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In vitro antibacterial activity of Tamarind (*Tamarindus Indica*) seed extracts against *Staphylococcus aureus* and *Klebsiella pneumoniae*

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Abstract

Despite the discovery of antibiotics, infectious diseases remain a serious concern due to the rise of antibiotic resistance. This situation necessitates the urgent search for alternative antimicrobial agents from various sources. Plants are a priority because of their bioactive components, which have potential in combating multi-drug resistant microorganisms. In Ethiopia, communities have traditionally used the seeds of Tamarind (Tamarindus indica) for wound healing, snake bites, abdominal pain, inflammation, helminth infections, antimicrobial effects, and diabetes management. However, scientific studies on this plant remain limited. This study aimed to investigate the antibacterial effects of Tamarindus indica seed extracts collected from Itang Woreda, Gambella, Ethiopia, against Staphylococcus aureus and Klebsiella pneumoniae. The maceration technique was used for extraction, employing acetone and ethanol as solvents. The antibacterial activity of each extract was evaluated at concentrations of 100, 200, and 300 mg/mL using the disk diffusion method. Pathogenic strains of Staphylococcus aureus (ATCC 25923) and Klebsiella pneumoniae (ATCC 700603) were obtained from the Ethiopian Biodiversity Institute and cultured on selective media. The findings indicated that neither the acetone nor ethanol extracts were effective against S. aureus or K. pneumoniae at any tested concentration. No significant differences were observed between the extracts and the negative control ($P > 0.05$). However, results were significantly different from the positive control ($P < 0.05$). These results suggest that Tamarindus indica seed extracts do not exhibit antibacterial activity against the tested bacteria. Further research on other pathogenic bacteria and fungi is recommended for more comprehensive conclusions.

Keywords/Phrases: Antibacterial activity, Disk diffusion, Maceration, Pathogenic bacteria, *T. indica*

1 Introduction

Infectious diseases have long posed a significant threat to humanity, and the discovery of antibiotics was initially considered a major victory against them. However, the increasing incidence of multi-drug resistance among pathogenic bacteria has intensified the struggle, seemingly favoring the bacteria (Aminov, 2010; Rios *et al*., 2016; Reygaert, 2018; Kim and Song, 2019; Talebi *et al*., 2019).

Attempts to combat infectious diseases through advancements in medicine have targeted not only bacteria but also fungi, viruses, and parasites. Unfortunately, these efforts often appear futile due to the widespread resistance to chemical antibiotics, which has reached alarming levels and poses a significant threat to global health (Reygaert, 2018; Stokes *et al*., 2020).

Despite significant efforts to manage infectious diseases with antibiotics, the rise of antimicrobial resistance, along with the high costs and widespread side effects of conventional drugs, highlights the ur-

gent need for new antimicrobial agents. This necessity drives scientists to explore alternative sources, particularly plants, which are prioritized for their bioactive components that may effectively combat multi-drug resistant microorganisms (Rahman *et al*., 2018; Stokes *et al*., 2020). Literature reviews indicate that plants possess bioactive compounds that support their use in traditional medicine and can serve as sources for pharmaceutical products.

Historical records show that the use of medicinal plants to alleviate human suffering dates back thousands of years, originating with early human civilization (Muluken *et al*., 2017; Helen *et al*., 2019). Phytochemicals produced during the secondary metabolism of plants—such as tannins, alkaloids, phenolic compounds, and flavonoids—are scientifically recognized for their therapeutic effectiveness (Belayhun *et al*., 2024; Njeru *et al*., 2013; Pagare *et al*., 2015). These compounds are valuable in traditional medicine for treating chronic and common microbial infections (Yuan *et al*., 2016; Salmerón-Manzano *et al*., 2020).

Ethiopia boasts a rich traditional healthcare system based on plants, with roots extending back several millennia. This long history has made it a vital part of Ethiopian culture as a source of therapeutics (Kebede *et al*., 2007; Netsanet *et al*., 2020). However, knowledge about medicinal plants varies among Ethiopian communities; a plant valued in one area may be underutilized in another due to a lack of documentation regarding its therapeutic properties (Behailu *et al*., 2021). While studies have acknowledged this diverse traditional knowledge, there has yet to be a systematic investigation into the antimicrobial effects of each species to enhance indigenous practices.

