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Research Article

Evaluation Of Synergistic Anti-Bacterial Effect of *Azadirachta Indica* and *Solanum Trilobatum* through In-Vitro Studies

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ABSTRACT

The emergence of antibiotic-resistant bacteria has become a significant global health concern, necessitating the exploration of alternative therapeutic approaches. This study investigates the synergistic antibacterial potential of *Azadirachta indica* and *Solanum trilobatum* against selected bacterial strains. The individual and combined extracts of these plants were evaluated using the disc diffusion method against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. The results demonstrate that both plant extracts exhibit antibacterial activity, with the combination of *Azadirachta indica* and *Solanum trilobatum* showing enhanced efficacy compared to individual treatments. The synergistic effect of the combined extracts was found to be significant, with a notable increase in the zone of inhibition. This study highlights the potential of synergistic antibacterial combinations, particularly between *Azadirachta indica* and *Solanum trilobatum*, as a promising strategy to combat antibiotic-resistant bacterial strains and improve therapeutic outcomes.

INTRODUCTION

Pharmacognosy plays a significant role in the modern era of drug discovery, especially in the search for new antibacterial agents. The importance of pharmacognosy in the development of antibacterial compounds cannot be overstated, especially considering the global issue of antibiotic resistance. Antibiotic resistance has become one of the most pressing challenges in

medicine today. With the continuous emergence of multidrug-resistant (MDR) bacteria, there is an urgent need to explore alternative treatments. This has led researchers to focus on natural compounds that have demonstrated antibacterial properties as potential candidates for therapeutic development. The role of synergy in antibacterial therapy is especially significant in addressing bacterial resistance. Synergistic antibacterial activity refers

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to the combined effect of two or more substances, where their interaction results in an enhanced antibacterial effect compared to when used separately. This strategy is particularly useful in combating bacteria that have developed resistance to conventional antibiotics. By combining different compounds that target multiple bacterial pathways, synergistic therapy can enhance efficacy, reduce toxicity, and delay the development of resistance.

Mechanisms of Synergistic Antibacterial Activity

Synergistic antibacterial combinations work through various mechanisms. Some of the primary mechanisms include:

- **Disruption of cell wall synthesis:** Several bioactive compounds from natural sources can weaken the bacterial cell wall, which is essential for maintaining the integrity and survival of the bacteria.
- **Inhibition of protein synthesis:** Certain compounds target bacterial ribosomes, halting protein production, which is crucial for bacterial growth and survival.
- **Disruption of nucleic acid synthesis:** Other compounds interfere with bacterial DNA replication and transcription processes, preventing bacterial reproduction.
- **Prevention of biofilm formation:** Many bacteria form biofilms, protective layers that shield them from the effects of antibiotics. Synergistic agents can help prevent or disrupt biofilm formation, making bacteria more vulnerable to treatment. These mechanisms of action can be harnessed effectively in synergistic combinations, enhancing the overall antibacterial activity. The combination of multiple agents that target different bacterial functions increases the likelihood of a successful therapeutic outcome.

Importance of Synergistic Combinations in Antimicrobial Therapy The combination of multiple antibacterial agents holds great potential

in combating bacterial infections. By using synergistic formulations, it is possible to reduce the overall dosage of each individual agent while maintaining or even increasing the therapeutic effect. This not only helps in minimizing potential side effects but also makes it less likely for bacteria to develop resistance against the combined agents. As bacterial resistance to conventional antibiotics continues to rise, synergistic antibacterial combinations represent a promising approach to overcoming this challenge. Research into the synergistic effects of different bioactive compounds, especially from natural sources, has shown that such combinations can be more effective than single-agent treatments. These combinations may offer broader spectrum activity, targeting a range of bacterial species, including those that are resistant to multiple drugs.

Aim: To evaluate the synergistic antibacterial effect of *Azadirachta indica* and *Solanum trilobatum* through in-vitro studies against selected bacterial strains.

Objectives: To assess the individual and synergistic antibacterial effect of the combined extracts against bacterial strains using disc diffusion method.

MATERIALS AND METHODS:

1. Collection of plant material

Procurement of fresh *Solanum trilobatum* leaves and *Azadirachta indica* leaves from a reliable source.

2. Identification and authentication of plant material

The collected specimens were botanically identified and authenticated by Dr.N.K. Sunil Kumar, Research officer, Department of Pharmacognosy, Siddha Central Research institute, Chennai - 600106. The samples were identified as *Azadirachta indica* belongs to the family Meliaceae and *Solanum trilobatum* belongs to the family Solanaceae.

