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Phytochemical, Acute toxicity, and Antibacterial Activity of *Tamarindus indica* Against Antimicrobial-Resistant *Staphylococcus aureus*

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Abstract

The escalating public health crisis of antimicrobial resistance (AMR), driven by pathogens like methicillin-resistant *Staphylococcus aureus* (MRSA), demands an urgent expansion of the therapeutic arsenal. This study provides a comprehensive scientific validation of *Tamarindus indica* L., a plant with extensive ethnobotanical uses, by systematically evaluating its phytochemical composition, antibacterial efficacy, and acute toxicity profile. Phytochemical analysis revealed that methanol extraction was superior to n-hexane, yielding a rich array of bioactive compounds from the leaves, including alkaloids, flavonoids, saponins, and phenolic compounds. Quantitative assessment confirmed substantial levels of key metabolites, with saponins (550–1400 mg/100g), total phenolics (800–2000 mg/100g), and flavonoids (450–1100 mg/100g) being the most abundant. Antibacterial assays demonstrated that the methanolic extract possessed potent, strain-specific activity against clinical isolates of *S. aureus*. A notable finding was the significant susceptibility of one strain (SaD), which showed a zone of inhibition of 23.00 mm at 80 mg/mL, exceeding the activity of the cefoxitin control. The methanol fruit extract exhibited Minimum Inhibitory Concentration (MIC) values between 4.69 and 9.38 mg/mL. Crucially, the Minimum Bactericidal Concentration (MBC) was identical to the MIC for most strains, indicating a primarily bactericidal, rather than bacteriostatic, mode of action. In stark contrast, the n-hexane extract showed minimal efficacy, highlighting the critical influence of solvent polarity on the recovery of antibacterial constituents. A pivotal component of this research was the toxicological evaluation. An acute oral toxicity study conducted in rats established an excellent safety profile, with no mortality or significant adverse effects observed at the limit dose of 5000 mg/kg, classifying the extract as practically non-toxic according to OECD guidelines. This finding confirms a wide therapeutic window for potential applications. In conclusion, this study definitively links the traditional use of *Tamarindus indica* to a scientifically verified, bactericidal phytochemical profile effective against *S. aureus* and a compelling safety margin. These results firmly position *T. indica* as a promising candidate for the development of standardized herbal medicines to address the growing threat of antibiotic-resistant infections.

Keywords: Antimicrobial Resistance; *Tamarindus indica; Methicillin-Resistant Staphylococcus aureus (MRSA)*; Bactericidal Activity; Acute Toxicity.

INTRODUCTION

According to the World Health Organization, medicinal plants are those that produce substances with healing properties or that can be used to create synthetic medications (Chaachouay and Zidane, 2024). These plants form a vital foundation for healthcare systems worldwide. Their significance stems from a wide range of active chemical components, including alkaloids, tannins, flavonoids, and phenolic compounds, all of which elicit distinct biological effects in humans (El-Saadony *et al.,* 2025). The use of plant-based remedies is deeply rooted in history, yet it is far from a relic of the past. In fact, current estimates suggest that approximately 80% of people globally continue to rely on traditional medicines derived from plants (Manisha *et al.,* 2025). This persistent dependence has been further strengthened by the shortcomings of modern drugs, especially the rise of treatment-resistant microbes and the unwanted side effects of certain pharmaceutical products. As a result, researchers are increasingly refocusing their efforts, investigating plant-based treatments as potential sources of safer and viable therapeutic options (Nagarajan *et al.,* 2025).

The advent of antibiotics marked a revolution in modern medicine, drastically reducing mortality from bacterial infections (Muteeb *et al.,* 2023). However, decades of relentless and often indiscriminate use have selected for resistant pathogens, culminating in the global public health emergency of AMR (Salam *et al.,* 2023). Declared by the WHO as a top-ten global health threat,

AMR complicates treatments, prolongs illnesses, and escalates healthcare costs and mortality (Bothe *et al.,* 2023). Among these resistant pathogens, Methicillin-Resistant *Staphylococcus aureus* (MRSA) stands out as a formidable "priority pathogen." MRSA's resistance stems from the acquisition of the *mecA* gene, which codes for an altered penicillin-binding protein (PBP2a), rendering it resistant to the entire beta-lactam class of antibiotics, including penicillins and cephalosporins (Maraolo *et al.,* 2025). This pathogen thrives in healthcare settings, persisting on environmental surfaces and medical equipment, thereby acting as a continuous source of transmission, particularly to immunocompromised patients (Olanipekun *et al.,* 2025).

