EJED 2025 7(1):40–47
Ethiopian Journal of Environment and Development
http://journal.du.edu.et/index.php/ejed

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

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Received: 03 February 2025 *Accepted: 25 June 2025* *Published: 30 June 2025*

Article DOI:**10.20372/ejed.v07i1.05**

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. Tamarind (Tamarindus indica) seeds have long been used by Ethiopian communities to treat wounds, snake bites, abdominal pain, inflammation, helminth infections, antibacterial effects, and diabetes. Scientific research on this plant is very scarce, nevertheless. The purpose of this study was to examine the antibacterial properties of Tamarindus indica seed extracts obtained from Itang Woreda, Gambella, Ethiopia, against Klebsiella pneumoniae and Staphylococcus aureus. Acetone and ethanol were employed as solvents in the maceration method of extraction. The disk diffusion method was used to assess each extract's antibacterial activity at doses of 100, 200, and 300 mg/mL. 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. The extracts and the negative control did not differ significantly ($P > 0.05$). The outcomes, however, differed considerably from the positive control ($P < 0.05$). These findings imply that the examined bacteria are not susceptible to the antibacterial action of Tamarindus indica seed extracts. For more thorough findings, greater investigation into other harmful bacteria and fungi is advised.

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). Tannins, alkaloids, phenolic compounds, and flavonoids are examples of phytochemicals that are formed during the secondary metabolism of plants and are known to have medicinal properties (Belayhun *et al*., 2024; Njeru *et al*., 2013; Pagare *et al*., 2015). Traditional medicine uses these substances to treat common and chronic 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.

According to earlier studies (Kuru, 2014; Menezes *et al*., 2016; Gomathinayagam *et al*., 2017; Pramila and Jirekar, 2021), *Tamarindus indica* contains a variety of bioactive phytoconstituents that are linked to a number of health benefits in traditional medicine, such as wound healing, snake bites, abdominal pain, inflammation, helminth infections, antimicrobial qualities, and antidiabetic effects. The current work, which assesses the antibacterial properties of *Tamarindus indica* crude seed extracts against *S. aureus* and *K. pneumoniae*, is based on this background.

Tamarind is the common name for *Tamarindus indica* L., a member of the Caesalpinioideae subfamily and the Fabaceae (Leguminosae) family. 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). Although many Ethiopian communities have long used tamarind seeds for medical purposes, little research has been done on the extracts' potential as antibacterial agents against drug-resistant bacteria. The purpose of this study is to investigate the antibacterial activity of tamarind seed extracts, which are typically regarded as trash, in order to offer scientific proof that they can be used as alternatives to treat infections that are resistant to antibiotics.

2 Material and methods

2.1 Seed sample collection

In January 2022, dry *Tamarindus indica* pods were gathered from Itang Special Woreda, which is around 801 kilometers from Addis Ababa, Ethiopia, and 35 kilometers from Gambella city. The samples were then transported to the microbiology laboratory at Dilla University, Ethiopia. This Woreda is situated between latitudes 8º4N to 8º5N and longitudes 34º30E to 33º55E, 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 18.09ºC to a maximum of 39.34ºC, 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.

Following full drying, the seeds were ground with a 0.5 mm mesh in a general-purpose grinder to the proper size for extraction (Geremew *et al*., 2018). Before being used, the resultant powder was labeled and kept at -20ºC in a firmly sealed glass bottle.

2.2 Crude extraction and yield

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

In summary, 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 filtered using double-layer filter paper (Fisher brand) to produce filtrates and residues, which were then macerated in ethanol for an additional 24 hours.

The filtrates were evaporated using a rotary evaporator (Merk, UK) at 45ºC 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 -20ºC. 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

For this investigation, two bacterial strains from the American Type Culture Collection (ATCC) were utilized: *Staphylococcus aureus* (ATCC 25923), a Gram-positive strain, and *Klebsiella pneumoniae* (ATCC 700603), a Gram-negative strain. These strains were chosen because of their toxicity and correlation with recurrent and severe human illnesses. The Ethiopian Biodiversity Institute in Addis Ababa, Ethiopia, graciously supplied the bacterial samples.

