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Evaluation of Antibacterial Activities of *Spondias mombin* and *Thaumatococcus daniellii* Leaf Extracts against Multidrug-Resistant Clinical Isolates

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Abstract

The rise of multidrug-resistant (MDR) bacteria has rendered the treatment of infectious diseases less effective, leading to significant economic burdens and highlighting the urgent need for new antimicrobial agents. This study aimed to evaluate the antibacterial activities of Spondias mombin and Thaumatococcus daniellii against the MDR clinical isolates such as Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, and Proteus mirabilis. The plant materials were extracted using the maceration method with acetone, ethanol, and chloroform as solvents. The phytochemical screening, and antibacterial activity of the extracts against the clinical isolates, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) were determined with standard procedure. Phytochemicals (saponins, sugars, tannins, flavonoids, alkaloids, steroids, triterpenes, terpenoids, and cardiac glycosides) were present in both plants' acetone, chloroform and ethanol crude extract. However, anthraquinones were absent in both acetone and ethanol extracts, whereas tannins, flavonoids, and anthraquinones were absent in the chloroform extract. The results showed that ethanol extract from T. daniellii exhibited the greatest antibacterial activity of 32.00 mm against E. coli. The minimum MIC and MBC for S. mombin ethanol extract against P. mirabilis were 3.125 mg/ml and 6.25 mg/ml, respectively. These findings support the potential of plant extracts as alternative antimicrobial agents against multidrug-resistant bacteria.

Keywords: Antibacterial activity, Spondias mombin, Thaumatococcus daniellii, Multidrug-resistant bacteria

INTRODUCTION

Antibiotic resistance has become a global threat and menace making the treatment of infectious diseases ineffective with great economic implications (WHO, 2022). The emergence of multidrug-resistant clinical isolates has been triggered by the abuse (misuse and overuse) of antibiotics in the treatment of man and excessive use in agriculture and activities involving aquaculture (Dadgostar, 2019; WHO, 2022). Despite documented antibacterial properties of *Spondias mombin* and *Thaumatococcus daniellii* (Ogunro *et al.*, 2023; Ukwubile *et al.*, 2017), limited studies have explored their efficacy against MDR clinical isolates, which this study aims to address. The emergence of superbugs has been fueled by antibiotic resistance, making their treatment challenging, contributing to a decrement in productivity and expensive treatments (Dadgostar, 2019; CDC, 2020; WHO, 2023).

Furthermore, different infections and health challenges involving pneumonia, sepsis, typhoid fever, infections from wounds and the urinary tract are caused by multi-drug-resistant organisms involving *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, and *Klebsiella* pneumoniae. In the same vein, the production of extended-spectrum ß-lactamases (ESBLs) and carbapenemases by these infections as a way of promoting antibiotic resistance and making classical antibiotics ineffective have greatly encouraged researchers to search for new agents from *Spondias mombin* and *Thaumatococcus daniellii* and other medicinal plants (Munita and Arias, 2016; Hamid *et al.*, 2017; Osumah *et al.*, 2021; Chaachouay and Zidane, 2024).

According to Ogunro *et al.* (2023), *Spondias mombin* is a medicinal plant that grows in the tropical regions and is popularly called in English yellow mombin or hog plum while the Yorubas called it *Iyeye*, Igbos referred to it as *Ijikara* but the Hausa called it *Tsardar masar*. *S. mombin*, a fruit-bearing tree originated from Africa, Central and South America. Historically, the leaves, fruit, and bark of *S. mombin* have been used traditionally to treat wounds, fever, urinary tract infections, diarrhoea, gastrointestinal disorders, and stomachache (Adebayo-Tayo *et al.*, 2014; Osei *et al.*, 2018; Adebola and Musa, 2021; Ogunro *et al.*, 2023).

