SHORT COMMUNICATION

Study of Antibacterial Activity of Silver Nanoparticles Prepared Using *Terminalia arjuna* and its Synergistic Association with Tetracycline

Pratibha Shah* and Samay Dwivedi

DOI: 10.18811/ijpen.v10i04.25

Abstract

The rising menace of antimicrobial resistance has invigorated the hunt for new lead molecules from natural products and furthered their application in synthesizing nanoparticles from them. In this study, the methanolic extract of *Terminalia arjuna* (TAE) was evaluated for its antibacterial potential, and its combined activity with tetracycline was screened. The antibacterial potency of TAE was confirmed by evaluating the minimum inhibitory concentration values (MIC). The MIC value recorded for TAE was 3.5 mg/mL, and for tetracycline, it was noted as 8.5 µg/mL by the agar dilution technique. The checkerboard method validated the synergistic association between TAE and Tetracycline after determining the Fractional inhibitory concentration index. Further, phytochemical screening of TAE was performed, and TAE was used to synthesize silver nanoparticles (AgNPs). The characterization of the AgNPs formed was done using the UV-vis spectrophotometric technique. The topology of TAE-AgNPs formed was studied using the scanning electron microscopy (SEM) technique at IIT-Bombay, and it was observed that the particles formed were spherical, with a size range of 50 to 60 nm. The selected four test strains were inhibited by TAE-AgNPs, exhibiting inhibition zones with a range of 14.7 to 28.2 mm. Thus, it can be suggested that AgNPs formed using TAE had potent antibacterial activity.

Keywords: Synergistic, *Terminalia arjuna*, Tetracycline, Silver nanoparticles, SEM.

Highlights

- The present study investigates the antibacterial activity of *Terminalia arjuna* extract, its combination effect with tetracycline and its application in green synthesis of silver nanoparticles.
- The average MIC of *T. arjuna* extract was 3.5 mg/mL and tetracycline was 8.5 µg/mL, against test strains.
- A synergistic association was established between *T. arjuna* extract and tetracycline for all four bacterial samples.
- The silver nanoparticles (AgNPs) formed using *T. arjuna* extract were characterized and all the test cultures were inhibited by the AgNPs, indicating AgNPs had potent antibacterial activity.

International Journal of Plant and Environment (2024); **ISSN:** 2454-1117 (Print), 2455-202X (Online)

Introduction

In the 21st century, there have been rising cases of infectious diseases, especially caused due to drug-resistant microbes. 25% of the 57 million annual deaths are due to pathogenic microbes, with a higher percentage in underdeveloped countries, as per the WHO report (WHO, 2022). The increase in multidrug-resistant pathogens has endangered the clinical efficacy of prevailing chemotherapeutic agents. The increased incidence of antimicrobial resistance by pathogens has fuelled the examination of several natural products for their likely antibacterial action. This has further directed the hunt for lead molecules to develop new antimicrobial compounds. Natural products have been applied in traditional Indian therapies since the ancient era. However, there is a need to corroborate the use of plants by screening the active components and using them in drug development. This will help reiterate the data that natural compounds are the original source of numerous antimicrobials (Anand *et al*., 2019).

Different studies have documented the most commonly found 24 species of *Terminalia*, including *T. arjuna*. Various indigenous therapies have used components of *T. arjuna* plant for decades, as they contain glycosides, flavonoids, and tannins as the main phytochemical compounds. It may be due to the phytochemical compounds in these products that exhibit antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory activities (Aneja *et al*., 2012).

The increasing hazard of bacterial resistance against antibiotics has inspired the preparation of nanoparticles (size 1–100 nm). The antimicrobial properties of nanoparticles formed depend on their topology, like the shape and size

Department of Microbiology, Kishinchand Chellaram College, Churchgate, Mumbai, Maharashtra, India.

***Corresponding author:** Pratibha Shah, Department of Microbiology, Kishinchand Chellaram College, Churchgate, Mumbai, Maharashtra, India, Email: pratibha.shah@kccollege.edu.in

How to cite this article: Shah, P., Dwivedi, S. (2024). Study of Antibacterial Activity of Silver Nanoparticles Prepared Using *Terminalia arjuna* and its Synergistic Association with Tetracycline. International Journal of Plant and Environment. 10(4), 219-223.

