Volume 6, Issue 4, 2025

Trends in
Natural Products Research

Solvent Fractions of *Terminalia Superba* Engl. and Diels (Combretaceae) Leaves Extract Exhibit Broad-Spectrum Antibacterial Activity

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Abstract

Amid the escalating global crisis of antibiotic resistance, *Terminalia superba*, an African traditional medicinal plant, was investigated for antibacterial activity. The methanol extract of the leaves was fractionated in ethylacetate, *n-butanol*, and *n-hexane* to afford the respective fractions. Using Fourier-transform infrared (FTIR), UV-Vis, and Gas Chromatography-Mass Spectrometry (GC-MS), the bioactive compounds were characterized. Antibacterial activity was evaluated via cup-plate agar diffusion and Minimum Inhibitory Concentration (MIC) tests against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Serratiam arcescens, Proteus mirabilis,* and *Candida albicans*. The ethylacetate fraction (EF) demonstrated broad-spectrum activity against five pathogens (MICs: 5.33–10.67 mg/ml), while the *n-butanol* fraction (BF) inhibited mostly *S. aureus and P. mirabilis* (MICs: 5.33–10.67 mg/ml). The *n-hexane* fraction (HF) showed no activity. P-Cymene, Dodecane, and Undecane derivatives, were characterized as the antimicrobial constituents in the leaf extract.

Keyword*s*: *Terminalia superba*, antibacterial activity, antibiotic resistance, Inhibition Zone Diameters (IZD).

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08135171291
https://doi.org/10.61594/tnpr.v6i4.2025.148
Page No.: 346-354
Volume: Volume 6 Issue 4, 2025
Trends in Natural Products Research

Received 4/10/25, Revised 25/10/25, Accepted 27/10/25, Published Online 22/12/25

Introduction

The escalating crisis of antibiotic resistance, responsible for over 1.2 million deaths annually (Aslam *et al*., 2019), has intensified the search for novel antimicrobial agents. This global health challenge is particularly acute in developing nations, where an estimated 80% of the population, including 81.6% in Nigeria, rely on traditional remedies (World Health Organization, 2019). This reliance has brought attention to plants such as *T. superba*, which is widely used in African traditional medicine for treating infections and wounds. The widespread use of synthetic antibiotics has fueled resistance and their limited availability in resource-poor regions necessitates the exploration of accessible and affordable treatments (Ventola, 2019; Anand *et al*., 2020). *T. superba* is known to be rich in bioactive compounds, including flavonoids, terpenoids, and alkaloids, which have been reported to possess antimicrobial, anti-inflammatory, and antioxidant properties (Ani *et al*., 2023; Kougnimon *et al*., 2018). Existing literature has documented the significant antimicrobial activity of other *Terminalia* species, such as *T. catappa* and *T. arjuna* (Chanda *et al*., 2019; Dwivedi and Chopra, 2020). Similar antimicrobial effects have been observed in other African medicinal plants, including *Zanthoxylum zanthoxyloides*, where phenolics and ellagitannins were responsible for their antibacterial properties (Eloff, 2019; Ngemenya *et al*., 2019). A persistent challenge in this field is the standardization of plant-based medicines and their transition to clinical applications, primarily due to their natural variability (Egbuna *et al*., 2020; Tlili and Sarikurkcu, 2020). This study investigated the antimicrobial potential of leaf extracts from *T. superba* against resistant bacteria.

Materials and Methods

Materials

Solvents used included methanol, *n-hexane*, ethyl acetate (from Sigma-Aldrich, CDH), and Dimethyl Sulfoxide (DMSO). All reagents were of analytical grade. Equipment used included a rotary evaporator, FTIR spectrometer (Thermo Scientific Nicolet iS10), UV-Vis spectrophotometer (Thermo Scientific Genesys 10S), and GC-MS (Agilent 6890/5975C).

Methods

Plant Preparation and Extraction

Fresh *T. superba* leaves were collected in Nsukka, Enugu State, Nigeria, and verified on May 14, 2024, by Alfred Ozioko at the International Centre for Ethnomedicine and Drug Development (voucher: InterCEDD/203). The leaves were air-dried for one week, ground, and then subjected to maceration in methanol at a 1:5 ratio for 72 hours with continuous shaking. Following filtration, the methanol extract was concentrated using a rotary evaporator. This concentrated extract was then partitioned in n-hexane (HF), ethyl acetate (EAF), and n-butanol (BF).

