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A Comprehensive Evaluation of Synergistic and Antagonistic Interaction Patterns between Natural Plant-Derived Extracts and Conventional Antibiotic Agents in Enhancing Antibacterial Efficacy

Bhanupratap Vishwakarma[1*], Shruti Mishra[1], Shivani Pandey[1], Vidya Bagul[2], Neha Jadhav[1], Sahil Pandey[1] and Sweta Dwivedi[1]

[1]Department of Microbiology, Zsct's Thakur Shyam Narayan Degree College, Kandivali East, Mumbai – 400101, India
[2]Department of Microbiology, Guru Nanak Khalsa College of Arts, Science & Commerce (Autonomous), Matunga, Mumbai- 400019, India

**Corresponding author*

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A B S T R A C T

Antimicrobial resistance (AMR), which remains a challenge in the effectiveness of traditional antibiotics, thus compels an urgent search for alternative or adjunctive therapeutic approaches. Interaction of extracts from four herbal medicinal plants (*Randia aculeata, Ixora coccinea, Capsella bursa-pastoris and Portulaca oleracea*) with antibiotics commonly used against *Escherichia coli* and *Staphylococcus aureus* is studied in this paper. Aqueous extracts were obtained from the same plant material and its antibacterial activity was evaluated against bacteria through agar well diffusion test both combinatively as well as individually (1:1) with antibiotics. Species- and combination-specific results were obtained corresponding to synergy and/or antagonism. Substantial synergistic increase in the antibacterial activity was also noted for *Capsella bursa-pastoris* + ciprofloxacin and *Ixora coccinea* + erythromycin against *E. coli and S.aureus*, respectively indicating that plant phytochemicals either enhance the antibiotic uptake or temper bacterial defense system. On the other hand, that is not always true, as mixed effects such as *Randia* + ampicillin or *Portulaca* + amoxicillin showed antagonistic or indifferent additive interactions between plants and drugs indicating plant–drug combinations are more complicated. Altogether, the results point to the possibility of combined plant–antibiotic treatment as a tool to either recover or enhance antibacterial efficacy and raise further attention for mechanical-based, MIC disinfectants applied on resistant strains. This study provides evidence for the up-and-coming potentialities of plant products as natural supplements in the management of AMR

Introduction

Antimicrobial resistance (AMR) has emerged as one of the most pressing global health challenges of the 21st century, threatening the efficacy of conventional antibiotics and jeopardizing the successful treatment of infectious diseases worldwide 1). The uncontrolled, excessive, and often irrational use of antibiotics in human medicine, agriculture, aquaculture, and livestock production has accelerated the natural evolution of bacteria, enabling them to develop complex resistance mechanisms 2). Multidrug-resistant (MDR) strains, such as extended-spectrum β-lactamase (ESBL) producers, carbapenem-resistant organisms, and methicillin- or vancomycin-resistant pathogens, have become increasingly prevalent, significantly limiting treatment options and contributing to increased morbidity, mortality, prolonged hospitalization, and healthcare costs 3). Alarmingly, infections that were once easily treatable with first-line antibiotics now often require higher doses of more toxic or expensive drugs, and in severe cases, no effective therapies remain available. The global pipeline for new antibiotic discovery is slow, costly, and fraught with scientific challenges, further emphasizing the urgent need for innovative, complementary, and sustainable approaches to enhance or restore the antibacterial potency of existing drugs 4).

One promising strategy to address this challenge is the exploration of medicinal plant-derived extracts as adjuncts to conventional antibiotic therapy. Medicinal plants have been a cornerstone of traditional medicine for centuries, and modern research has confirmed that they are rich sources of bioactive secondary metabolites, including alkaloids, flavonoids, tannins, terpenoids, saponins, coumarins, phenolic acids, and glycosides. These compounds exhibit a wide spectrum of biological activities, including antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant, and immunomodulatory effects 5). Importantly, plant-derived compounds can interact with conventional antibiotics in complex ways, resulting in synergistic, additive, indifferent, or antagonistic effects 6). Synergistic interactions occur when the combined effect of the plant extract and antibiotic exceeds the sum of their individual effects. Mechanistically, synergy may result from increased bacterial membrane permeability, inhibition of efflux pumps, disruption of biofilm formation, interference with bacterial quorum sensing, or complementary targeting of metabolic pathways. Such interactions can significantly enhance the therapeutic potential of antibiotics, reduce the required dosage, lower toxicity, and even restore the activity of drugs that have become less effective due to resistance 7). Conversely, antagonistic interactions caused by chemical inactivation, binding interference, or competition at bacterial target sites can reduce antibiotic efficacy and compromise treatment outcomes. Additive or indifferent interactions, while less dramatic, are also important to characterize, as they inform whether plant extracts can be safely combined with antibiotics without affecting their activity 8).

