JCARM
Journal of Contemporary Academic Research and Methodologies

An Online Peer Reviewed / Refereed Journal
Volume 1 | Issue 4 | May 2026
Website: www.journal.jcarm.in

Research

Phytochemical Screening and Antibacterial Activity of Tamarind (Tamarindus Indica) Against Escherichia coli Isolated from Random Stool Sample

Suleiman Shafiu Ango[1]*, Chikwendu Lilian[2], Jimoh Mustapha Titilope[3]

[1]Department of Microbiology, Federal University Gusau, Zamfara State, Nigeria.
https://orchid.org/0009-0005-9282-772X2
[2]Department of Microbiology, Federal University Gusau, Zamfara State, Nigeria.
https://orchid.org/0009-0001-0804-9330
[3]Department of Microbiology, Federal University Gusau, Zamfara State, Nigeria.
https://orchid.org/0009-0005-9179-1542

Correspondence should be addressed to: suleimanshafiu08@gmail.com

Abstract: Tamarindus indica, commonly known as tamarind, is a tropical tree that has been traditionally used in various medicinal preparation. This present study was aimed to investigate the photochemical constituents and antibacterial activity of aqueous and ethanolic extract of Tamarindus indica leaves extract against Escherichia coli. The phytochemical analysis revealed the presence of alkaloids, saponins, flavonoids, terpenoids, and absence of glycoside and steroids. The antibacterial activity of the extract was carried out using agar well diffusion method. The antibacterial activities of aqueous and ethanolic extracts of Tamarindus indica leaf at concentrations of 100, 50, 25, 12.5 mg/ml showed the zone of inhibition for Escherichia coli in response to the extracts ranged from 14-9 mm and 18-10 mm respectively. The ethanolic extract shows the maximum zone of inhibition of (18mm at concentration of 100mg/ml) while the aqueous extract shows the least inhibitory effect on test isolates (9 mm at concentration of 12.5 mg/ml). The MIC of aqueous and ethanolic leaves extract on E. coli is 12.5 mg/ml. The MBC of aqueous and ethanolic leaves extract on E. coli is 100 mg/ml and 50 mg/ml respectively. This result indicates that Tamarindus indica leave have antibacterial activities against Escherichia coli due to some secondary metabolites it possesses. Therefore, T. indica leaves could be promising natural antibacterial agent with potential applications in the pharmaceutical industries for controlling of pathogenic bacteria used in this work.

Keywords: Phytochemical, Antibacterial, Bactericidal, Resins.

https://doi.org/10.5281/zenodo.20604774

Introduction

Tamarindus indica, commonly known as the tamarind tree, is a monotypic leguminous species within the subfamily Detarioideae (family Fabaceae). The name is a medieval Latinization of the Arabic *tamr hindi*, meaning 'Indian date' (Lal *et al.*, 2023). While likely indigenous to tropical Africa, the tree has been cultivated in the Indian subcontinent for millennia, leading to its widespread distribution across the tropics, including Australia, Oceania, Southeast Asia, and China. Following its introduction by Spanish and Portuguese colonists in the 16th century, it became a dietary staple in Mexico, Central America, and parts of South America (Avalos *et al.*, 2024). Today, India remains the leading global producer (Mishra *et al*., 2024).

Morphologically, *T. indica* is a large, widely spreading tree reaching heights of 60–80 feet. It features dark, rough bark, paripinnate leaves, and yellow, boat-shaped flowers (Xue *et al*., 2026). The fruit is characterized by a moist, reddish-brown, sticky pulp with a sweet-acidic flavor and distinctive yellowish-brown fibers (Alizadeh and Habibzadeh, 2024).

The plant is highly valued across the pharmaceutical, food, chemical, and textile industries. Traditionally, various parts of the tree have been utilized to treat ailments ranging from abdominal pain, diarrhea, and dysentery to malaria, inflammation, and skin infections (Poddar *et al.*, 2020). Phytochemical analyses have confirmed its diverse therapeutic properties, including antimicrobial, antioxidant, hepatoprotective, and anti-hyperlipidemic activities.

