

Assessment of combined antibacterial activities of *Spondias mombin* and *Thaumatococcus daniellii* leaf extracts and their synergistic potentialsJaphet E. Aisoni^{1*}, Stephen D. Oloninefa², Muhammed Yusha'u¹, Jamilu Isyaku³, Innocent A. Jesse⁴, Victor K. Fadayomi⁵, Victoria Bakare⁶, Abosede A. Adewumi⁷¹Department of Microbiology, Bayero University, Kano, Nigeria²Department of Biological Sciences, Kogi State University, Kabba, Nigeria³Department of Microbiology, Kano University of Science and Technology, Wudil, Nigeria⁴Department of Biological Sciences, Taraba State University, Jalingo, Nigeria⁵Department of Microbiology, Federal University, Lafia, Nigeria⁶Department of Biological Sciences, Nigerian Defence Academy, Kaduna, Nigeria⁷Department of Plant and Environmental Biology, Kwara State University, Malete, Nigeria**ABSTRACT**

The menace of antimicrobial resistance (AMR) has become a public threat and global burden, making conventional antibiotics ineffective in combating diseases and necessitating the search for new phytochemicals with antimicrobial potential. This study investigated the combined antibacterial activities of *Spondias mombin* and *Thaumatococcus daniellii* leaf extracts against clinical bacterial isolates, including *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Escherichia coli*. Phytochemical extraction was performed by maceration using acetone, ethanol, and chloroform. The antibacterial activities at varying concentrations (40-100 mg/ml), minimum inhibitory concentration (MIC), and synergistic effects of the extracts were assessed using the agar well diffusion and broth dilution techniques. Major Phytochemicals identified were saponins, tannins, flavonoids, alkaloids, terpenoids, and cardiac glycosides, while anthraquinones were absent in all the extracts. Combined acetone and ethanolic extracts of *S. mombin* and *T. daniellii* demonstrated the strongest antibacterial activity, with a zone of inhibition of 36.00 mm against *E. coli*, but the lowest MIC of 3.125 mg/ml was recorded against *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *S. aureus* in the synergistic effects of the antibacterial activity of *S. mombin* and *T. daniellii* acetone, chloroform and ethanolic leaf extracts, respectively. Synergistic antibacterial effects of combined extracts showed fractional inhibitory concentration index (FICI) values between 0.16 and 0.38 indicating synergy. The findings revealed a synergy between the extracts of *S. mombin* and *T. daniellii*, suggesting the possibility of developing novel, effective, and affordable drugs in developing countries like Nigeria, while further studies on the isolation, purification, and characterization of the structures of specific phytochemicals responsible for the antibacterial and synergistic effects should be carried out.

Keywords: Combined antibacterial activities, Synergistic effects, *Spondias mombin*, *Thaumatococcus daniellii*, Potentials

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The menace of antimicrobial resistance (AMR) has become a public threat and burden globally, making conventional antibiotics ineffective in combating diseases. The search for new phytochemicals from medicinal plants as alternatives to conventional antibiotics is necessitated as a result of the challenge of AMR.¹ Medicinal plants are plants in which one or more of their organs contain substances useful for therapeutic purposes or serve as precursors for synthesizing useful drugs.² They contain different bioactive compounds, which include phenolics, terpenoids, saponins, alkaloids, flavonoids, cardiac glycosides, and tannins that show antibacterial activities against infectious diseases and also provide eco-friendly, sustainable, and affordable solutions, especially in low-and-middle-income countries where there are limitations to conventional medicines.³

The antibacterial activities of the phytochemicals are facilitated through nucleic acid synthesis inhibition, biofilm formation suppression, disruption of the cell membranes of bacteria, and quorum sensing interference.^{4,5}

