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RESEARCH ARTICLE

In Vitro Evaluation of the Antibacterial Potential of Crude Tamarind (*Tamarindus indica*) Seed Extracts Against *Staphylococcus aureus* and *Klebsiella pneumonia*

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ABSTRACT: Infectious diseases remain a significant global health challenge despite the discovery of antibiotics, primarily due to the increasing prevalence of antibiotic-resistant bacterial strains. This has necessitated the exploration of alternative therapeutic agents, particularly plant-derived bioactive compounds, which have demonstrated antimicrobial properties in traditional medicine. The present study aimed to assess the antibacterial efficacy of crude extracts from Tamarindus indica seeds against two clinically relevant pathogens, Staphylococcus aureus and Klebsiella pneumoniae, given the plant's extensive ethnomedicinal applications. The seeds were subjected to maceration using acetone and ethanol as extraction solvents, yielding crude extracts that were tested at concentrations of 100, 200, and 300 mg/mL via the disk diffusion method. Tetracycline (2.5 mg/mL) and Tween 20 (1 mL) served as positive and negative controls, respectively. The results revealed that neither the acetone nor ethanol extracts exhibited inhibitory effects on S. aureus or K. pneumoniae at any concentration tested. Statistical analysis indicated no significant difference between the crude extracts and the negative control ($P = 1.00$), whereas a highly significant difference was observed compared to the positive control ($P = 0.00$). These findings suggest that Tamarindus indica seed extracts, under the tested conditions, lack antibacterial activity against the selected pathogens. However, given the variability in phytochemical composition influenced by extraction methods and solvents, further investigations involving different plant parts, broader microbial strains, and alternative extraction techniques are warranted to conclusively determine their antimicrobial potential.

Keywords: Antibacterial activity, *Tamarindus indica*, Disk diffusion assay, Phytochemical extraction, Antimicrobial resistance, Pathogenic bacteria.

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1. INTRODUCTION

Infectious diseases have been a persistent threat to human health throughout history, with bacterial infections posing a particularly significant challenge due to their rapid evolution and adaptability [1-10]. The discovery of antibiotics in the early 20th century marked a turning point in medical science, offering effective treatments for previously fatal infections [11-19]. However, the overuse and misuse of these drugs have led to the emergence of multidrug-resistant (MDR) bacterial strains, rendering many conventional antibiotics ineffective [18, 7, 20-24]. This alarming trend has escalated into a global public health crisis, with the World Health Organization (WHO) identifying antibiotic resistance as one of the top ten threats to global health [21, 25].

The growing crisis of antimicrobial resistance has prompted intensified research into alternative therapeutic

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agents, with medicinal plants representing a particularly promising avenue due to their extensive historical use and diverse phytochemical composition. Traditional medicine systems across various cultures have long employed plant-based remedies for treating infections and inflammatory conditions, with documented efficacy spanning millennia [12, 5]. This therapeutic potential stems from plants' complex secondary metabolite profiles, which include structurally diverse compounds such as alkaloids, flavonoids, tannins, and phenolic substances [14, 15, 26]. These phytochemicals demonstrate broad-spectrum pharmacological activities through multiple mechanisms of action, including cell membrane disruption, enzyme inhibition, and interference with microbial nucleic acid synthesis. Importantly, the multi-target mode of action characteristic of plant-derived compounds reduces the likelihood of resistance development compared to single-target synthetic antibiotics [27, 20]. The synergistic interactions between different phytoconstituents in crude extracts often enhance antimicrobial efficacy while potentially mitigating toxicity, making plant-based medicines particularly valuable in addressing resistant pathogens. This pharmacological complexity, combined with centuries of ethnomedical validation, positions medicinal plants as crucial resources in the ongoing search for novel antimicrobial agents to combat increasingly resistant pathogens.

Ethiopia, with its rich biodiversity and long-standing tradition of herbal medicine, offers a vast repository of medicinal plants with untapped antimicrobial potential [6, 13]. However, the lack of systematic documentation and scientific validation of these plants has limited their integration into modern healthcare systems. One such plant, Tamarindus indica, is widely cultivated in tropical regions and is renowned for its nutritional and medicinal properties. While the pulp of T. indica is commonly consumed for its dietary benefits, the seeds—often discarded as waste—have been less explored despite their reported ethnomedicinal uses, including the treatment of diarrhea, dysentery, and wound infections [8, 10].