Herbal medicine remains a cornerstone of healthcare for 75–90% of the world's rural population (Sen *et al.,* 2011). In recent years, the limitations of synthetic drugs specifically the rise of antibiotic-resistant pathogens have sparked a global resurgence in the use of medicinal plants (AlSheikh *et al*., 2020). *T. indica* is particularly notable in this regard; its leaves are rich in proteins, fats, fibers, and vitamins, alongside bioactive flavonoids and polyphenols that provide significant antimicrobial potential (Kuru, 2014).

In Northern Nigeria, the stem bark and leaves are frequently prepared as decoctions, often combined with potash, to address conditions such as jaundice, yellow fever, stomach disorders, and general body pain, as well as to serve as blood tonics and skin cleansers (Osuntokun, 2021). As modern science continues to validate these traditional practices, *Tamarindus indica* remains a vital candidate for new drug development, offering a natural and accessible alternative to synthetic antibacterial agents.

Methodology

Study area

This study was conducted in Gusau, the capital of Zamfara State, located at latitude 13°349' N and longitude 5°1489' E, at an elevation of 272 meters above sea level. The local economy is primarily driven by agriculture, trade, fishing, and leather craftsmanship. Geographically, the area is situated within a dry Sudan savannah region characterized by sandy landscapes and scattered hills. The climate follows a distinct seasonal pattern, with rainfall occurring from June to early September, occasionally extending into October; the region receives an average annual rainfall of 550 mm, with peak precipitation in August. The hottest period occurs between November and February, when temperatures can reach a maximum of 45°C.

Plant sample collection

Tamarindus indica leaves were harvested from the campus of the Federal University Gusau. The samples were immediately placed in sterile polythene bags to ensure integrity and transported to the university microbiology laboratory for processing and analysis.

Preparation of plant material

The harvested fresh leaves were thoroughly cleaned under running tap water to remove surface dust, followed by a final rinse with distilled water. The samples were then air-dried at room temperature for two weeks. Once completely dried, the leaves were pulverized into a fine powder using a laboratory mortar and pestle. The resulting powder was stored in a sealed polythene bag for subsequent analysis (Różyło *et al*., 2022).

Preparation of Leaf extracts

Approximately 40 grams of the powdered leaves were macerated separately in 400 ml of distilled water and ethanol at room temperature for four days, with the mixtures agitated consistently (Vongsak *et al.,* 2013). The resulting preparations were then decanted and filtered through muslin cloth. The filtrates were evaporated to dryness using a water bath, and the residues were stored in clean, sterile plastic containers, shielded from sunlight until needed for experimental procedures (Abubakar and Haque, 2020). This prepared material served as the basis for both the phytochemical and antibacterial analyses.

Phytochemical Screening

Phytochemical screening was performed at the Microbiology Laboratory of the Federal University Gusau to identify the presence or absence of specific bioactive compounds, including alkaloids, saponins, glycosides, flavonoids, terpenoids, and tannins, following the methodology established by Alamgir (2018).

Phytochemical Protocol

Test for Tannins

Two (2) ml of filtrate was added in a clean test tube, few drops (5-6) of 0.1 % ferric chloride solution were added. The reaction mixture was observed for a brownish green or blue-black coloration for the confirmation of the presence of tannins.

Test for Saponin

Ten (10) ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth and then observed for the formation of an emulsion, indicative of the presence of saponin.

Test for Terpenoids

Small portion of filtrate was mixed with 2ml of chloroform, and concentrated H2SO4, (3) ml was carefully added to form a layer. A reddish-brown coloration formed at the interface indicated the presence of terpenoids.

Test for Cardiac Glycosides

Small portion of filtrate was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was overlaid with 1ml of concentrated H_2SO_4, a brown ring at the interface indicates the glycosides.

Test for Flavonoids

Small portion of filtrate was treated with 5ml of dilute ammonia solution, followed by the addition of concentrated H_2SO_4. A yellow colouration indicates the presence of flavonoids.

Test for Alkaloids (Mayer's test)

Two drops of Mayer's reagent were added to 5cm3 of the leaf extract and the solution was shaken vigorously in a test tube. A cream-colored precipitate indicates the presence of alkaloids.