In addition, previous research by Osumah *et al.* (2021) revealed that the antibacterial activities of *Spondias mombin n*-hexane and *n*-butanol leaf extracts against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhimurium* increased as the concentration increases except against *Escherichia coli*. Similarly, antibacterial activity was recorded against *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus* using aqueous and ethanol extracts (Adebayo-Tayo *et al.*, 2014). Ukwubile *et al.*, (2017) opined that *Thaumatococcus daniellii*, a rhizomatous plant also known as a sweet prayer plant and belonging to the family Marantaceae grows in the tropical forest and coastal areas in Nigeria, Cote d'Ivoire and Ghana. Osei *et al.* (2018) reported that *T. daniellii* gained wider recognition because of the production of a natural sweetener called thaumatin, which helps modify and enhance flavor in drinks and foods. *T. daniellii* has been very useful traditionally to treat infections, inflammation, and gastrointestinal disorders (Osei *et al.*, 2018). Ukwubile *et al.*, (2017) reported the antibacterial susceptibility of *T. daniellii* leaf extracts against *Bacillus subtilis*, *Streptococcus pyogenes*, *Shigella dysenteriae*, *Campylobacter jejuni*, *Salmonella typhimurium* and *Staphylococcus aureus*. Therefore, this study focuses on the evaluation of antibacterial activities of *Spondias mombin* and *Thaumatococcus danielli* extracts against multidrug-resistant clinical isolates.

MATERIALS AND METHODS

Sample Collection

Plant Materials

The *Spondias mombin* and *Thaumatococcus Daniel* authenticated leaves were collected from a forest in Oke-Opa along Barrack Road, Ondo West Local Government Area in Ondo State of Nigeria on July 2nd, 2023. Vouchers numbers: NIPRD/H/7358 and NIPRD/H/7359 for *Thaumatococcus danielli* and *Spondias mombin* respectively, were issued after the leaves collected were identified and authenticated by the National Institute for Pharmaceutical Research and Development (NIPRD) in Idu-Abuja and the vouchers deposited in the Herbarium Department of NIPRD.

Clinical Isolates

In this study, *Proteus mirabilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli* were used as clinical isolates. They were collected from the Microbiology Laboratory, at National Hospital Abuja and confirmed as multi-drug-resistant isolates using the Kirby-Bauer diffusion method (CLSI, 2020).

Preparation of Plant Materials

The identified and authenticated leaves were washed thoroughly under running tap water to remove any dirty or extraneous materials. They were air-dried at room temperature for 3 weeks, then pulverized and ground into powder using an electronic blender, and then stored in air-tight containers for further analyses.

Extraction of Plant Materials

The leaves *of Thaumatococcus danielli* and *Spondias mombin* were extracted using a modified maceration method (Ingle *et al.*, 2017; Hidayat and Wulandari, 2021). Ninety-two grams (92 g) of powdered leaves of *T. daniellii* were weighed into a conical flask, and 828 ml of acetone was added, kept at room temperature, and shaken every 24 h and for 7 days. The extract was filtered at the end of 7 days with Whatman filter paper under a vacuum and dried at room temperature (25°C) using a watch glass dish. The extract obtained was weighed, and the percentage yield was calculated as shown in Equation 1. The above procedure was repeated using ethanol and chloroform. The extraction of *S. mombin* leaves was done using the same procedure with acetone, ethanol, and chloroform.

$$\text{Percentage Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Powdered Plant Material}} \times 100 \quad \text{Eq. 1}$$

Screening for the Presence of Phytochemicals in Crude Extracts

As reported in the study Oloninefa *et al.*, (2024), the standard procedure was used to screen the phytochemicals in the crude extracts. The phytochemicals screened include Alkaloids, flavonoids, terpenoids, tannins, cardiac glycosides, anthraquinones, steroids, triterpenes, and carbohydrates.

Standardization of Clinical Isolates

A 0.5 McFarland Turbidity Standard was used to standardize the selected clinical isolates (Murray *et al.*, 2007).

Extracts Concentrations and Preparations

A modified method of Oloninefa *et al.* (2024) was used to prepare 40 mg/ml of the extract concentration. A 200 mg of acetone, ethanol, and chloroform crude extracts each was weighed into 5 ml of 20% dimethyl sulfoxide (DMSO) to obtain 40 mg/ml concentrations for acetone, ethanol, and chloroform, respectively. The remaining concentrations (100 mg/ml, 80 mg/ml, and 60 mg/ml) were prepared with a similar procedure.