FTIR Analysis

Fourier-transform infrared (FTIR) spectra of the fractions were recorded in the mid-infrared region (650–4000 cm^{-1} with 32 scans at a resolution of 16 cm^{-1}. Functional groups were identified using Omnic software.

UV-Vis Spectroscopy

The fractions were diluted to a 1:10 ratio, centrifuged, and scanned from 200–1100 nm to determine their absorption peaks.

GC-MS Analysis

GC-MS analysis was done as described by Ikeh *et al.,* (2025). An Agilent GC-7890A/MS-5975C gas chromatograph with an HP-5MS column was utilized. Helium was used as the carrier gas at a flow rate of 1 mL/min. Compounds were identified by their retention times and by matching their mass spectra to the instrument's library.

Antimicrobial Tests

The cup-plate agar diffusion method was used to evaluate the antimicrobial activity of the fractions (Okoli et al., 2008). Concentrations of 80–10 mg/mL were used for the EAF and BF, while the HF was tested at 100–12.5 mg/mL against selected bacteria and *Candida albicans*. Minimum inhibitory concentrations (MICs) were determined by agar dilution (Nwodo *et al*., 2019), with concentrations ranging from 10.67–0.67 mg/mL. Bacterial cultures were incubated at 37°C for 24 hours, and fungal cultures at 25°C for 48 hours.

Statistical Analysis

The statistical analysis of phytoconstituents characterization was done using PubChem, an approach involving the identifying of compounds based on their mass spectra, relevant quantitative and categorical data, such as retention times, peak intensities, molecular weights, chemical functional group, molecular formulae and chemical structure.

Results

Results of FTIR Analysis of the fractions

An infrared spectroscopy analysis of three fractions revealed distinct functional groups. The EF (Figure 1)

displayed characteristic peaks for alcohols or phenols O-H at 3209.2 cm^{-1}, alkanes C-H at 2922.2 and 2851.4 cm^{-1}, and carboxylic acids C=O at 1703.4 cm^{-1}. The BF (Figure 2) showed similar alcohol/phenol O-H peaks at 3339.7 cm^{-1} and alkane C-H peaks at 2922.2, 2959.5, and 2873.8 cm^{-1}, but its carbonyl peak C=O at 1703 cm^{-1} suggests the presence of an aldehyde. Lastly, the HF (Figure 3) contained alcohol/phenol O-H groups at 3336.0 cm^{-1}, alkane C-H groups at 2922.2 and 2855.1 cm^{-1}, and a carbonyl group C=O at 1707.1 cm^{-1}, which is indicative of a carboxylate (Ikeh *et al*., 2025).

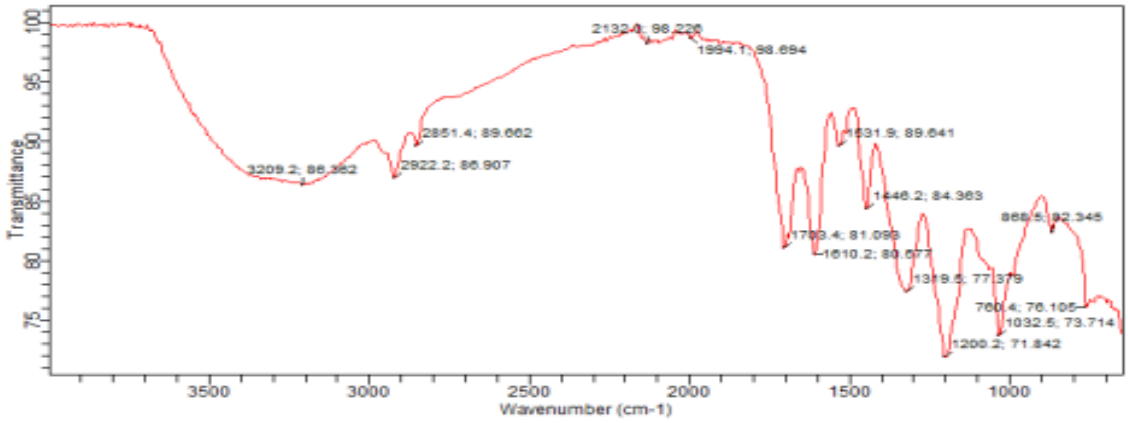

Figure 1: FTIR Spectrum of EAF showing distinct peaks.