Spondias mombin, also referred to as yellow mombin, is of the family Anacardiaceae and a native of tropical America but grown widely across West Africa.⁶ The edible fruits from *S. mombin* are very rich in carbohydrates, minerals, and vitamin C while its leaves are useful in traditional medicine to treat digestive disorders, infectious diseases, and inflammation.⁷ On the other hand, previous studies showed the inhibitory activities of leaf and bark extracts of *S. mombin* against Gram-positive and Gram-negative bacteria.^{8,9} *Thaumatococcus daniellii*, which is well known for a natural protein sweetener (Thaumatococin), is of the family Marantaceae from rain forests of West Africa.¹⁰ The leaves of *T. daniellii* contain flavonoids and phenolics, and other phytochemicals with anti-inflammatory, antioxidant, and antimicrobial activities while the leaves are used for wrapping food and as roofing materials, whereas its rhizomes and fruits are used traditionally.^{11,12}

Furthermore, the combination of crude extracts obtained from medicinal plants that have gained attention from researchers has led to a reduction in the emergence of AMR, enhancement in the efficacy of antibacterial activities, reduction in the required concentrations for treatment, and widening of the antimicrobial spectrum.^{4,12} However, there are limited empirical findings despite the available evidence showing the combined antibacterial activities of both plants and

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possibly synergistic potential. Hence, this study assesses the combined antibacterial activities of *Spondias mombin* and *Thaumatococcus daniellii* leaf extracts with synergistic potentials.

Materials and Method

Collection of Crude Extracts and Clinical Isolates

The crude extracts of *Spondias mombin* and *Thaumatococcus daniellii*, comprising acetone, chloroform, and ethanol leaf extracts, were collected from the Microbiology Laboratory of Bayero University, Kano. On the other hand, clinical isolates (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Escherichia coli*) used for the study were collected from the Microbiology Laboratory, National Hospital, Abuja and kept on nutrient agar slants at 4°C until further use. The reconfirmation of the isolates was achieved by cultural characteristics (colony morphology on selective and differential media), Gram staining, and conventional biochemical tests such as urease, triple sugar iron, catalase, citrate utilization, coagulase, indole reactions, and motility tests.¹³

Determination of Qualitative Phytochemical Constituents of the Crude Extracts

The qualitative phytochemical constituents such as terpenoids, tannins, alkaloids, steroids, flavonoids, cardiac glycosides, anthraquinones, triterpenes and carbohydrates in the acetone, ethanol and chloroform crude extracts of both plants was determined using standard procedures.¹⁴⁻¹⁷

Clinical Isolates Standardization

A 0.5 McFarland Turbidity Standard was prepared to standardize the isolates. Ten-fold dilutions (10^{-1} to 10^{-6}) of overnight-cultured clinical isolates in Nutrient broths was done, and the absorbance of the dilutions was measured at 540 nm with the aid of a UV/Visible spectrophotometer, with comparison with the absorbance of the 0.5 McFarland Turbidity Standard previously obtained.¹⁸

Combined Extract Concentration Preparation

A separate preparation of acetone leaf extract of *Spondias mombin* and *Thaumatococcus daniellii* was done. A 40 mg/ml of the acetone leaf extract concentration of *Spondias mombin* was prepared by dissolving 200 mg of acetone extract into 5 ml of 20% dimethyl sulfoxide (DMSO). In the same vein, 40 mg/ml of the acetone leaf extract concentration of *Thaumatococcus daniellii* was obtained by dissolving 200 mg of acetone leaf extract of the same plant into 5 ml of 20% DMSO. Equal volumes of the two extract concentrations (1:1 or 20:20) were mixed to obtain 40 mg/ml of combined acetone leaf extract. The same procedure was used to obtain the concentrations of 60 mg/ml, 80 mg/ml, and 100 mg/ml, respectively.¹⁷

Determination of Antibacterial Susceptibility of the Combined Extracts

The antibacterial susceptibility of the combined extracts against the clinical isolates was carried out using Agar well diffusion method.^{17,19-21} Mueller Hinton Agar (MHA) was prepared in line with the manufacturer's instruction. The standardized clinical isolates were streaked on the prepared MHA. A 6 mm sterilized cork borer was used to make the wells while a molten agar was used to seal the bottom of the wells to prevent extract leakage. The combined extract (100 µL/0.10 mL) of varying concentrations was introduced into the wells. Dimethyl sulfoxide (DMSO) of 100 µL (0.10 mL) and 5 µg/ml Ciprofloxacin (a standard drug) served as the negative and positive controls, respectively. The incubation of the plates at 37°C for 24 h was done at the end of the pre-diffusion time (30 minutes) while the zones of inhibition were measured.^{17,19-22}