Studying plant–antibiotic interactions is scientifically and clinically significant. Identifying synergistic combinations provides a strategy to combat multidrug-resistant pathogens while minimizing antibiotic consumption and associated side effects. Furthermore, integrating plant-derived compounds with conventional antibiotics aligns with current global priorities, including sustainable and eco-friendly drug development, cost-effective healthcare solutions, and reduction of AMR spread 9). Systematic evaluation of these interactions addresses critical gaps in the literature, where the mechanisms and consistency of plant–antibiotic effects are often poorly understood. Evidence-based data on these combinations can guide rational therapeutic applications, especially in regions where medicinal plants are abundant and antibiotic resistance is prevalent 10).

In this study, four selected medicinal plants *Randia aculeata*, *Ixora coccinea*, *Capsella bursa-pastoris*, and *Portulaca oleracea* were evaluated for their potential to modulate the activity of commonly used antibiotics, including ampicillin, amoxicillin, erythromycin, and ciprofloxacin. The antibacterial activity of plant extracts alone, antibiotics alone, and plant–antibiotic mixtures (1:1 ratio) was assessed using standardized agar well diffusion assays against representative Gram-positive and Gram-negative bacteria. By systematically comparing these treatments, the study aimed to identify synergistic, additive, indifferent, and antagonistic interaction patterns. The findings are expected to provide a comprehensive understanding of how natural plant-derived compounds can enhance or influence antibiotic efficacy and highlight the potential of integrating traditional botanical knowledge with modern antimicrobial strategiesc 11).

Ultimately, this research contributes to the global effort to combat AMR by exploring novel, sustainable, and practical combination therapies. It emphasizes the relevance of natural products in complementing

conventional drugs, supports the rational selection of plant–antibiotic combinations, and lays the groundwork for future studies that could lead to the development of effective, low-cost, and environmentally friendly antibacterial therapies. By bridging traditional medicine with modern pharmacological research, the study reinforces the importance of interdisciplinary approaches in addressing one of the most urgent public health challenges of our time.

Materials and Methods

Plant Material Collection and Extract Preparation

Four medicinal plants *Randia aculeata*, *Ixora coccinea*, *Capsella bursa-pastoris*, and *Portulaca oleracea* were collected, thoroughly washed with distilled water, and air-dried. The plant materials were cut into small pieces for aqueous extract preparation. Ten grams of each plant sample were boiled in 100 mL of sterile distilled water for 20 minutes. After cooling to room temperature, the extracts were clarified by centrifugation at 10,000 rpm for 10 minutes at 4 °C to remove debris and particulate matter. The clear supernatants were collected in sterile tubes and stored at 4 °C for use within 48 hours. No microfiltration was performed, but sterility was confirmed by plating aliquots of the extracts on nutrient agar prior to testing.

Antibiotic Selection and Preparation

Four commonly used antibiotics ampicillin, amoxicillin, erythromycin, and ciprofloxacin were utilized in this study. Stock solutions were prepared to achieve a final working concentration of 30 µg/mL for each antibiotic. These antibiotics were used as positive controls and in combination with plant extracts to evaluate potential interaction effects.

Bacterial Cultures and Inoculum Preparation

Representative Gram-positive and Gram-negative bacteria were selected for antibacterial testing, including *Escherichia coli*, *Staphylococcus aureus*. Each bacterial strain was cultured overnight in nutrient broth, and turbidity was adjusted to match the 0.5 McFarland standard to ensure uniform inoculum density.

Agar Well Diffusion Assay and Combination Testing

The antibacterial activity of the plant extracts, antibiotics, and their combinations was evaluated using the agar well diffusion method. Mueller-Hinton agar plates were swabbed uniformly with each bacterial culture. For each organism, three treatments were applied per plate: (1) a well containing plant extract alone (control), (2) a well containing antibiotic alone at 30 µg/mL (control), and (3) a well containing a 1:1 mixture of plant extract and antibiotic (test sample). A total of 8 plates were prepared to cover all combinations of the four plant extracts with four antibiotics across the selected bacterial strains. The plates were incubated at 37 °C for 24 hours, after which zones of inhibition were measured in millimeters.

Data Recording and Interpretation

Zones of inhibition from individual and combined treatments were recorded and compared to determine interaction patterns synergistic, additive, indifferent, or antagonistic. All experiments were conducted under aseptic conditions, following standard laboratory safety protocols. The results provided insights into the potential of plant extracts to enhance or modulate the antibacterial activity of conventional antibiotics, highlighting combinations with promising therapeutic potential.

