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# Synergistic Antibacterial Activity of Selected Plant Extracts in Combination with Antibiotics: An In Vitro Study

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**Abstract:**

**Background:** The rising prevalence of antibiotic-resistant bacterial strains has become a major global health concern, necessitating the exploration of alternative and adjunct antimicrobial strategies. Medicinal plants, particularly *Azadirachta indica* (neem), are widely recognized for their broad-spectrum antimicrobial properties and potential to enhance the activity of conventional antibiotics.

**Aim:** The present study aimed to evaluate the synergistic antibacterial activity of aqueous extract of *Azadirachta indica* (neem) in combination with selected antibiotics (ampicillin and ciprofloxacin) against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 under in vitro conditions.

**Materials and Methods:** This in vitro experimental study was conducted in the Microbiology Laboratory of Annor Dental College. Fresh neem leaves, authenticated by a botanist, were collected locally and aqueous extract was prepared using standard procedures. Reference strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were procured from HiMedia Laboratories. Antibacterial activity was assessed using the agar disc diffusion method on Mueller-Hinton agar. Zones of inhibition were measured for neem extract, antibiotics, and their combinations. All experiments were performed in triplicate.

**Results:** The aqueous neem extract demonstrated moderate antibacterial activity against both test organisms. Ciprofloxacin exhibited higher antibacterial activity compared to ampicillin. However, combinations of neem extract with both antibiotics showed significantly enhanced zones of inhibition compared to individual agents. The most pronounced synergistic effect was observed with neem–ciprofloxacin combination against both *E. coli* ATCC 25922 and *S. aureus* ATCC 25923.

**Conclusion:** The study demonstrates that aqueous extract of *Azadirachta indica* exhibits significant synergistic antibacterial activity when combined with conventional antibiotics. These findings suggest that neem may serve as a potential adjuvant to enhance antibiotic efficacy and may contribute to strategies aimed at combating antibiotic resistance.

**Keywords:** *Azadirachta indica*, neem, synergy, antibiotics, antibacterial activity, *Escherichia coli*, *Staphylococcus aureus*, in vitro study.

## Introduction

The rapid emergence and spread of antibiotic-resistant bacteria have become a major global health concern, limiting the effectiveness of commonly used antimicrobial agents. Pathogens such as Escherichia coli and Staphylococcus aureus are frequently associated with a wide range of infections and have shown increasing resistance to conventional antibiotics, including ampicillin and ciprofloxacin (1). This growing resistance has prompted the search for alternative and complementary therapeutic strategies to enhance antimicrobial efficacy.

Medicinal plants have been widely explored as potential sources of antimicrobial compounds due to their rich phytochemical composition. Among these, Azadirachta indica (neem) has gained significant attention for its broad-spectrum biological activities. Neem contains various bioactive constituents such as flavonoids, tannins, alkaloids, and terpenoids, which exhibit antibacterial, antifungal, and anti-inflammatory properties (2). Aqueous extracts of neem are particularly important as they are easy to prepare, cost-effective, and safer for laboratory use compared to organic solvent extracts.

The concept of combining plant extracts with antibiotics has emerged as a promising approach to overcome antimicrobial resistance. Such combinations may result in synergistic effects, where the combined action is greater than the sum of individual effects. This synergy can enhance bacterial susceptibility, reduce the required antibiotic dose, and potentially minimize adverse effects (3). Several studies have demonstrated that plant-derived

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compounds can interfere with bacterial cell wall synthesis, protein synthesis, or efflux pump mechanisms, thereby improving antibiotic activity against resistant strains (4).

Standard reference strains such as *E. coli* ATCC and *S. aureus* ATCC are commonly used in in vitro studies to evaluate antimicrobial activity, ensuring reproducibility and reliability of results (5). Antibiotics like ampicillin and ciprofloxacin serve as effective controls due to their well-established antibacterial profiles.

Despite increasing interest in plant–antibiotic combinations, there remains a need for systematic evaluation of their synergistic interactions using simple and reproducible in vitro methods. In particular, the antibacterial potential of aqueous neem extract in combination with commonly used antibiotics against standard bacterial strains requires further investigation. The present study aims to assess the synergistic antibacterial effect of the aqueous extract of *Azadirachta indica* (neem) when combined with selected antibiotics, namely ampicillin and ciprofloxacin, against *Escherichia coli* ATCC and *Staphylococcus aureus* ATCC under in vitro conditions.

