Molecular Docking Insights: Unveiling Plant Extracts' Potential to Amplify Antibiotic Efficacy

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ABSTRACT

Introduction: *Acinetobacter baumannii*, Carbapenem-resistant Enterobacteriaceae, *Staphylococcus aureus*, Streptococcus species, and other MDR pathogens have developed resistance mechanisms against several types of antibiotics. As a result, there is a higher chance of morbidity and mortality, higher healthcare delivery costs, and the possibility of infectious illness reemergence. The prevalence of AMR is particularly high in Pakistan, and various studies have reported resistance as high as 90% among common bacterial strains. This study aims to cater these life-threatening challenges of antimicrobial resistance by identifying synergistic interactions of edible plant extracts with antibiotics to identify potential bioactive compounds that can target the drug resistance mechanisms to restore antibiotic efficacy. Understanding how natural compounds may play a role in combating the AMR will potentially contribute towards preliminary stages in the development of new interventions against the menace.

Objective: The objective of this study is to investigate options for targeting antibiotic-resistant mechanisms in multidrug-resistant bacteria, rendering them sensitive to antibiotics to which they have developed resistance.

Methodology: The synergistic effects of edible plant extracts in combination with antibiotics were analyzed using extracts of *Punica granatum, Citrus paradisi-grapefruit peel, Malus domestica-apple, Lepidium sativum, Artemisia dracunculus, Murraya koenigii, Hyssopus officinalis, Foeniculum vulgare,* and *Salvia officinalis*. These extract-antibiotic combinations were evaluated against MDR bacterial strains. High-water-content items were filtered and lyophilized, and dried herbs and spices were powdered, soaked in 70% methanol, filtered, evaporated, and dissolved in DMSO. MHA and MHB were prepared for antimicrobial analysis. MHA plates were separated into control and treatment arms, and bacterial culture, after adjusting the OD_{595} to 0.2, was spread for lawn formation. Antibiotic-impregnated discs with broad-spectrum activity were placed to investigate antibacterial and synergistic interaction. Plates were then incubated at 37°C for 24 h and the zones of inhibition were measured. All experiments were performed in triplicate to ensure reproducibility.

To study their possible mechanism of actions, the molecular structures of Punicic Acid from pomegranate, Pinocamphone from hyssop, and Trans-Anethole from fennel seeds were docked against NorA, AcrB TolC and MdtB efflux pump proteins of *Staphylococcus aureus, Acinetobacter baumannii, Klebsiella spp., and E. coli,* respectively. The structures of the proteins obtained from Protein Data Bank were docked using GalaxyWeb; then, the binding affinities and hydrogen bonding, hydrophobic, and π–π interactions were studied in Discovery Studio Visualizer to describe the mechanism of inhibition.

Result: The plant extracts in combination with antibiotics demonstrated several synergistic interactions against MDR pathogens. Hyssop extract suppressed the growth of *MRSA* and *A. baumannii* when treated in combination with pipercillin/tazobactum, however, its combination with chloramphenicol, inhibited growth of *MRSA* only. Amikacin and ciprofloxacin effectively reduced the propagation of *MRSA* ($p < 0.01$) and *A. baumannii* ($p < 0.05$) when treated with the pomegranate extract. Apple extract shown a substantial

synergistic effect against *MRSA* and *E. coli* when combined with tetracycline and chloramphenicol. Fennel extract enhanced the antibiotic activity of gentamicin ($p < 0.05$) and cefoxitin ($p < 0.01$) against *A. baumannii*. These results were endorsed by docking studies, which showed that Punicic Acid, the bio-active ingredient in pomegranate extract, docked with the NorA efflux pump of *MRSA* with a binding energy of -7.2 kcal/mol, potentially inhibiting the efflux and boosting the effectiveness of amikacin/ciprofloxacin. Similarly, Pinocamphone from hyssop exhibited strong binding to AcrB pump of *A. baumannii* at -6.9 kcal/mol, in concurrence with the synergy observed with TZP. Trans-Anethole from fennel seeds showed binding energy to the drug-efflux pump at -7.0 kcal/mol, correlate with increased effectiveness of cefoxitin and gentamicin.

Conclusion: In antimicrobial assays, pomegranate, hyssop, and fennel seed extracts shows distinct synergy and increased antibiotic activity against MDR pathogens such as *MRSA* and *A. baumannii*. Docking results supported their effectiveness in overcoming resistance by demonstrating their robust interactions with bacterial efflux pump proteins. These extracts have considerable potential as supplemental treatments and should be studied further for potential clinical uses.

Keywords: Antimicrobial Resistance (AMR), Efflux Pump Inhibitor (EPI), Natural Product Synergy, Molecular Docking, Multidrug-resistant (MDR), Antibiotic Efficacy Restoration.

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