Submitted: 22/03/2024 **Accepted:** 23/10/2024 **Published:** 31/12/2024

of the nanoparticles formed. Nanoparticles have dissimilar physical and chemical characteristics in contrast to their bulk components. The use of nanoparticles in therapeutics is of the utmost significance since several studies have demonstrated its broad spectrum and potent antibacterial activity (Tyagi *et al*., 2017, Wang *et al*., 2017).

The reduction of silver ions causes the formation of silver nanoparticles, and this reduction may be either by chemical or biological techniques. The toxicity caused by the chemical reduction methods of silver ions for the formation of AgNPs is damaging to the environment. Hence, biological synthesis is a more economical and environment-safe approach for AgNP synthesis, in comparison to chemical methods. Microbes, plant extracts and enzymes can be used for the biological synthesis of nanoparticles. The organic compounds found in these extracts can reduce silver salts and also act as capping agents (Xu *et al*., 2020). These nanoparticles can also be used as an adjunct in antibacterial therapies along with the existing antibiotics and thus aid in effective therapeutics.

The study described herein aims to assess the antibacterial activity of *T. arjuna* bark extract, evaluate its synergistic association with tetracycline and analyze its application to synthesize nanoparticles.

Methodology

Methanolic plant extract preparation

T. arjuna bark was procured from a local market in Mumbai, India and was powdered by mechanical methods. Further, 10 gm of the obtained powder was extracted using absolute methanol by the soxhlet process. The *T. arjuna* crude extract (TAE) obtained was dried and preserved in a sterile container at 4^0C.

Qualitative phytochemical studies of TAE

TAE was screened for saponins by foam test, phytosterols by Salkowsi test, flavonoids by lead acetate test, glycosides by Borntrager's test and tannins by ferric chloride test, as per standard protocols (Mandal *et al*., 2013).

Study of Antioxidant property of TAE by DPPH analysis

Varying concentrations of ascorbic acid (standard), using methanol as a diluent, were prepared. The test sample included 1-mL solution of 0.004% DPPH with TAE. The absorbance at 517 nm was noted after 30 minutes. The blank solution comprised of methanol and a control sample contained DPPH along with methanol.

The below formula was used to calculate the antioxidant activity -

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test)/absorbance of control] * 100.

Absorbance control: absorbance of DPPH solution (without TAE),

Absorbance test: absorbance of TAE (Mohammad *et al*., 2012).

Bacterial strains

The four test strains used in the current study were procured from the Microbiology department of the college. The four test strains used were *Escherichia coli*, *Corynebacterium diphtheriae*, *Staphylococcus aureus*, and *Salmonella typhi*. The four test strains were sub-cultured periodically and preserved at 4^0C.

Antibacterial activity of *Terminalia arjuna* extract (TAE)

TAE was dissolved in 10% dimethyl sulfoxide (DMSO) and evaluated for inhibiting the bacterial test strain by agar diffusion method, as per guidelines (CLSI, 2012), using Mueller Hinton agar. Wells were punched into the inoculated MHA plates using a sterile cork borer. Sterile distilled water was used to prepare varying concentrations of TAE (25, 50, 75 and 100%, w/v). These dilutions of TAE were then added to the punched wells. Two controls were also set up - Tetracycline (10 µg – positive control) and 10% DMSO (Negative control). The test was carried out in triplicates and the mean zone of inhibition (ZOI) was measured in millimeters (Debnath *et al*., 2013).

Evaluation of minimum inhibitory concentration (MIC) of TAE and Tetracycline

CLSI prescribed agar dilution technique was used to note the MIC of TAE and tetracycline. Both the components were added to MH agar separately, to obtain a concentration range, which would help to determine MIC. The range prepared for TAE was 1 to 10 mg/mL and for tetracycline, it was 1 to 10 µg/mL, using molten MH agar. The plates were inoculated with four test strains using the spot inoculation technique and incubated at 37°C for 24 hours and the MIC was noted (Nakamura *et al*., 2000).