Determination of Antibacterial Susceptibility of the Crude Extracts against the Clinical Isolates

The agar well diffusion method was used to determine the antibacterial activity of the crude extracts against the clinical isolates (CLSI, 2015). Mueller Hinton Agar (MHA) was prepared as stated by the manufacturer. Standardized cultures of the clinical isolates were streaked on the Petri dishes containing about 20 ml MHA using a sterilized wire inoculating loop. A 6 mm sterile cork borer was used to make wells which were sealed with a molten agar to prevent the leakage of extracts. Each well received 100 µL (0.10 ml) of different concentrations of plant extracts. Dimethyl sulfoxide (DMSO) served as negative control, while Ciprofloxacin, a standard drug, served as the positive control. The plates were incubated at 37ºC for 24 h at the expiration of 30 minutes pre-diffusion time. The zones of inhibition were measured directly with a metre rule while the above method was carried out in triplicates, and the mean of the triplicate result was determined.

Determination of Minimum Inhibitory Concentration (MIC) by Broth Dilution Method

The MIC of the plant extracts was determined using a two-fold broth dilution method as Mu *et al.* (2021) outlined. Different concentrations (100, 50, 25, 12.5, 6.25, and 3.125mg/ml) of the effective plant extracts were prepared by two-fold serial dilution of the stock solution in test tubes. Test organisms were inoculated into test tubes containing the extracts and incubated for 24 h at 37ºC. The result was determined based on the clear and turbidity tubes. The negative control contained the plant extracts without the standardized isolates.

Determination of Minimum Bactericidal Concentration (MBC)

The tubes with no growth were sub-cultured onto fresh plates to determine the MBC (Sampaio *et al.*, 2019). At the end of 24 h of incubation period, the lowest concentration without bacteria growth was regarded as MBC.

Data Analysis

IBM SPSS Statistics Version 23 was utilized to analyze the data obtained in the work. The level of significance was tested at $p<0.05$ (IBM Corp., 2015).

RESULTS

Phytochemical Components in *Spondias mombin* and *Thaumatococcus daniellii* Leave Extracts

The phytochemicals from *Spondias mombin* and *Thaumatococcus daniellii* extracts were revealed in Tables 1 and 2. The results showed that saponins, carbohydrates, tannins, flavonoids, alkaloids, steroids, triterpenes, terpenoids, and cardiac glycosides were present in acetone and ethanol crude extract for both plants, whereas anthraquinones were absent. On the other hand, saponins, carbohydrates, alkaloids, steroids, triterpenes, terpenoids, and cardiac glycosides were present in the chloroform crude extract of both plants but tannins, flavonoids and anthraquinones were absent (Tables 1 and 2).

Table 1: Qualitative Phytochemical Components in *Spondias mombin* Leaf Extracts

Phytochemicals	Acetone	Ethanol	Chloroform
Saponins	+	+	+
Carbohydrates	+	+	+
Tannins	+	+	-
Flavonoids	+	+	-
Alkaloids	+	+	+
Anthraquinones	-	-	-
Steroids	+	+	+
Triterpenes	+	+	+
Terpenoids	+	+	+
Cardiac glycosides	+	+	+

Key: + : Present; - : Absent

Table 2: Qualitative Phytochemical Components in *Thaumatococcus daniellii* Leaf Extracts

Phytochemicals	Acetone	Ethanol	Chloroform
Saponins	+	+	+
Carbohydrates	+	+	+
Tannins	+	+	-
Flavonoids	+	+	-
Alkaloids	+	+	+
Anthraquinones	-	-	-
Steroids	+	+	+
Triterpenes	+	+	+
Terpenoids	+	+	+
Cardiac glycosides	+	+	+

Key: + : Present; - : Absent

Antibacterial Activities of *Spondias mombin* and *Thaumatococcus daniellii* Extracts

The results showed that *Spondias mombin* and *Thaumatococcus daniellii* leaf extracts were active against the selected organisms as shown in Tables 3-6 (Table 3, 4, 5 & 6). The results revealed significant activities against all the bacteria. Acetone extract of *S. mombin* was active against the isolates as follows: *E. coli* (12.00-14.00); *K. pneumoniae* (12.00-22.00), *P. mirabilis* (12.00-18.67) and *S. aureus* (8.00-10.40); the results of chloroform extract of *S. mombin* against the isolates were: *E. coli* (8.00-14.00); *K. pneumoniae* (12.00-20.00), *P. mirabilis* (12.00-26.00) and *S. aureus* (8.00-10.40) meanwhile ethanol extract of *S. mombin* against the organisms were: *E. coli* (10.00-12.00), *K. pneumoniae* (10.00-16.00): *P. mirabilis* (16.07-21.00) and *S. aureus* (18.00-26.00). On the other hand, Ciprofloxacin activity against *E. coli* recorded 38.17; *K. pneumoniae* (50.00), *P. mirabilis* (36.00), and *S. aureus* (40.67) (Tables 3 & 4).