Determination of Microbiological properties

Media Preparation

The culture medium employed was Eosin-Methylene Blue (EMB) agar. To prepare it, 15 grams of the agar powder were dissolved in sterile distilled water within a conical flask, brought to a boil, and autoclaved at 121°C. Once sufficiently cooled, the medium was poured into sterile Petri dishes and left to solidify.

Sample Collection and Inoculation

Three fresh stool samples were collected in sterile containers. A small portion of each sample was transferred using a sterile inoculating loop and spread across the surface of the EMB agar plates using the streaking method. The plates were then incubated at 37°C for 24 hours. Following incubation, the resulting colonies were sub-cultured onto fresh media to facilitate precise identification and characterization.

Bacterial Identification

Organisms were identified based on their growth characteristics, Gram reaction, and biochemical profiles (Bullock and Aslanzadeh, 2012).

Gram staining procedure

A smear of the isolate was air-dried on a grease-free slide and heat-fixed. Following the Gram staining protocol crystal violet, Lugol's iodine, alcohol decolorization, and safranin. The slide was examined under a 100x objective lens.

Biochemical test

To confirm the identity of the bacterial isolates, a series of biochemical tests were conducted which include: Indole test, Methyl Red Test, Voges Proskauer and Citrate utilization test.

Antimicrobial activity

Preparation of Extract Concentrations

To prepare the test solutions, 0.2 grams of the plant extract was dissolved in 2 ml of dimethyl sulfoxide (DMSO) to create the stock solution. From this, four serial concentrations—12.5 mg/ml, 25 mg/ml, 50 mg/ml, and 100 mg/ml were prepared for use (Naz *et al*., 2017).

Antibacterial Susceptibility Testing (Agar Well Diffusion)

The agar well diffusion method was employed to evaluate the antibacterial activity of the extracts, with amoxicillin serving as the positive control. Bacterial suspensions were adjusted to a 0.5 McFarland turbidity standard and uniformly seeded onto Mueller-Hinton agar plates using sterile swabs. Once the agar surface was set, 8 mm wells were created using a sterile cork borer. These wells were filled with the various extract concentrations and the antibiotic control. The plates were left for one hour to allow for pre-diffusion before being incubated at 37°C for 24 hours. Following incubation, the zones of inhibition were measured in millimeters using a ruler (Naz *et al*., 2017).

Minimum Inhibitory Concentration (MIC)

The MIC was determined using the broth dilution technique to identify the lowest concentration of extract capable of preventing visible bacterial growth. A two-fold serial dilution of the extract was prepared in a liquid medium, resulting in concentrations of 50, 25, 12.5, 6.25, and 3.125 mg/ml. Each tube was inoculated with 0.2 ml of the standardized bacterial suspension (0.5 McFarland) and incubated at 37°C for 24 hours. The lowest concentration demonstrating no turbidity (growth) was recorded as the MIC for *Escherichia coli*.

Minimum Bactericidal Concentration (MBC)

The MBC test was conducted to determine if the extracts were bacteriostatic (inhibitory) or bactericidal (lethal). Following the MIC assay, the contents of the tubes showing no growth were sub-cultured onto nutrient agar plates and incubated at 37°C for 24 hours. The MBC was defined as the lowest concentration of the extract that yielded no visible bacterial colonies upon sub-culturing (Naz *et al*., 2017).

Results

The phytochemical screening of *Tamarindus indica* leaves (Table 1) revealed the presence of flavonoids, saponins, tannins, terpenoids, alkaloids, and phenols, while resins, steroids, and glycosides were absent. The bacterial isolate, identified as *E. coli* through Gram reaction and biochemical testing (Table 2), was subsequently subjected to susceptibility testing. The antibacterial efficacy of the leaf extracts was assessed using the agar well diffusion method (Table 3). The results demonstrated that *Tamarindus indica* inhibits *E. coli* growth, with ethanolic extracts consistently exhibiting higher activity than aqueous extracts across all tested concentrations. The potency of these extracts was further quantified by determining the Minimum Inhibitory Concentration (MIC) required to prevent bacterial growth (Table 4) and the Minimum Bactericidal Concentration (MBC) required to achieve complete bacterial elimination (Table 5).

Table 1: Phytochemical screening of Tamarindus indica leave extract.

