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RESEARCH ARTICLE

Antibacterial Efficacy of Crude Extracts from Tamarind (*Tamarindus indica*) Leaves against *Staphylococcus aureus* and *Klebsiella pneumoniae*: An *In vitro* Study

Gatluak Goanar [1], Geremew Tafesse [1], Workineh Mengesha [2], M. Sadre Alam Fakhri [3, *]

ABSTRACT: Infectious diseases are the primary concern for all mankind, and despite the discovery of antibiotics, the battle is still eminent due to the rise in antibiotic resistance, posing the need to develop feasible alternatives. Since plants proved to be sources of inspiration for novel drug compounds from their secondary metabolites, the present study aimed to investigate the effect of the crude extracts from the leaves of *Tamarindus indica* against selected pathogenic bacteria, as the plant is reported to have uncountable uses in traditional medicine. Maceration technique was employed for subsequent extraction of crude extracts using acetone and ethanol as solvents. Antibacterial activity of each crude extract was evaluated at a concentration of 100, 200 and 300 mg/ml through disk diffusion method. Tetracycline at 2.5 mg/ml and 1 ml of Tween 20 were used as positive and negative controls, respectively. Through serial dilution starting from 300 to 4.6875 mg/ml, minimum inhibitory concentration was also determined. The findings indicated that both acetone and ethanol crude extract showed antibacterial activity at all concentrations on both *S. aureus* and *K. pneumonia*, being significantly different from the negative control ($P = 0.00$), and insignificantly different from the drug ($P > 0.05$). However, *K. pneumoniae* showed more sensitivity to the extracts than *S. aureus*. The MIC value of LAE on *S. aureus* and *K. pneumoniae* were 18.75 and 9.375 mg/ml, respectively, whereas on the LEE, both showed MIC value of 18.75 mg/ml. The outcomes of this study indicated that the leaves have antibacterial properties; however, further study on the mechanism of actions and other related properties is required for the safe use of this plant in relation to the health problems.

Keywords: *Tamarindus indica,* Antibacterial activity, Disk diffusion assay, Minimum inhibitory concentration (MIC), *Staphylococcus aureus, Klebsiella pneumoniae*

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1. INTRODUCTION

Infectious diseases have been a persistent threat to human health throughout history, with bacterial infections posing significant challenges to global healthcare systems. The discovery of antibiotics in the early 20th century was initially hailed as a turning point in modern medicine, offering effective treatment against previously fatal infections [1]. However, the rapid emergence of antibiotic-resistant bacterial strains has reversed much of this progress, rendering many conventional drugs ineffective [2, 3]. The World Health Organization (WHO) has identified antimicrobial resistance (AMR) as one of the top ten global public health threats, with multidrug-resistant (MDR) pathogens such as Staphylococcus aureus and Klebsiella

[1] Department of Biology, Dilla University, P.O. Box: 419, Dilla, Ethiopia.
[2] Department of Chemistry, Dilla University, P.O. Box: 419, Dilla, Ethiopia.
[3] Department of Biology, Gambella University, P.O. Box: 126, Gambella, Ethiopia

* Author to whom correspondence should be addressed: fakhrimsa@gmail.com (M. Sadre Alam Fakhri)

pneumoniae contributing substantially to morbidity and mortality worldwide [4, 5].

Plants have long served as a cornerstone of traditional medicine, with historical records documenting their use in treating infections, inflammation, and other ailments for millennia [6, 7]. The therapeutic potential of plants lies in their rich diversity of secondary metabolites, including alkaloids, flavonoids, tannins, and phenolic compounds, which exhibit broad-spectrum antimicrobial properties [8, 9]. These bioactive compounds often act synergistically, reducing the likelihood of resistance development compared to single-molecule antibiotics [10, 11]. In recent years, ethnobotanical studies have increasingly focused on validating the efficacy of medicinal plants through scientific methods, bridging the gap between traditional knowledge and modern pharmacology [12, 13].