Furthermore, the activity of acetone extract of *T. daniellii* against the isolates was as follows: *E. coli* (14.00-16.40); *K. pneumoniae* (12.00-19.47), *P. mirabilis* (19.93-31.70) and *S. aureus* (8.00-10.73); chloroform extract of *T. daniellii* against the isolates showed the following results: *E. coli* (14.00-16.40); *K. pneumoniae* (8.00-16.47); *P. mirabilis* (8.00-14.80) and *S. aureus* (10.00-13.71) while ethanol extract activity against *E. coli* ranged from 24.00-32.00, *K. pneumoniae* (10.00-13.80): *P. mirabilis* (12.00-18.00) and *S. aureus* (10.10-17.80). The activity of Ciprofloxacin against the isolates were *E. coli* (44.00), *K. pneumoniae* (42.00), *P mirabilis* (36.13), and *S. aureus* (36.07) (Tables 5-6). However, the DMSO was not active against all the isolates in both plants, but there were significant differences in the results obtained in both plants at $p<0.05$ as shown in Tables 3-6 (Table 3, 4, 5 & 6).

Table 3: Antibacterial Activity of Leaf Extracts of *Spondias mombin* (mm)

Extracts/Control		DMSO	Ciprofloxacin	Ethanol	Chloroform	Acetone
Escherichia coli	40 mg/ml	0.00±0.00^{a}	38.17±0.09^{d}	10.00±1.15^{b,c}	8.00±1.15^{b}	12.00±1.15^{c}
	60 mg/ml	0.00±0.00^{a}	38.17±0.09^{c}	10.40±1.15^{b}	10.00±1.15^{b}	13.00±1.15^{b}
	80 mg/ml	0.00±0.00^{a}	38.17±0.09^{c}	10.00±1.15^{b}	12.00±1.15^{b}	13.40±1.15^{b}
	100 mg/ml	0.00±0.00^{a}	38.17±0.09^{d}	12.00±1.13^{b}	14.00±1.15^{c}	14.00±1.15^{c}
Klebsiella pneumoniae	40 mg/ml	0.00±0.00^{a}	50.00±0.00^{c}	10.00±1.15^{b}	12.00±1.15^{b}	12.00±1.15^{b}
	60 mg/ml	0.00±0.00^{a}	50.00±0.00^{d}	12.00±1.15^{b}	12.80±1.15^{b}	16.00±1.15^{c}
	80 mg/ml	0.00±0.00^{a}	50.00±0.00^{d}	14.00±1.15^{b}	14.00±1.15^{b}	18.00±1.15^{c}
	100 mg/ml	0.00±0.00^{a}	50.00±0.00^{d}	16.00±1.15^{b}	20.00±1.15^{c}	22.00±1.15^{c}

Results represent mean ± standard error of mean of triplicate determination. Values with the same superscript in the same column are not significantly different at $p<0.05$

Table 4: Antibacterial Activity of Leaf Extracts of *Spondias mombin* (mm)

Extracts/Control		DMSO	Ciprofloxacin	Ethanol	Chloroform	Acetone
Proteus mirabilis	40 mg/ml	0.00±0.00^{a}	36.00±1.15^{d}	16.07±0.67^{c}	12.00±1.15^{b}	12.00±1.15^{b}
	60 mg/ml	0.00±0.00^{a}	36.00±1.15^{d}	17.60±0.61^{c}	14.00±1.15^{b}	14.00±1.15^{b}
	80 mg/ml	0.00±0.00^{a}	36.00±1.15^{c}	19.00±1.15^{b}	16.00±1.15^{b}	16.00±1.15^{b}
	100 mg/ml	0.00±0.00^{a}	36.00±1.15^{d}	21.00±1.15^{b}	26.00±1.15^{c}	18.67±0.67^{b}
Staphylococcus aureus	40 mg/ml	0.00±0.00^{a}	40.67±1.15^{d}	18.00±1.15^{c}	8.00±1.15^{b}	8.00±1.15^{b}
	60 mg/ml	0.00±0.00^{a}	40.67±1.15^{d}	19.00±1.15^{c}	10.00±1.15^{b}	10.00±1.15^{b}
	80 mg/ml	0.00±0.00^{a}	40.67±1.15^{d}	22.00±1.15^{c}	10.20±1.15^{b}	10.20±1.15^{b}
	100 mg/ml	0.00±0.00^{a}	40.67±1.15^{d}	26.00±1.15^{c}	10.40±1.15^{b}	10.40±1.15^{b}

