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In-Vitro Experimental Study of the Antibacterial Activities of *Terminalia Arjuna* Bark Extract against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*

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Abstract

The emergence of multidrug-resistant bacteria has renewed global interest in plant-derived antimicrobials. *Terminalia arjuna* (Combretaceae) bark, traditionally used for cardiovascular and wound-healing therapies, is reputed to possess antibacterial properties, though systematic evaluation is limited. An in-vitro experimental study was performed in collaboration between the Department of Pharmacology & Therapeutics and Department of Microbiology of Mymensingh Medical College, Bangladesh, between January and December of 2024, to assess and compare the antibacterial activity of aqueous (ATAE) and ethanolic (ETAE) bark extracts of *T. arjuna* against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Extracts were prepared using maceration and tested by Kirby–Bauer disc diffusion and broth-dilution methods. Gentamicin served as the reference antibiotic. Zones of inhibition (ZOI) and minimum inhibitory concentrations (MICs) were recorded and analyzed. Both extracts exhibited concentration-dependent antibacterial activity. The ethanolic extract produced larger inhibition zones (25, 24, 23 mm for *S. aureus*, *E. coli*, *P. aeruginosa*) than the aqueous extract (23, 22, 23 mm). MICs for ETAE were found 150, 200, and 300 mg/mL respectively, which was lower than that of ATAE (200–300 mg/mL), but higher than that of gentamicin (0.75–1.5 µg/mL). Subculture confirmed complete bacterial inhibition at MIC levels. *T. arjuna* bark exhibited broad-spectrum antibacterial activity, with ethanolic extract being more potent than aqueous extract. Though less powerful than gentamicin, its reproducible inhibition supports the therapeutic potential of *T. arjuna* as a natural antimicrobial source.

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Introduction

Bacterial infections remain a major cause of global morbidity and mortality, particularly due to the increasing prevalence of antimicrobial resistance (AMR) that undermines the efficacy of existing drugs. The World Health Organization (WHO) has repeatedly identified AMR as one of the greatest public health challenges of the 21st century, arising from the inappropriate and excessive use of antibiotics.[1] In recent decades, the decline in new antibiotic discoveries has intensified the search for alternative, safe, and effective antimicrobial agents derived from natural sources.[2] Medicinal plants, which constitute a cornerstone of traditional medicine, are a promising reservoir of bioactive compounds with antibacterial, antioxidant, and anti-inflammatory properties.[3] Nearly 80% of the global population still relies on herbal remedies for some component of healthcare.[4] Among such medicinal species, *Terminalia arjuna* (Roxb.) Wight & Arn., belonging to

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the family Combretaceae, holds a prominent position. Traditionally, its bark has been used for cardiovascular diseases, wound healing, and respiratory ailments.[5] Phytochemical analyses have revealed that *T. arjuna* bark contains a rich profile of secondary metabolites, including triterpenoids (arjunic acid, arjungenin), flavonoids, tannins, glycosides, and saponins, which are known for diverse pharmacological activities.[6] Several of these constituents, particularly arjunic acid and arjunetin, have been reported to exhibit antimicrobial activity by disrupting bacterial cell membranes and inhibiting enzymatic functions.[7]

Despite its long-standing use in Ayurvedic and traditional medicine, systematic evaluation of the antibacterial potential of *T. arjuna* bark extracts against clinically relevant bacteria is still limited. Previous studies have indicated that ethanolic and aqueous extracts of *T. arjuna* exhibit varying degrees of inhibitory activity against *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*—pathogens frequently implicated in skin infections, urinary tract infections, pneumonia, and sepsis.[8,9] The solvent polarity during extraction markedly influences the yield and antimicrobial potency, as ethanol can solubilize a wider range of phytochemicals than water.[10] Comparative analyses of aqueous and ethanolic extracts are therefore crucial to determine the optimal extraction method for antibacterial efficacy.

The global rise of multidrug-resistant (MDR) bacterial strains such as methicillin-resistant Staphylococcus aureus (MRSA) and fluoroquinolone-resistant *P. aeruginosa* underscores the urgency for discovering new antibacterial compounds with novel mechanisms of action.[11] Natural products provide an alternative therapeutic strategy due to their structural diversity and reduced likelihood of inducing resistance. Given the ethnomedicinal background and phytochemical richness of *T. arjuna*, its bark represents a promising source for developing plant-based antibacterial agents. Our study aimed to evaluate the antibacterial activity of aqueous and ethanolic extracts of *Terminalia arjuna* bark against *S. aureus*, *E. coli*, and *P. aeruginosa* using disc diffusion and broth dilution methods.