Previous studies on *T. indica* have yielded conflicting results regarding its antimicrobial efficacy. Some researchers [2, 22] have reported significant activity against Gram-positive and Gram-negative bacteria, while others [23] found negligible effects. These discrepancies may arise from variations in extraction methodologies, solvent polarity, or the geographical origin of plant samples. For instance, non-polar solvents like hexane may extract different compounds compared to polar solvents like ethanol, leading to varying bioactivity profiles [25, 26]. Additionally, the genetic diversity of bacterial strains and differences in experimental protocols (e.g., agar diffusion vs. broth dilution methods) can influence outcomes.

Given these gaps, the present study aimed to systematically evaluate the antibacterial activity of T. indica seed extracts against S. aureus and *K. pneumoniae*, two pathogens associated with hospital-acquired infections and high resistance rates. S. aureus, a Gram-positive bacterium, is a leading cause of skin infections, pneumonia, and sepsis, while *K. pneumoniae*, a Gram-negative pathogen, is notorious for causing urinary tract infections, pneumonia, and bloodstream infections [7, 24]. The study employed maceration-based extraction using acetone and ethanol to assess the influence of solvent polarity on antimicrobial activity.

The findings of this research contribute to the growing body of knowledge on plant-derived antimicrobials and highlight the need for standardized protocols in phytochemical research. By elucidating the potential (or lack thereof) of *T. indica* seeds as antibacterial agents, the study provides a foundation for future investigations into alternative plant-based therapeutics. Such efforts are critical in the face of escalating antibiotic resistance and the urgent need for sustainable treatment options.

2. EXPERIMENTAL DETAILS

The present investigation was conducted to evaluate the antibacterial potential of *Tamarindus indica* seed extracts through a systematic experimental approach involving plant material collection, extraction procedures, antibacterial testing, and statistical analysis. The methodology was designed to ensure scientific rigor while adhering to established protocols in phytochemical and microbiological research.

2.1. Plant Material Collection and Preparation

The collection of *Tamarindus indica* pods was conducted in January 2022 from Itang Special Woreda, Ethiopia, a region situated approximately 35 km from Gambella city and 801 km from Addis Ababa. The collection site was geographically documented at coordinates 8°4'N to 8°5'N latitude and 34°30'E to 33°55'E longitude, with an elevation range of 350–480 meters above sea level. This area experiences a hot, humid tropical climate, with annual temperatures varying between 18.09°C and 39.34°C and substantial rainfall averaging 1500–2000 mm during the wet season [3]. The climatic conditions of the region are known to influence the phytochemical composition of medicinal plants, making the selection of collection time and location crucial for ensuring optimal bioactive compound content.

Following collection, the pods were carefully transported to the Department of Biology at Dilla University (DU) in sealed plastic bags to minimize exposure to environmental contaminants and preserve sample integrity. Taxonomic authentication was performed by a certified botanist to confirm the identity of *Tamarindus indica*, ensuring the correct species was used for subsequent analyses. The fruits were manually processed under sterile conditions, where stainless steel knives were employed to separate the seeds from the pulp, minimizing the risk of microbial contamination. The extracted seeds were then subjected to controlled air-drying in the laboratory at a

consistent temperature of 25±2°C for approximately two weeks. This drying process was essential to reduce moisture content, thereby preventing fungal or bacterial growth while preserving the stability of bioactive compounds.

Once fully dried, the seeds were finely ground using an electric grinder fitted with a 0.5 mm mesh sieve to produce a homogenous powder, which is critical for ensuring uniform extraction efficiency. The resulting powder was accurately weighed using a high-precision electronic balance (0.001 g accuracy) to standardize subsequent extraction procedures. To maintain sample integrity and prevent degradation, the processed material was stored in airtight amber glass containers at room temperature, shielded from light and humidity until extraction commenced [3]. This meticulous preparation protocol was designed to minimize variability and ensure reproducibility in the extraction and bioactivity assessment phases of the study. The documented procedures align with established phytochemical research methodologies, reinforcing the reliability of the experimental outcomes.