Results represent mean ± standard error of mean of triplicate determination. Values with the same superscript in the same column are not significantly different at $p<0.05$

Table 5: Antibacterial Activity of Leaf Extracts of *Thaumatococcus daniellii* (mm)

Extracts/Control		DMSO	Ciprofloxacin	Ethanol	Chloroform	Acetone
Escherichia coli	40 mg/ml	0.00±0.00^{a}	44.00±1.15^{d}	24.00±1.15^{c}	14.00±1.15^{b}	14.00±1.15^{b}
	60 mg/ml	0.00±0.00^{a}	44.00±1.15^{d}	25.00±1.15^{c}	16.00±1.15^{b}	16.00±1.15^{b}
	80 mg/ml	0.00±0.00^{a}	44.00±1.15^{d}	28.00±1.15^{c}	16.20±1.15^{b}	16.20±1.15^{b}
	100 mg/ml	0.00±0.00^{a}	44.00±1.15^{d}	32.00±1.15^{c}	16.40±1.15^{b}	16.40±1.15^{b}
Klebsiella pneumoniae	40 mg/ml	0.00±0.00^{a}	42.00±1.05^{d}	10.00±1.15^{b,c}	8.00±1.15^{b}	12.00±1.15^{c}
	60 mg/ml	0.00±0.00^{a}	42.00±1.05^{c}	10.47±1.16^{b}	10.00±1.15^{b}	13.23±1.18^{b}
	80 mg/ml	0.00±0.00^{a}	42.00±1.05^{c}	12.33±1.21^{b}	14.33±1.09^{b}	15.43±1.10^{b}
	100 mg/ml	0.00±0.00^{a}	42.00±1.05^{d}	13.80±1.78^{b}	16.47±1.05^{b,c}	19.47±1.21^{c}

Results represent mean ± standard error of mean of triplicate determination. Values with the same superscript in the same column are not significantly different at $p<0.05$

Table 6: Antibacterial Activity of Leaf Extracts of *Thaumatococcus daniellii* (mm)

Extracts/Control		DMSO	Ciprofloxacin	Ethanol	Chloroform	Acetone
Proteus mirabilis	40 mg/ml	0.00±0.00^{a}	36.13±1.27^{e}	12.00±1.15^{c}	8.00±1.15^{b}	19.93±0.59^{d}
	60 mg/ml	0.00±0.00^{a}	36.13±1.27^{e}	14.00±1.15^{c}	10.00±1.15^{b}	23.13±0.55^{d}
	80 mg/ml	0.00±0.00^{a}	36.13±1.27^{d}	14.00±1.15^{b}	12.87±0.59^{b}	27.07±0.48^{c}
	100 mg/ml	0.00±0.00^{a}	36.13±1.27^{e}	18.00±1.15^{c}	14.80±1.15^{b}	31.70±0.60^{d}
Staphylococcus aureus	40 mg/ml	0.00±0.00^{a}	36.07±1.21^{c}	10.10±1.18^{b}	10.00±1.15^{b}	8.00±1.15^{b}
	60 mg/ml	0.00±0.00^{a}	36.07±1.21^{c}	12.37±1.18^{b}	10.60±1.27^{b}	10.07±1.10^{b}
	80 mg/ml	0.00±0.00^{a}	36.07±1.21^{c}	14.53±1.16^{c}	12.07±1.16^{b,c}	10.60±1.27^{b}
	100 mg/ml	0.00±0.00^{a}	36.07±1.21^{c}	17.80±0.61^{c}	13.17±1.21^{b}	10.73±1.21^{b}

Results represent mean ± standard error of mean of triplicate determination. Values with the same superscript in the same column are not significantly different at $p<0.05$

