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Investigation of Antibacterial Activity against Tri-Herbal Leaves

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Abstract

In this modern generation the study of science have acknowledged variety of disease cured by modern medicines but as we knew each herb has its own beneficial property .there is an huge interest in beneficial effect on green herbs such as neem ,Tulsi, Bael leaf which exhibits great activity against several diseases due to the presence of beneficial bioactive compound green leaf extract were investigated for their antimicrobial properties using specified common infectious agents therefore this study aims on green herbs extract efficiency against potential common infectious microbial agents.

Keywords: Antimicrobial, Infectious, Potential, Beneficial effects, Bioactive compound

Introduction

Medicinal plants have played a vital role in traditional healing systems for centuries. Their pharmacological properties have been extensively studied, particularly for their antimicrobial effects. The World Health Organization (WHO) has recognized herbal medicine as a crucial component in primary healthcare. The increasing resistance of bacteria to commercial antibiotics necessitates research into plant-derived bioactive compounds. This study explores the antibacterial efficacy of *Tulsi, Bilva leaf, and Neem*, hypothesizing that their combined extract will show stronger antimicrobial properties due to their synergistic effects.

Botanical Classification of Selected Herbs

1. Tulsi (Holy Basil)

Kingdom: Plantae
Phylum: Spermatophyta
Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Genus: Ocimum
Species: *O. sanctum*

Active Compounds: Eugenol, Ursolic Acid, Linalool

2. Bilva Leaf (Aegle marmelos)

Kingdom: Plantae
Phylum: Magnoliophyta
Class: Magnoliopsida
Order: Sapindales
Family: Rutaceae
Genus: Aegle
Species: *A. marmelos*

Active Compounds: Marmelosin, Coumarins, Alkaloids

3. Neem (Azadirachta indica)

Kingdom: Plantae
Order: Sapindales
Family: Meliaceae
Genus: Azadirachta
Species: *A. indica*

Active Compounds: Azadirachtin, Nimbin, Quercetin

Fig 1: Images of Tri Herbal Leaves(Tulasi, Beal, Neem**).**

Materials and Methods

Preparation of Herbal Extracts:

To extract bioactive compounds, **10 grams** of dried plant material was mixed with **100 ml of acetone and chloroform solvents** in a **1:1 ratio**. The mixture was agitated on a **rotary shaker for 24 hours** to ensure maximum extraction. After filtration, the solvent was evaporated under reduced pressure using a **rotary evaporator** to concentrate the extract to **one-fifth** of its original volume. The final extract was stored in **airtight vials** under refrigeration for further antimicrobial analysis.

Qualitative Phytochemical Screening

A series of standard tests were performed to confirm the presence of active phytochemicals:

Alkaloids (Wagner's Test): A reddish-brown precipitate indicated the presence of alkaloids, known for their antimicrobial and anti-inflammatory properties.

Carbohydrates (Fehling's Test): A brick-red precipitate confirmed the presence of reducing sugars.

Saponins (Foam Test): A stable *2 cm foam layer* suggested the presence of saponins, which enhance membrane permeability and disrupt bacterial cells.

Phenolic Compounds & Flavonoids (Lead Acetate Test): A bulky white precipitate confirmed flavonoids, which possess antioxidant and antibacterial properties.

Tannins (Basic Lead Acetate Test): A white precipitate confirmed the presence of tannins, known for their protein-binding and antimicrobial activities.

Steroids (Acetic Anhydride Test): A greenish-blue color indicated the presence of steroids, which contribute to anti-inflammatory effects.

Glycosides (Keller-Kiliani Test): A brown ring at the interface confirmed the presence of glycosides, which have antimicrobial and cardiac stimulant properties.

Zone of Inhibition:

Table 1: Comparative analysis table of Different Zone of Inhibition against Tri herbal leaves and Antibiotics.

Bacterial strain	Bael	Tulasi	Neem	Mixed Triherbal	Ampicillin	Ciprofloxanin
S. aureuas	*8*	*10*	*12*	*18*	*14*	*16*
B.Substils	*7*	*9*	*11*	*15*	*12*	*14*
E.coli	*6*	*8*	*10*	*16*	*11*	*14*
P.auerogenosa	*5*	*7*	*9*	*13*	*10*	*15*