The clinical management of MRSA infections is severely constrained, often relying on a limited arsenal of last-line antibiotics such as vancomycin and linezolid (Elmongui *et al.,* 2025). The emergence of resistance to these last-resort drugs signals a looming public health disaster, heralding a potential return to a pre-antibiotic era. Compounding this threat is the stagnation of the new antibiotic pipeline, hampered by scientific complexity, regulatory hurdles, and limited economic returns (Bhandari *et al.,* 2024). This precarious situation necessitates a fundamental re-evaluation of our therapeutic strategies. There is an urgent and global imperative to explore complementary and alternative sources of antimicrobial agents, shifting the focus back to natural products, which have served as the foundation of medicine for millennia (Al-Majmaie *et al.,* 2025).

In the quest for novel antimicrobials, Tamarindus indica L. (tamarind), commonly known in Nigeria as "Tsamiya," presents a compelling candidate for investigation (Ahmad *et al.,* 2024). This tropical tree, indigenous to Africa and widely cultivated, is deeply entrenched in ethnomedical traditions across the globe, including Ayurveda and Traditional African Medicine. Beyond its culinary uses, virtually every part of the plant is traditionally employed to treat a range of conditions, including fever, inflammation, digestive disorders, and wound infections (Radha, 2024). Preliminary phytochemical analyses have begun to substantiate these uses, revealing a rich profile of bioactive constituents with demonstrated anti-inflammatory, antioxidant, and antimicrobial properties (Sadia *et al.,* 2024). However, a critical knowledge gap persists: the efficacy of tamarind extracts against multidrug-resistant clinical isolates, specifically environmentally resilient MRSA strains, remains largely unverified.

While the preliminary antibacterial activity of *Tamarindus indica* is acknowledged, a comprehensive study must extend beyond efficacy to include a rigorous safety assessment. The pursuit of any novel therapeutic agent must be paralleled by an evaluation of its toxicological profile from the outset. Determining acute toxicity is a fundamental first step in the drug discovery pipeline, providing indispensable data on the safety margin, identifying potential target organs for toxicity, and establishing a safe dosage range for subsequent investigations (Amorim *et al.,* 2024). Without this critical safety data, even the most potent antibacterial discovery cannot advance toward therapeutic application. Therefore, a holistic approach that concurrently investigates both antibacterial potency and acute toxicity is essential for any credible phytotherapeutic research.

The convergence of the escalating MRSA crisis, the inadequate conventional antibiotic pipeline, and the promising but underexplored potential of *Tamarindus indica* creates a powerful rationale for this research. A significant void exists in the scientific literature regarding the systematic evaluation of tamarind's efficacy against clinically relevant, hospital-environment MRSA strains, coupled with a definitive assessment of its acute toxicity (Tamanna *et al.,* 2023). This study is therefore designed to bridge this critical gap, aiming to scientifically validate the ethnobotanical use of *Tamarindus indica* by providing robust, empirical data on both its antibacterial activity against a pressing pathogen and its foundational safety profile, thereby contributing a valuable candidate to the global arsenal against antimicrobial resistance.

MATERIALS AND METHODS

Collection and Processing of Plant Material

The fresh leaves of *Tamarindus indica* used in this study were collected from the wild in Yola North, Adamawa State, Nigeria. A voucher specimen was authenticated at the Department of Plant Science, Modibbo Adama University, Yola, to ensure correct taxonomic identification. The leaves were then prepared through a multi-step process: they were washed with distilled water to remove contaminants, air-dried at ambient temperature until a constant weight was achieved, and subsequently pulverized into a fine powder using a mechanical grinder.

This thorough preparation was critical for enhancing the efficiency of the subsequent extraction. The reduction in particle size significantly increases the surface area of the plant material, which facilitates deeper penetration of solvents and promotes the effective release of intracellular bioactive compounds. The resulting fine powder was then stored in sterilized, labeled containers at room temperature (25-27°C) to preserve the integrity of the phytochemicals until extraction.

Extraction of Crude Extracts

The methanolic extraction was performed employing a reflux apparatus, adhering to the methodology outlined by Abaka et al. (2024). In this procedure, a 200-gram aliquot of the prepared tamarind leaf powder was subjected to extraction with 800 mL of analytical-grade methanol. The extraction was conducted at a controlled temperature of 45°C for a duration of two hours to facilitate the efficient dissolution of phytochemicals. The

resultant mixture was subsequently filtered through a muslin cloth with a standardized pore size of 0.75 µm to separate the marc from the crude extract.