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of *Spondias mombin* and *Thaumatococcus daniellii* Leaf Extracts

Tables 7-8 revealed the results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Spondias mombin* and *Thaumatococcus daniellii* leaf extracts. The MIC values for *Spondias mombin* acetone extract were: *E. coli* (25 mg/ml), *K. pneumoniae* (12.5 mg/ml), *P. mirabilis* (6.25 mg/ml) *and S. aureus* (25 mg/ml); *S. mombin* chloroform extract had the following MIC values: *E. coli* (50 mg/ml), *K. pneumoniae* (25 mg/ml), *P. mirabilis* (25 mg/ml) *and S. aureus* (100 mg/ml) while the *S. mombin* ethanol extract had the following MIC values: *E. coli* (50 mg/ml), *K. pneumoniae* (25 mg/ml), *P. mirabilis* (3.125 mg/ml) *and S. aureus* (6.25 mg/ml) (Table 7).

The MBC values for *S. mombin* acetone extract were: *E. coli* (50 mg/ml), *K. pneumoniae* (25 mg/ml), *P. mirabilis* (12.5 mg/ml) *and S. aureus* (50 mg/ml); *S. mombin* chloroform extract had the following MBC values: *E. coli* (100 mg/ml), *K. pneumoniae* (50 mg/ml), *P. mirabilis* (50 mg/ml) *and S. aureus* (Above the concentration used) while the *S. mombin* ethanol extract had the following MBC values: *E. coli* (100 mg/ml), *K. pneumoniae* (50 mg/ml), *P. mirabilis* (6.25 mg/ml) *and S. aureus* (12.5 mg/ml) (Table 7).

Furthermore, the MIC values for *T. daniellii* acetone extract were: *E. coli* (25 mg/ml), *K. pneumoniae* (25 mg/ml), *P. mirabilis* (25 mg/ml) *and S. aureus* (100 mg/ml); *T. daniellii* chloroform extract had the following MIC values: *E. coli* (25 mg/ml), *K. pneumoniae* (50 mg/ml), *P. mirabilis* (50 mg/ml) *and S. aureus* (50 mg/ml) while the *T. daniellii* ethanol extract had the following MIC values: *E. coli* (12.5 mg/ml), *K. pneumoniae* (25 mg/ml), *P. mirabilis* (25 mg/ml) *and S. aureus* (25 mg/ml) (Table 8).

The MBC values for *T. daniellii* acetone extract were: *E. coli* (50 mg/ml), *K. pneumoniae* (50 mg/ml), *P. mirabilis* (50 mg/ml) *and S. aureus* (Above the concentration used); *T. daniellii* chloroform extract had the following MBC values: *E. coli* (50 mg/ml), *K. pneumoniae* (100 mg/ml), *P. mirabilis* (100 mg/ml) *and S. aureus* (100 mg/ml) while the *T. daniellii* ethanol extract had the following MBC values: *E. coli* (25 mg/ml), *K. pneumoniae* (50 mg/ml), *P. mirabilis* (50 mg/ml) *and S. aureus* (50 mg/ml) (Table 8).

Table 7: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the Extracts of *Spondias mombin* leaf (mm)

Bacterial Isolates	Acetone		Chloroform		Ethanol	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Escherichia coli	25	50	50	100	50	100
Klebsiella pneumoniae	12.5	25	25	50	25	50
Proteus mirabilis	6.25	12.5	25	50	3.125	6.25
Staphylococcus aureus	25	50	100	*	6.25	12.5

KEY:
MIC- Minimum inhibitory concentration. **MBC**- Minimum bactericidal concentration. ***-** MBC above concentration use

Table 8: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the Extracts of *Thaumatococcus daniellii* leaf (mm)

Bacterial Isolates	Acetone		Chloroform		Ethanol	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Escherichia coli	25	50	25	50	12.5	25
Klebsiella pneumoniae	25	50	50	100	25	50
Proteus mirabilis	25	50	50	100	25	50
Staphylococcus aureus	100	*	50	100	25	50

KEY: MIC- Minimum inhibitory concentration. **MBC**- Minimum bactericidal concentration. ***-** MBC above concentration use