Study of association between TAE and Tetracycline by checkerboard assay

The synergistic effect of tetracycline (stock- 10 µg/mL) and *T. arjuna* extract (stock-5 mg/mL) was evaluated using MH agar plates by checkerboard technique. As per CLSI guidelines, the fractional inhibitory concentration index (FIC) was calculated (CLSI, 2012).

FIC was formulated by-

∑FIC = Fractional inhibitory concentration (A) + Fractional inhibitory concentration (B)

Where Fractional inhibitory concentration (A) is MIC of TAE (when it is added with tetracycline)/MIC of TAE (when it is added alone),

Where Fractional inhibitory concentration (B) is MIC of Tetracycline (when it is added with TAE)/ MIC of tetracycline (when it is added alone).

The interpretation of results was done as per Nakamura *et al*., (2000), which indicated synergy when the FIC index value was 0.5 or less than it. Further, when the FIC index was 2 or more than 2, then it was designated as antagonism. A FIC index between 0.5 and 1 was termed additive, and between 1 and 2 was indicated as indifference.

Green synthesis of AgNPs using TAE

TAE can act as a capping and reducing agent for the formation of silver nanoparticles. In the current study, to synthesise AgNPs, silver nitrate was used as a precursor. For the preparation, 0.3 millimolar aqueous silver nitrate solution was added dropwise to

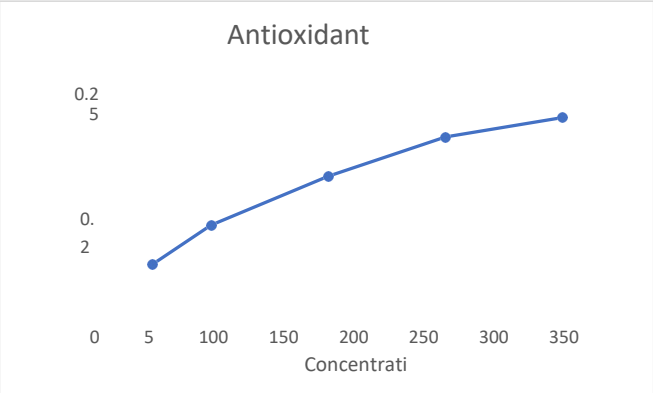

Fig. 1: DPPH assay (For quantitative antioxidant activity of *T. arjuna)*

5 ml of the TAE, with constant stirring at 50 to 60°C. The symbolic construction of TAE-silver nanoparticles may be indicated due to the conversion of the solution to a brown color (Palanisamy *et al*., 2014).

The construction of TAE-AgNPs was further confirmed using UV-vis spectrophotometric technique (400–600 nm) and field emission gun-scanning electron microscope (FEG-SEM). The SEM techniques were performed to study the topology of TAE-AgNPs at IIT- Bombay.

Analysis of TAE-AgNPs antibacterial activity

As per the standard procedures prescribed by CLSI, 50 µL of AgNPs synthesized were added into the wells and the ZOI was measured in millimeters, in triplicates and the mean ZOI was determined (CLSI, 2012).

Results and Discussions

As the world is facing increasing antimicrobial resistance, it is imperative to search for different strategies based on traditional therapeutics to contest microbial infections. In the existing study, TAE (bark) was analyzed for its antibacterial properties using four test strains. TAE exhibited the occurrence of phytochemicals, which is generally credited with the reason for antimicrobial properties. These molecules also are categorized as secondary metabolites (Haroun *et al*., 2016; Kumar *et al*., 2018). Bioactive molecules found in herbal plants are said to have therapeutic implications and are of great importance in novel drug design. TAE harbored various phytochemicals, which include flavonoids, glycosides, tannins, phytosterols and phenolic compounds (Table 1). The ability to reduce the DPPH by TAE, designated free radical scavenging activity (Mohammad *et al*., 2012) (Fig. 1).