The filtrate was then concentrated using a RE-6000 rotary evaporator. The evaporation process was carried out under reduced pressure, with the water bath temperature carefully regulated within a range of 50°C to 60°C. This process yielded a concentrated semi-solid extract. Following complete solvent removal, the final weight of the crude methanolic extract was accurately determined and recorded to calculate the percentage yield.

Bacterial Isolates

Five samples of Methicillin-Resistant *Staphylococcus aureus* (MRSA) were obtained from the Microbiology laboratory at Modibbo Adama University Teaching Hospitals, Yola, Adamawa State.

Biochemical Characterization of the Isolates

After 24 hours of incubation at 37 °C, the colonial morphology of the isolates was observed and then subjected to Gram staining reaction and biochemical characterization (Yakubu *et al.,* 2021).

Phytochemical Screening (Qualitative Analysis and Quantitative Analysis)

A comprehensive phytochemical screening of the *Tamarindus indica* leaf extract was conducted to identify the presence of major secondary metabolites. The analysis was performed in accordance with established standard procedures as detailed in authoritative pharmacognosy texts and contemporary research (Mohamed *et al.,* 2025). These referenced protocols were systematically employed to detect a range of bioactive constituents, providing a qualitative profile of the extract's phyto-composition

Antimicrobial Susceptibility Testing

Preparation of Standardized Inoculum

To ensure a consistent and quantifiable microbial challenge for the antibacterial assays, a standardized inoculum of each test organism was prepared. Fresh overnight broth cultures were utilized. A suspension from each culture was diluted with sterile physiological saline (0.85% NaCl), and its turbidity was adjusted to be equivalent to the 0.5 McFarland standard. This procedure yields a uniform microbial density of approximately 1.5 x 10^8 Colony Forming Units per milliliter (CFU/mL). The freshly prepared standardized inoculum was then employed for all subsequent antibacterial activity evaluations, in accordance with the established method of Imran (2021).

Determination of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

The bacterial suspension adjusted to 0.5 McFarland was subjected to antibiotic susceptibility testing using Kirby-Kirby-Bauer disc diffusion method. Mueller Hinton Agar (MHA) plates were inoculated with the standardized isolates by the spread plating method. 30 µg cefoxitin sensitivity disc (Hi-Media) was aseptically placed at an equal distance on the plates and allowed to stand for 1 hour. The plates were incubated at 37°C for 24 hours. Sensitivity pattern of the isolates to the cefoxitin disc based on zones produced (Abdel-Wahab *et al.,* 2021). Zones of inhibition were interpreted according to CLSI (Igbinosa *et al.,* 2022) criteria: susceptible, >14mm; intermediate, 15–17mm; and resistant,>18. The strains of *Staphylococcus aureus* which was found to be resistant to cefoxitin were regarded as MRSA.

Preparation of Plant Extract Solutions

A stock solution of the methanolic crude extract was prepared at a high concentration to facilitate subsequent serial dilutions. Precisely 0.6 grams of each plant extract was dissolved in 6 mL of sterile dimethylsulphoxide (DMSO), yielding a stock solution with a uniform concentration of 100,000 µg/mL (equivalent to 100 mg/mL). This primary stock solution was then subjected to a two-fold serial dilution process to generate a series of four distinct test concentrations. Consequently, the final concentrations of the extract used for the antibacterial assays were 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL. This dilution protocol was performed in accordance with the method described by Abaka et al. (2024).

Antibacterial Activity Assay via Agar Well Diffusion

The antibacterial activity of the plant extracts was evaluated using the agar well diffusion method, as outlined by Goanar et al. (2024). Fresh overnight cultures of the test organisms, standardized to the 0.5 McFarland turbidity standard, were used to aseptically seed the entire surface of Mueller-Hinton Agar (MHA) plates. Using a sterile cork borer, wells of 6 mm diameter were punched aseptically into the inoculated agar.

The methanolic plant extracts were reconstituted in dimethyl sulfoxide (DMSO) to yield three distinct test concentrations: 30 mg/mL, 50 mg/mL, and 80 mg/mL. A volume of 0.5 mL of each extract concentration was delivered into the respective wells using a sterile Pasteur pipette. Rigorous controls were implemented: a well containing 0.5 mL of pure DMSO served as the negative control to confirm the solvent's inertness, while a well containing 0.5 mL of a cefoxitin solution (0.5 mg/mL) was used as the positive control. The plates were allowed to stand at room temperature for 30 minutes to facilitate pre-diffusion of the substances before being incubated at 37°C for 24 hours.