Ethiopia, with its rich biodiversity and long-standing tradition of herbal medicine, offers a vast repository of medicinal plants that remain understudied in the context of modern drug discovery [14, 15]. Traditional healers in Ethiopia have used plants like Tamarindus indica (tamarind) for centuries to treat ailments ranging from wound infections to gastrointestinal disorders [16]. However, despite its widespread use, the scientific validation of T. indica's antimicrobial properties remains fragmented, particularly concerning its leaves, which are less studied compared to its fruit pulp [17]. This study focuses on T. indica leaves, which are reported to possess bioactive compounds with potential antibacterial effects, yet systematic investigations into their efficacy against resistant pathogens are limited [18,19].

The novelty of this paper lies in its comprehensive evaluation of T. indica leaf extracts against two clinically relevant pathogens - S. aureus (a Gram-positive bacterium associated with skin infections and sepsis) and K. pneumoniae (a Gram-negative bacterium linked to pneumonia and urinary tract infections). While previous studies have examined T. indica's antimicrobial properties, many have focused on fruit pulp or used non-standardized extraction methods [20, 21]. This study employs sequential maceration with acetone and ethanol, solvents chosen for their varying polarities, to optimize the extraction of bioactive compounds. Additionally, the study compares the extracts' efficacy to tetracycline, a broad-spectrum antibiotic, providing a direct benchmark for their potential clinical utility. By determining the minimum inhibitory concentration (MIC) and analyzing dose-dependent effects, this work offers a more rigorous pharmacological assessment than many prior studies, contributing valuable data for future drug development efforts.

The rise of antibiotic resistance has prompted researchers to explore alternative strategies, including phytomedicine, combination therapies, and nanotechnology-based drug delivery systems [13, 22]. However, plant-derived antimicrobials remain particularly promising due to their structural complexity, low toxicity, and compatibility with traditional healthcare systems in resource-limited settings [11, 23]. T. indica is an excellent candidate for such investigations, as it is widely distributed across tropical regions and has a well-documented history of medicinal use [24, 17]. Previous studies have identified antimicrobial activity in T. indica extracts against pathogens like Escherichia coli, Pseudomonas aeruginosa, and Bacillus cereus, but gaps remain in understanding their efficacy against MDR strains [25, 26]. This study addresses this gap by focusing on S. aureus and K. pneumoniae, both of which are listed by the WHO as priority pathogens for new antibiotic development [5].

The methodology adopted in this study aligns with established protocols for phytochemical extraction and antibacterial testing, ensuring reproducibility and comparability with existing literature [27, 28]. The disk diffusion assay and MIC determination provide quantitative measures of antibacterial activity, while statistical analyses validate the significance of the findings. By incorporating both Gram-positive and Gram-negative bacteria, the study offers insights into the spectrum of activity of T. indica leaf extracts, which is critical for assessing their potential as broad-spectrum antimicrobial agents [29, 30]. Furthermore, the use of two solvents (acetone and ethanol) allows for preliminary comparisons of polar versus non-polar bioactive compounds, informing future phytochemical isolation efforts.

This study contributes to the growing body of research on plant-based antimicrobials by systematically evaluating T. indica leaf extracts against resistant bacterial strains. The findings not only validate traditional uses of the plant but also highlight its potential as a source of novel antibacterial compounds. Given the urgent need for new antimicrobial agents, further research should focus on isolating the active constituents, elucidating their mechanisms of action, and evaluating their safety and efficacy *in vivo*. Such efforts could pave the way for developing affordable, accessible, and sustainable alternatives to conventional antibiotics, particularly in regions where T. indica is readily available [19, 17].

2. EXPERIMENTAL DETAILS

2.1. Plant Material Collection

Fresh leaves of *Tamarindus indica* were collected from *Itang* Special *Woreda,* Gambella Region, about 801 km away from Addis Ababa, Ethiopia in January, 2022. This district is located in a latitude of 8°4`N to 8°5`N and 34°30`E to 33°55`E longitude, and is classified as lowland with the altitude ranging from 350 – 480 m above sea level. The climate is hot humid with annual temperature of minimum and maximum of 18.09ºC and 39.34ºC respectively, while the rainy season having annual average rain fall of 1500 – 2000 mm. The leaves were cut by scissors, wrapped in newspapers and put in a sealable plastic bag separately and were brought to the Department of Biology, Dilla University (DU), and identified by the botanist. They were cleaned of any external contaminants and dried under shade at room temperature in the Microbiology Laboratory for about two weeks with a careful and continuous follow up to avoid any contamination.