Results and Discussion

The antibacterial activity of four medicinal plant extracts namely Randia aculeata, Ixora coccinea, Capsella bursa-pastoris and Portulaca oleracea were explored against Escherichia coli and Staphylococcus aureus when tested alone and in combination each two as 1:1 with some antibacterial agents (Ampicillin - Amp, Amoxicillin - Amox, Erythromycin - Ery and Ciprofloxacin - Cipro). Results The results were indicative of a wide range of antibacterial responses, interspecies interaction types (i.e. synergy to antagonism), and species-specificity associated with plant-antibiotic interactions.

Three plants (Randia, Ixora and Portulaca) revealed significant intrinsic inhibition against E. coli with the production of zone of inhibitions (23 mm, 18 mm and 20 mm), respectively while Capsella did not show any activity. The Ciprofloxacin remained as the most effective antibiotic (32 mm), followed by Amoxicillin (20 mm), Ampicillin (18 mm) and Erythromycin (16

mm). A synergy was found in only one combination: Capsella + Ciprofloxacin. After supplementation with the extract, a significant increase of 36 mm against control but no activity was observed when tested alone. This is indicative of some phytochemicals that are otherwise non-bioactive, may facilitate transport of antibiotic or even inhibit efflux in bacteria. On the contrary, combination of Randia + Ampicillin, Ixora + Erythromycin and Portulaca + Amoxicillin produced lesser inhibition zones suggests antagonism.

Figure.1 Antibacterial activity of plantt extract and antibiotic against *E.coli*

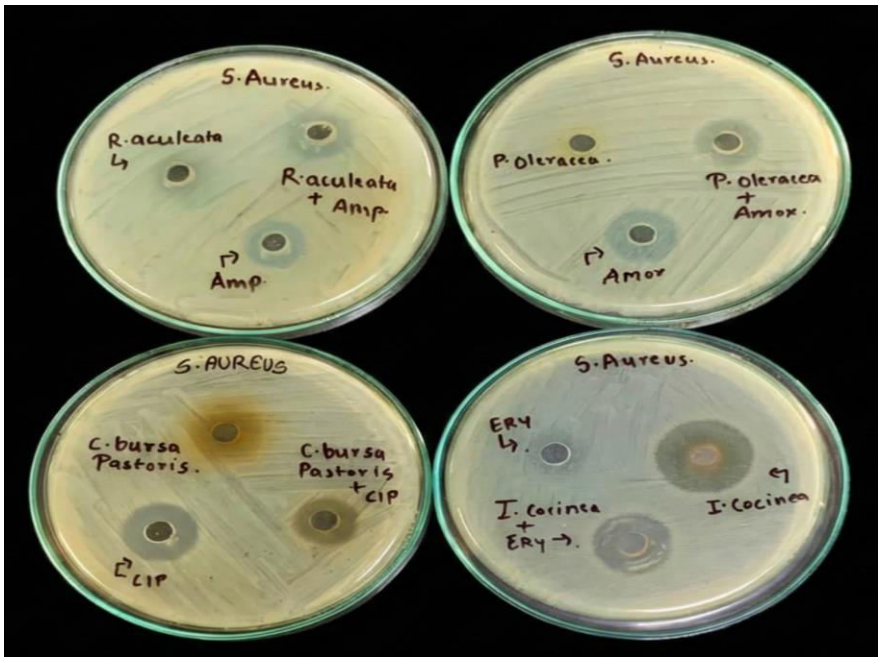

Figure.2 Antibacterial activity of plantt extract and antibiotic against *S.aureus*

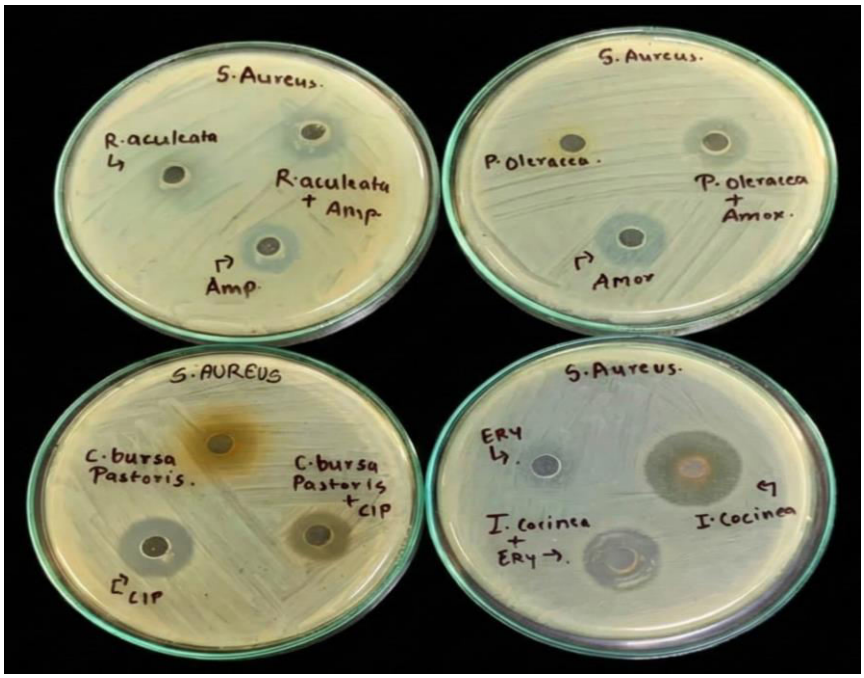

Table.1 The antibacterial activity of the four plant extracts against *E. coli*, tested alone and with their respective antibiotics