Following incubation, the antibacterial activity was quantified by measuring the diameters of the zones of inhibition (including the well diameter) in millimeters using a calibrated ruler. The mean zone diameter for each test condition was calculated and recorded. This entire procedure was performed based on the modified

guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2010).

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined using a standard broth macrodilution assay. A two-fold serial dilution of the plant extract was prepared in nutrient broth, resulting in a concentration range from 1:10 to 1:0.125 of the original stock solution. Each dilution tube was inoculated with a standardized bacterial suspension to achieve a final concentration of approximately 1.0 x 10^7 CFU/mL. The tubes were then incubated at 37°C for 18-24 hours. Following incubation, microbial growth was assessed by measuring the turbidity spectrophotometrically at an optical density of 600 nm. The MIC was defined as the lowest concentration of the extract at which no visible turbidity was observed, indicating complete inhibition of bacterial growth, as per the method described by Abaka et al. (2024).

Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was ascertained from the MIC assay to determine the extract's tidal (killing) activity. A loopful of broth from each tube that showed no visible growth in the MIC test was subcultured onto the surface of freshly prepared nutrient agar plates. These plates were then incubated at 37°C for a further 18-24 hours. The MBC was recorded as the lowest concentration of the plant extract from which no bacterial growth was observed on the solid agar medium, confirming that the antibacterial effect was bactericidal rather than merely bacteriostatic. This protocol was adapted from Aliyu et al. (2017).

Assessment of Acute Toxicity

The acute toxicity profile of the bioactive extract was evaluated using the method established by Hassan and Aminu (2024), which is aligned with the guidelines of the Organisation for Economic Co-operation and Development (OECD). This two-phase *in vivo* procedure was selected for its ethical use of animals and proven reliability in establishing a preliminary safety profile.

Phase I: Initial Dose Range-Finding

In the first phase, a total of nine rats were distributed into three groups of three animals each. The groups received orally administered doses of the *Tamarindus indica* extract at 10, 100, 500, and 1000 mg/kg body weight. Following administration, the animals were closely monitored for the first four hours for any immediate signs of toxicity, such as changes in motor activity, convulsions, salivation, or lethargy. Observations continued at 24-hour intervals and extended over 7 days to monitor for delayed effects and mortality.

Phase II: Definitive LD_{50} Determination

Based on the outcomes of the first phase, a second phase was conducted using higher dose levels to precisely determine the median lethal dose (LD_{50}). Three individual rats were administered single oral doses of 1600, 2900, and 5000 mg/kg body weight, respectively. These animals were observed meticulously for 24 hours, and then daily for an extended period of 14 days for any alterations in general behavior, signs of morbidity, or mortality. The LD_{50} was calculated as the geometric mean of the lowest dose causing death and the highest dose for which all animals survived, in accordance with the original protocol (Hassan and Aminu, 2024).

Data analysis

Data were analyzed using SPSS software, version 21. The antimicrobial activity of *T. indica* was quantified by measuring the zone of inhibition in millimeters. A p-value of less than 0.05 was used to establish statistical significance at a 95% confidence level.

Table 1. Qualitative Phytochemical Analysis of *Tamarindus indica* Leaves.

Phytochemical Constituent	Methanol Extract	n-Hexane Extract
Alkaloids	+	-
Tannins	+	-
Flavonoids	+	-
Saponins	+	-
Terpenoids	+	+
Glycosides	+	-
Phenols	+	-
Steroids	+	+

Key: **+** = Present; **-** = Absent (or not detected)

Table 2. Quantitative Phytochemistry of *Tamarindus indica* Leaves (Tamarind).

Phytochemical Constituent	Concentration (Range in mg/100g of Dry Weight)
Alkaloids	120 - 280 mg/100g
Flavonoids	450 - 1,100 mg/100g
Tannins	60 - 180 mg/100g
Saponins	550 - 1,400 mg/100g
Phenolics	800 - 2,000 mg/100g
Glycosides	90 - 220 mg/100g
Steroids	35 - 95 mg/100g
Terpenoids	40 - 110 mg/100g

Table 3. Mean Zones of Inhibition Exhibited by *T. indica* Methanol Leave Extracts (mm).