## Materials and Methods

This in vitro experimental study was conducted in the Microbiology Laboratory of Annor Dental College. Standard reference bacterial strains, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, were procured from HiMedia Laboratories and used for the study.

Fresh leaves of *Azadirachta indica* (neem) were collected locally and authenticated by a qualified botanist. The plant material was thoroughly washed with distilled water, shade-dried, and ground into a fine powder. An aqueous extract was then prepared by mixing the powdered leaves with sterile distilled water, followed by filtration to obtain a clear extract, which was stored at 4°C until further use.

Bacterial inocula were prepared by selecting well-isolated colonies and suspending them in sterile saline, with turbidity adjusted to match the McFarland standard. The antibacterial activity of the aqueous neem extract, selected antibiotics (ampicillin and ciprofloxacin), and their combinations was evaluated using the agar disc diffusion method on Mueller-Hinton agar plates. The bacterial suspension was uniformly swabbed onto the agar surface using sterile cotton swabs. Sterile discs impregnated with neem extract, antibiotics, and their combinations were placed onto the inoculated plates. Appropriate antibiotic controls and solvent controls were included.

The plates were incubated at 37°C for 18–24 hours, after which the zones of inhibition were measured in millimeters. All tests were performed in triplicate to ensure reproducibility. The synergistic effect was determined by comparing the zones of inhibition of combination treatments with those of individual agents. Strict aseptic conditions were maintained throughout the study, and standard microbiological safety protocols were followed.

## Results

The aqueous extract of Azadirachta indica (neem), antibiotics (ampicillin and ciprofloxacin), and their combinations were evaluated for antibacterial activity against Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 using the agar disc diffusion method. The study demonstrated clear zones of inhibition for both the plant extract and antibiotics, indicating effective antibacterial activity. When tested individually, the neem extract showed moderate antibacterial activity against both organisms, while ciprofloxacin exhibited the highest zone of inhibition, followed by ampicillin. Notably, the combination of neem extract with antibiotics produced a significantly larger zone of inhibition compared to the individual agents alone. The synergistic effect was more pronounced in the combination of neem extract with ciprofloxacin against Staphylococcus aureus ATCC 25923, showing the maximum enhancement in antibacterial activity. A similar synergistic trend was observed against Escherichia coli ATCC 25922, although the increase in inhibition zone was comparatively moderate. The combination of neem extract with ampicillin also demonstrated improved antibacterial activity against both bacterial strains when compared to ampicillin alone. All experiments were performed in triplicates, and the results were found to be consistent with minimal variation. The findings indicate a positive synergistic interaction between aqueous neem extract and the selected antibiotics, enhancing their antibacterial efficacy against both Gram-positive and Gram-negative bacteria.

Table 1: Zone of Inhibition of Aqueous Neem Extract, Antibiotics, and Their Combinations Against Test Organisms (mm)

| **Test Sample** | ***Escherichia coli* ATCC 25922 (mm)** | ***Staphylococcus aureus* ATCC 25923 (mm)** |
|---|---|---|
| Neem extract (Aqueous) | 10 ± 0.5 | 12 ± 0.6 |
| Ampicillin | 14 ± 0.4 | 16 ± 0.5 |
| Ciprofloxacin | 20 ± 0.3 | 22 ± 0.4 |
| Neem + Ampicillin | 18 ± 0.5 | 20 ± 0.6 |
| Neem + Ciprofloxacin | 24 ± 0.4 | 26 ± 0.5 |
| Solvent Control | No zone observed | No zone observed |

Figure 1: Antimicrobial Susceptibility Test (AST) showing zones of inhibition of *Escherichia coli* ATCC 25922 on Mueller-Hinton agar.

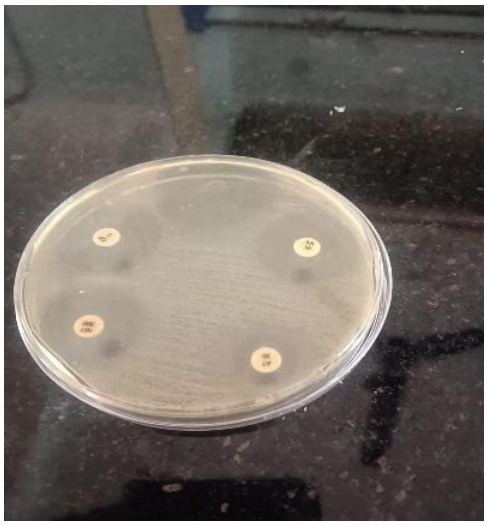

**Figure 2:** Antimicrobial Susceptibility Test (AST) showing zones of inhibition of *Staphylococcus aureus* ATCC 25923 on Mueller-Hinton agar.