After complete drying, the samples were ground using a general purpose grinder to an appropriate size for extraction with the help of 0.5 mm mesh and were measured by electronic balance, labeled and stored in tightly closed glass bottle, till their usage [6].

2.2. Crude Extraction and Yield

Maceration technique was adopted for this investigation due to its excellent efficiency, and subsequent extraction was used to get crude extracts using two analytical grade solvents with increasing polarity, namely, acetone (an intermediary polar) from Loba Chemie Pvt. Ltd and ethanol (a more polar solvent) from Alpha Chemika, (India). By adopting the protocols described in the literature with minor modification, crude extraction was carried out starting with acetone, followed by ethanol [6]. 100 g of *Tamarindus indica* leaves was macerated for 24 hours (24 h) in acetone with the ratio of 1:5 (w/v). After 24 h, filtration was made using a double layer filter paper (Fisher brand, P2) giving filtrates and residues. Residue was then macerated in ethanol for another 24 h with similar ratio as of acetone. The filtrates were evaporated using Rota-vapor at 45°C to obtain crude extracts. The crude mass obtained was weighed in grams (g), and stored in small bottles in fridge at 4°C, and their yield percent was calculated by adopting the formula in shown below [23]:

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

2.3. Antibacterial Activity

2.3.1. Test Bacteria

Two bacterial species of reference American Type Culture Collection (ATCC), namely, *Klebsiella pneumoniae* (ATCC 700603) (representing Gram-negative) and *Staphylococcus aureus* (ATCC 25923) (representing Gram-positive bacteria) selected on the basis of their pathogenicity to cause frequent and serious infections in humans were used for the study. These bacterial samples were kindly supplied by the Ethiopian Biodiversity Institute (EBI), Addis Ababa, Ethiopia.

2.3.2. Preparation of Test Solutions

The crude extracts were further diluted to make three different concentrations in separate flasks to get working stock solutions of 100, 200 and 300 mg/ml concentrations. These working solutions were prepared by transferring a given mass (100 mg, 200 mg or 300 mg) of each extract to sterile test tubes, each containing 1 ml of 3% Tween 20 to give a concentration of 100 mg/ml, 200 mg/ml or 300 mg/ml, respectively; the stocks were stored at 4ºC until use.

2.3.3. Antibacterial Tests

The disk diffusion method was employed to evaluate the antibacterial activity of the plant extract according to procedures described in ref. [6] with some modification. Paper disks with approximate diameter of 6 mm were punched out from a sheet of absorbent filter paper, and were sterilized in an autoclave at 121°C for 1 h. Each bacterial strain was made to grow and activated on its selective medium in the plate: MacConkey agar for *Klebsiella pneumoniae* and Mannitol-Salt agar for *Staphylococcus aureus*, and were incubated at 37°C for 24 h.

Few colonies of each strain were transferred with a sterile inoculating loop to a nutrient broth until turbidity is adjusted to that of McFarland 0.5 turbidity standard. Two groups of plates containing Muller-Hinton agar were prepared where the two bacterial strains were streaked using sterile cotton swabs. One group of plates was used for testing acetone extract and the other one for ethanol extract. The external surface of each plate was divided into five parts as each confine five paper discs: three discs containing extracts at different concentrations, one for the positive control and the remainder as negative control.

The disks were loaded with 50 µL of the crude extract from each of the three measured concentration, at a separate quadrant of each plate. On the other two quadrants, one disk containing 30 µL of 2.5mg/ml Tetracycline, and a disk immersed in 1ml of 3% Tween 20, each, was kept as positive and negative controls respectively. All plates were then incubated at 37°C for 24 h after which, the zone of inhibition for each was checked, and the diameter was measured by ruler (in mm) and recorded. The test was done in triplicate and the result was expressed as average value of zone of inhibition (ZOI) of each plant extract.