Determination of the Minimum Inhibitory Concentration (MIC) of *Spondias mombin* and *Thaumatococcus daniellii* Leaf Extracts

The MIC of the *S. mombin* and *T. daniellii* leaf extracts was determined following a two-fold broth dilution procedure.²² The

different concentrations ranging between 100 and 3.125 mg/ml were prepared in test tubes from a two-fold serial dilution of the stock solution. This was followed by the inoculation of the isolates, and the incubation was carried out at 37°C for 24 h. Two controls containing the media were set up (that is, a control tube containing the extract minus the isolate and the second tube containing the extract with the isolate). The lowest concentration of the extract that prevented visible growth was taken as the MIC.

Determination of the Minimum Inhibitory Concentration (MIC) of Combined Extracts

The MIC of the combined extracts with antibacterial activities was determined using a two-fold broth dilution procedure.^{21,23} The preparation of varying concentrations between 100 and 3.125 mg/ml was obtained in test tubes from a two-fold serial dilution of the stock solution, followed by the inoculation of the isolates, while incubation was done at 37°C for 24 h. Two controls containing the media were set up (that is, a control tube containing the extract without the isolate and the second tube containing the extract with the isolate). The lowest concentration of the extract that prevented visible growth was taken as the MIC.

Determination of Combined Effects of Antibacterial Activity of the Extracts

The interactions of extract-extract were determined using the fractional inhibitory concentration index (FICI) based on the Loewe additivity zero interaction theory.²⁴ Equation (E) 1 was used to calculate the FIC index values.

$$FICI = \sum FIC = \frac{MIC \text{ of extract A in Combination}}{MIC \text{ of extract A alone}} + \frac{MIC \text{ of extract B in combination}}{MIC \text{ of extract B alone}}$$

MIC of extract A alone/MIC of extract B alone

A FICI of 0.5 shows synergism; 0.5 to 1 shows additive effects; 1 to 4 indifferences and 4 shows antagonism.^{25,26}

Statistical Analysis

The data obtained from antibacterial activities were subjected to analysis of variance using IBM SPSS Statistics Version 23, and significance difference was determined at $P < 0.05$ from the values with different superscripts along the same column.²⁷

Results and Discussion

Qualitative Phytochemical Constituents in *Spondias mombin* and *Thaumatococcus daniellii* Leaf Extracts

The phytochemical constituents present in acetone and ethanol extracts of *Spondias mombin* and *Thaumatococcus daniellii* leaves were: Saponins, carbohydrates, tannins, flavonoids, alkaloids, steroids, triterpenes, terpenoids, and cardiac glycosides, while anthraquinones were absent. Meanwhile, the chloroform extracts of the two plants had saponins, carbohydrates, alkaloids, steroids, triterpenes, terpenoids, and cardiac glycosides, but there was an absence of tannins, flavonoids, and anthraquinones (Tables 1-2). The key phytochemicals (alkaloids, terpenoids, saponins and steroids) present in all the extracts; tannins and flavonoids in both acetone and ethanol extracts, with the absence of anthraquinones in all the extracts, are in agreement with the previous findings that reported that medicinal plants contain phytochemicals with antimicrobial, antioxidant and inflammatory activities while the choice of extracting solvent and its polarity influence the presence or absence of phytochemicals.²⁸⁻³⁰