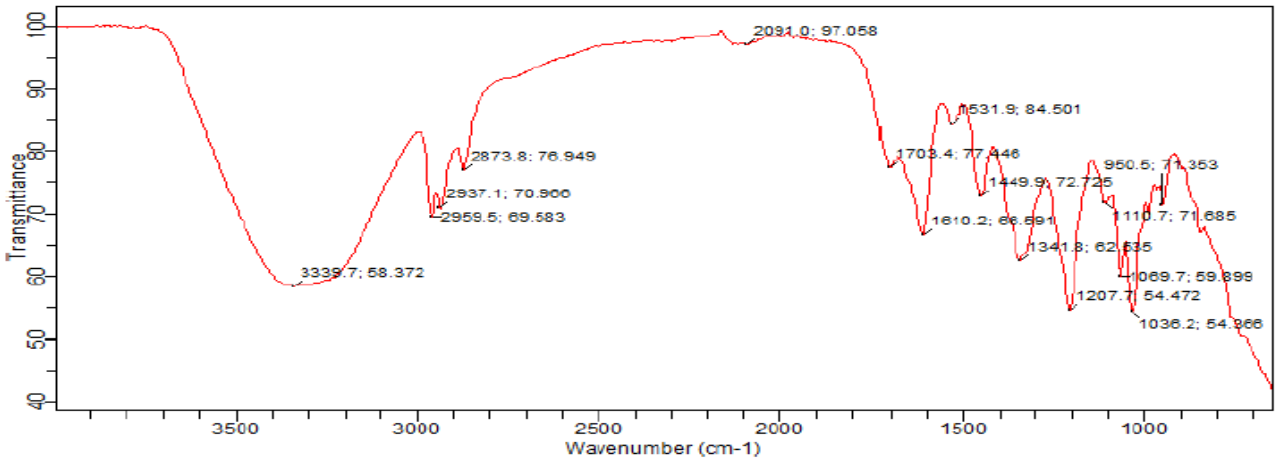

Figure 2: FTIR Spectrum of BF showing distinct peaks.

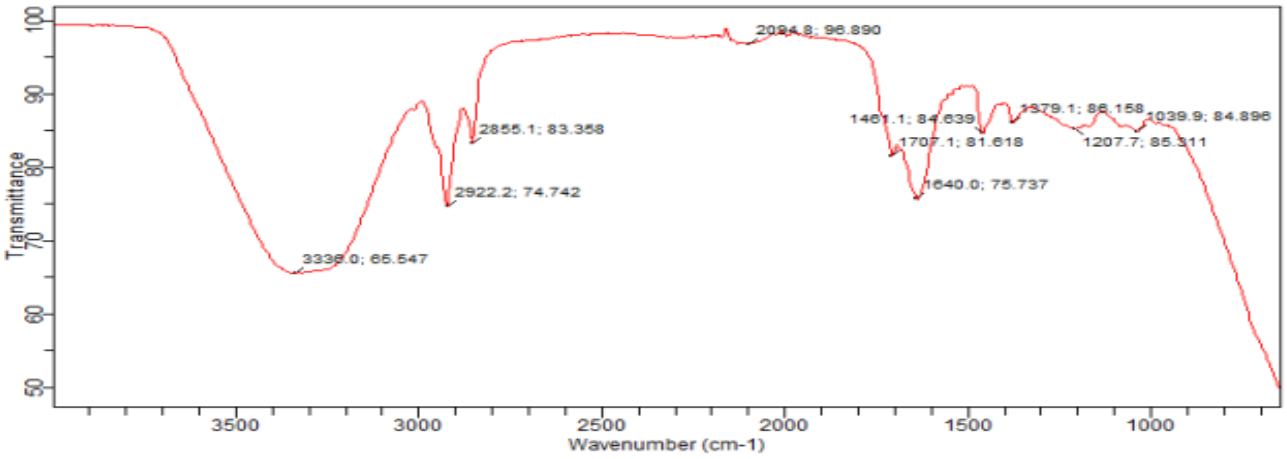

Figure 3: FTIR Spectrum of HF showing distinct peaks.