S/N	PHYTOCHEMICAL CONSTITUENTS	ETHANOL	AQUEOUS
1.	Alkaloids	+	+
2.	Flavonoids	+	+

3.	Saponins	+	+
4.	Tannins	+	+
5.	Terpenoids	+	+
6.	Steroids	-	-
7.	Glycoside	-	-
8.	Resins	+	-

Key: + = Presence of phytochemicals, - = Absence of phytochemicals

Table 2: Gram reaction and biochemical tests of the isolate

s	**l red**	**proskauer**	**:**	**n/Shape**
: 1				
: 2				

Table 3: Antibacterial activity of aqueous and ethanolic leaf extract of Tamarindus indica against E. coli

Concentrations (mg/ml) / Zones of Inhibition (mm)

Isolate	**Extracts**	**12.5**	**25**	**50**	**100**	**Amoxicillin(10πg)**
E. coli 1	Aqueous	9	11	12	14	26
E. coli 2		9	10	13	15	28
E. coli 1	Ethanol	10	14	15	17	26
E. coli 2		10	12	15	18	28

Table 4: The minimum Inhibitory Concentrations(mg/ml) of Tamarindus indica (Tamarind) leaves extract against E. coli

Test Extracts	**Concentration of extracts (mg/ml)**					
Isolates	100	50	25	12.5	6.5	3.125
E. coli						
Ethanol Extract	_	_	_	ox	+	+
Aqueous Extracts	_	_	_	ox	+	+
E. coli2						
Ethanol Extract	_	_	_	ox	+	+
Aqueous Extracts	_	_	ox	+	+	+

Key: - = Absence of growth, + = Presence of growth, mg = Milligram, ml = Milliliter, ox = MIC.

Table 5: Minimum bactericidal concentrations MBC of the extracts of Tamarindus indica (Tamarind) leaves extracts against Escherichia coli.

Test Extracts	**Concentration of extracts (mg/ml)**					
Isolates	100	50	25	12.5	6.25	3.125
E. coli 1						
Ethanol Extract	+	Ox	+	+	+	+
Aqueous Extracts	Ox	+	+	+	+	+
E. coli 2						

Ethanol Extract	_	O x	+	+	+	+
Aqueous Extracts	Ox	+	+	+	+	+

Key: - =Absence of growth, + = Presence of growth, Ox = MBC, mg = Milligram, ml = Milliliter.

Discussion

This study evaluated the phytochemical profile and antibacterial efficacy of *Tamarindus indica* leaf extract against *Escherichia coli*. Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, tannins, and terpenoids compounds widely recognized for their antimicrobial properties Abdallah and Muhammad (2018). These results align with findings by Abdallah and Muhammad (2018), who noted that *T. indica* is highly effective against gastrointestinal pathogens, including *E. coli*. Regarding extraction efficacy, the ethanolic extract demonstrated superior antibacterial activity compared to the aqueous extract. This difference is likely due to ethanol's enhanced capacity to dissolve and extract bioactive secondary metabolites, a finding consistent with established research on solvent-dependent extraction Abdallah *et al*., (2018).

The agar well diffusion assay revealed clear zones of inhibition, indicating sensitivity to the extracts. Following the criteria set by Cheesbrough (1984), where a zone of inhibition >3mm indicates sensitivity, the *E. coli* isolate showed significant susceptibility. The activity was dose-dependent, with the 100mg/ml concentration yielding the largest zone of inhibition and the 12.5mg/ml concentration the smallest. Furthermore, MIC and MBC results confirmed that the extracts possess both bacteriostatic and bactericidal capabilities, reinforcing the potential of *T. indica* as a viable alternative against Gram-negative pathogens.

Conclusion

This research confirms that *Tamarindus indica* leaf extract contains significant bioactive compounds specifically alkaloids, flavonoids, saponins, tannins, and terpenoids that provide potent antibacterial activity against *E. coli*. The study demonstrates a clear dose-dependent relationship, where higher concentrations (up to 100mg/ml) result in more substantial inhibitory and bactericidal effects. The determination of MIC and MBC values further validates that the extract can effectively inhibit and kill *E. coli*. These findings underscore the clinical potential of *T. indica* as a natural, plant-based therapeutic agent. By validating traditional uses through scientific analysis, this study highlights the importance of continued research into botanical remedies as a sustainable strategy for managing microbial infections in the face of rising antibiotic resistance.