Treatment	Organism - *E. coli* (Zone of Inhibition) mm
Randia aculeata – Extract Alone	23
Ampicillin (AMP) (30 µg/mL) Alone	18
Randia aculeata + Ampicillin (1:1)	20
Capsella bursa-pastoris – Extract Alone	0
Ciprofloxacin (CIP) (30 µg/mL)	32
Capsella bursa-pastoris + Ciprofloxacin (1:1)	36
Ixora coccinea – Extract Alone	18
Erythromycin (ERY) (30 µg/mL)	16
Ixora coccinea + Erythromycin (1:1)	14
Portulaca oleracea – Extract Alone	20
Amoxicillin (AMOX) (30 µg/mL)	20
Portulaca oleracea + Amoxicillin (1:1)	18

Table.2 The antibacterial activity of the four plant extracts against *S. aureus* tested alone and with their respective antibiotics

Treatment	Organism - *S.aureus* (Zone of Inhibition) mm
Randia aculeata – Extract Alone	0
Ampicillin (AMP) (30 µg/mL) Alone	14
Randia aculeata + Ampicillin (1:1)	14
Capsella bursa-pastoris – Extract Alone	0
Ciprofloxacin (CIP) (30 µg/mL)	19
Capsella bursa-pastoris + Ciprofloxacin (1:1)	14
Ixora coccinea – Extract Alone	22
Erythromycin (ERY) (30 µg/mL)	0
Ixora coccinea + Erythromycin (1:1)	19
Portulaca oleracea – Extract Alone	0
Amoxicillin (AMOX) (30 µg/mL)	16
Portulaca oleracea + Amoxicillin (1:1)	16

These antagonistic interactions may derive from the interference to antibiotic binding sites, membrane permeability modification or chemical interaction of phytochemicals that can counteract the effect the effect of antibiotics.

In the case of S. aureus, only Ixora coc-cinea displayed good intrinsic antibacterial activity (22 mm), indicative of its broad spectrum potential. It was obvious that the others had no appreciable inhibition effect. Ciprofloxacin (19 mm) and Amoxicillin (16 mm) were moderately active while Erythromycin was completely inactive. More interestingly, Ixora + Erythromycin resulted in an inhibition zone of 19 mm (Figure (Figure55); thus the antibiotic lost activity was indeed brought to its active level. This synergy-like effect indicates that bioactive compounds present in Ixora may serve to improve the penetration of antibiotic or block staphylococcal defense systems, thereby causing erythromycin to become active again. Randia + Ampicillin, and Portulaca + Amoxicillin exhibited indifferent interactions, followed by Capsella with Ciprofloxacin, the zone activity 19 mm to 14 mm suggesting antagonism. These contradictory findings illustrate the fact that plant extracts do not uniformly improve antibiotic efficacy and rather that interactions vary according to the composition of phytochemicals used, bacterial physiology, and modes of actions.

In conclusion, the data presented in this work indicate that it is possible to modulate the activity of antibiotics positively or negatively, depending on the combination and organism. Modest synergistic interactions such as Capsella combination + ciprofloxacin against E. coli and Ixora combination + erythromycin against S. aureus hint the direction for planting compounds to act jointly, bolster or reinstate antibiotics action. Nevertheless, the presence of an opposite outcome emphasizes the necessity for rigorous assessment before such combinations can be promoted for therapeutic purpose Future investigations should target to achieve minimum inhibitory concentrations (MICs) concerning every extract-antibiotic partnership, epiphanize the acting phytochemicals and argue about these by means using efflux pump assays, membrane-permeability measurements and biofilm inhibition models. Future work on MDR strains and pure plant compounds can provide a better understanding of natural resources in combating the global crisis of antibiotic resistance. These efforts would eventually contribute to the safe, effective, and sustainable combination therapy between phytochemicals and antibiotics.

Author Contributions

Bhanupratap Vishwakarma: Investigation, formal analysis, writing—original draft. Shruti Mishra: Validation, methodology, writing—reviewing. Shivani Pandey:—Formal analysis, writing—review and editing. Vidya Bagul: Investigation, writing—reviewing. Neha Jadhav: Resources, investigation writing—reviewing. Sahil Pandey: Validation, formal analysis, writing—reviewing. Sweta Dwivedi: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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