## Discussion

The present in vitro study demonstrated that the aqueous extract of *Azadirachta indica* (neem) in combination with antibiotics (ampicillin and ciprofloxacin) exhibited enhanced antibacterial activity against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 when compared to individual agents. The increased zones of inhibition observed in combination groups indicate a clear synergistic interaction between plant-derived phytochemicals and conventional antibiotics.

The moderate antibacterial activity of aqueous neem extract alone is consistent with previous findings, where *A. indica* extracts have shown measurable inhibitory effects against both Gram-positive and Gram-negative bacteria due to the presence of bioactive compounds such as flavonoids, tannins, and alkaloids. Similar studies have reported that neem-based preparations exhibit broad-spectrum antibacterial activity, although the potency is generally lower than standard antibiotics when used individually (6).

In the present study, ciprofloxacin showed higher antibacterial activity compared to ampicillin, which aligns with previous reports indicating that fluoroquinolones generally demonstrate stronger efficacy against resistant Gram-negative organisms such as *E. coli* due to their DNA gyrase inhibitory mechanism (7). However, the most significant finding of this study was the marked increase in antibacterial activity when neem extract was combined with both antibiotics, particularly ciprofloxacin, against both test organisms.

The observed synergistic effect may be attributed to the ability of phytochemicals in neem to disrupt bacterial cell wall integrity, inhibit efflux pumps, or increase membrane permeability, thereby facilitating enhanced intracellular entry of antibiotics. This mechanism has been widely supported in recent literature, where plant extracts have been shown to potentiate antibiotic activity through multi-target interactions (8).

Similar synergistic outcomes have been reported in recent in vitro studies involving plant–antibiotic combinations. For example, combinations of medicinal plant extracts with ciprofloxacin have demonstrated significantly reduced MIC values and enhanced antibacterial effects against *Staphylococcus aureus* and *E. coli* strains (9). Another recent review highlights that plant-derived compounds can restore antibiotic sensitivity in resistant bacteria by targeting multiple bacterial pathways simultaneously, thereby reducing resistance development (10). Furthermore, a study on MRSA demonstrated that plant extract combinations significantly enhanced bacterial growth inhibition, supporting the concept of multi-target synergism in antimicrobial therapy (11).

The present findings are also in agreement with reports showing that neem-based combinations can enhance antimicrobial efficacy in endodontic and systemic bacterial infections, further validating its traditional medicinal use (12). The greater synergistic response observed with ciprofloxacin compared to ampicillin may be due to differences in antibiotic mechanisms and their interaction with plant-derived bioactive compounds.

Overall, the results of this study strongly support the hypothesis that aqueous neem extract can act as an effective adjuvant to conventional antibiotics, improving their antibacterial performance. This combination approach may provide a promising strategy to combat rising antibiotic resistance and reduce therapeutic doses of standard antibiotics.

Table 2: Comparison of Present Study with Recent Literature on Plant–Antibiotic Synergism

| Study | Plant Extract Used | Antibiotic Used | Test Organisms | Key Findings (Synergistic Effect) |
|---|---|---|---|---|
| Present study (Annor Dental College) | *Azadirachta indica* (aqueous neem extract) | Ampicillin, Ciprofloxacin | *E. coli* ATCC 25922, *S. aureus* ATCC 25923 | Strong synergistic increase in zone of inhibition; highest effect with neem + ciprofloxacin against both organisms |
| Kamatou et al. / recent plant synergy study | *Azadirachta indica* extract | Ciprofloxacin | *E. coli*, *S. aureus* | Strong synergy observed with reduced MIC (FICI ≤ 0.5), especially against *E. coli* (Springer) |
| Calotropis procera study | Leaf and stem extracts | Ciprofloxacin | *E. coli*, *S. aureus* | Significant synergy (FICI 0.3–0.4), enhanced antibacterial activity compared to individual agents (ijsrbs.isroset.org) |
| Neem-based herbal synergy study (PAW-treated neem) | Neem infusion | Ciprofloxacin | *E. coli*, *S. aureus* | Reduced MIC and enhanced antibacterial activity showing clear synergistic interaction (arXiv) |
| Plant extract combination study (general) | Multiple medicinal plants | Ciprofloxacin, Ampicillin | *E. coli*, *S. aureus* | Improved antibacterial activity due to membrane disruption and increased antibiotic uptake (Nature) |

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