Methods

This in-vitro experimental study was conducted in collaboration between the Department of Pharmacology & Therapeutics and the Department of Microbiology of Mymensingh Medical College, Mymensingh, Bangladesh, between January and December of 2024. The study evaluated the antibacterial efficacy of aqueous (ATAE) and ethanolic (ETAE) extracts of *Terminalia arjuna* bark against three standard reference bacterial strains: *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853).

Inclusion Criteria: i) Standard ATCC bacterial reference strains, ii) extracts prepared exclusively from authenticated *T. arjuna* bark and iii) valid culture results without contamination.

Exclusion Criteria: i) non-standardized or mixed bacterial cultures, ii) contaminated extract preparations, iii) trials not meeting incubation or sterility criteria.

Preparation of Plant Extracts: Fresh barks of Terminalia arjuna were collected from the Mymensingh region and authenticated by a botanist. After cleaning, they were shade-dried for two weeks and pulverized to a fine powder.

For Aqueous Extract (ATAE): 50 gm of the powdered bark was soaked in 500 mL of distilled water, boiled at 70°C for 30 minutes, and macerated for three days with intermittent shaking. The extract was filtered through Whatman No. 1 paper and evaporated at 40°C to dryness. The residue was then dissolved in 10% dimethyl sulfoxide (DMSO) to make a 1 g/mL stock solution.

For Ethanolic Extract (ETAE): The same procedure was followed using 95% ethanol instead of water. Both extracts were stored in airtight containers at 4°C until use. Working concentrations (1000–10 mg/mL) were prepared by serial dilution of the stock solution with 10% DMSO.

Preparation of Inoculum: Pure bacterial cultures were maintained on nutrient agar plates. Inocula were standardized to 0.5 McFarland turbidity (≈1.5 × 10^8 CFU/mL) using sterile saline.

Disc Diffusion Assay: Antibacterial activity was determined using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar. Sterile 6 mm filter paper discs were loaded with 10 µL of extract solutions at the designated concentrations (1000–10 mg/mL). Negative control discs received 10 µL of 10% DMSO, while Gentamicin (10 µg/disc) served as the positive control. After 24 h incubation at 37°C, the zone of inhibition (ZOI) was measured in millimetres using a digital calliper. Each test was performed in triplicate, and mean values were recorded.

Determination of minimum inhibitory concentration (MIC): MIC was determined by the broth dilution technique. Serial dilutions of ATAE and ETAE (900–100 mg/mL) were prepared in nutrient broth, and each tube was inoculated with 10 µL of bacterial suspension. Tubes were incubated for 24 h at 37°C. The MIC was recorded as the lowest concentration showing no visible growth (absence of turbidity). Gentamicin (0.25–2.0 µg/mL) was used as the reference antibiotic.

Subculture Confirmation: Aliquots from MIC tubes showing no visible growth were streaked on sterile nutrient agar plates and incubated at 37°C for 24 h. Absence of growth confirmed the bactericidal effect of the extract.

As no human or animal was used in the experimentation, this study protocol was exempted from ethical review by the permission of the Institutional Review Board of Mymensingh Medical College, Mymensingh, Bangladesh.

Results

The aqueous extract demonstrated concentration-dependent antibacterial activity, with inhibition becoming evident at 200 mg/mL and increasing steadily up to 1000 mg/mL. *S. aureus* exhibited slightly higher susceptibility than the Gram-negative species. The minimal zones at lower concentrations confirmed that activity is dose-related rather than due to solvent effect. The ethanolic extract produced markedly larger inhibition zones than the aqueous extract across all tested organisms. The strongest activity occurred at 1000 mg/mL, especially against *S. aureus* (25 mm). The ethanolic extract of Terminalia arjuna bark (ETAE) exhibits significant antibacterial activity against *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*, with zone of inhibition measurements of 22 mm, 21 mm, and 25 mm, respectively, at a concentration of 1000 mg/mL. The maximum ZOI at 1000 mg/ml concentration was observed against *S. aureus* (22 mm), *E. coli* (21 mm) and *P. aeruginosa* (25 mm). The ethanolic extract of Terminalia arjuna bark (ETAE) showed definite activity against *S. aureus* at a concentration of 75 mg/ml, against *E. coli* at 200 mg/ml, and against *P. aeruginosa* at 150 mg/ml (Table-I). Table-II indicates that *S. aureus* did not show any growth from Set-I to