2.2. Crude Extraction and Yield Determination

The maceration technique was selected for extraction due to its demonstrated efficiency in recovering phytochemical constituents from plant matrices [3]. Sequential extraction was performed using two analytical grade solvents with increasing polarity: acetone (Loba Chemie Pvt. Ltd, India) followed by ethanol (Alpha Chemika, India). Exactly 100 g of seed powder was initially macerated in 500 mL acetone (1:5 w/v ratio) for 24 hours with periodic agitation to enhance compound dissolution. The mixture was filtered through double-layered Whatman No. P2 filter paper (Fisher Scientific) to separate the filtrate from residual plant material. The marc was then subjected to secondary extraction using 500 mL ethanol under identical conditions to ensure comprehensive phytochemical recovery [3].

Both filtrates were concentrated separately using a rotary evaporator (Buchi R-300) at 45°C under reduced pressure to obtain solvent-free crude extracts. The resultant extracts were weighed to determine percentage yields using the standard formula:

$$\text{Extract yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant material}} \text{ X } 100$$

The acetone extract (SAE) and ethanol extract (SEE) were stored in sterile glass vials at 4°C to prevent degradation prior to antibacterial testing [9].

2.3. Microbial Strains and Culture Conditions

The study employed two reference bacterial strains obtained from the American Type Culture Collection (ATCC): *Staphylococcus aureus* (ATCC 25923) and *Klebsiella pneumoniae* (ATCC 700603). These microorganisms were selected based on their clinical relevance as causative agents of nosocomial infections and demonstrated antibiotic resistance patterns. The bacterial cultures were procured from the Ethiopian Biodiversity Institute (EBI) and maintained on nutrient agar slants at 4°C. Prior to experimentation, each strain was revitalized by subculturing on selective media - Mannitol Salt Agar (MSA) for *S. aureus* and MacConkey Agar (MCA) for *K. pneumoniae* - followed by incubation at 37°C for 24 hours [3,11].

2.4. Preparation of Test Solutions

Stock solutions of crude extracts were prepared at concentrations of 100, 200, and 300 mg/mL by dissolving respective quantities in 3% Tween 20 (v/v). The solutions were sterilized by filtration through 0.22 µm membrane filters (Millipore) and stored at 4°C. Tetracycline hydrochloride (2.5 mg/mL) and 3% Tween 20 served as positive and negative controls, respectively. All solutions were prepared under aseptic conditions in a laminar flow hood to prevent microbial contamination [11].

2.5. Antibacterial Susceptibility Testing

The disk diffusion method (Kirby-Bauer technique) was employed to assess antibacterial activity following standardized protocols with minor modifications [3]. Sterile 6 mm diameter filter paper disks (Whatman No. 1) were impregnated with 50 µL of each test concentration (100-300 mg/mL) and allowed to dry under sterile conditions. Mueller-Hinton Agar (MHA) plates were inoculated with standardized bacterial suspensions adjusted to 0.5 McFarland turbidity (≈1.5×10^8 CFU/mL). The inoculated plates were divided into five sectors, with three sectors containing extract-loaded disks, one sector for tetracycline (30 µL), and one for Tween 20 (50 µL). All plates were incubated aerobically at 37°C for 24 hours, after which zones of inhibition (ZOI) were measured using digital calipers (0.01 mm precision). Each assay was performed in triplicate to ensure reproducibility [3,11].

2.6. Data Analysis and Statistical Interpretation

Quantitative data were expressed as mean ± standard error of mean (SEM) of triplicate measurements. Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post-hoc test (SPSS v26). Differences were considered statistically significant at $p<0.05$ with 95% confidence intervals. The absence of inhibition zones was recorded as 0 mm for analytical purposes [3, 9, 11].

2.7. Ethical Considerations

The study protocol received ethical approval from the Department of Biology, Dilla University (Ref. No. DU/BIO/ERC/2022-01). Additional clearance for bacterial strain procurement was obtained from the Ethiopian Biodiversity Institute (EBI/MLS/2022/004). All chemicals and culture media were acquired from authorized suppliers (Kezen Trading PLC) following institutional procurement guidelines [3, 11].

3. RESULTS AND DISCUSSION

The global challenge of antimicrobial resistance has necessitated the exploration of alternative therapeutic agents, with medicinal plants emerging as promising candidates due to their diverse array of bioactive compounds. This study evaluated the antibacterial potential of *Tamarindus indica* seed extracts against two clinically significant pathogens, *Staphylococcus aureus* and *Klebsiella pneumoniae*, through a systematic investigation of extraction yields and antimicrobial efficacy. The findings present both confirmatory and contradictory evidence when compared with existing literature, warranting detailed discussion of the potential factors influencing these outcomes.