The presence of saponins and alkaloids in all the extracts shows that these phytochemicals are much in abundance in both *Spondias mombin* and *Thaumatococcus daniellii* and they have anti-inflammatory, antimicrobial, immunomodulatory, analgesic and anti-cancer properties respectively.^{28,31} However, tannins and flavonoids which were absent in chloroform extracts align with previous studies that reported that they are better extracted by polar solvents like ethanol and acetone.^{29,31} The findings from this study show that ethanol efficiently extracted key phytochemicals (Flavonoids, saponins, terpenoids, and tannins) from both plants, making the plants

alternative sources of antimicrobial and antioxidant agents, especially

in traditional medicine and pharmaceutical industries.^{28,30,31}

Table 1: Qualitative Phytochemical Constituents 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 Constituents in *Thaumatococcus daniellii* Leaf Extracts

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

Key: +: Present; -: Absent

Combined Antibacterial Susceptibilities of *Spondias mombin* and *Thaumatococcus daniellii* Leaf Extracts

The antibacterial susceptibilities of the combined extracts at varying concentrations (40-100 mm) revealed the following results: acetone extract (32.00-36.00 mm), chloroform extract (18.00-28.00 mm), ethanol extract (30.00-36.00 mm) and ciprofloxacin (44.00 mm) against *Escherichia coli*; acetone extract (28.00-35.00 mm), chloroform extract (18.00-32.00 mm), ethanol extract (30.00-34.00 mm) and ciprofloxacin (42.00 mm) against *Klebsiella pneumoniae* (Table 3).

Table 4 shows antibacterial susceptibilities of the combined extracts at varying concentrations (40-100 mm). The results were: acetone extract (30.00-34.00 mm), chloroform extract (20.00-34.00 mm), ethanol extract (26.00-34.00 mm) and ciprofloxacin (36.13 mm) against *Proteus mirabilis*; acetone extract (30.00-35.00 mm), chloroform extract (22.00-32.00 mm), ethanol extract (22.00-34.00 mm) and ciprofloxacin (36.07 mm) against *Staphylococcus aureus*. Meanwhile, no antibacterial activity recorded against the isolates by DMSO (Table 4).

The results of antibacterial susceptibilities of the combined extracts against the isolates were concentration dependent while the larger zones of inhibition produced by the acetone and ethanolic extracts suggest more antibacterial constituents were extracted by the two solvents. This result is in agreement with the previous findings that both *S. mombin* and *T. daniellii* contain bioactive components such as flavonoids, tannins, alkaloids, saponins, and terpenoids with antibacterial potential that is broad-spectrum.³³⁻³⁵ On the other hand, the absence of antibacterial activity recorded from DMSO showed that the antibacterial activities were orchestrated by the combined extracts and not the solvents.

Minimum Inhibitory Concentration (MIC) *Spondias mombin* and *Thaumatococcus daniellii* Leaf Extracts

Table 5 shows the MIC values of acetone, chloroform, and ethanol extracts of *S. mombin* against the tested isolates. Acetone extract had for: *E. coli* (25 mg/ml), *K. pneumoniae* (12.5 mg/ml), *P. mirabilis* (6.25 mg/ml) and *S. aureus* (25 mg/ml); chloroform extract recorded: *E. coli* (50 mg/ml), *K. pneumoniae* (25 mg/ml), *P. mirabilis* (25 mg/ml) and *S. aureus* (100 mg/ml) while the ethanol extract the followings: *E. coli* (50 mg/ml), *K. pneumoniae* (25 mg/ml), *P. mirabilis* (3.125 mg/ml) and *S. aureus* (6.25 mg/ml).

Furthermore, MIC values of acetone, chloroform, and ethanol extracts of *T. daniellii* against the tested isolates were shown in Table 6. Acetone extract had for: *E. coli* (25 mg/ml), *K. pneumoniae* (25 mg/ml), *P. mirabilis* (25 mg/ml) and *S. aureus* (100 mg/ml); chloroform extract had these values: *E. coli* (25 mg/ml), *K. pneumoniae* (50 mg/ml), *P. mirabilis* (50 mg/ml) and *S. aureus* (50 mg/ml) while the ethanol extract had: *E. coli* (12.5 mg/ml), *K. pneumoniae* (25 mg/ml), *P. mirabilis* (25 mg/ml) and *S. aureus* (25 mg/ml).