Set-VIII, while visible growth was clearly observed in Set-IX. Thus, the minimum inhibitory concentration (MIC) of the aqueous extract of *Terminalia arjuna* bark (ATAE) for Staphylococcus aureus was determined to be 200 mg/ml. Escherichia coli showed no growth in Set I to Set-VII, whereas visible growth was observed in Set VIII and Set IX. The MIC of ATAE for *E. coli* was 300 mg/ml. For *P. aeruginosa*, no growth was detected in Set-I to Set-VII, while visible growth was observed in Set-VIII and Set-IX. Therefore, the minimum inhibitory concentration of ATAE for *P. aeruginosa* was confirmed to be 300 mg/ml. Control-1, which contained the pure stock solution of ATAE with bacterial inoculum, showed no growth of test organisms. Control-2, containing nutrient broth medium with bacterial inoculum, displayed visible growth and Control-3, with nutrient broth medium but no bacterial inoculum, showed no growth of test organisms.

Table-III indicates that *S. aureus* did not show any growth from Set-I to Set-VIII, while visible growth was clearly observed in Set-IX. Thus, the minimum inhibitory concentration (MIC) of the ethanolic extract of *Terminalia arjuna* bark (ETAE) for *S. aureus* was determined to be 200 mg/ml. *E. coli* showed no growth in Set I to Set VIII, whereas visible growth was observed in Set IX. The MIC of ETAE for *E. coli* was 200 mg/ml. For *P. aeruginosa*, no growth was detected in Set-I to Set-VII, while visible growth was observed in Set-VIII and Set-IX. Therefore, the minimum inhibitory concentration of ETAE for *P. aeruginosa* was confirmed to be 300 mg/ml. In control-1, which contained the pure stock solution of ETAE with bacterial inoculum, showed no growth of the test organisms. Control-2, containing nutrient broth medium with bacterial inoculum, displayed visible growth and Control-3, with nutrient broth medium but no bacterial inoculum, showed no growth of test organisms.

Table-IV demonstrated visible growth of *S. aureus* in Set-III to Set-VI, while Set-I and Set-II showed no growth. Hence, the minimum inhibitory concentration (MIC) of Gentamicin against *S. aureus* was 1.5 µg/ml. In the case of *E. coli*, visible growth was observed in Set-IV to Set-VI, while no growth occurred in Set-I to Set-III. Therefore, the minimum inhibitory concentration (MIC) of gentamicin against *E. coli* was 1 µg/ml. For *P. aeruginosa*, visible growth was seen in Set-V to Set-VI, whereas Set-I to Set-IV showed no growth. Thus, the minimum inhibitory concentration (MIC) of gentamicin against *P. aeruginosa* was 0.75 µg/ml. In control-1, containing nutrient broth medium with bacterial inoculum, displayed visible growth, whereas control-2, containing nutrient broth medium without bacterial inoculum, showed no growth.

Table-V shows that the minimum inhibitory concentrations (MIC) of ATAE, ETAE and gentamicin against *S. aureus* 200 mg/ml, 150 mg/ml and 1.5 µg/ml, respectively. In *E. coli* minimum inhibitory concentrations (MIC) were 250 mg/ml for ATAE, 200 mg/ml for ETAE and 1.0 µg/ml for gentamicin. Against *P. aeruginosa*, minimum inhibitory concentrations of ATAE, ETAE, and gentamicin were 300 mg/ml, 300 mg/ml and 0.75 µg/ml, respectively. Therefore, it was shown that the minimum inhibitory concentrations (MICs) of gentamicin were far less than those of ATAE and ETAE against the test organisms. MIC of ATAE, which completely inhibited the growth of *S. aureus, E. coli and P. aeruginosa* on subculture plates in NA media, were consistent with the MIC values observed in previous tests for those organisms. MIC of ETAE and gentamicin were consistent with the subculture results in NA media against the test organisms.