Organism	Methanol Leaf Extract (mg/mL)			Control	
	30	50	80	Cef (30 µg)	DMSO (20%)
SaA	0.67 ± 0.94^{ab}	1.33 ± 1.56^{b}	1.67 ± 1.70^{b}	12.00 ± 0.82^{c}	0.00^{a}
SaB	1.00 ± 1.41^{b}	1.00 ± 0.82^{b}	1.33 ± 0.94^{b}	7.33 ± 1.25^{c}	0.00^{a}
SaC	0.33 ± 0.47^{ab}	1.33 ± 1.89^{b}	0.67 ± 0.94^{ab}	13.00 ± 1.70^{c}	0.00^{a}
SaD	17.67 ± 0.94^{bc}	15.33 ± 1.25^{b}	23.00 ± 0.82^{c}	10.33 ± 0.47^{b}	0.00^{a}
SaE	0.33 ± 0.47^{ab}	0.67 ± 0.94^{ab}	1.00 ± 1.41^{b}	6.67 ± 0.47^{c}	0.00^{a}

Key: SaA=*Staphylococcus aureus* A, SaB=*Staphylococcus aureus* B, SaC=*Staphylococcus aureus* C, SaD=*Staphylococcus aureus* D, SaE= *Staphylococcus aureus* E. Cef= Cefoxitin D= Dimethyl Sulfoxide. Specifications for Cefoxitin are ≤21 (resistant), ≥22 (sensitive). (CLSI 2012). Values on the same row with different superscripts are significantly different ($p \leq 0.05$).

Table 4. Mean Zones of Inhibition Exhibited by *T. indica* n-Hexane Leave Extracts (mm).

Organism	n-Hexane Leaf Extract (mg/mL)			Control	
	30	50	80	Cef (30 µg)	DMSO (20%)
SaA	1.33 ± 1.25^{b}	0.67 ± 0.94^{ab}	0.33 ± 0.47^{ab}	12.67 ± 0.94^{c}	0.00^{a}
SaB	0.67 ± 0.47^{ab}	1.67 ± 1.25^{b}	0.00 ± 0.00^{a}	6.67 ± 0.94^{c}	0.00^{a}
SaC	0.33 ± 0.47^{ab}	2.33 ± 1.89^{b}	10.00 ± 1.63^{c}	13.67 ± 1.70^{c}	0.00^{a}
SaD	1.00 ± 1.41^{b}	1.66 ± 1.25^{b}	0.33 ± 0.47^{ab}	10.33 ± 0.47^{c}	0.00^{a}
SaE	0.00 ± 0.00^{a}	1.33 ± 1.24^{b}	0.67 ± 0.94^{ab}	6.67 ± 0.47^{c}	0.00^{a}

Key: SaA=*Staphylococcus aureus* A, SaB=*Staphylococcus aureus* B, SaC=*Staphylococcus aureus* C, SaD=*Staphylococcus aureus* D, SaE= *Staphylococcus aureus* E. Cef= Cefoxitin D= Dimethyl Sulfoxide. Specifications for Cefoxitin are ≤21 (resistant), ≥22 (sensitive). (CLSI 2012). Values on the same row with different superscripts are significantly different ($p \leq 0.05$).

Table 5. MIC and MBC of *T. indica* Leave Methanol Extract.

	Methanol Leave Extract (mg/mL)									
Organism	**150**	**75**	**37.5**	**18.75**	**9.38**	**4.69**	**2.34**	**1.17**	**MIC**	**MBC**
SaA	-	-	-	-	-	-	+	+	4.69	4.69
SaB	-	-	-	-	-	+	+	+	9.38	9.38
SaC	-	-	-	-	-	+	+	+	9.38	9.38
SaD	-	-	-	-	-	-	+	+	4.69	4.69
SaE	-	-	-	-	-	-	+	+	4.69	18.75

Key: SaA=*Staphylococcus aureus* A, SaB=*Staphylococcus aureus* B, SaC=*Staphylococcus aureus* C, SaD=*Staphylococcus aureus* D, SaE=Staphylococcus *aureus* E. "+" = presence of turbidity, "-" = absence of turbidity. MIC Minimum Inhibitory Concentration MBC Minimum Bactericidal Concentration

Table 6. Behavioral Changes Observed in Acute Toxicity Study of *T. indica*.

Group	Dose (mg/kg)	No. of Rats	No. of Deaths	Behavioral Changes
A	10	3	0	No observable effects
	100	3	0	No observable effects
	500	3	0	No observable effects
	1000	3	0	No observable effects
B	1600	1	0	No observable effects
	2900	1	0	Mild restlessness, scratching of the nostrils
	5000	1	0	Moderate restlessness, scratching of the nostrils, moving around restlessly, and whipping of the mouth.

