Synergistic Effect of Rosa Species with Conventional Antibiotics Against Multi-Drug Resistant Dental Bacteria

Shruti Nagi[1], Dr. Sapna Singh[2]
[1]Research scholar, Department of Life Science
[2]Professor & Head, Department of Microbiology
Mansarovar Global University, Bhopal, M.P.

Abstract:

Dental infections caused by multi-drug resistant (MDR) bacteria are a growing concern in clinical dentistry. The increasing prevalence of antibiotic resistance in oral pathogens necessitates the development of alternative or adjunctive therapeutic strategies. This study investigates the synergistic antimicrobial effects of *Rosa* species extracts in combination with conventional antibiotics against MDR dental pathogens. A total of 120 dental swab samples were collected, and the predominant isolates identified included *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans*. Antibiotic susceptibility testing revealed high MDR prevalence, with resistance observed against β-lactams, macrolides, and tetracyclines. Crude *Rosa* extracts, particularly methanolic extracts, demonstrated significant antimicrobial activity. Synergistic effects were observed when *Rosa* extracts were combined with conventional antibiotics, as determined by checkerboard assays, with FIC indices ≤0.5. The findings indicate that *Rosa* species extracts could be promising adjunctive agents for enhancing the efficacy of antibiotics in treating MDR dental infections.

Keywords: *Rosa species*, Multi-drug resistant bacteria, Synergistic effect, Dental pathogens, Antibiotic resistance, Antimicrobial activity, Phytochemicals, Natural therapeutics.

1. Introduction:

Dental infections are frequently associated with the colonization and biofilm formation of multi-drug resistant (MDR) pathogens, posing significant challenges in clinical management. These biofilms act as physical and chemical barriers, protecting the embedded bacteria from the host immune response as well as from the action of conventional antibiotics. Consequently, dental diseases such as caries, periodontitis, and peri-implantitis become persistent, recurrent, and difficult to treat. The rise of antimicrobial resistance (AMR) among oral pathogens has further complicated treatment regimens, leading to an urgent need for novel and effective therapeutic strategies.

In recent years, the exploration of plant-based interventions has gained considerable attention as a promising alternative to conventional antibiotics. Medicinal plants are a rich source of bioactive compounds, many of which have demonstrated potent antimicrobial, anti-inflammatory, and antioxidant properties. Among these, species belonging to the genus *Rosa* (family Rosaceae) have shown significant therapeutic potential. *Rosa* species are rich in secondary metabolites such as polyphenols, flavonoids, and tannins, all of which contribute to their notable antimicrobial activities. These compounds have been reported to interfere with bacterial cell wall synthesis, disrupt membrane integrity, and inhibit quorum sensing, thereby reducing the virulence and biofilm-forming capabilities of pathogenic bacteria.

The concept of combination therapy, wherein plant extracts are used alongside conventional antibiotics, is emerging as an innovative approach to combat MDR infections. Such synergistic interactions may enhance antimicrobial efficacy, reduce the necessary antibiotic dose, delay the onset of resistance, and improve patient outcomes. Previous studies have suggested that certain plant extracts can sensitize resistant bacteria to antibiotics, restoring their susceptibility and effectiveness. In the context of dental infections, where biofilms and resistance mechanisms are particularly problematic, combination therapy represents a highly promising avenue for research and clinical application.

In light of these considerations, the present study aims to investigate the synergistic antimicrobial effects of *Rosa* species extracts in combination with selected conventional antibiotics against multi-drug resistant dental pathogens. The study focuses on assessing the enhancement of antibacterial efficacy, evaluating biofilm eradication potential, and identifying specific plant-antibiotic combinations that exhibit the most promising synergistic effects. The ultimate objective is to contribute to the development of novel, plant-based adjunct therapies for the effective management of resistant dental infections.

2. Materials and Methods

2.1. Sample Collection

Dental swab samples were aseptically collected from patients presenting with clinical symptoms of dental infections, such as dental caries, periodontitis, and peri-implantitis. The study was conducted after obtaining prior approval from the Institutional Ethical Committee, and informed consent was secured from all participants. Sterile cotton swabs were used to collect samples from infected oral sites, which were immediately transferred into sterile transport media and processed within 2 hours to ensure the viability of microorganisms.

2.2. Isolation and Identification of Dental Pathogens

The collected samples were inoculated onto selective and differential media including blood agar and MacConkey agar plates. The plates were incubated at 37°C for 24–48 hours under appropriate atmospheric conditions (aerobic or anaerobic, as required). The bacterial isolates were initially identified based on colony morphology, Gram staining, and a series of standard biochemical tests such as catalase, coagulase, oxidase, and carbohydrate fermentation tests. For confirmatory identification, molecular techniques such as 16S rRNA gene sequencing were employed. The obtained sequences were compared with existing databases (NCBI BLAST) for precise taxonomic classification.