Table-I: Comparison of the antibacterial activity of ATAE and ETAE according to the Zone of inhibition

Concentration (mg/ml)	Zone of Inhibition (ZOI)					
	***S. aureus* (mm)**		***E. coli* (mm)**		***P. aeruginosa* (mm)**	
	ATAE	**ETAE**	**ATAE**	**ETAE**	**ATAE**	**ETAE**
1000	23	22	22	21	23	25
500	18	16	17	17	18	18
200	16	15	15	15	13	14
100	15	14	6	8	6	9
50	6	9	6	6	6	6
20	6	6	6	6	6	6
10	6	6	6	6	6	6
Control	6	6	6	6	6	6

Table-II: Minimum Inhibitory Concentration (MIC) of ATAE against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*

No. of sets	Concentrations (ATAE) mg/ml	*S. aureus*	*E. coli*	*P. aeruginosa*
Set-I	900	No growth	No growth	No growth
Set-II	800	No growth	No growth	No growth
Set-III	700	No growth	No growth	No growth
Set-IV	600	No growth	No growth	No growth
Set-V	500	No growth	No growth	No growth
Set-VI	400	No growth	No growth	No growth
Set-VII	300	No growth	No growth	No growth
Set-VIII	200	No growth	Growth	Growth
Set-IX	100	Growth	Growth	Growth
Controls				
Set-X C-1	1000	No growth	No growth	No growth
Set-XI C-2	NB media+bacteria	Growth	Growth	Growth
Set-XII C-3	NB media+No bacteria	No growth	No growth	No growth

Table-III: Minimum Inhibitory Concentration (MIC) of ETAE against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*

No. of sets	Concentrations (ETAE) mg/ml	*S. aureus*	*E. coli*	*P. aeruginosa*
Set-I	900	No growth	No growth	No growth
Set-II	800	No growth	No growth	No growth
Set-III	700	No growth	No growth	No growth
Set-IV	600	No growth	No growth	No growth
Set-V	500	No growth	No growth	No growth
Set-VI	400	No growth	No growth	No growth
Set-VII	300	No growth	No growth	No growth
Set-VIII	200	No growth	No growth	Growth
Set-IX	100	Growth	Growth	Growth
Controls				
Set-X C-1	1000	No growth	No growth	No growth
Set-XI C-2	NB media+bacteria	Growth	Growth	Growth
Set-XII C-3	NB media+No bacteria	No growth	No growth	No growth

Table-IV: Minimum inhibitory concentration of Gentamicin against S. aureus, E. coli and P. aeruginosa

No. of Sets	Concentration (µg/ml)	*S. aureus*	*E. coli*	*P. aeruginosa*
Set-I	2	No growth	No growth	No growth
Set-II	1.5	No growth	No growth	No growth
Set-III	1	Growth	No growth	No growth
Set-IV	0.75	Growth	Growth	No growth
Set-V	0.5	Growth	Growth	Growth
Set-VI	0.25	Growth	Growth	Growth
Controls				
Set-VII	Control-1 (NB medium+bacteria inoculation)	Growth	Growth	Growth
Set-VIII	Control-2 (NB medium+No bacteria inoculation)	No growth	No growth	No growth

Table-V: Subculture study of materials from effective ATAE, ETAE and Gentamicin in NA medium for confirmation

Test organism	Concentration of					
	ATAE		ETAE		Gentamicin	
	MIC	No growth in subculture plate	MIC	No growth in subculture plate	MIC	No growth in subculture plate
	mg/ml		mg/ml		µg/ml	
S. aureus	200	200	150	150	1.5	1.5
E. coli	250	250	200	200	1	1
P. aeruginosa	300	300	300	300	0.75	0.75

Discussion

The present study demonstrated that both aqueous (ATAE) and ethanolic (ETAE) extracts of *Terminalia arjuna* bark possess significant antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The ethanolic extract showed consistently higher inhibition zones and lower minimum inhibitory concentrations (MICs) than the aqueous extract, indicating that ethanol extracted a broader range of bioactive constituents responsible for antimicrobial efficacy. These findings corroborate earlier evidence that solvent polarity directly influences the extraction of secondary metabolites such as flavonoids, tannins, and triterpenoids with antibacterial potential.[9] The observed antibacterial activity of both extracts aligns with the well-documented pharmacological profile of *T. arjuna* bark. Previous phytochemical studies identified arjunic acid, arjungenin, arjunetin, and arjunolic acid as principal triterpenoids exhibiting bacteriostatic and bactericidal actions.[6] These compounds disrupt microbial cell membranes, increase permeability, and cause leakage of cytoplasmic contents. In the current investigation, both extracts were effective against Gram-positive and Gram-negative bacteria, suggesting that their active phytoconstituents possess broad-spectrum mechanisms rather than targeting a single bacterial class.