DISCUSSION

The study showed that different solvents used for the extraction pave the way for the emergence of diverse phytochemicals (Steroids, cardiac glycosides, tannins, flavonoids, saponins, alkaloids, triterpenes, and carbohydrates)

obtained from the two plants (*Spondias mombin* and *Thaumatococcus daniellii*). Previous studies by Akintola *et al.*, (2021) and Okoro *et al.* (2022) supported this result as captured in their reports that the polarity of solvents influences different phytochemicals obtained. Akintola *et al.* (2021), Okoro *et al.* (2022), Ahmad *et al.* (2022), and Oladeji *et al.* (2023) went further to state that because of the polarity of ethanol and acetone they have been used to extract different types of bioactive components that have antimicrobial, antioxidant, anti-inflammatory and immunomodulatory potentials. However, the absence of anthraquinones could suggest that these compounds were insufficient in concentration or needed specific solvents for their extraction, as opined by Adeyemi and Lawal (2021), while the absence of flavonoids and tannins in chloroform extracts suggests that they may require specific solvents for effective extraction as reported by Ibrahim *et al.*, (2020) and Akpan *et al.*, (2023).

Furthermore, previous studies by Okeke and Chukwudi (2020), Oluwaseun *et al.* (2021), Ajayi *et al.* (2022) and Eze *et al.* (2023) agreed with the antibacterial activity of acetone extracts from both *S. mombin* and *T. daniellii* against the selected clinical isolates because of the antimicrobial properties of flavonoids, tannins, saponins, and terpenoids. Similarly, the highest antibacterial activity obtained from ethanol extract of *T. daniellii* against *E. coli* agrees with the previous work by Njoku *et al.* (2021) that reported the efficacy of ethanol to extract flavonoids. Generally, the study revealed the efficacy of the extracts obtained from the two plants against Gram-negative and Gram-positive bacteria, possibly going a long way to tackling multi-drug-resistant bacteria instead of conventional antibiotics (Smith and Jones, 2020).

However, despite the high efficacy of the antibacterial activities of Ciprofloxacin against all the isolates yet, *S. mombin* and *T. daniellii* could serve as alternative antibacterial agents against the MDR bacteria (Olaniyi and Mustapha, 2022).

The antibacterial activity results obtained for dimethyl sulfoxide (DMSO) are expected as it contains no antibacterial agents. The study showed that significant differences in antibacterial efficacy at $p<0.05$ suggest that the extract's potency depends on the solvent, plant species, and extraction methods used, as Adebayo *et al.* (2023) reported.

The low MIC results from acetone extract of *S. mombin* against *Proteus mirabilis* and *Klebsiella pneumoniae* in this study agree with the previous results obtained by Oluwole *et al.* (2021), while the corresponding MBC values reveal higher concentrations are required to achieve bactericidal effects, particularly for *E. coli* and *S. aureus*. On the other hand, the chloroform extract of *S. mombin*, which required relatively higher MIC values for *E. coli* and *S. aureus*, agreed with the previous studies by Adebayo and Johnson (2022), suggesting that non-polar solvents like chloroform may be less effective in extracting polar bioactive compounds. Similarly, MBC values for the chloroform extract showed limited bactericidal potency, with the concentration needed to kill *S. aureus* exceeding the study's concentration range. Likewise, the lowest MIC values recorded from ethanol extract of *S. mombin* against *P. mirabilis* and *S. aureus* revealing potent bioactive compounds with high efficacy agreed with previous studies by Njoku *et al.* (2023) and Adebola and Musa (2021) while the MBC results support the effectiveness of ethanol for extraction as revealed by its bactericidal effects at low concentrations against *P. mirabilis* and *S. aureus*.

CONCLUSION

This study showed the significant antibacterial activity of *S. mombin* and *T. daniellii* leaf extracts against multidrug-resistant bacteria isolates due to the presence of bioactive compounds in the extracts (especially the ethanol extracts), suggesting possible use of these plants could serve as valuable sources of new antimicrobial agents and alternative to tackle the rising global threat of MDR bacteria.

Conflict Of Interest

The authors declare no conflict of interest.

Recommendations

The following recommendations were made from the outcomes of this study:

(a) Medicinal plants such as *S. mombin* and *T. daniellii* should be sought as promising alternatives for treating bacterial infections.

(b) Further studies should be carried out on elucidating and purifying the phytochemicals in both *S. mombin* and *T. daniellii*.

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