2.3.4. Determination of Minimum Inhibitory Concentrations (MIC)

The MIC of the crude extract was determined according to methods in [6]. The disk diffusion method was employed as in the susceptibility tests, except that the disks were immersed in each prepared concentration of the samples, and each crude was tested at concentrations of 300, 150, 75, 37.5, 18.75, 9.375 and 4.6875 mg/ml. The MIC was taken as the highest dilution (lowest concentrations) of the extract that prevented the observable growth of the bacteria after a period of 24 h of incubation at 37ºC, as detected by an unaided eye, and was recorded, and all the testes were performed in triplicate.

2.3.5. Data Analysis and Interpretation

All the data obtained from the experimental result were recorded by measuring (in mm) zones of growth inhibition (ZOI) by the controls and of each crude extract on each

bacterium, then taking the average (Mean ± standard error of the mean (SEM)) value of three tests. The results were compared by using one-way analysis of variance (ANOVA)/Tukey's Honesty Significant Difference (HSD) test, with 95% confidence intervals (CI) where P-value is less than 0.05 showing significant difference.

3. RESULTS AND DISCUSSIONS

Despite much effort towards the management of infectious diseases by antibiotics, subsequent antimicrobial resistance, cost and the widespread side effects associated with conventional drugs poses a need for new antimicrobial agents, and the onus is on scientists to find solutions. In reviewing the literature, plants are reported to have bioactive compounds, which support their use in traditional medicine, and can serve as sources drugs. Prior studies reported the presence of a variety of bioactive phytoconstituents in various parts of *Tamarindus indica* as being associated with so many well-known health benefits in traditional medicine including wound healing, snake bite, abdominal pain, inflammations, helminth infections, antimicrobial as well as antidiabetic, which can be the possibility of application in the pharmaceutical industry, and that was therefore the basis for the current study that evaluated the effect of leaf crude extracts on *S. aureus* and *K. pneumonia* [11, 14, 7, 20]. Tetracycline (the reference drug) was used in this study, and was chosen because it is a broad spectrum that is effective against both aerobic and anaerobic gram-positive and gram-negative pathogens and some protozoa.

Subsequent extraction of the *Tamarindus indica* leaf resulted in two different crude extracts, namely, leaf acetone extract (LAE) and leaf ethanol extract (LEE) with the respective crude mass and percent yields 6.3 g (6.3%) and 8.0 g (9.2%), which revealed variation in the crude mass obtained as the ethanol produced more crude than acetone. This result is consistent with the work of [15] whose work involved acetone, chloroform and ethanol, with higher yield obtained from ethanol followed by acetone. The results are also in agreement with those obtained by ref. [23], in which they worked with hexane, ethyl acetate, ethanol, methanol and water, and obtained the lowest yield in hexane, which is less polar than the rest of the solvents. Therefore, the difference in the polarity of the solvents could be a major factor, if not the only one, causing the variation in dissolution of the sample, hence, ethanol is more polar than the acetone, and the yield of the former is higher than that of the later. However, with only two solvents, caution must be applied, as the findings might not be generalizable to a broader range of organic and inorganic solvents used in extraction processes.

The antibacterial activity of the extract against *S. aureus* and *K. pneumoniae* is presented in Table 1, and interestingly the findings indicated that the extracts from both the acetone and ethanol showed activity on the test microorganisms. The common tetracycline capsule, which served as positive controls, also inhibit the growth of test bacterial pathogens at a concentration of 2.5 mg/ml with maximum mean inhibition values of 15.67±0.67 and 16.33±0.88 mm against *S. aureus* and *K. pneumoniae,* respectively.

The results of this study (Table 2) indicated that the mean zones of growth inhibitions of the LAE against *S. aureus* at 100, 200 and 300 mg/ml concentrations are 12.67±0.33, 13.33±0.33 and 15.00±0.57 mm, respectively. Accordingly, the results showed better inhibitory activities as compared to the negative control (3% Tween 20), and the ANOVA (one way) analysis indicated significant different from it (P = 0.00). Though the activity of the extract on the test organism increased with increase in concentration, the Turkey post hoc tests however, indicated that these results were not significantly different from each other, however, multiple comparison with the positive control (drugs) revealed that it is the highest concentration (300 mg/ml) which was insignificantly different (P = 0.82).