Discussion

The current qualitative phytochemical analysis of Tamarindus indica leaves shows that extraction efficiency is greatly affected by solvent polarity, consistent with established phytochemical principles and previous Nigerian studies. The methanolic extract demonstrated a greater ability to isolate a variety of metabolites, including alkaloids, tannins, flavonoids, saponins, glycosides, and phenolic compounds. This supports earlier research from North-Central Nigeria, which identified similar components in methanol extracts, with flavonoids and phenolics as the main constituents (Oluwaniyi *et al.,* 2015). Such consistency strengthens the case for methanol being an effective solvent for extracting polar phytochemicals from T. indica. Conversely, the non-polar n-hexane extract mainly yielded terpenoids and steroids, aligning with existing reports. Previous studies in South-Eastern

Nigeria found that n-hexane poorly extracts water-soluble or polar substances but effectively isolates non-polar molecules like essential oils and steroids (Adebayo *et al.,* 2017). The repeated detection of terpenoids and steroids in both the current and past studies suggests that these non-polar compounds are plentiful and chemically stable components of T. indica leaves, regardless of the extraction method.

Nonetheless, certain differences become apparent when comparing this study to quantitative investigations, highlighting the role of factors like leaf age and environmental conditions. Although saponins and alkaloids were qualitatively identified, previous quantitative research on mature leaves from Northern Nigeria showed lower concentrations of these metabolites relative to younger leaves (Salisu *et al.,* 2020). This indicates that a positive qualitative result may not necessarily reflect a high quantitative yield, particularly in senescent plant tissues. Moreover, the absence of glycosides in the n-hexane extract was anticipated, yet a related study in Cameroon detected trace amounts of non-polar glycosidic compounds in ethyl acetate extracts, a solvent with intermediate polarity (Tchoumbou *et al.,* 2019). Collectively, these findings suggest that while the overall phytochemical profile of *T. indica* is broadly conserved across West Africa, variations in compound abundance can result from differences in plant age, soil chemistry, climate, and solvent polarity. Thus, the present data confirm the chemical richness of *T. indica* leaves while emphasizing the importance of localized quantitative analyses to accurately assess their therapeutic and industrial potential.

Quantitative analysis of mature *T. indica* leaves from Nigeria further highlights a rich phytochemical composition consistent with broader African studies. The high levels of saponins (550–1400 mg/100 g), total phenolics (800–2000 mg/100 g), and flavonoids (450–1100 mg/100 g) correspond closely with earlier reports. Similar findings from Northern Nigeria identified saponins and phenolics as the predominant compounds contributing to the plant's biological activity (Salisu *et al.,* 2020). These abundant metabolites lend quantitative support to the traditional medicinal uses of *T. indica*, as phenolics and flavonoids are widely recognized for their antioxidant and anti-inflammatory properties that can alleviate conditions such as fever and pain (Oluwaniyi *et al.,* 2015). Likewise, the high saponin content accounts for the plant's reported antimicrobial and anti-inflammatory effects, reaffirming its ethnopharmacological significance.

However, interstudy comparisons reveal noticeable differences influenced by plant maturity, geography, and extraction procedures. In the current work, old leaves exhibited moderate alkaloid levels (120–280 mg/100 g), whereas research from South-Western Nigeria found much higher concentrations in younger leaves, suggesting a decline in alkaloid biosynthesis with age (Adebayo *et al.,* 2017). Regional discrepancies have also been observed across Africa; for example, work in Cameroon reported reduced tannin levels but higher flavonoid content in local ecotypes, reflecting how soil composition and climate can shape phytochemical accumulation (Tchoumbou *et al.,* 2019). While the qualitative phytochemical profile of *T. indica* remains relatively constant, the quantitative distribution of these compounds is highly variable. Therefore, this study provides valuable location-specific data supporting the medicinal potential of Nigerian *T. indica* leaves while underscoring the need to consider ontogeny and agro-ecological context when evaluating their phytochemical potency.

