on Gram-negative bacteria [45]. Higher concentration (200 mg/mL) of the extract showed an increase in zones of inhibition. Previous studies on the leaves, root, and bark extracts of *S. mombin* have shown susceptibility to *E. coli*, *S. aureus*, and *P. Aeruginosa* [19, 46-47]. This study further evaluated the effect of the methanol extract of *S. mombin* seed against these bacteria. *Proteus vulgaris* was shown to be susceptible. *P. vulgaris* is an opportunistic pathogen that causes cystitis and pyelonephritis. It is differentiated from other enterobacteriaceae and providencia by its ability to remove the amino group from lysine and phenylalanine; rapid and abundant production of the enzyme (urease) [48]. MIC was subsequently determined at lower concentrations of 50 mg/mL to 3.125 mg/mL against the selected bacteria. Gram-positive bacterium (*S. aureus*) showed MIC at a concentration of 6.25 mg/mL, while Gram-negative bacteria (*P. vulgaris* and *P. aeruginosa*) showed MIC at 25 mg/mL. These values are indicative that the extract from *S. mombin* seeds is more potent against *S. aureus*. Minimum bactericidal concentration (MBC) was further determined while trying to assess the concentration of the extract that will cause the death of 99 % of the bacteria within 24 hr at a temperature of 37 °C.

P. vulgaris and *S. aureus* showed bactericidal concentration of 25 mg/mL, while *P. aeruginosa* showed MBC of 50 mg/mL. These values showed that 99 % of *S. aureus* and *P. vulgaris* could be killed at a concentration of 25 mg/mL, while 50 mg/mL of the extract will be required to kill *P. aeruginosa*. The minimum bactericidal concentration to minimum inhibitory concentration (MBC/MIC) ratios for *S. aureus*, *P. vulgaris* and *P. aeruginosa* were 4, 1, and 2, respectively. These values may be indicative of bactericidal effect, as ratios within the range of 1-4 are typically associated with killing activity [28]. Nonetheless, additional studies (particularly those assessing membrane integrity and cell wall alterations) are required to substantiate this proposed mode of action.

Plants owe much of their antibacterial and antioxidant power to a rich mix of tannins, flavonoids, phenolics, alkaloids, and saponins. Research shows that when flavonoids and alkaloids are abundant, plants become natural defenders against microbes and inflammation, while also fighting oxidative stress [49-50]. Terpenoids stand out for their ability to neutralize free radicals, and steroids play a remarkable variety of roles, from combating cancer to supporting heart health and fighting infections [7]. These compounds act through multiple mechanisms, often working synergistically, and their abundance in plant extracts is a key factor in the observed bioactivities [51].

Conclusions

This study has shown that the seed of *S. mombin* is rich in phytochemicals, especially polyphenols with documented evidence of antioxidant activity. The antioxidant potential of the seed extract was assessed via DPPH, TAC, and FRAP assays, and it confirmed the ability of the extract to exert significant radical inhibition that is comparable to the standard used in the assay. Methanol extract of *S. mombin* seed showed antibacterial activity against *S. aureus*, *P. vulgaris*, *P. aeruginosa*, and *E. coli* at 100 mg/mL up to a final concentration of 200 mg/mL. The antibacterial evaluation was performed, and the killing dose of the extract were further determined for each bacterium, and the mechanism of action was determined to be bactericidal.

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Conflict of Interest

The authors declare there is no conflict of interest.

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Authors' Contributions

Anthony E. Aiwonegbe: Conceptualization, supervision, writing and revision of the original draft; Gift O. Nkulo, Anita E. Ekhasomi and Imuentinyan G. Iyonmana: Data collection, data analysis, and project handling; Emmanuel E. Odion: Data analysis, writing and editing of final draft.

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