Prior research (Kuru, 2014; Menezes *et al*., 2016; Gomathinayagam *et al*., 2017; Pramila and Jirekar, 2021) has identified various bioactive phytoconstituents in *Tamarindus indica*, associated with numerous health benefits in traditional medicine, including wound healing, snake bites, abdominal pain, inflammation, helminth infections, antimicrobial properties, and antidiabetic effects. This background serves as the basis for the current study, which evaluates the antibacterial effects of crude seed extracts of *Tamarindus indica* against *S. aureus* and *K. pneumoniae*.

Tamarindus indica L., belonging to the Fabaceae (Leguminosae) family and the Caesalpinioideae subfamily, is commonly known as tamarind. It is believed to be native to tropical Africa, particularly Sudan and surrounding regions, including Ethiopia, but has been widely naturalized and cultivated across tropical and subtropical areas of the world (Orwa *et al*., 2009). While not originally native to Ethiopia, tamarind has been cultivated extensively, particularly in lowland and arid regions such as Afar, Somali, Eastern Hararge, South Omo Zone, Benshangul-Gumuz, Gambella, and the Rift Valley (Abdulrazak and Tadesse, 2016).

Though tamarind (*Tamarindus indica*) is primarily valued for the nutritional aspects of its fruit pulp, its seeds are traditionally used to treat various diseases, often discarded as waste. In a previous study, we explored the antibacterial activities of tamarind fruit pulp extracts, yielding encouraging results (Gatluak *et al*., 2024). Despite the traditional use of tamarind seeds for medicinal purposes across various Ethiopian communities, the potential of these extracts as antimicrobial agents against drug-resistant bacteria has not been thoroughly investigated. This study aims to examine the antibacterial activity of tamarind seed extracts, traditionally considered waste, to provide scientific evidence supporting their use as alternatives for treating antibiotic-resistant pathogens.

2 Material and methods

2.1 Seed sample collection

Dry pods of *Tamarindus indica* were collected from Itang Special Woreda, located 35 km from Gambella city and approximately 801 km from Addis Ababa, Ethiopia, in January 2022. The samples were then transported to the microbiology laboratory at Dilla University, Ethiopia. This Woreda is situated between latitudes 8^o4N to 8^o5N and longitudes 34^o30E to 33^o55E, classified as lowland with an altitude ranging from 350 to 480 meters above sea level. The climate is hot and humid, with annual temperatures ranging from a minimum of 18.09^oC to a maximum of 39.34^oC, and an average annual rainfall of 1500 to 2000 mm during the rainy season.

Upon arrival at the microbiology laboratory, the collected *Tamarindus indica* pods were cut with scissors, wrapped in newspaper, and placed in a sealable plastic bag. They were then taken to the Department of Biology at Dilla University, where a botanist identified the samples, which were kept under voucher number GG-002. The pulp was manually peeled off using a stainless-steel knife, and the seeds were allowed to dry at room temperature in the laboratory for about two weeks, with careful monitoring to prevent contamination.

After complete drying, the seeds were ground using a general-purpose grinder to an appropriate size for extraction, utilizing a 0.5 mm mesh (Geremew *et al*., 2018). The resulting powder was labeled and stored in a tightly sealed glass bottle at -20ºC until use.

2.2 Crude extraction and yield

The maceration technique was adopted for extraction due to its high efficiency. Crude extracts were obtained using two analytical-grade solvents with increasing polarity: acetone from Loba Chemie Pvt. Ltd and ethanol from Alpha Chemika, India. The extraction protocols were based on Jundi *et al*. (2021) with minor modifications.

Briefly, 100 grams of *Tamarindus indica* seed powder were macerated in acetone for 24 hours at a ratio of 1:5 (w/v). The mixture was then filtered using double-layer filter paper (Fisher brand), yielding filtrates and residues. The residue was subsequently macerated in ethanol for another 24 hours using the same ratio as with acetone.

The filtrates were evaporated using a rotary evaporator (Merk, UK) at 45^{o}C to obtain the crude extracts. The resulting mass was weighed in grams using an electronic balance and stored in small bottles in a refrigerator at -20^{o}C. The yield percentage was calculated using the formula provided by Mariah *et al*. (2021), as shown in Eq. 1.

$$Extract\ yield(\%) = (\frac{Dry\ weight\ of\ extract}{Dry\ weight\ of\ plant\ seed\ powder})x100$$

2.3 Test bacteria

Two bacterial strains from the American Type Culture Collection (ATCC) were used for this study: one Gram-negative strain, *Klebsiella pneumoniae* (ATCC 700603), and one Gram-positive strain, *Staphylococcus aureus* (ATCC 25923). These strains were selected due to their pathogenicity and their association with frequent and serious infections in humans. The bacterial samples were kindly provided by the Ethiopian Biodiversity Institute in Addis Ababa, Ethiopia.