The results obtained from the MIC showed that the two plants possess bioactive compounds (flavonoids, alkaloids, tannins, steroids, triterpenes, terpenoids, and cardiac glycosides) with antibacterial potential, even though there is a variation in potency due to different solvents and isolates, and in agreement with previous MICs obtained between 9.75-50 mg/ml except MIC value obtained for acetone extract against *S. aureus* which possibly may be due to the solvent used.^{31, 32, 34}

Table 3: Combined Antibacterial Activities of Leaf Extracts of *Thaumatococcus daneillii* and *Spondias mombin* (40-100 mg/ml) (mm)

Extracts/Control	<i>Escherichia coli</i>				<i>Klebsiella pneumoniae</i>			
	40 mg/ml	60 mg/ml	80 mg/ml	100 mg/ml	40 mg/ml	60 mg/ml	80 mg/ml	100 mg/ml
Acetone (AB)	32.00±1.15 ^d	32.00±1.15 ^c	34.00±1.15 ^c	36.00±1.15 ^c	28.00±1.15 ^c	30.00±1.15 ^c	32.00±1.15 ^c	35.00±1.15 ^b
Chloroform (AB)	18.00±1.15 ^b	22.00±1.15 ^b	26.00±1.15 ^b	28.00±1.15 ^b	18.00±1.15 ^b	24.00±1.15 ^b	28.00±1.15 ^b	32.00±1.15 ^b
Ethanol (AB)	30.00±1.15 ^c	34.00±1.15 ^d	34.67±1.15 ^c	36.00±1.15 ^c	30.00±1.15 ^d	32.00±1.15 ^d	32.00±1.15 ^c	34.00±1.15 ^b
Ciprofloxacin	44.00±1.15 ^e	44.00±1.15 ^e	44.00±1.15 ^d	44.00±1.15 ^d	42.00±1.05 ^e	42.00±1.05 ^e	42.00±1.05 ^d	42.00±1.05 ^c
DMSO	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

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

Key: A= *Spondias mombin*; B =*Thaumatococcus daneillii***Table 4:** Combined Antibacterial Activities of Leaf Extracts of *Thaumatococcus daneillii* and *Spondias mombin* (40-100 mg/ml) (mm)

Extracts/Control	<i>Proteus mirabilis</i>				<i>Staphylococcus aureus</i>			
	40 mg/ml	60 mg/ml	80 mg/ml	100 mg/ml	40 mg/ml	60 mg/ml	80 mg/ml	100 mg/ml
Acetone (AB)	30.00±1.15 ^d	32.00±1.15 ^c	33.00±1.15 ^b	34.00±1.15 ^b	30.00±1.15 ^c	32.00±1.15 ^c	34.00±1.15 ^c	35.00±1.15 ^{b,c}
Chloroform (AB)	20.00±1.15 ^b	28.00±1.15 ^b	30.00±1.15 ^b	34.00±1.15 ^b	22.00±1.15 ^b	26.00±1.15 ^b	30.00±1.15 ^b	32.00±1.15 ^b
Ethanol (AB)	26.00±1.15 ^c	30.00±1.15 ^{b,c}	32.00±1.15 ^b	34.00±1.15 ^b	22.00±1.15 ^b	28.00±1.15 ^b	30.00±1.15 ^b	34.00±1.15 ^b
Ciprofloxacin	36.13±1.27 ^e	36.13±1.27 ^d	36.13±1.27 ^c	36.13±1.27 ^c	36.07±1.21 ^d	36.07±1.21 ^d	36.07±1.21 ^d	36.07±1.21 ^c
DMSO	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

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

Key: A= *Spondias mombin*; B =*Thaumatococcus daneillii*

Table 5: Minimum Inhibitory Concentration of the Extracts of *S. mombin* Leaf

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

KEY: MIC- Minimum inhibitory concentration

Table 6: Minimum Inhibitory Concentration of the Extracts of *T. daniellii* Leaf

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

KEY: MIC- Minimum inhibitory concentration

Synergistic Effects of the Antibacterial Activity of *Spondias mombin* and *Thaumatococcus daniellii* Leaf Extracts