The antioxidant value of *T. arjuna* by DPPH method, after plotting the graph, was estimated as 350 mg/ml. Since the *T. arjuna* extract was diluted to 1:512 dilutions, so 512 is the dilution factor. By graph, the amount of antioxidants was found to be 350 mg/mL = 350 × 512 = 1,79,200 mg/mL. The antioxidant activity was found to be significant, and the percentage value was estimated to be 47.22%, as per the formula described earlier.

Table 1: Phytochemical profile of *T. arjuna* extract

Phytochemical compounds	*Results*
Saponins	+
Phenolic compound & Tannins	+
Glycoside	+
Phytosterols	+
Flavonoids	+

The average ZOI of 50% TAE against the four bacterial strains was found to be in the range of 15.8 to 21.5 mm, and for 100%, it ranged between 18.5 to 25 mm, indicative of its potent broad-spectrum antibacterial activity (Table 2). 10% DMSO and antibiotic Tetracycline were used as controls. Previous studies also indicated similar results (Aneja *et al*., 2012; Debnath *et al*., 2013). In this study against the four test strains, the mean MIC of *T. arjuna* bark extract was recorded as 3.5 mg/mL and for tetracycline, it was estimated as 8.5 µg/mL (Table 3). Similar results were observed in the study carried out by combined association between TAE and tetracycline. The activity was evaluated by Checkerboard test and the FIC index for the four bacterial strains was found to be 0.5 or less for all the strains. This indicates a synergistic association between TAE and Tetracycline for all four bacterial cultures (Table 3). These results were in accordance with the study carried out by Aneja *et al*., 2012. Thus, the combination of *T. arjuna* extract along with tetracycline exhibited a synergistic association against all the selected test strains.

The current study highlighted that the *T. arjuna* extract and tetracycline, if combined, can lead to solving the problem of Tetracycline resistance or lower its dose in therapeutics. Hence, patients with serious infections may benefit from this combination. Though the antibacterial activity of *T. arjuna* has been reported earlier, there is a paucity of data published about its synergistic effectiveness along with antibiotics against bacterial strains. The existing study established the synergistic association of *T. arjuna* bark extract with the antibiotic tetracycline.

Initially, in the current study, visual detection was used to confirm the formation of AgNPs, followed by further characterization. By studying the plasmon peak formed at 430 nm, the formation of TAE-AgNPs was indicated (Fig 2). Another advanced technique, named field emission gun-scanning electron microscope, was also used for confirmation of silver nanoparticle formation, wherein spherical-shaped nanoparticles

Table 2: Zone Of Inhibition (mean±SD in mm) of TAE against test cultures by agar cup method

Concentration	*S. aureus*	*E. coli*	*C. diphtheriae*	*S. typhi*
50% extract	17.5±0.58 mm	18±0.58 mm	21.5±1.0 mm	15.8±1.52 mm
100% extract	21.2±1.52 mm	20.6±0.58 mm	25±1.52 mm	18.5±0.5mm
Tetracycline	25±0 mm	27±0 mm	27±0 mm	23±0 mm

Table 3: MIC and Combined activity of TAE (stock-5 mg/ml) and tetracycline (stock- 10µg/ml) by Agar dilution method

Bacterial strains	*MIC of TAE (mg/ml)*	*MIC of TAE in combination with sub-MIC of tetracycline (mg/ml)*	*MIC of Tetracycline (µg/ml)*	*MIC of Tetracycline with sub-MIC of TAE(µg/ml)*	*∑FIC (FIC Index)*
Salmonella typhi	4	1	9	2.0	0.47
Escherichia coli	4	1	9	2.5	0.52
Staphylococcus aureus	3	0.5	8	1.5	0.35
C. diphtheriae	3	0.5	8	2.0	0.42