3. Plant profile



Azadirachta indica**Vernacular Names****Table: 1 Indian Names**

Indian Names of <i>Azadirachta indica</i>	
Hindi	Nim, Nimb, Nimgachh, Balnimb, Ninb
Telgu	Vepa, Kondavepa, Turakavepa, Nimbamu, Vemu, Yapa
Bengali	Nim, Nimb, Nimgachh
Urdu	Neem
Tamil	Vepa, Veppu, Vembu, Veppan, Arulundi, Vempu
Malayalam	Nimbam, Rajaveppu
Gujrati	Limba, Danujhada, Kohumba, Limbado, Limbra

Table :2 International Names

International names <i>Azadirachta indica</i>	
English	Margosa tree, Indian Lilac
Sanskrit	Arishta, Ravipriya, Vranashodhakari, Nimba, Prabhadr, Arkapadapa, hhardana, Kitaka, Malaka, Shita, Subhadra, Yavaneshta
Burma	Tamabin, Ta-mar bin, Kamakha, Bawtamaka, Kamaka, Thamaka, Thin, Thinborotamakha
French	Lilas de chine, Margosier
German	Grossblaettiger, Zedrach.

Taxonomical Classification**Table 3: Taxonomical Classification**

Kingdom	Plantae
Phylum	Magnoliophyta
Species	Indica
Class	Rutales
Order	Dipsacales
Family	Meliaceae
Genus	<i>Azadirachta</i>

**Figure 1: Leaves of *Azadirachta indica******Solanum Trilobatum*****Vernacular Names****Table:4 Indian Names**

Indian Names of <i>Solanum trilobatum</i>	
Hindi	Bhatia, Tati, Sainka
Tamil	Thumbai, Kaatraan, ManjalThumbai
Bengali	Kantakari
Telugu	Gokarakaya
Malayalam	Kattarvazha
Urudu	Kundur
Gujarati	Kunduru, Bheedi

Table:5 International names

International Names of <i>Solanum trilobatum</i>	
English	Climbing Brinjal.
Sanskrit	Alarka, Vallikantakaarika, Kantakaari-lata.

Taxonomical Classification**Table 6: Taxonomical Classification**

Kingdom	Plantae
Phylum	Magnoliophyta
Species	Trilobatum
Class	Magnoliopsida
Order	Solanales
Family	Solanaceae
Genus	<i>Solanum</i>

**Figure 2: Leaves of *Solanum trilobatum*****4. Extraction of plant material**

The leaves of plant were collected from a healthy tree, ensuring that only mature, disease-free leaves are selected. The leaves are washed and dried under shade to prevent photochemical degradation. The dried leaves are ground into a fine powder using a mechanical grinder. Weigh 50gm of coarsely powdered air-dried *Azadirachita*

indica and *Solanum trilobatum* leaves and transferred into the beaker. It was treated with (50%) ethanol i.e 1:1 ethanol and distilled water and macerate for 48-72 hours, with occasional stirring. The extract was filtered using Whatman filter paper no.1, and concentrated under water bath at 40-45°C.

5. Phytochemical Screening

Preliminary qualitative analysis of bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and phenols.

6. Antibacterial Testing

Selection of Bacterial Strains: Gram-positive and Gram-negative bacteria will be selected for testing.

Disc Diffusion Method: Individual and combined extracts will be tested for antibacterial activity.

Synergistic Effect Analysis: Combination studies of *Solanum trilobatum* and *Azadirachta indica* extracts to evaluate enhanced antibacterial activity. That can be compared with streptomycin.

Antibacterial Assay-Disc Diffusion method

Antibacterial screening

Medium: Mueller Hinton agar.

Composition of Mueller Hinton agar

1. Beef infusion – 30g/l
2. Casein and hydrolysate - 17.5g/l
3. Starch - 1.5g/l
4. Agar - 17g/l

Preparation of agar medium

3.8g of Mueller Hinton agar was weighed and dissolved in 100ml of distilled water and adjust the pH 7.3±0.2 sterilized by autoclave at 121°C for 15mins and used for the sensitivity test.

Preparation of sterile swabs

Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or dry heat (only for wooden swabs) by packing the swabs in culture tubes, papers or tins etc.,

Sterilization of forceps

Sterilize forceps by dipping in alcohol and burning off the alcohol.