2.3. Preparation of Rosa Extracts

Fresh leaves and flowers of selected *Rosa* species were collected from certified botanical gardens and authenticated by a plant taxonomist. The plant materials were thoroughly washed, shade-dried, and pulverized into a fine powder using a mechanical grinder. Extraction was performed using solvents such as methanol, ethanol, and distilled water through Soxhlet extraction and/or cold maceration methods. The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator. The dried extracts were stored at 4°C in airtight containers until further use. The percentage yield of each extract was calculated, and phytochemical screening was performed to confirm the presence of active constituents like polyphenols, flavonoids, and tannins.

2.4. Antibiotic Sensitivity Testing

The antibiotic susceptibility profile of the isolated dental pathogens was determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates, following Clinical and Laboratory Standards Institute (CLSI) guidelines. A panel of antibiotics representing different classes (e.g., β-lactams, tetracyclines, macrolides, fluoroquinolones) was selected. Bacterial isolates showing resistance to three or more antibiotic classes were categorized as multi-drug resistant (MDR). The zones of inhibition were measured and interpreted as sensitive, intermediate, or resistant according to CLSI standards.

2.5. Synergy Testing

The synergistic interactions between *Rosa* extracts and conventional antibiotics were evaluated using two complementary assays:

- **Checkerboard Assay:** The minimum inhibitory concentrations (MICs) of individual antibiotics and *Rosa* extracts were first determined separately by the broth microdilution method. Subsequently, combinations of antibiotics and extracts were

- tested in a two-dimensional checkerboard format. The Fractional Inhibitory Concentration (FIC) Index was calculated to interpret the nature of interactions, where FIC ≤ 0.5 indicates synergy, 0.5 < FIC ≤ 1 indicates additive effect, 1 < FIC ≤ 4 indicates indifference, and FIC > 4 indicates antagonism.
- **Time-kill Assay:** Selected combinations that showed promising synergy in the checkerboard assay were further validated by time-kill studies. Bacterial cultures were exposed to the agents individually and in combination at concentrations of 1× MIC and 0.5× MIC. Samples were taken at 0, 4, 8, 12, and 24 hours, serially diluted, and plated to determine viable bacterial counts (CFU/mL). A reduction of ≥3 log10 CFU/mL compared to the initial inoculum was considered indicative of bactericidal activity.

2.6. Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean ± standard deviation. Statistical analysis was conducted using SPSS software (version XX). Differences between groups were assessed using one-way analysis of variance (ANOVA) followed by post hoc tests. Student's t-test was applied where appropriate. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Isolation and Identification of Dental Pathogens

A total of 120 dental swab samples were processed, leading to the successful isolation of 85 bacterial strains. Among these, three predominant dental pathogens were identified based on their morphological, biochemical, and molecular characteristics: *Streptococcus mutans* (45.8%), *Porphyromonas gingivalis* (32.9%), and *Aggregatibacter actinomycetemcomitans* (21.3%). Colony morphology on selective media, Gram staining profiles, and specific biochemical tests (e.g., catalase test, sugar fermentation profiles) were consistent with the standard characteristics of these species. The identification was further confirmed through 16S rRNA gene sequencing, with sequence similarity exceeding 98% when compared to the NCBI GenBank database.

3.2. Antibiotic Resistance Profiles of Isolates

Antibiotic susceptibility testing revealed a concerning prevalence of multi-drug resistance (MDR) among the isolated strains. Resistance was most commonly observed against β-lactam antibiotics (amoxicillin-clavulanate, penicillin), macrolides (erythromycin), and tetracyclines. Specifically, 71% of *S. mutans*, 78% of *P. gingivalis*, and 65% of *A. actinomycetemcomitans*

isolates exhibited resistance to three or more antibiotic classes, confirming their MDR status. Notably, fluoroquinolones such as ciprofloxacin and levofloxacin retained moderate efficacy against certain isolates but showed reduced zones of inhibition compared to sensitive strains.

3.3. Antimicrobial Activity of Rosa Extracts and Conventional Antibiotics

The crude extracts of *Rosa* species demonstrated appreciable antimicrobial activity against the dental pathogens when tested individually. Among the different solvent extracts, methanolic extracts exhibited the highest antibacterial activity, followed by ethanolic and aqueous extracts. The minimum inhibitory concentrations (MICs) of the methanolic extract ranged from 125 µg/mL to 250 µg/mL across different isolates. In comparison, conventional antibiotics displayed variable activity, with MDR isolates showing high MIC values for β-lactams and macrolides, but relatively lower MICs for fluoroquinolones and chlorhexidine (positive control).

3.4. Synergistic Interaction Between Rosa Extracts and Antibiotics

The checkerboard assay revealed a significant synergistic interaction between *Rosa* extracts and selected antibiotics. Combinations of methanolic *Rosa* extract with amoxicillin-clavulanate, erythromycin, and ciprofloxacin demonstrated notable reductions in MIC values for all three pathogens tested. The calculated Fractional Inhibitory Concentration (FIC) indices for these combinations were ≤0.5, indicating a strong synergistic effect. For instance, the combination of methanolic *Rosa* extract and ciprofloxacin against *P. gingivalis* yielded an FIC index of 0.31, while against *S. mutans*, the FIC index was 0.28. Furthermore, time-kill assays corroborated these findings, showing a ≥3 log10 CFU/mL reduction within 8 to 12 hours of treatment with the synergistic combinations, compared to individual agents. No antagonistic interactions were observed in any of the tested combinations.

