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Original Research Article

Synergistic Antibacterial Activity of *Azadirachta indica* Bark Extract Combined with *Curcuma longa* Rhizome Extract Against Multidrug Resistant Bacteria

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Abstract

The emergence of multidrug resistant (MDR) bacteria has become a major global health concern due to the reduced effectiveness of conventional antibiotics. The present study evaluated the synergistic antibacterial activity of *Azadirachta indica* (neem) bark extract combined with *Curcuma longa* (turmeric) rhizome extract against selected multidrug resistant bacterial isolates. Ethanolic extracts of neem bark and turmeric rhizome were prepared and tested individually as well as in combination (1:1 ratio) using the disc diffusion method on Mueller-Hinton agar. The antibacterial activity was assessed against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Staphylococcus aureus*. Chloramphenicol was used as the positive control, while ethanol served as the negative control. The combined extract demonstrated enhanced antibacterial activity compared to the individual extracts, indicating a synergistic effect between neem and turmeric. Maximum inhibition was observed against *S. aureus* (20 mm), followed by *B. cereus* (18 mm), *E. coli* (10 mm), and *P. aeruginosa* (8 mm). Individual extracts showed comparatively lower inhibition zones. The results suggest that the synergistic interaction of phytochemicals such as curcumin, flavonoids, tannins, and azadirachtin may contribute to the improved antibacterial effect. This study highlights the potential of combined medicinal plant extracts as natural alternative antimicrobial agents against multidrug resistant pathogens.

Keywords: *Azadirachta indica*, *Curcuma longa*, Synergistic antibacterial activity, Multidrug resistant bacteria, Medicinal plants, Disc diffusion method, Phytochemicals, Natural antimicrobial agents.

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the major global public health threats due to the rapid development of multidrug resistant (MDR) bacterial pathogens. The increasing resistance of microorganisms toward commonly used antibiotics has reduced the effectiveness of conventional antimicrobial therapy, resulting in prolonged illness, increased mortality, and higher healthcare costs (Ventola, 2015; Davies and Davies, 2010). Pathogenic bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus cereus* are frequently associated with resistant infections and foodborne diseases (Silva and Fernandes Júnior, 2010).

Medicinal plants have been widely used in traditional systems of medicine for the treatment of infectious diseases because they contain numerous bioactive phytochemicals including alkaloids, flavonoids, tannins, terpenoids, phenolics, and essential oils (Cowan, 1999). These compounds possess antimicrobial, antioxidant, anti-inflammatory, and therapeutic properties. In recent years, the synergistic use of medicinal plant extracts has gained scientific attention because combined plant extracts may produce enhanced antibacterial activity compared to individual extracts alone (Eloff, 1998).

Azadirachta indica (Neem), belonging to the family Meliaceae, is an important medicinal plant known for its broad spectrum antimicrobial activity. Neem bark contains active compounds such as nimbidin, azadirachtin, flavonoids, tannins, and polyphenols that exhibit inhibitory effects against both Gram-positive and Gram-negative bacteria (Subapriya and Nagini, 2005; Biswas *et al*., 2002).

Curcuma longa (Turmeric), a member of the family Zingiberaceae, is another medicinal plant extensively used in traditional medicine. The rhizome contains curcumin, a biologically active polyphenolic

compound possessing antimicrobial, antioxidant, anti-inflammatory, and anticancer properties (Gupta *et al*., 2012). Previous studies have reported that turmeric extracts exhibit antibacterial activity against several pathogenic microorganisms (Singh *et al*., 2002).

The present study was undertaken to evaluate the synergistic antibacterial activity of *Azadirachta indica* bark extract combined with *Curcuma longa* rhizome extract against selected multidrug resistant bacterial isolates using the disc diffusion method.

MATERIALS AND METHODS

Fresh bark of *Azadirachta indica* and rhizomes of *Curcuma longa* were collected, washed thoroughly with distilled water, shade dried, powdered, and extracted using ethanol as the solvent according to standard phytochemical extraction procedures (Eloff, 1998). The extracts were concentrated using a water bath and stored at 4°C until further use.