3.1. Extraction Yields and Solvent Efficiency

The sequential extraction process yielded two distinct crude extracts: seed acetone extract (SAE) with a 5% yield (5.0 g) and seed ethanol extract (SEE) with an 8% yield (7.1 g). The higher extraction efficiency of ethanol compared to acetone aligns with previous studies demonstrating the influence of solvent polarity on phytochemical recovery [11]. Research by Mariah et al. [9] similarly reported increased yields with polar solvents (methanol, water) compared to non-polar alternatives (hexane), supporting the observation that ethanol's intermediate polarity facilitates superior extraction of bioactive constituents from plant matrices. This phenomenon can be attributed to ethanol's ability to dissolve a broader range of secondary metabolites, including polar glycosides and moderately non-polar compounds like flavonoids and terpenoids [25]. However, the limited solvent spectrum examined in this study (only acetone and ethanol) precludes definitive conclusions about optimal extraction conditions for *T. indica* seeds, suggesting the need for future investigations incorporating a wider range of solvents with varying polarities.

3.2. Antibacterial Activity Assessment

Contrary to expectations based on traditional medicinal uses, neither SAE nor SEE demonstrated measurable inhibitory effects against the test organisms at any concentration (100-300 mg/mL) (Table 1). The complete absence of inhibition zones (0.00 ± 0.00 mm) contrasted sharply with the positive control tetracycline, which exhibited robust activity (15.33-16.33 mm inhibition zones). Statistical analysis confirmed no significant difference between the extracts and negative control (Tween 20) (P=1.00), while highly significant differences existed versus tetracycline (P=0.00) (Table 2). These results mirror findings by [23], who reported null antibacterial activity of hexane-extracted *T. indica* seed oil against *S. aureus* and *E. coli*. The universal lack of activity across both Gram-positive and Gram-negative bacteria in our study suggests either absence of antimicrobial phytochemicals in the seed extracts under these experimental conditions, or presence of compounds in concentrations below the minimum inhibitory threshold.

3.3. Comparative Analysis with Previous Studies

The observed results present intriguing contradictions with several published works.

Table 1. Qualitative presentation of growth inhibitory level of seed crude extracts of *T. indica* on the tested bacterial pathogens as compared to the antibiotic.

Test material	Concentration	Effect level		Remarks
		Test bacteria		
		Staphylococcus aureus	***Klebseilla pneumonia***	
Tween 20	1 ml	-	-	Drug used: Tetracycline
Drug	2.5 mg/ml	++++	++++	
SAE	100 mg/ml	-	-	
	200 mg/ml	-	-	
	300 mg/ml	-	-	
SEE	100 mg/ml	-	-	
	200 mg/ml	-	-	
	300 mg/ml	-	-	

Key: SAE = Seed Acetone Extract, SEE = Seed Ethanol Extract; - = No effect, ++++ = Strong effect

Table 2. Quantitative presentation of the inhibitory activity of the crude extract on the test bacteria as compared to the drug.

Test material	Concentration level	Inhibitory activity in mm			
		Test bacteria			
		Staphylococcus aureus		*Klebsiella pneumoniae*	
		Mean ± SEM	P-value	Mean ± SEM	P-value
Drug	2.5 mg/ml	15.67 ± 0.67[a]		16.33 ± 0.33[a]	
	00 mg/ml	0.00 ± 0.00[b]	0.00	0.00 ± 0.00[b]	0.00
	200 mg/ml	0.00 ± 0.00[b]	0.00	0.00 ± 0.00[b]	0.00
SAE	300 mg/ml	0.00 ± 0.00[b]	0.00	0.00 ± 0.00[b]	0.00
Drug	2.5 mg/ml	15.33 ± 0.33[a]		16.33 ± 0.33[a]	
	100 mg/ml	0.00 ± 0.00[b]	0.00	0.00 ± 0.00[b]	0.00
	200 mg/ml	0.00 ± 0.00[b]	0.00	0.00 ± 0.00[b]	0.00
SEE	300 mg/ml	0.00 ± 0.00[b]	0.00	0.00 ± 0.00[b]	0.00

Key: SAE = Seed Acetone Extract; SEE = Seed Ethanol Extract. Mean values with different superscripts in the same column are significantly different.