UV-Vis Analysis

UV-Vis analysis of the fractions revealed distinct characteristics. While the EF (Figure 4) showed very busy peaks, the BF (Figure 5) showed two peaks, one at 288 nm and another at 320 nm, which is indicative of unsaturated groups within the compounds. The HF (Figure 6) presented a strong peak at approximately 273 nm, suggesting the presence of conjugated systems.

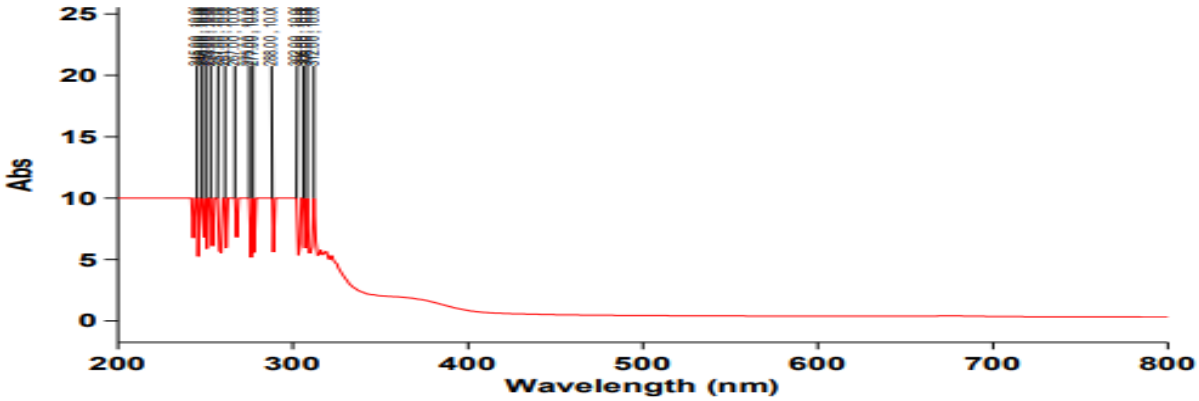

Figure 4: UV-Visible spectrum of the EAF showing multiple peaks.

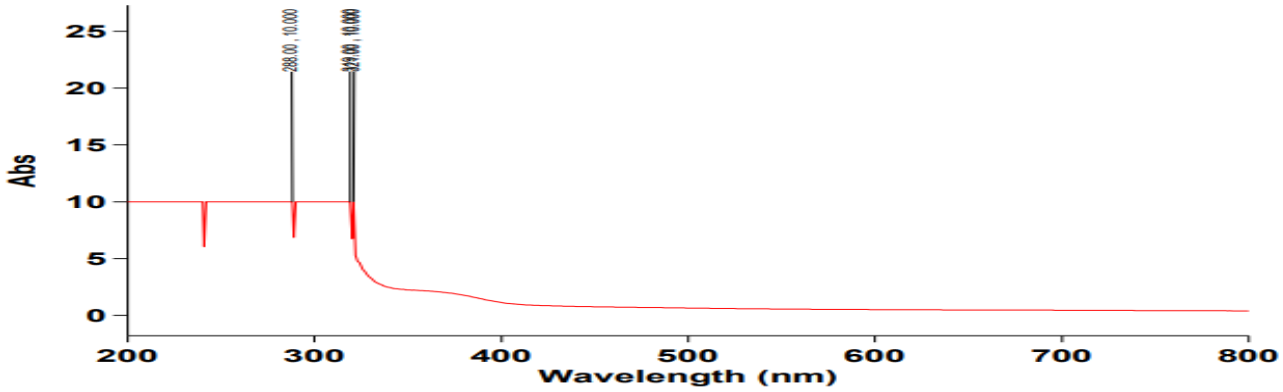

Figure 5: UV-Visible spectrum of the BF showing some peaks.