References

1. Abdallah, M. S. and 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. J Anal Pharm Res, 7(5): 606-609.

2. Abubakar, A. R. and Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences, 12(1): 1-10.

3. Alamgir, A. N. M. (2018). Secondary metabolites: Secondary metabolic products consisting of C and H; C, H, and O; N, S, and P elements; and O/N heterocycles. In Therapeutic use of medicinal plants and their extracts: volume 2: phytochemistry and bioactive compounds (Pp. 165-309). Cham: Springer International Publishing.

4. Alizadeh, M. and Habibzadeh, L. (2024). Pomegranate grafting: Optimization of technique and evaluation of fruit traits affected by cultivated and wild rootstocks. Scientia Horticulturae, 338: 113786.

5. AlSheikh, H. M. A., Sultan, I., Kumar, V., Rather, I. A., Al-Sheikh, H., Tasleem Jan, A., & Haq, Q. M. R. (2020). Plant-based phytochemicals as possible alternative to antibiotics in combating bacterial drug resistance. Antibiotics, 9(8): 480.

6. Avalos, G. L., Carpena, J. G. R., Paz, W. O. B., Flores, C. L. and Gasca, C. A. C. (2024). Exploring the genetic of three Hairless Pig breed populations in Mexico. Revista De La Facultad De Agronomia De La Universidad Del Zulia, 41(3).

7. Bullock, N. O. and Aslanzadeh, J. (2012). Biochemical profile-based microbial identification systems. In Advanced techniques in diagnostic microbiology (pp. 87-121). Boston, MA: Springer US.

8. Kuru, P. (2014). Tamarindus indica and its health-related effects. Asian Pacific Journal of Tropical Biomedicine, 4(9): 676-681.

9. Lal, R. K., Chanotiya, C. S. and Kumar, A. (2023). The prospects and potential of the horticultural and pharmacological medicinal herb senna (Cassia angustifolia Vahl.): A review. Technology in Horticulture, 3(1).

10. Mishra, P., Alhussan, A. A., Khafaga, D. S., Lal, P., Ray, S., Abotaleb, M., ... and El-Kenawy, E. S. M. (2024). Forecasting production of potato for a sustainable future: global market analysis. Potato Research, 67(4): 1671-1690.

11. Naz, R., Ayub, H., Nawaz, S., Islam, Z. U., Yasmin, T., Bano, A., ... and Roberts, T. H. (2017). Antimicrobial activity, toxicity and anti-inflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. BMC complementary and alternative medicine, 17(1): 302.

12. Osuntokun, O. T. (2021). Efficacy, properties and therapeutic use of some major medicinal plants for human health. Biopesticides: Botanicals and microorganisms for improving agriculture and human health, 179.

13. Poddar, S., Sarkar, T., Choudhury, S., Chatterjee, S., and Ghosh, P. (2020). Indian traditional medicinal plants: A concise review. International Journal of Botany Studies, 5(5): 174-190.

14. Różyło, R., Piekut, J., Dziki, D., Smolewska, M., Gawłowski, S., Wójtowicz, A., and Gawlik-Dziki, U. (2022). Effects of wet and dry micronization on the GC-MS identification of the phenolic compounds and antioxidant properties of freeze-dried spinach leaves and stems. Molecules, 27(23): 8174.

15. Sen, Saikat, Raja Chakraborty, and Biplab De (2011). "Challenges and opportunities in the advancement of herbal medicine: India’s position and role in a global context." Journal of Herbal medicine 1.3-4: 67-75.

16. Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y., and Gritsanapan, W. (2013). Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringa oleifera leaf extract by the appropriate extraction method. Industrial crops and products, 44: 566-571.

17. Xue, Y., Geng, X., Liang, X., Lu, G., Smagghe, G., Wei, L., ... and Liu, B. (2026). Cylindrocladium Black Rot of Peanut and Red Crown Rot of Soybean Caused by Calonectria ilicicola: A Review. Agronomy, 16(1): 111.