2.4 Preparation of test solutions

The crude seed extracts were diluted to prepare three different concentrations in separate flasks, following the methods of Mesay *et al*. (2020). Specifically, working stock solutions of 100, 200, and 300 mg/mL were prepared by transferring 100, 200, or 300 mg of each extract into sterile test tubes, each containing 1 mL of 3% Tween 20. The resulting concentrations were 100, 200, and 300 mg/mL, respectively. The stock solutions were stored at -20^{o}C until further investigation.

2.5 Antibacterial activity

The disk diffusion method was employed to evaluate the antibacterial activity of the extracts, following the procedures described by Gatluak *et al*. (2024) and Workineh *et al*. (2024). Briefly, paper disks with an approximate diameter of 6 mm were punched from a sheet of absorbent filter paper and sterilized in an autoclave at 121^{o}C for 1 hour.

Each bacterial strain was grown on its selective medium: *Klebsiella pneumoniae* on MacConkey agar and *Staphylococcus aureus* on mannitol-salt agar and incubated at 37^{o}C for 24 hours. A few colonies of each strain were then transferred with a sterile inoculating loop to nutrient broth, adjusting the turbidity to match the McFarland 0.5 turbidity standard.

Two groups of plates containing Mueller-Hinton agar were prepared, with the two bacterial strains streaked using sterile cotton swabs. One group was used to test acetone extracts, while the other was for ethanol extracts. The surface of each plate was divided into five sections, each accommodating five paper disks: three disks containing extracts at different concentrations, one for the positive control, and one for negative control. Tetracycline, a broad-spectrum antibiotic effective against both aerobic and anaerobic

Gram-positive and Gram-negative pathogens, was used as the positive control (Nguyen *et al*., 2014; Pancu *et al*., 2021).

Each disk was loaded with 50 μL of the crude extract at the specified concentrations in separate quadrants of each plate. In the other two quadrants, one disk contained 30 μL of a 2.5 mg/mL Tetracycline solution (positive control), and another disk was immersed in 1 mL of 3% Tween 20 (negative control). All plates were incubated at 37^{o}C for 24 hours, after which the zone of inhibition was measured in millimeters using a ruler and recorded. The test was conducted in triplicate, and the results were expressed as the average zone of inhibition (ZOI) for each plant extract.

2.6 Data analysis

All data obtained from the experimental results were recorded by measuring the zone of inhibition (ZOI) in millimeters for each control and crude extract against each bacterium. The average values were calculated as the mean $\pm$ standard error of the mean (SEM) from the triplicate tests. The results were compared using one-way analysis of variance (ANOVA) with Tukey's Honest Significant Difference (HSD) test, with 95% confidence intervals (CI). A P-value of less than 0.05 was considered statistically significant.

2.7 Ethical Consideration

Ethical clearance was obtained from the ethical committee of Dilla University after securing a letter from the Department of Biology.

2.8 Results and Discussions

The study results indicated that the extraction of *Tamarindus indica* seed powder yielded two different crude extracts: the acetone extract and the ethanol extract, with respective crude masses and percent yields of 5.0 g (5%) and 7.1 g (8%). These results show variation in the crude mass obtained from the extraction, with ethanol yielding a higher crude mass than acetone. This finding aligns with the work of Mesay *et al*. (2020), which reported higher yields from ethanol compared to acetone and chloroform. Similarly, Mariah *et al*. (2021) found that among various solvents, hexane yielded the least due to its lower polarity.

The variation in solvent polarity appears to be a significant factor influencing the extraction efficiency. Since ethanol has higher polarity than acetone, it is expected to extract more soluble compounds, resulting in a higher yield. However, given the limited sample size (using only two solvents), caution is warranted, as these findings may not be generalizable across a broader range of organic and inorganic solvents used in extraction processes.

In terms of antibacterial activity, the crude extracts from the seeds of *T. indica* showed unexpected results against *Staphylococcus aureus* and *Klebsiella pneumoniae*. None of the three concentrations (100, 200, and 300 mg/mL) of either acetone or ethanol extracts demonstrated inhibitory effects on the growth of both tested pathogenic bacteria (Table 1).