The synergistic effects of the antibacterial activity of the leaf extracts of *S. mombin* and *T. daniellii*. Fractional inhibition concentration index (FICI) values obtained for acetone extracts ranged from 0.16 to 0.38, where three synergistic effects and one additive effect were

recorded, respectively. The FICI values recorded for chloroform extracts ranged from 0.19 to 0.38, and the four values obtained had synergistic effects; meanwhile, FICI values obtained for ethanol extracts were between 0.31 and 2.25, in which two values had synergistic effects, one with indifference effect and one with additive effect (Table 7).

Table 7: Synergistic Effects of the Antibacterial Activity of *Spondias mombin* and *Thaumatococcus daniellii* Leaf Extracts

Bacterial Isolates	Acetone			Chloroform			Ethanol		
	MIC	FICI	Effect	MIC	FICI	Effect	MIC	FICI	Effect
<i>Escherichia coli</i>	3.125	0.25	S	6.25	0.38	S	3.125	0.31	S
<i>Klebsiella pneumonia</i>	3.125	0.38	S	3.125	0.19	S	3.125	0.25	S
<i>Proteus mirabilis</i>	6.25	1.25	I	3.125	0.19	S	6.25	2.25	I
<i>Staphylococcus aureus</i>	3.125	0.16	S	3.125	0.19	S	3.125	0.63	A

KEY:

MIC: Minimum Inhibition Concentration

FICI: Fractional Inhibition Concentration Index

S: Synergy

A: Additive

I: Indifference

The FICI is based on the Loewe additivity zero interaction theory. A FICI of ≤ 0.5 indicates synergism (S); > 0.5 to 1 indicates additive effects (A); > 1 to ≤ 4 indicates indifference (I) and > 4 is considered to be antagonism (G).

The synergistic potentials demonstrated in the combined antibacterial activities of *S. mombin* and *T. daniellii* extracts, particularly against *Escherichia coli* and *Staphylococcus aureus*, may possibly serve as alternatives to tackle bacteria responsible for antibacterial resistance that are on the increase daily. As a result of this, bacterial susceptibility will be greatly enhanced through the combinations of different phytochemicals with different mechanisms of actions thereby de-escalating the possibility of the development of antibacterial resistance.^{37,38} The strong synergistic effect shown by acetone extracts against *E. coli*, *S. aureus* and *Klebsiella pneumoniae* possibly suggest that the solvent was better in extracting phytochemicals such as flavonoids, tannins and terpenoids, which have the capacity to disrupt the membranes of the bacteria, inhibition of important enzymes and interference with the synthesis of nucleic acid.^{32,38} In the same vein, chloroform extracts also demonstrated synergistic capability against *S. aureus* and *K. pneumoniae*, thus contributing significantly to the inhibition of bacteria, while the extracts' synergistic activity against *E. coli* helps to break the outer membranes, which often restrict the penetration of antimicrobial agents.³⁹

Furthermore, the ethanol extracts showed extraction efficiency in extracting wide range of polar phytochemicals, which greatly contribute to their antibacterial activities coupled with the synergistic activity against *K. pneumoniae* and *E. coli* while the additive effect on *S. aureus* possibly suggest the partial overlapping of the extracted phytochemicals mechanism of action.²⁹ Meanwhile, the result of indifferent activity recorded from the combined extracts against *Proteus mirabilis* may be due to the formation of biofilm and efflux pumps, which limit susceptibility to antimicrobial agents.³⁸

Conclusion

The outcomes of the study showed that the combined *S. mombin* and *T. daniellii* leaf extracts had synergistic potential, suggesting the possibility of developing novel, effective, and affordable drugs in developing countries like Nigeria, while further studies on the isolation, purification, and characterization of the structures of specific phytochemicals responsible for the antibacterial and synergistic effects should be carried out.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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