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Title: Exploring the Potential of Phyto-molecules from Indian Medicinal Plants to Suppress the Multidrug Resistance in Bacteria Targeting the β-lactamase Enzyme

Abstract

Antibiotics have been identified as a powerful source for the treatment of bacterial disease. However, their effectiveness reduced over time due to the development of antibiotic resistance in bacteria. Further, their non-judicial usage impacted the environment negatively, emphasizing the need for a novel ecofriendly way of treating bacterial infections. Among the various mechanisms through which bacteria resist antibiotics, β-lactamase is considered a key factor because of its wide distribution and high variability. β-lactamase production is mainly responsible for drug failure against bacteria, it weakens β-lactam antibiotics which responsible for inhibiting bacterial cell wall synthesis. CDC and WHO also reported that humans are moving to post antibiotic era and unsafe in this condition. The need of the hour is to break this antibiotic resistance in bacteria to restore the activity of existing antibiotics or invent newer generation of antibiotics. The current study explores the potential of plant-derived secondary metabolites (PSMs), as β-lactamase inhibitors to combat antibiotic resistance in bacteria through a multi-objective experimental approach. Secondary metabolites were extracted from thirty medicinal plants using six solvents of increasing polarity. These extracts were screened for β-lactamase inhibition using a chromogenic nitrocefin assay. The active extracts were further assessed for their β-lactam potentiating effects using the checkerboard method against three MDR bacterial strains (*Bacillus cereus*, *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*), showing synergistic and additive interactions. Kinetic analysis revealed uncompetitive inhibition, suggesting that these extracts bind to the enzyme-substrate complex. High-resolution LC-MS analysis identified 77 metabolites dominated by polyphenols and lipids. VIP analysis and Pearson correlation identified key inhibitory candidates. The acetone extract of *Syzygium cumini* bark was selected for bioactivity-guided purification, using RP-TLC and Combi-flash chromatography. Bioactive fractions were analyzed using UV-Vis, HPLC, and LC-MS, revealing potent β-lactamase inhibitory constituents. Further, computational tools were used to evaluate the inhibitory potential of selected metabolites. Molecular docking was performed on multiple conformations of four β-lactamase enzymes (SHV1, TEM1, CTX-M, and KPC-2), followed by 100 ns molecular dynamics simulations using GROMACS. MMPBSA binding energy analysis from the last 10 ns revealed that these metabolites had the most stable and lowest energy interactions with the enzymes, indicating strong inhibitory potential. These findings open avenues for further *in vitro* and *in-vivo* research to support the development of plant-based therapeutics against antibiotic-resistant bacteria.