According to this data, this study found no statistically significant difference between the crude extracts and the negative control ($P > 0.05$). However, the results were significantly different from the positive control ($P < 0.05$) (Table 2). The significant difference between the crude extracts and the drug (positive control), along with the lack of difference between the crude extracts and the negative control (3% Tween 20), suggests that the extracts have no antibacterial activity against the test bacteria.

Table 1. Qualitative growth inhibitory level of *T. indica* seed extracts on the tested pathogenic bacteria compared to the Tetracycline antibiotic (positive control) and Tween 20 (negative control)

Solvent	Extract concentration (mg/mL)	Test bacteria	
		Staphylococcus aureus	*Klebseilla pneumoniae*
Tween 20	1 mL	-	-
Tetracycline	2.5	++++	++++
Acetone	100	-	-
	200	-	-
	300	-	-
Ethanol	100	-	-
	200	-	-
	300	-	-

Note: - = No effect, ++++ = Strong effect

Table 2. Quantitative growth inhibitory activity (mm) of *T. indica* seed extracts against pathogenic bacteria compared to both positive (Tetracycline antibiotic) and negative (Tween 20) controls

Solvent	Extract concentration (mg/mL)	*S. aureus*		*K. pneumoniae*	
		Mean ± SEM	P-value	Mean ± SEM	P-value
Tween 20	1 mL	0.00 ± 0.00^{b}	< 0.05	0.00 ± 0.00^{b}	< 0.05
Tetracycline	2.5	15.67 ± 0.67^{a}	> 0.05	16.33 ± 0.33^{a}	> 0.05
	100	0.00 ± 0.00^{b}	< 0.05	0.00 ± 0.00^{b}	< 0.05
Acetone	200	0.00 ± 0.00^{b}	< 0.05	0.00 ± 0.00^{b}	< 0.05
	300	0.00 ± 0.00^{b}	< 0.05	0.00 ± 0.00^{b}	< 0.05
Tetracycline	2.5	15.33 ± 0.33^{a}		16.33 ± 0.33^{a}	
	100	0.00 ± 0.00^{b}	< 0.05	0.00 ± 0.00^{b}	< 0.05
Ethanol	200	0.00 ± 0.00^{b}	< 0.05	0.00 ± 0.00^{b}	< 0.05
	300	0.00 ± 0.00^{b}	< 0.05	0.00 ± 0.00^{b}	< 0.05

a; b = showing significant differences of the extracts with the positive control,
the mean values with different superscripts in the same column are significantly different.

In accordance with the present results, these findings mirror those of Sutrisno *et al*. (2019), which demonstrated that the hexane crude oil extract from *Tamarindus indica* seeds showed no inhibition against *Staphylococcus aureus* and Escherichia coli. Conversely, the outcomes presented here contrast with the findings of Das *et al*. (2014), who reported varying degrees of antimicrobial activity from the methanolic extract of *T. indica* seeds against Salmonella paratyphi A, Salmonella typhi, E. coli, *S. aureus*, methicillin-resistant *S. aureus*, Vibrio cholerae, S. paratyphi B, Pseudomonas aeruginosa, Bacillus subtilis, Proteus alcalifaciens, Proteus mirabilis, C. fulvum, Neurospora crassa, and Aspergillus niger.

Furthermore, the levels of activity observed in this investigation were significantly lower than those reported by Sujith *et al*. (2015), who noted good activity against some Gram-positive bacteria with the seed coat extract, although not against Gram-negative bacteria.

The reasons for these contradictory results are unclear, but they may relate to the nature of the solvents used, as different solvents can produce varying phytochemicals (secondary metabolites) from the same plant sample (Tiwari and Rana, 2015; De Castro *et al*., 1998; Twaij and Hasan, 2022). Additionally, the specific bacterial strains tested (*S. aureus* and *K. pneumoniae*) and possible interference from the extraction solvents cannot be ruled out.

3 Conclusion

The findings of this study indicate that the *Tamarindus indica* seed extract did not exhibit antibacterial activity. Therefore, these results do not support strong recommendations for its use by indigenous communities for treating various diseases, as the implications of both the solvent used and the specific bacterial strains should be considered. Further research involving other pathogenic bacteria and fungi is necessary to draw more convincing conclusions.

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Conflict of Interest

The authors declare that there is no competing interest in relation to this work.

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