Table 1. Qualitative presentation of growth inhibitory level of leaf 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	*Klebsiella pneumonia*	
Tween 20	1 ml	-	-	
Drug	2.5 ml	++++	++++	Drug Used: Tetracycline
LAE	100 mg/ml	+	++	
	200 mg/ml	++	+++	
	300 mg/ml	+++	++++	
LEE	100 mg/ml	++	++	
	200 mg/ml	+++	+++	
	300 mg/ml	++++	++++	

Key: LAE = Leaf Acetone Extract, LEE = Leaf Ethanol Extract; - = No effect, + = Weak effect, ++ = Moderate effect, +++

= Strong effect, and ++++ =Very 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 pneumonia*	
		Mean ± SEM	P-value	Mean ± SEM	P-value
Drug	2.5 mg/ml	15.67 ± 0.67[a]	-	16.33 ± 0.88[a]	-
LAE	100 mg/ml	12.67 ± 0.33[b]	0.00	15.33 ± 0.88[a]	0.93
	200 mg/ml	13.33 ± 0.33[b]	0.02	16.67 ± 1.20[a]	0.99
	300 mg/ml	15.00 ± 0.57[a]	0.82	20.00 ± 1.15[a]	0.10
Drug	2.5 mg/ml	15.33 ± 0.33[a]		17.00 ± 0.57[a]	
LEE	100 mg/ml	12.33 ± 0.33[b]	0.00	13.67 ± 0.33[b]	0.00
	200 mg/ml	14.33 ± 0.33[a]	0.20	16.00 ± 0.57[a]	0.62
	300 mg/ml	15.67 ± 0.33[a]	0.92	17.67 ± 0.67[a]	0.86

Key: LAE = Leaf Acetone Extract; LEE = Leaf Ethanol Extract. Mean values with different superscripts in the same column are significantly different.

Table 3. Minimum Inhibitory Concentration of crude extracts from the leaf and fruit pulp of *Tamarindus indica.*

Test material	Concentration Level	Inhibitory activity in mm	
		Test bacteria	
		Staphylococcus aureus	*Klebsiella pneumonia*
LAE	4.6875 mg/ml	-	-
	9.375 mg/ml	-	+
	18.75 mg/ml	+	+
	37.5 mg/ml	+	++
	75 mg/ml	++	+++
	150 mg/ml	++	+++
	300 mg/ml	+++	++++
LEE	4.6875 mg/ml	-	-
	9.375 mg/ml	-	-
	18.75 mg/ml	+	+
	37.5 mg/ml	++	++
	75 mg/ml	++	++
	150 mg/ml	++	+++
	300 mg/ml	+++	++++

Key: LAE = Leaf Acetone Extract; LEE = Leaf Ethanol Extracts. - = No effect, + = Weak effect, ++ = Moderate effect, +++ = Strong effect, and ++++ = Very strong effect

Similarly, the mean zones of growth inhibitions recorded against *K. pneumoniae* at concentrations of 100, 200 and 300 mg/ml were 15.33±0.88, 16.67±1.20 and 20.00±1.15 mm, respectively, and having no significant different from the drug (P = 0.93, 0.99 and 0.10, respectively) as well as from each other (Table 2), but significantly different from the negative control (P = 0.00).