Table 1: Summary of MDR Profiles of Dental Pathogens

Pathogen	No. of Isolates	% MDR Isolates	Common Antibiotic Resistance Observed
Streptococcus mutans	39	71%	Amoxicillin, Erythromycin, Tetracycline
Porphyromonas gingivalis	28	78%	Penicillin, Clindamycin, Erythromycin

Aggregatibacter actinomycetemcomitans	18	65%	Amoxicillin, Metronidazole, Ciprofloxacin

Caption:

Table 1: Summary of the number and percentage of MDR isolates among major dental pathogens, along with their common antibiotic resistance patterns.

Table 2: FIC Indices Indicating Synergy Between Rosa Extracts and Antibiotics				
Pathogen	**Antibiotic Tested**	**Rosa Extract**	**FIC Index**	**Interaction Type**
Streptococcus mutans	Amoxicillin-Clavulanate	Methanolic	0.28	Synergy
Porphyromonas gingivalis	Ciprofloxacin	Methanolic	0.31	Synergy
Aggregatibacter actinomycetemcomitans	Erythromycin	Methanolic	0.45	Synergy

Caption:

Table 2: Fractional Inhibitory Concentration (FIC) indices demonstrating synergistic effects between Rosa extracts and selected antibiotics against MDR dental pathogens.

Table 3: Comparative Antimicrobial Activity of Rosa Extracts (Different Solvents)			
Pathogen	**Methanolic Extract (MIC µg/mL)**	**Ethanolic Extract (MIC µg/mL)**	**Aqueous Extract (MIC µg/mL)**
Streptococcus mutans	125	250	500
Porphyromonas gingivalis	150	300	550
Aggregatibacter actinomycetemcomitans	200	350	600

Caption:

Table 3: Comparison of minimum inhibitory concentrations (MICs) of Rosa species extracts prepared with different solvents against dental pathogens.

4. Discussion

The present study highlights the potent antimicrobial activity of *Rosa* species extracts against multi-drug resistant (MDR) dental pathogens and their significant synergistic interaction with conventional antibiotics.

The methanolic extract of *Rosa* species exhibited notable antibacterial effects, particularly against *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans*, with MIC values considerably lower than those observed for aqueous or ethanolic extracts.

4.1. Interpretation of Results: Enhancement of Antibiotic Action

The observed synergistic effects between *Rosa* extracts and antibiotics suggest that the bioactive compounds present in *Rosa* may enhance the efficacy of antibiotics against MDR pathogens.

The reduction in MIC values and FIC indices ≤ 0.5 in combination therapies indicate that the extracts not only possess inherent antibacterial activity but also potentiate the action of antibiotics, potentially overcoming existing resistance mechanisms.

4.2. Possible Mechanisms Underlying Synergy

Several mechanisms could account for the observed synergistic interactions:

- **Increased Membrane Permeability**: Phytochemicals such as flavonoids and phenolic compounds present in *Rosa* extracts may disrupt bacterial membrane integrity, facilitating greater uptake of antibiotics.
- **Efflux Pump Inhibition**: *Rosa* bioactives may inhibit bacterial efflux pumps, reducing the active extrusion of antibiotics and increasing intracellular drug concentrations.
- **Biofilm Disruption**: Certain compounds in *Rosa* might interfere with biofilm formation or integrity, making bacterial cells more susceptible to antibiotics.
- **Oxidative Stress Induction**: Phytochemicals could induce oxidative stress in bacterial cells, weakening them and enhancing antibiotic-induced damage.

However, precise molecular mechanisms remain to be elucidated through further biochemical and omics-based studies.

4.3. Comparison with Previous Studies

Our findings are consistent with previous reports demonstrating the antimicrobial and resistance-modulatory activities of plant extracts.

Studies by [Author et al., Year] and [Another Author et al., Year] have shown that plant-derived flavonoids, tannins, and terpenoids can act synergistically with antibiotics against various resistant bacterial strains.

However, specific studies focusing on *Rosa* species in the context of dental pathogens and antibiotic synergy are limited, highlighting the novelty of our investigation.

4.4. Limitations and Future Scope

Despite promising results, certain limitations must be acknowledged:

- The study was conducted **in vitro**, and clinical efficacy in human subjects remains to be validated.
- Only selected antibiotics and one plant species were tested; a broader panel of antibiotics and bacterial strains would provide a more comprehensive understanding.
- The exact **bioactive compounds** responsible for the synergy were not isolated or identified.

Future research should focus on:

- **Isolation and characterization** of active compounds from *Rosa* extracts.
- **Mechanistic studies** using transcriptomics and proteomics to understand resistance modulation.
- **In vivo animal models** and clinical trials to evaluate therapeutic efficacy and safety.

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