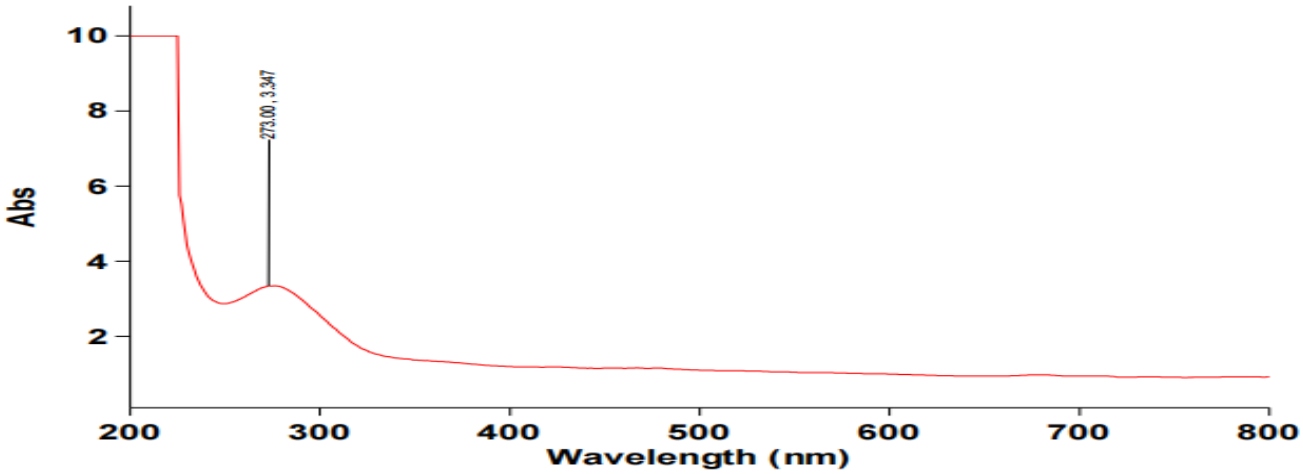

Figure 6: UV-Visible spectrum of the HF showing a clear single peak.

Phytoconstituents of the fractions as detected by the GC-MS Analysis

The major compounds identified in the EF fractions were P-Cymene, Dodecane, 2,6,11-trimethyl-, Undecane, 3,7-dimethyl-, and 1-Octadecene (Table 1), while BF and HF fractions contained O-Cymene, Nonane, Dodecane, 2,6,10-trimethyl, 1-Docosene, Decanoic acid 3-methyl-, Hexadecanoic acid methyl ester, and cis-Vaccenic acid (Tables 2 and 3).

Table 1: Major compounds in EAF with known pharmacological activities.

Peak Number	Retention Time	Area %	Compound	Pharmacological Action
6	7.201	1.38	P-Cymene	Antibacterial, antioxidant, anti-inflammatory
10	8.382	3.51	Dodecane, 2,6,11-trimethyl-	Antibacterial activity
13	8.700	1.55	Undecane, 3,7-dimethyl-	Antibacterial, antioxidant
35	27.258	3.45	1-Octadecene	Antibacterial, antioxidant, anticancer

Table 2: Major compounds in BF with known pharmacological activities.

Peak Number	Retention Time	Area %	Compound	Pharmacological Action
5	7.199	0.38	O-Cymene	Antioxidant, anti-inflammatory
10	8.534	3.23	Nonane	Disrupt bacterial cell membrane
9	8.382	3.45	Dodecane, 2,6,10-trimethyl	Antibacterial properties
41	32.970	0.78	1-Docosene	Antibacterial

Table 3: Major compounds in HF with known pharmacological activities.

Peak Number	Retention Time	Area %	Compound	Pharmacological Action
2	12.460	5.32	Decanoic acid, 3-methyl-	Antibacterial, antifungal
8	16.982	2.78	Hexadecanoic acid, methyl ester	Antimicrobial, anti-inflammatory
14	19.425	12.64	cis-Vaccenic acid	Anti-inflammatory, Antibacterial

Antimicrobial activities of the fractions

The EAF exhibited broad-spectrum activity against five bacterial species—*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa,* and *Proteus mirabilis*—with minimum inhibitory concentrations (MICs) ranging from 5.33 to 10.67 mg/ml. The BF demonstrated potent inhibitory effects on S. aureus and P. mirabilis. Conversely, the HF showed minimal antimicrobial activity, highlighting the critical role of solvent polarity in the extraction of active compounds (Table 4).