The bacterial isolates used in the study included *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Staphylococcus aureus*. Pure cultures were maintained on nutrient agar slants at refrigerated conditions.

The antibacterial activity of the individual extracts and their combination (1:1 ratio) was evaluated by the disc diffusion method on Mueller-Hinton agar plates following standard microbiological procedures (Bauer *et al*., 1966). Sterile discs impregnated with plant extracts at a concentration of 50 mg/ml were placed on inoculated agar plates. Chloramphenicol (30 µg/ml) was used as the positive control, while ethanol served as the negative control.

The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured in millimeters. The antibacterial activity was determined by comparing the diameter of inhibition zones produced by individual extracts, combined extracts, and the positive control.

RESULTS

The antibacterial activity of individual extracts of neem bark (*Azadirachta indica*) and turmeric (*Curcuma longa*), as well as their combined extract (1:1 ratio), was evaluated against multidrug resistant bacterial isolates using the disc diffusion method. Chloramphenicol (30 µg/ml) was used as the positive control, while ethanol served as the negative control. No inhibition zone was observed in the negative control.

The combined extract exhibited enhanced antibacterial activity against all tested organisms compared to the individual extracts alone, indicating a synergistic effect between neem bark and turmeric extracts.

Among the tested organisms, maximum inhibition by the combined extract was observed against *Staphylococcus aureus* (20 mm), followed by *Bacillus cereus* (18 mm), *Escherichia coli* (10 mm), and *Pseudomonas aeruginosa* (8 mm). The positive control showed the highest inhibition against all bacterial isolates (Table 1, Figures 1-4).

Table 1: Zone of inhibition of neem bark extract, turmeric extract, combined extract, and positive control against multidrug resistant bacteria

Test Organism	Turmeric Rhizome Extract (50 mg/ml)	Neem Bark Extract (50 mg/ml)	Combined Extract (1:1) (50 mg/ml)	Positive Control Chloramphenicol (30 µg/ml)
Pseudomonas aeruginosa	6 mm	5 mm	8 mm	10 mm
Bacillus cereus	10 mm	14 mm	18 mm	18 mm
Staphylococcus aureus	13 mm	15 mm	20 mm	25 mm
Escherichia coli	7 mm	7 mm	10 mm	17 mm