Procedure

After preparing and solidifying the Mueller-Hinton Agar (MHA) plates, the bacterial culture is evenly spread across the agar surface using a sterile cotton swab. The swabbing is done in three different directions to ensure uniform distribution of the bacterial suspension. The plates are then left undisturbed for 5 minutes to allow proper absorption of the inoculum into the agar. Next, sterile filter paper discs (6 mm diameter) are impregnated with the extracts at the desired concentrations and carefully placed on the agar surface using sterile forceps. The plates are then incubated at 37°C for 18–24 hours, allowing the test compounds to diffuse into the agar and exert their antibacterial effects. After incubation, the zones of inhibition (clear areas around the discs indicating bacterial growth inhibition) are measured using a millimeter ruler or caliper. The diameter of these inhibition zones is recorded, and the antibacterial activity of the extracts is evaluated by comparing the results with the standard antibiotic.

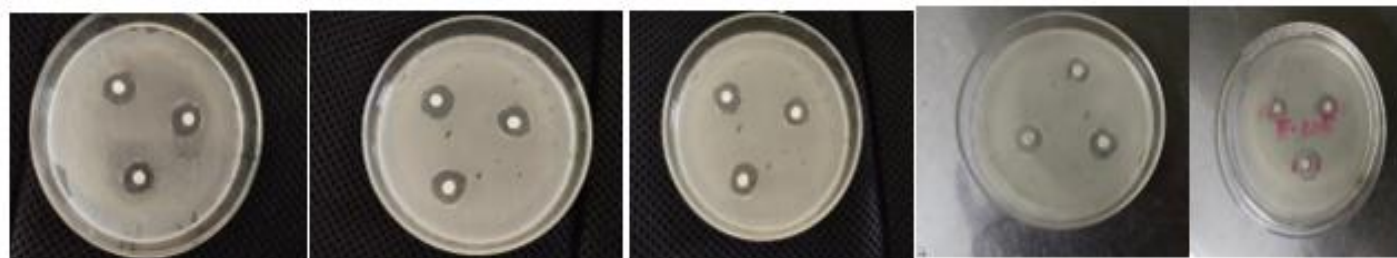


Figure 3: Zone Of Inhibition

Table 7: List Of Bacteria Used for This Study

S. No	Gram positive bacteria	Gram negative bacteria
1.	Staphylococcus aureus	Escherichia coli
2.	-	Pseudomonas aeruginosa

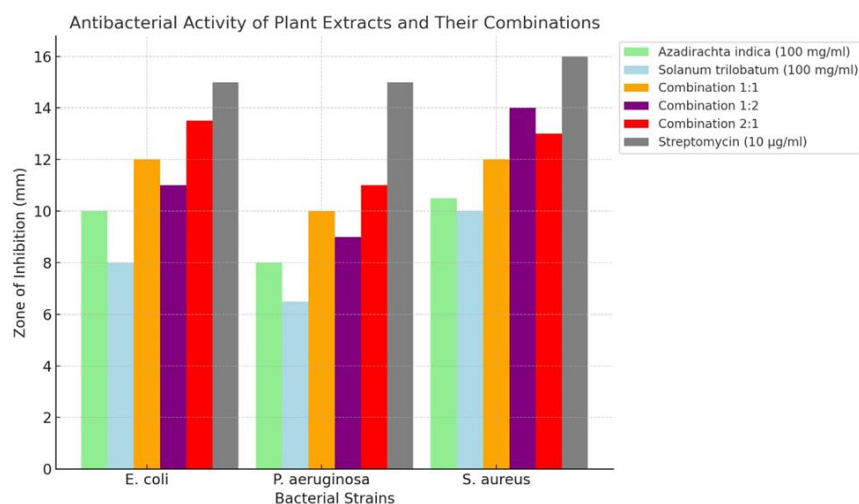
Invitro Antimicrobial Studies

Antibacterial Screening by Disc Diffusion

Method

Table 8: Antibacterial screening by disc diffusion method

Organism	Synergistic effect on combination of <i>azadirachta indica</i> with <i>solanum trilobatum</i> leaf extract			Azadirachta indica (100mg/ml)	Solanum trilobatum (100mg/ml)	Streptomycin (10µg/ml)
	1:1	1:2	2:1			
E. coli	12	11	13.5	10	8	15
Pseudomonas aeruginosa	10	9	11	8	6.5	15
Staphylococcus aureus	12	14	13	10.5	10	16



CONCLUSION

The antibacterial activity of *Azadirachta indica* and *Solanum trilobatum* leaf extracts, both individually and in combination, was assessed against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* using the disc diffusion method. The results demonstrated that both plant extracts possess antibacterial properties, with *Staphylococcus aureus* showing the highest susceptibility to the combination treatment. The 2:1 ratio of *Azadirachta indica* and *Solanum trilobatum* exhibited the greatest synergistic effect.

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