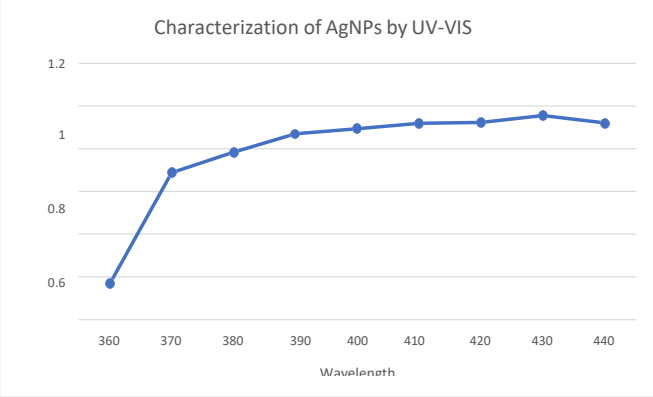

Fig. 2: Line graph showing absorbance by AgNPs by UV-VIS spectrophotometer

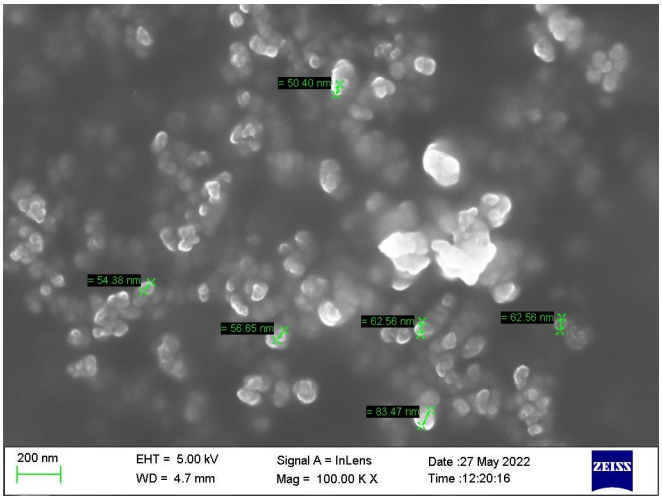

Fig. 3: Characterization of AgNPs by Field emission gun-Scanning Electron Microscope

Table 4: Zone Of Inhibition of AgNPs (mean±SD) against test cultures by agar well diffusion method

Test culture	*Mean Zone of inhibition ± SD (mm) AgNP-TAE*
S. aureus	17.7 ±1.52
E. coli	14.7 ±0.58
C. diphtheriae	28.2 ±1.04
S. typhi	16.7 ±0.76

(50–60 nm) were observed, along with some clumping of AgNPs (Fig 3).

The screening of the antibacterial activity of TAE assisted silver nanoparticles formed revealed powerful antibacterial activity. All the selected test strains were significantly inhibited, and the ZOI ranged between 14.7 to 28.2 mm. The largest ZOI was seen against *Corynebacterium diphtheriae* at 100% concentration, while *Salmonella typhi* and *Escherichia coli*, exhibited the minimum ZOI (Table 4). The results were similar to a previous study by Debnath *et al*. (2013).

Conclusion

The findings of the current study led to a hypothesis that gram-positive and gram-negative organisms are inhibited by TAE, the former being more inhibited in comparison. The present study also suggests the possibility of the use of a synergistic drug-herb (Tetracycline-*T. arjuna* extract) combination to combat bacterial infections. This study also demonstrates a lucrative and environment-caring method for generating silver nanoparticles by TAE. The findings of the study indicate that the combination of antibiotics and medicinal plants may significantly aid the detection of novel antimicrobials against organisms resistant to antibiotics.

Acknowledgment

The authors convey their heartfelt gratitude towards the head of the Microbiology department from Kishinchand Chellaram College along with Mr. Vishal Ramkar from IIT Bombay, for helping in SEM analysis.

Author Contribution

Dr. Pratibha Shah conceptualized the current study. She was also the guide and supervisor for the project. Mr. Samay Dwivedi performed the various tests practically. Both authors carried out the statistical analysis and data interpretation.

Conflict of Interest

None

References

Anand, U., Jacobo-Herrera, N., Altemimi, A., & Lakhssassi, N. (2019). A comprehensive review on medicinal plants as antimicrobial therapeutics: Potential avenues of Biocompatible Drug Discovery. *Metabolites*, 9(11), 258. doi:10.3390/metabo9110258

Aneja, K. R., Sharma, C., & Joshi, R. (2012). Antimicrobial activity of Terminalia Arjuna Wight & Arn.: An ethnomedicinal plant against pathogens

causing ear infection. *Brazilian Journal of Otorhinolaryngology*, 78(1), 68–74. doi:10.1590/s1808-86942012000100011

CLSI, Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, 7th ed., CLSI document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, 2012.