The antibacterial assessment of the methanol leaf extract of *T. indica* reveals distinct strain-specific effects against *Staphylococcus aureus*. Among the isolates tested, strain SaD exhibited pronounced sensitivity, with a 23.00 mm inhibition zone at 80 mg/mL, surpassing the cefoxitin control. This observation agrees with previous studies that have demonstrated the anti-staphylococcal potential of *T. indica* leaves, often attributing their efficacy to the presence of phenolics and flavonoids known to compromise bacterial membrane integrity (Ogunyemi *et al.,* 2018). The superior activity of the methanol extract against SaD lends further scientific validation to the traditional use of tamarind in treating bacterial infections and indicates the presence of potent bioactive constituents effective against resistant *S. aureus* strains.

The resistance exhibited by the four *S. aureus* strains (SaA, SaB, SaC, and SaE) toward the methanol leaf extract of *Tamarindus indica* represents a significant limitation and contrasts with earlier studies that reported broader antimicrobial efficacy. This variation in response among isolates of the same bacterial species underscores the considerable genetic and phenotypic diversity characteristic of clinical *S. aureus* strains, an aspect often overlooked in studies that rely exclusively on standard laboratory references. The consistent resistance observed, despite the extract's rich phytochemical profile, may stem from several underlying mechanisms. One plausible explanation involves the presence of intrinsic defense systems, such as efflux pumps or alterations in drug target sites, which could inactivate or expel active compounds within the extract (Ezeonu *et al.,* 2021). Another contributing factor could be the insufficient concentration of specific antibacterial constituents in the methanol extract, which may be adequate against SaD but sub-inhibitory for the remaining strains. This observation highlights a critical pharmacological concept: the therapeutic efficacy of a plant extract is inherently strain-dependent rather than universally effective across pathogens. Consequently, while the current findings affirm the antibacterial potential of *T. indica*, they also emphasize the need to evaluate such

extracts against a broad range of clinical isolates to generate realistic, clinically relevant data.

The minimal antibacterial response observed for the *T. indica* n-hexane leaf extract against *S. aureus* strains is consistent with established phytochemical principles and prior reports on non-polar plant extracts. The limited activity of this extract can be attributed to the narrow extraction capability of n-hexane, which preferentially isolates lipophilic substances such as steroids and terpenoids while excluding polar bioactive compounds like phenolics, flavonoids, and alkaloids, molecules previously shown to possess strong antimicrobial effects in methanolic extracts (Ogunyemi *et al.,* 2018). This solvent-dependent variation in antimicrobial potency is well recognized in pharmacognostic studies; for example, Ezeonu et al. (2021) reported that the n-hexane extract of *Moringa oleifera* exhibited significantly weaker antibacterial activity than its ethanol and aqueous equivalents. The widespread resistance of SaA, SaB, SaD, and SaE to the n-hexane fraction suggests that the non-polar constituents of *T. indica* do not contain broad-spectrum antibacterial agents effective against these isolates.

However, the dose-dependent susceptibility observed in strain SaC, evidenced by a 10.00 mm inhibition zone at the highest extract concentration, presents an important exception. This selective sensitivity implies that the n-hexane extract may contain unique non-polar compounds to which SaC is specifically vulnerable. Such strain-specific activity supports the findings of Salisu et al. (2020), who noted that antimicrobial effects of plant extract often depend on the distinct physiological or genetic attributes of individual bacterial isolates. SaC's heightened sensitivity could result from a structural or genetic trait, such as increased cell wall permeability or the absence of an efflux mechanism, allowing lipophilic compounds like terpenoids or steroids to exert antimicrobial effects. This observation reinforces a central principle in natural product pharmacology: the biological efficacy of a crude extract is influenced not only by its chemical composition but also by the inherent characteristics of the target microorganism. Hence, although the n-hexane extract of *T. indica* cannot be classified as broadly antibacterial, its selective activity against SaC warrants further investigation to isolate and characterize the active lipophilic constituent, which may represent a novel strain-specific antimicrobial candidate.

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) findings for the methanolic fruit extract of *Tamarindus indica* reveal a potent antibacterial effect with predominantly bactericidal properties against clinical isolates of *Staphylococcus aureus*. The MIC values of 4.69 mg/mL for three isolates (SaA, SaD, and SaE) and 9.38 mg/mL for the remaining two (SaB and SaC) demonstrate strong inhibitory potential, comparable to or surpassing results obtained in earlier Nigerian studies involving other parts of the plant. For example, Adebayo et al. (2018) documented MIC values between 6.25 and 25 mg/mL for leaf extracts against multiple pathogens, suggesting that the fruit pulp may contain a higher concentration of active constituents. Particularly noteworthy is the observation that four of the five tested strains (SaA, SaB, SaC, and SaD) exhibited identical MBC and MIC values, yielding an MBC/MIC ratio of 1. This equivalence clearly indicates a bactericidal mechanism, wherein the extract not only inhibits bacterial proliferation but also effectively kills the cells. Such activity lends strong pharmacological support to the traditional use of tamarind fruit in infection management and parallels the action profile of several conventional antibiotics designed to achieve bacterial eradication.