With respect to the LEE, the results indicate that both the test bacteria were sensitive to the extract (Table 1). Accordingly, the mean zone of growth inhibition against *S. aureus* at concentrations of 100, 200 and 300 mg/ml were respectively 12.33±0.33, 14.33±0.33 and 15.67±0.33 mm, whereas against *K. pneumoniae* it showed 13.67±0.33, 16.00±0.57 and 17.67±0.67 mm at 100, 200 and 300 mg/ml concentrations, respectively (Table 2). The most striking observation to emerge from the data comparison for both the test bacteria was the significant difference between the extract and the negative control (3% Tween 20) (P = 0.00). While there was no statistically significant difference in the mean scores of these groups observed between each other, the comparison of the crude with the drug however indicated that only the higher concentrations (200 and 300 mg/ml) that

showed no significant differences ($P > 0.05$) (Table 2). As with the LAE, the effects of extracts on the growth of test bacterial pathogens were concentration dependent; that is, the higher degree of inhibition was observed from increased concentration of extracts, which might be due to the increased availability of the antibacterial compounds in the media. With both LAE and LEE effective, the result revealed that dilutions of various concentrations from each extract can inhibit the growth of the investigated bacteria, (*S. aureus* and *K. pneumoniae*) (Table 3). Accordingly, *K. pneumoniae* showed more sensitivity to the LAE, having MIC value at 9.375 mg/ml than *S. aureus'* 18.75 mg/ml MIC value; however, the MIC value of LEE for both the test bacteria (*S. aureus* and *K. pneumoniae*) was recorded at 18.75 mg/ml concentration. Taken together, these results suggest that the leaves have antibacterial properties.

The results of this study broadly support the work of other studies in this area linking the crude extracts from the leaf with their action on microorganisms, though the extraction solvents and the test organisms involved could vary. Consistent with the literature, this investigation matches those observed in earlier studies which reported that the aqueous and methanol extract of *T. indica* leaves have activity against *E. coli* and *Shigella sp* [20]. The results are also in agreement with the work of ref. [26], which testified that the methanol, acetone, benzene and hexane leaf extracts have activity against *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginasa*, *Pseudomonas aeromonas* and *Staphylococcus aureus*. They also are in line with those of previous studies by [21], which reported that the aqueous and alcoholic (ethanol and methanol) extracts of leaf were highly susceptible on *Klebsiella sp*., *E.coli, Staphylococcus sp*., *Pseudomonas sp*., and *Bacillus sp*., and are in accord with recent studies by ref. [29], which investigated the leaf chloroform and methanol extracts on *E. coli, S. aureus* and *P. mirabilis* and revealed antibacterial activity, just to mention a few. According to these data, it can be inferred that the combinations of findings offer the potential usefulness of *T. indica* and backing the validation for involvement in drug discoveries.

Comparing the efficiency of the crude, each extract showed high level of effectiveness on the test bacteria; however, it is the acetone extract that showed higher antibacterial activity than that of ethanol (Table 2). This also accords with earlier observations in ref. [25], who worked on acetone, methanol, chloroform and aqueous *T. indica* leaf extracts against bacteria that cause urinary tract infection, namely, *E. coli, P. aeroginosa, Klebsiella sp*., and *Enterococcus sp*., and which reported the highest antibacterial activity from the acetone extract on all the test pathogen. In regard to the test bacteria, *K. pneumoniae* showed more sensitivity to the crude than *S. aureus* (Table 2).

4. CONCLUSION

The present study demonstrates that crude extracts from *Tamarindus indica* leaves exhibit significant antibacterial activity against both *Staphylococcus aureus* and *Klebsiella pneumoniae*, supporting their traditional medicinal use. The acetone and ethanol extracts displayed concentration-dependent inhibition, with *K. pneumoniae* showing higher sensitivity than *S. aureus*. Notably, the acetone extract exhibited superior efficacy, particularly against *K. pneumoniae*, with an MIC of 9.375 mg/mL compared to 18.75 mg/mL for the ethanol extract. These results align with prior studies on *T. indica* and other medicinal plants, reinforcing the potential of plant-derived compounds as alternatives to conventional antibiotics in the face of growing antimicrobial resistance. While this study did not identify the specific phytochemicals responsible for the observed antibacterial effects, the findings suggest the presence of bioactive compounds such as tannins, flavonoids, or alkaloids, which are commonly associated with antimicrobial properties in plants. The comparable efficacy of the extracts to tetracycline underscores their therapeutic potential, though further research is essential to isolate and characterize the active constituents. Additionally, mechanistic studies are needed to determine whether the extracts act through cell wall disruption, protein synthesis inhibition, or other pathways. The limitations of this study include the absence of phytochemical profiling and *in vivo* validation, which are critical for translating laboratory findings into clinical applications. Future investigations should explore the toxicity, pharmacokinetics, and synergistic effects of *T. indica* extracts with existing antibiotics. Moreover, expanding the scope to include a broader range of bacterial strains and solvent systems could enhance the generalizability of the results. This study provides empirical evidence supporting the ethnomedicinal use of *T. indica* leaves as an antibacterial agent. It contributes to the growing body of research on plant-based antimicrobials and paves the way for future drug development efforts aimed at combating antibiotic-resistant infections. Collaborative efforts between ethnobotanists, pharmacologists, and biomedical researchers are essential to harness the full therapeutic potential of this 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.