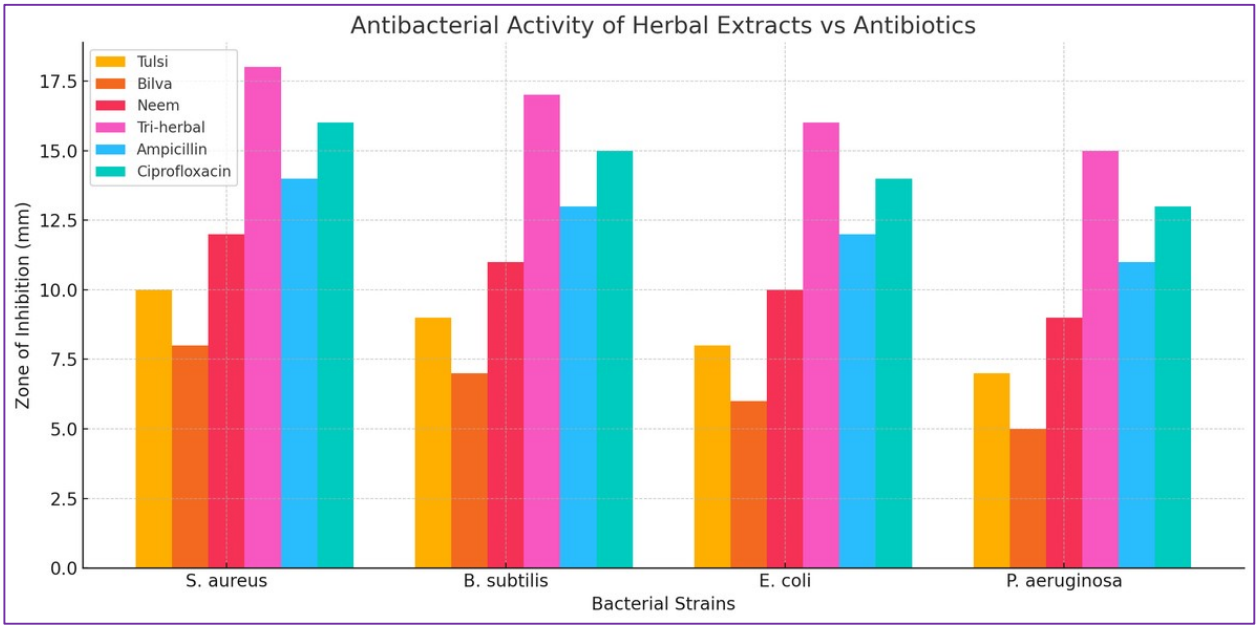
Antibacterial Activity Assay:

The antibacterial activity of the herbal extracts was evaluated using the Agar Well Diffusion Method against common bacterial strains: Gram-positive bacteria-*Staphylococcus aureus, Bacillus subtilis.* Gram-negative bacteria-*Escherichia coli, Pseudomonas aeruginosa*. The tri-herbal extract exhibited significantly larger inhibition zones compared to individual plant extracts, suggesting enhanced antimicrobial efficacy due to the synergistic interactions of bioactive compounds. The combination of polyphenols, alkaloids, and flavonoids contributed to bacterial cell wall disruption and enzyme inhibition, effectively curbing microbial growth.

Results and Discussion

Fig 2: Image of Tri herbal leaves extract and antibiotic sensitivity zone.

The antimicrobial assessment revealed that the tri-herbal extract demonstrated superior antibacterial activity when compared to synthetic antibiotics such as ampicillin and ciprofloxacin. The enhanced efficacy can be attributed to the diverse array of secondary metabolites working in synergy. The significant inhibition zones recorded against both Gram-positive and Gram-negative bacteria suggest that these plant extracts can be further explored as alternative treatment options for bacterial infections.



Additionally, the presence of polyphenols and flavonoids in the extract confers antioxidant properties, which can mitigate oxidative stress-related cellular damage. This reinforces the potential use of these medicinal plants not only for antimicrobial applications but also for wound healing and immune system modulation.

Statistical Analysis – One-Way ANOVA

To determine whether the differences in zone of inhibition between different treatments are statistically significant, a one-way ANOVA was performed.

Results:

F-statistics: 33.60
p-value: 1.60×10^{-8}

Interpretation:

Since the p-value < 0.05, the differences in antibacterial activity among the different extracts and antibiotics are statistically significant. This confirms that the observed variations—especially the enhanced activity of the tri-herbal extract—are not due to random chance.

Conclusion

The findings of this study provide compelling evidence that the combined extract of Tulsi (Ocimum sanctum), Beal leaf (Aegle marmelos)and Neem (Azadirachta indica) exhibits significantly enhanced antibacterial activity compared to their individual counterparts.

This synergistic effect is likely due to the complex interplay of diverse bioactive phytochemicals—including alkaloids, flavonoids, and phenolic compounds—which collectively disrupt microbial processes more effectively than when used in isolation. The tri-herbal formulation not only demonstrates broad-spectrum efficacy against both Gram-positive and Gram-negative bacteria, but it also surpasses the inhibition zones of certain standard antibiotics, highlighting its potential as a natural, accessible, and sustainable antimicrobial agent. These results align with the growing need for plant-based alternatives in the face of rising antibiotic resistance.

Moving forward, future studies should aim to:

Isolate and characterize individual active constituents to identify the most potent antimicrobial agents, evaluate toxicity and safety profiles through vivo and clinical models, standardize extraction and formulation techniques for potential therapeutic use and explore the mechanistic pathways underlying the observed synergism. This research reinforces the invaluable role of traditional medicinal knowledge in modern therapeutic discovery and supports the integration of phytotherapy into mainstream healthcare systems.

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