Debnath, S., Dey, D., Hazra, S., Ghosh, S., Ray, R., Hazra, B. (2013). Antibacterial and antifungal activity of Terminalia arjuna Wight & Arn. bark against ATCC strains and multi-drug-resistant clinical isolates. *Journal of Coastal Life Medicine*. 1(4): 315-321. doi:10.12980/JCLM.1.2013C757

Gul, R., Jan, S. U., Faridullah, S., Sherani, S., & Jahan, N. (2017). Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from ephedra intermedia indigenous to Balochistan. *The Scientific World Journal*, 2017, 1–7. doi:10.1155/2017/5873648

Haroun, M. F., and Al-Kayali, R., S. (2016). Synergistic effect of Thymbra spicata L. extracts with antibiotics against multidrug- resistant Staphylococcus aureus and Klebsiella pneumoniae strains. Iranian Journal of Basic Medical Sciences.19(11):1193-1200. PMID: 27917275; PMCID: PMC5126220.

Kumar, V., Sharma, N., Sourirajan, A., Khosla, P. K., & Dev, K. (2018). Comparative evaluation of antimicrobial and antioxidant potential of ethanolic extract and its fractions of bark and leaves of Terminalia arjuna from north-western Himalayas, India. *Journal of Traditional and Complementary Medicine*, 8(1), 100–106. doi: 10.1016/j.jtcme.2017.04.002.

Mandal, S., Patra, A., Samanta, A., Roy, S., Mandal, A., Mahapatra, T. D., … Nandi, D. K. (2013). Analysis of phytochemical profile of Terminalia arjuna bark extract with antioxidative and antimicrobial properties. *Asian Pacific Journal of Tropical Biomedicine*, 3(12), 960–966. doi:10.1016/s2221-1691(13)60186-0

Mohammad, S., Sadika, A., Md, I. H., Md, A. H., & Mohiuddin, A. B. (2012). Evaluation of in vitro antioxidant activity of bark extracts of terminalia Arjuna. *Journal of Medicinal Plants Research*, 6(39), 5286–5298. doi:10.5897/jmpr12.580

Nakamura, A. (2000). Combined effects of meropenem and aminoglycosides on pseudomonas aeruginosa in vitro. Journal of Antimicrobial Chemotherapy, 46(6), 901–904. doi:10.1093/jac/46.6.901

Palanisamy, K., Kalaiselvi, P. A., Gabriel, M., Thangavel, J., Sundaram, L.(2014). Emblica officinalis leaf extract mediated green synthesis of antibacterial silver nanoparticles against human pathogens. *World Journal of Pharmacy and Pharmaceutical Sciences*. 3(3): 2019-2032. doi/full/10.5555/20143148760

Tyagi, S. (2017). Comparative analysis of metal nanoparticles synthesized from Hibiscus Rosa SINESIS and their antibacterial activity estimation against nine pathogenic bacteria. *Asian Journal of Pharmaceutical and Clinical Research*, 10(5), 323. doi:10.22159/ajpcr. 2017.v10i5.17458

Wang, L., Hu, C., & Shao, L. (2017). The antimicrobial activity of nanoparticles: Present situation and prospects for the future. *International Journal of Nanomedicine*, 12, 1227–1249. doi:10.2147/ijn. s121956

WHO Traditional Medicine Strategy 2002–2005. May 2002. Available at: http://www.who.int/medicines/organization/trm/orgtrmmain.shtml.

Xu, L., Wang, Y.-Y., Huang, J., Chen, C.-Y., Wang, Z.-X., & Xie, H. (2020). Silver nanoparticles: Synthesis, medical applications and Biosafety. *Theranostics*, 10(20), 8996–9031. doi:10.7150/thno.45413