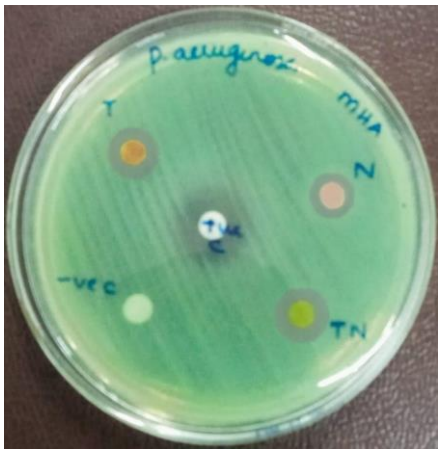

Fig. 1

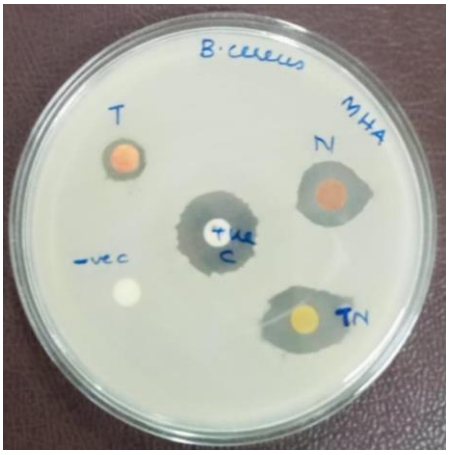

Fig. 2

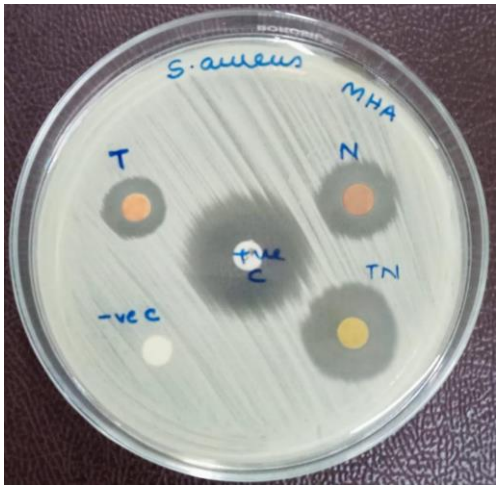

Fig. 3

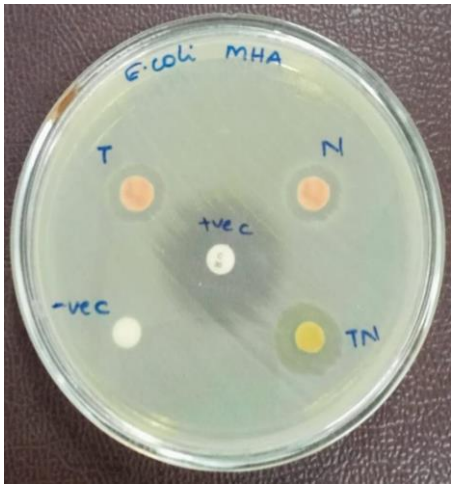

Fig. 4

Figures 1-4: Zone of inhibition of neem bark extract, turmeric extract, combined extract, positive and negative control against multidrug resistant bacteria 1. *P. aeruginosa*, 2. *B. cereus*, 3. *S. aureus*, 4. *E. coli*

DISCUSSION

The present study demonstrated that the combined extract of neem bark and turmeric possessed enhanced antibacterial activity against multidrug resistant bacterial isolates when compared with the individual extracts alone. The synergistic interaction between the two medicinal plants may be attributed to the combined action of bioactive phytochemicals such as curcumin, flavonoids, tannins, azadirachtin, and other phenolic compounds (Gupta *et al*., 2012; Biswas *et al*., 2002).

The combined extract showed improved inhibitory activity against both Gram-positive and Gram-negative bacteria. Among the tested organisms, *Staphylococcus aureus* showed maximum susceptibility, whereas *Pseudomonas aeruginosa* exhibited comparatively lower susceptibility. Similar observations were reported by Silva and Fernandes Júnior (2010), who noted that Gram-negative bacteria are generally more resistant due to the presence of an outer membrane that restricts penetration of antimicrobial compounds.

The individual turmeric extract showed moderate antibacterial activity, while neem bark extract demonstrated comparatively higher inhibition against *Bacillus cereus* and *Staphylococcus aureus*. Previous studies by Singh *et al*. (2002) also reported significant antibacterial activity of turmeric extracts against pathogenic bacteria.

The synergistic antibacterial effect observed in the present study may result from the interaction of multiple phytochemicals acting on different bacterial targets simultaneously. Eloff (1998) suggested that combinations of plant-derived compounds can enhance antibacterial efficiency through additive or synergistic mechanisms.

The positive control chloramphenicol exhibited greater antibacterial activity than the plant extracts, particularly against *Staphylococcus aureus* and *Escherichia coli*. However, the combined plant extract showed promising inhibitory activity, suggesting its potential as a natural alternative antimicrobial agent against resistant pathogens.

SUMMARY

The study evaluated the synergistic antibacterial activity of *Azadirachta indica* bark extract combined with *Curcuma longa* rhizome extract against multidrug resistant bacteria using the disc diffusion method. The individual extracts showed moderate antibacterial activity, whereas the combined extract exhibited enhanced inhibitory effects against all tested bacterial isolates. Maximum inhibition was observed against *Staphylococcus aureus*, followed by *Bacillus cereus*. The results demonstrated that the combination of neem bark and turmeric extracts possesses significant synergistic antibacterial activity.The findings suggest that combined medicinal plant extracts may serve as potential natural antimicrobial agents for the management of multidrug resistant bacterial infections.

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