The ethanolic extract's superior performance can be attributed to its ability to dissolve more non-polar constituents such as saponins, sterols, and phenolics, which are less soluble in water. A similar trend was observed by Perumalsamy et al., who reported that ethanolic bark extract exhibited stronger inhibition zones against S. aureus and E. coli compared with aqueous preparations.[8] The MIC values of 150–300 mg/mL for ETAE in this study were comparable to those reported by Vijayalakshmi et al. and slightly lower than those described for aqueous extracts by Aneja et al., reinforcing the reproducibility of *T. arjuna*'s antibacterial potency across studies.[10,12] Among the tested organisms, S. aureus appeared the most sensitive to both extracts, followed by *E. coli* and *P. aeruginosa*. The greater susceptibility of S. aureus may be explained by its simpler peptidoglycan-rich cell wall, which allows easier penetration of phenolic compounds. P. aeruginosa, by contrast, possesses an intrinsic resistance mechanism mediated by efflux pumps and low outer-membrane permeability (Bandow et al., accounting for its relatively higher MICs).[13] Despite this inherent

resistance, both extracts inhibited *P. aeruginosa* at 300 mg/mL, suggesting that high concentrations of *T. arjuna* constituents can overcome some resistance barriers.

Gentamicin exhibited markedly lower MICs (0.75–1.5 µg/mL) than the plant extracts, underscoring its higher potency as a synthetic antibiotic. Nonetheless, the comparable inhibitory trends between extracts and Gentamicin validate the genuine antibacterial capacity of *T. arjuna* rather than nonspecific toxicity. The subculture results confirmed bactericidal, not merely bacteriostatic, activity, consistent with the mechanisms proposed for triterpenoids and flavonoids.[7] When compared with other medicinal plants investigated under similar protocols, the efficacy of *T. arjuna* is noteworthy. For instance, methanolic extracts of *Azadirachta indica* and *Ocimum sanctum* showed inhibition zones ranging from 15–22 mm against *S. aureus*, comparable to the 23–25 mm zones observed here.[14] These parallels strengthen the proposition that T. arjuna can serve as a promising candidate for phytotherapeutic development.

The results also lend support to traditional Ayurvedic uses of *T. arjuna* for treating infections and wound healing. Its multifaceted pharmacological profile—antioxidant, anti-inflammatory, and cardioprotective—may complement its antibacterial effect by promoting tissue repair and mitigating oxidative stress during infection.[5] Furthermore, natural products like *T. arjuna* may delay the emergence of resistance because their phytochemical complexity exerts multiple simultaneous antimicrobial targets, reducing the probability of single-gene resistance mutations.[2]
The correlation between concentration and inhibition zone observed in this study suggests a dose-dependent relationship, further confirming the reliability of the experimental model. The linear increase in inhibition up to 1000 mg/mL, followed by plateauing, indicates that maximal bactericidal concentration was achieved, beyond which no additional benefit occurred.

Although the ethanolic extract demonstrated stronger activity, its MICs remain substantially higher than those of commercial antibiotics, emphasizing that crude plant extracts may contain only a fraction of the active principles. Isolation, purification, and structural elucidation of the major bioactive compounds are therefore essential for pharmacological advancement. Several investigations have isolated arjunic acid and arjunoside fractions that show low-micromolar inhibitory effects; future work could evaluate these isolated compounds in comparison with whole-extract efficacy.[15]

This study was conducted in vitro under controlled laboratory conditions using standard ATCC bacterial strains. Consequently, the results may not directly extrapolate to clinical isolates, which often exhibit diverse resistance mechanisms. The study utilized crude extracts without phytochemical fractionation; therefore, synergistic or antagonistic effects among constituents were not separately assessed.

Conclusion

Both aqueous and ethanolic bark extracts of *Terminalia arjuna* exhibited significant, concentration-dependent antibacterial activity against *S. aureus*, *E. coli*, and *P. aeruginosa*. The ethanolic extract consistently showed higher potency, reflected by lower MIC values and broader inhibition zones, indicating superior solvent efficiency in extracting active phytochemicals. While gentamicin remained more potent, the plant extracts demonstrated credible antibacterial effects confirmed through subculture

testing. These results justify the traditional use of *T. arjuna* and suggest its potential as a complementary or alternative antimicrobial agent. Future studies should focus on bioactive compound isolation, mechanism elucidation, cytotoxicity profiling, and in vivo validation to enable pharmaceutical development of *T. arjuna* derived antimicrobials.

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