2.4 Preparation of test solutions

In accordance with Mesay *et al*. (2020), the crude seed extracts were diluted to create three distinct concentrations in different flasks. In particular, 100, 200, or 300 mg of each extract were transferred into sterile test tubes with 1 mL of 3% Tween 20 to create working stock solutions of 100, 200, and 300 mg/mL. Concentrations of 100, 200, and 300 mg/mL were the outcomes. Until more research was conducted, the stock solutions were kept at -20ºC.

2.5 Antibacterial activity

The antibacterial activity of the extracts was assessed using the disk diffusion method, in accordance with the protocols outlined by Gatluak *et al*. (2024) and Workineh *et al*. (2024). In short, paper disks about 6 mm in diameter were punched out of a sheet of absorbent filter paper and autoclaved for an hour at 121ºC to sterilize them.

Each bacterial strain was grown on its selective medium: *Klebsiella pneumoniae* on MacConkey agar and Staphylococcus aureus on mannitol-salt agar, and incubated at 37ºC for 24 hours. After that, a few colonies of each strain were moved to nutritional broth using a sterile inoculating loop, and the turbidity was adjusted to meet the McFarland 0.5 turbidity criterion.

Sterile cotton swabs were used to streak the two bacterial strains on two sets of Mueller-Hinton agar plates. Acetone extracts were tested in one group and ethanol extracts in another. Each plate's surface was split into five portions, each of which could hold five paper disks: one for the positive control, one for the negative control, and three disks with extracts at various concentrations. The positive control was tetracycline, a broad-spectrum antibiotic that works against both aerobic and anaerobic Gram-positive and Gram-negative infections

In distinct quadrants of each plate, 50 μL of the crude extract at the designated concentrations were placed onto each disk. In the other two quadrants, one disk was submerged in 1 mL of 3% Tween 20 (negative control) and another disk held 30 μL of a

2.5 mg/mL Tetracycline solution (positive control). Following a 24-hour incubation period at 37ºC, the zone of inhibition was measured using a ruler and reported in millimeters. The average zone of inhibition (ZOI) for each plant extract was used to express the outcomes of the test, which was carried out in triplicate.

2.6 Data analysis

The zone of inhibition (ZOI) for each control and crude extract against each bacterium was measured in millimeters for all data derived from the experimental results. The mean ± standard error of the mean (SEM) from the triplicate tests was used to compute the average results. One-way analysis of variance (ANOVA) with Tukey's Honest Significant Difference (HSD) test and 95% confidence intervals (CI) was used to compare the outcomes. Statistical significance was defined as a P-value of less than 0.05.

2.7 Ethical Consideration

After obtaining a letter from the Department of Biology, the Dilla University ethical committee granted ethical approval.

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.

The crude extracts from *T. indica* seeds exhibited surprising antibacterial efficacy against *Klebsiella pneumoniae* and Staphylococcus aureus. The growth of both tested pathogenic bacteria was not inhibited by any of the three concentrations (100, 200, and 300 mg/mL) of either acetone or ethanol extracts (Table 1).

Based on these findings, the crude extracts and the negative control did not differ statistically significantly ($P > 0.05$). But compared to the positive control, the outcomes differed significantly ($P < 0.05$) (Table 2). The lack of difference between the crude extracts and the negative control (3% Tween 20) and the substantial difference between the crude extracts and the medication (positive control) indicate that the extracts have no antibacterial activity against the test bacteria.

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. In contrast, Das *et al*. (2014) found that the methanolic extract of *T. indica* seeds exhibited varying degrees of antimicrobial activity 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.

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.

3 Conclusion

The results of this investigation show that there was no antibacterial activity in the *Tamarindus indica* seed extract. 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.

Acknowledgments

The authors express their gratitude for the financial assistance provided by the Office of the Vice President for Research and Technology Transfer at Dilla University in Ethiopia. They also thank Dilla University's Department of Biology and Chemistry for providing access to lab facilities. The Ethiopian Biodiversity Institute provided the tested bacterial strains, for which the authors are also grateful.

Conflict of Interest

The authors affirm that they have no conflicting interests with regard to this work.

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