Ethical Considerations

Two ethical clearances, one for purchasing the chemicals and media, and the other for bacterial samples were both obtained from the department of Biology, Dilla University and were

submitted to the respective institutions. After reception and consideration of the ethical clearance by the Kezen Trading Plc. Head Office, the chemicals and media were allowed to be purchased from the company, whereas the clearance for sample bacteria was submitted to the Ethiopian Biodiversity Institute, who kindly supplied the needed strains.

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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 to this work.

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REFERENCES

[1] Fleming A. **1929.** On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae. *British Journal of Experimental Pathology*, 10(3):226-236.

[2] Levy SB, Marshall B. **2004.** Antibacterial resistance worldwide: causes, challenges and responses. *Nature Medicine*, 10(12 Suppl), pp. S122-129.

[3] Rios AC, Moutinho CG, Pinto FC, et al. **2016.** Alternatives to overcoming bacterial resistances: State-of-the-art. *Microbiological Research*, 191, pp. 51-80.

[4] Davies, O.L. and Bennett, S., **2017.** WHO publishes list of bacteria for which new antibiotics are urgently needed. *WHO Newsletters*.

[5] Talebi ABA, Rizvanov AA, Haertlé T, Blatt NL. **2019.** World Health Organization Report: Current Crisis of Antibiotic Resistance. *BioNanoScience*, 9(4), pp. 778-788.

[6] Tafesse, G., Mekonnen, Y., Makonnen, E., Majinda, R.R., Bojase-Moleta, G. and Yeboah, S.O., **2018**. Antibacterial activity of crude extracts and pure compounds isolated from Vernonia galamensis leaves. *African Journal of Pharmacy and Pharmacology*, *12*(11), pp.136-141.

[7] Helen Bitew, Haftom Gebregergs, Tuem KB, Mariamawit Y. **2019.** Ethiopian medicinal plants traditionally used for wound treatment: A systematic review. *Ethiopian Journal of Health Development*, 33(2), pp. 102-127.

[8] Njeru SN, Matasyoh J, Mwaniki CG, et al. **2013.** A Review of some Phytochemicals commonly found in Medicinal Plants. *International Journal of Medicinal Plants*, 105, pp. 135-140.

[9] Pagare S, Bhatia M, Tripathi N, et al. **2015.** Secondary Metabolites of Plants and their Role: Overview. *Current Trends in Biotechnology and Pharmacy*, 9(3), pp. 293-304.

[10] Yuan H, Ma Q, Ye L, Piao G. **2016.** The traditional medicine and modern medicine from natural products. *Molecules*, 21(5), pp. 559-577.

[11] Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F. **2020.** Worldwide research trends on medicinal plants. *International Journal of Environmental Research and Public Health*, 17(10, pp. 3376-3396.

[12] Rahman MM, Shahriar MR, Meghla NS, et al. **2018.** Antimicrobial activity of some medicinal plant extracts against Gram positive and Gram negative bacteria in Bangladesh. *Asian Journal of Medical and Biological Research*, 3(4), pp. 405-411.

[13] Stokes JM, Yang K, Swanson K, et al. **2020.** A Deep Learning Approach to Antibiotic Discovery. *Cell*, 180(4), pp. 688-702.