Table 4: Antibacterial activities of the fractions

Test Organism	EAF	BF	HF	EAF	BF	HF
	IZD (mm)			MIC (mg/ml)		
	80/40 mg/ml	80/40 mg/ml	100/50 mg/ml			
S. aureus	18/9	12/8	0/0	10.67	10.67	NA
B. subtilis	20/8	0/0	0/0	5.33	NA	NA
E. coli	10/8	0/0	0/0	10.67	NA	NA
P. aeruginosa	12/8	0/0	0/0	5.33	NA	NA
S. marcescens	0/0	0/0	0/0	NA	NA	NA
P. mirabilis	15/8	12/8	0/0	5.33	5.33	NA

Discussion

This study indicates that *T. superba* is a potent source of antimicrobial compounds, presenting a viable solution to the global antibiotic resistance crisis, which accounts for over 1.2 million deaths annually (Aslam *et al*., 2019). The need for new treatment of bacterial infection is highlighted by the rise of resistant pathogens like *P. aeruginosa* and *Serratiam arcescens* (Breijyeh *et al*., 2020). Compounds such as p-Cymene and Dodecane, which can disrupt bacterial membranes or inhibit quorum sensing, offer alternatives to the insights of traditional antibiotics (Barbieri *et al*., 2019; Porras *et al*., 2021). The efficacy of EAF and BF suggests that the active compounds are likely polar in nature, such as derivatives of p-Cymene and Dodecane, which are known to disrupt bacterial membranes (Balahbib *et al*., 2021; Ani *et al*., 2023). These findings corroborate traditional knowledge regarding medicinal plants used in Africa and contribute to the ongoing discussion about the potential of plant-based remedies as alternatives to synthetic antibiotics (Porras *et al.*, 2021).

This result holds significant implications for public health, particularly in regions like sub-Saharan Africa where access to conventional antibiotics is limited (World Health Organization, 2019). The active polar fractions of *T. superba* could be developed into affordable treatments for prevalent infections, including those caused by *P. aeruginosa*, a common nosocomial pathogen (Porras *et al*., 2021). Furthermore, the safety profile of the plant (Kouadio *et al*., 2021) suggests a lower risk of adverse effects compared to some synthetic antibiotics.

The inherent variability of plant-based medicines, that makes them unsuitable for mainstream medical applications (Egbuna *et al*., 2020). This study demonstrated that targeted solvent selection can produce consistent and potent compounds such as Dodecane and Undecane derivatives, which are often overlooked in favour of more widely studied flavonoids and terpenoids (Cunha *et al*., 2020; Hagaggi *et al*., 2024). The focus on novel compounds challenges the overreliance on synthetic antibiotics, which are becoming less effective against resistant pathogens (Breijyeh *et al*., 2020).

The antimicrobial properties of Dodecane and Undecane derivatives suggest that alkanes have been underestimated as antimicrobial agents (Khan *et al*., 2021; Oboh *et al*., 2021; Nyalo *et al*., 2022). Advanced analytical techniques, including FTIR, UV-Vis, and GC-MS, were crucial in identifying key functional groups such as O-H and C=O, which are essential for the biological activity of these compounds (Sharma *et al*., 2021). These insights could refine future extraction protocols and lead to a new focus on non-polar compounds, potentially through the use of technologies like nanoemulsions to enhance their bioavailability (Chakraborty *et al*., 2023).

There is need to investigate the synergistic effect of the *BF* and conventional antibiotics like ciprofloxacin or with extracts from other medicinal plants, such as those in the *Combretum* genus (Cheesman *et al*., 2019; Fyhrquist *et al*., 2020).

Conclusion

The present study established that the EAF and BF of *T. superba* leaf extract are potent antibacterial agents, validating their use in traditional medicine. These findings lay a strong foundation for the development of new antimicrobial therapies

Acknowledgement

Thanks to Enugu State University of Science and Technology for granting the access and opportunity to utilize the research facilities at the Faculty of Pharmaceutical Sciences. Thanks to Mr Goodnews Ikeh; the laboratory technician of the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, for his kind assistance in the course of this study.

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CITATION: Ani, NI (2025) Solvent-Partitioned Fractions of *Terminalia Superba* Leaves Exhibit Broad-Spectrum Antibacterial Activity: Phytochemical Characterization and MIC Determination
Trend Nat Prod Res Vol 6(4). 346-354. https://doi.org/10.61594/tnpr.v6i4.2025.148