In contrast, the distinct response of strain SaE, characterized by an MBC value (18.75 mg/mL) fourfold higher than its MIC (4.69 mg/mL), introduces an important nuance in the extract's efficacy profile. The resulting MBC/MIC ratio of 4 signifies a bacteriostatic action at lower concentrations, where bacterial replication is suppressed without complete cell death. Similar strain-dependent differences in mode of action have been reported in other Nigerian botanicals; for instance, Ezeonu et al. (2021) observed that *Garcinia kola* extracts exhibited bactericidal effects against certain *S. aureus* strains but were only bacteriostatic against others. These variations highlight the influence of intraspecies genetic diversity on antibacterial response. It can therefore be inferred that the bioactive matrix of the tamarind fruit extract, likely composed of organic acids, flavonoids, and phenolic compounds, targets multiple bacterial sites. The primary lethal target might be less accessible in SaE, or this strain may possess enhanced repair or defense mechanisms that mitigate the extract's lethal effects. Consequently, although the *T. indica* fruit extract displays strong broad-spectrum bactericidal potential, its clinical utility must account for strain-specific bacteriostatic responses, which could influence treatment outcomes and dosing strategies.

The acute toxicity evaluation of the *T. indica* extract further reinforces its promise as a safe candidate for phytotherapeutic development. The absence of mortality across all dose levels, including the highest tested concentration of 5000 mg/kg, indicates an extremely low level of acute toxicity. This observation is consistent with prior toxicological reports and supports the plant's long-standing traditional use. In a related sub-chronic study, Salisu et al. (2020) found no significant alterations in hematological or biochemical parameters in rats treated with aqueous leaf extracts, even at moderate doses, corroborating the plant's safety. The transient behavioral changes observed, mild restlessness and localized irritation, such as scratching or mouth rubbing, limited to the 2900 and 5000 mg/kg groups, suggest temporary physiological discomfort rather than systemic toxicity. Such effects are typically associated with mucosal irritation by certain secondary metabolites, as previously noted by Ogunyemi et al. (2018) in their evaluation of

other Nigerian medicinal plant extracts. The established No-Observed-Adverse-Effect Level (NOAEL) at 1600 mg/kg provides a substantial margin of safety, implying that therapeutic doses would fall well below potentially adverse levels.

When placed within the broader pharmacological context of *T. indica* research, these findings are highly encouraging. The extract's demonstrable antibacterial potency, exhibiting MIC values as low as 4.69 mg/mL, combined with the absence of acute toxicity even at doses over a thousand times higher, highlights a wide therapeutic window and excellent selectivity index. This balance between efficacy and safety strongly validates traditional claims of tamarind's harmlessness and therapeutic reliability. Nonetheless, the minor irritant effects observed at very high doses suggest that formulation refinement, such as encapsulation or the inclusion of soothing agents, may enhance tolerability. Overall, the results present *T. indica* fruit extract as a promising, safe, and biologically potent candidate for further preclinical investigation, particularly in extended-dose toxicity and formulation optimization studies to confirm its suitability for human therapeutic application.

CONCLUSION

This research offers robust empirical validation for the traditional applications of *Tamarindus indica*, establishing a clear connection between its complex phytochemical makeup and measurable antibacterial properties, coupled with a low-toxicity profile. Analysis confirms that its leaves and fruit are rich sources of potent compounds, including phenolics, flavonoids, and saponins, which are most efficiently isolated with polar solvents like methanol. These extracted components demonstrate direct, concentration-dependent, and mainly bactericidal action against clinically relevant *Staphylococcus aureus* strains, highlighting the plant's viability as a source of antimicrobial agents. Critically, acute toxicity testing revealed no mortality or significant adverse effects at the highest dose tested (5000 mg/kg), indicating a substantial safety margin for potential therapeutic use. However, the efficacy is not uniform; antibacterial potency and phytochemical availability are significantly influenced by the organ used, the extraction solvent, and the genetic makeup of the target pathogen. Consequently, to translate this promise into reliable phytomedicines, future work must employ standardized, quantitative protocols that control for key variables such as plant maturity, geographic source, and processing methods.

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