Das et al. [2] documented broad-spectrum antimicrobial activity of methanolic seed extracts against 14 microbial species, including methicillin-resistant *S. aureus* (MRSA) and *P. aeruginosa*, with inhibition zones ranging from 8-18 mm. Similarly, Sujith et al. [22] reported selective antibacterial effects of seed coat extracts, particularly against Gram-positive organisms. Several factors may account for these discrepancies:

3.3.1. Solvent Selection and Phytochemical Profile

The polarity-dependent extraction efficiency significantly influences the antimicrobial potential of plant extracts [25,26]. Methanol, employed by Das et al. [2], exhibits higher polarity than both acetone and ethanol, potentially extracting a distinct spectrum of bioactive compounds. Furthermore, sequential extraction in our study may have caused partitioning of active constituents between solvents, diluting their effective concentrations. Recent phytochemical analyses reveal that *T. indica* seeds contain antimicrobial compounds like lupeol and catechins that show preferential solubility in more polar solvents [16].

3.3.2. Bacterial Strain Variability

The use of reference ATCC strains in this study versus clinical isolates in other research may contribute to observed differences. Reygaert demonstrated that hospital-acquired *K. pneumoniae* strains frequently exhibit enhanced resistance mechanisms compared to reference strains, potentially making them more susceptible to plant-derived compounds that bypass conventional resistance pathways [18].

3.3.3. Extract Concentration and Bioavailability

While the tested concentrations (100-300 mg/mL) exceed those used in many antimicrobial studies (typically 5-50 mg/mL), the lack of activity suggests either insufficient bioactive compound concentration or interference from inhibitory matrix components. [26] reported that certain plant polysaccharides can bind antimicrobial phenolics, reducing their bioavailability in crude extracts.

The disk diffusion method, while standardized for antibiotic testing, may not optimally detect plant extract activity due to limited compound diffusion in agar. Studies employing broth dilution or bioautography methods frequently report lower minimum inhibitory concentrations (MICs) for the same extracts [21]. The null results despite *T. indica*'s ethnomedicinal reputation warrant careful interpretation. Traditional preparations often use water-based decoctions or prolonged extraction times that may liberate different phytochemical profiles compared to short-duration organic solvent extractions [6]. Additionally, synergistic effects between multiple plant components in whole extracts may be necessary for antimicrobial activity, as isolated compounds from *T. indica* seeds like tartaric acid exhibit pH-dependent bacteriostatic effects not detectable in standard assays [10]. The significant antibacterial activity of tetracycline (a broad-spectrum antibiotic inhibiting protein synthesis at 30S ribosomal subunit) confirms appropriate experimental conditions and bacterial susceptibility [24]. The extracts' inability to match this performance suggests they either lack compounds targeting essential bacterial processes (cell wall synthesis, nucleic acid replication) or contain them in subtherapeutic quantities.

4. CONCLUSION

This study provides evidence that *Tamarindus indica* seed extracts prepared with acetone and ethanol lack detectable antibacterial activity against *S. aureus* and *K.*

pneumoniae under standardized testing conditions. While contradicting some ethnopharmacological reports, these findings highlight the critical influence of extraction protocols and assay selection on antimicrobial evaluation outcomes. The results underscore the necessity of method standardization in phytotherapeutic research and suggest that *T. indica* seeds may require specific preparation methods or synergistic combinations to manifest their purported antimicrobial properties. Further multidisciplinary research integrating phytochemistry, microbiology and pharmacognosy approaches will be essential to fully elucidate the therapeutic potential of this widely used medicinal plant.

DECLARATIONS

Ethical Approval

We affirm that this manuscript is an original work, has not been previously published, and is not currently under consideration for publication in any other journal or conference proceedings. All authors have reviewed and approved the manuscript, and the order of authorship has been mutually agreed upon.

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Availability of data and material

All of the data obtained or analyzed during this study is included in the report that was submitted.

Conflicts of Interest

The authors declare that they have no financial or personal interests that could have influenced the research and findings presented in this paper. The authors alone are responsible for the content and writing of this article.

Authors' contributions

All authors contributed equally in the preparation of this manuscript.

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