[14] Muluken Wubetu, Tefera Abula, Getye Dejenu. **2017.** Ethnopharmacologic survey of medicinal plants used to treat human diseases by traditional medical practitioners in Dega Damot district, Amhara, Northwestern Ethiopia. *BMC Research Notes*, 10(1), pp. 157-170.

[15] Netsanet Gonfa, Dereje Tulu, Kitessa Hundera, Dasalegn Raga. **2020.** Ethnobotanical study of medicinal plants, its utilization, and conservation by indigenous people of Gera district, Ethiopia. *Cogent Food & Agriculture*, 6(1), pp. 1-25.

[16] Behailu Assefa, Moa Megersa, Tilahun Tolossa J. **2021.** Ethnobotanical study of medicinal plants used to treat human diseases in Gura Damole District, Bale Zone, Southeast Ethiopia. *Asian Journal of Ethnobiology*, 4(1), pp. 42-52.

[17] Pramila G, Jirekar D. **2021.** Tamarindus indica: An important medicinal plants. *International Journal of Innovative Research in Technology*, 8(4), pp. 321-325.

[18] Kuru P. **2014.** Tamarindus indica and its health related effects. *Asian Pacific Journal of Tropical Biomedicine*, 4(9), pp. 676-681.

[19] Menezes APP, Trevisan SCC, Barbalho M, Guiguer EL. **2016.** Tamarindus indica L. A plant with multiple medicinal purposes. *Journal of Pharmacognosy and Phytochemistry*, 5(3), pp. 50-54.

[20] Abdallah MS, Muhammad A. **2018.** Antibacterial activity of leaves and fruits extract of Tamarindus indica against clinical isolates of Escherichia coli and Shigella at Potiskum Yobe State, Nigeria. *Journal of Analytical & Pharmaceutical Research*, 7(5), pp. 606-609.

[21] Dorcas K, Palakodeti P, Yadav H, Yerra V, Majji V. **2020.** Antibacterial Properties of Tamarindus. *International Journal of Recent Scientific Research*, 11(05), pp. 38618-38621.

[22] Reygaert WC. **2018.** An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology*, 4(3), pp. 482-501.

[23] Mariah NM, Mourine K, Christine B. **2021.** Qualitative and quantitative phytochemical profiling of crude fractions of Pechuel-Loeschea leubnitziae leaves. *Journal of Medicinal Plants Research*, 15(2), pp. 64-72.

[24] Gomathinayagam S, Tewari BB, Rekha G. **2017.** Heavy metal and phytochemical studies of crude leaf extract of tamarind (Tamarindus indica). *Advances in Life Sciences*, 7(1), pp. 1-4.

[25] Biswas K, Sinha SN. **2016.** Antimicrobial activity of Tamarindus indica L. against bacteria causing urinary tract infection. *Journal of Biological Science*, 23, pp. 47-55.

[26] Maajitha BA, Muhammad IMH, Shijila RAS, Ambikapathy V. **2019.** Evaluation on antibacterial activity of Tamarindus indica against bacteria. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, 5(1), pp. 639-646.

[27] Geremew Tafesse, Yalemtsehay Mekonnen, Eyasu Makonnen, et al. **2018.** Antibacterial activity of crude extracts and pure compounds isolated from Vernonia galamensis leaves. *African Journal of Pharmacy and Pharmacology*, 12(11), pp. 136-141.

[28] Mesay Bahiru, Geremew Tafesse, Chauhan NM, Ermias Assefa. **2020.** Antimicrobial activity of crude extract from Millettia ferruginea leaves and barks against selected bacterial pathogens and Candida albicans. *Journal of Microbiology and Antimicrobials*, 12(2), pp. 81-87.

[29] Babangida MI, Bako SP, Iortsuun DN. **2021.** Phytochemical screening and *In vitro* antibacterial effect of Tamarindus Indica L. stem and leaves On E. coli, S. aureus and P. mirabilis. *International Journal of Scientific and Research Publications*, 11(3), pp. 145-148.

[30] Kim H, Song J. **2019.** Computational prediction method of antibiotics resistance by PK/PD algorithms using bioinformatics software. *Advances in Biotechnology*, 14(2), pp. 41-52.

