## Abstract

Since time immemorial plant extracts have been used for their antibacterial properties. In recent time, the active compounds have been isolated and their activities are established. However, considering the diversity of the plant kingdom and their secondary metabolites, further research needs to be done to identify potential metabolites having direct antibacterial and antibiotic potentiation ability. The identification of antibiotic potentiators is important considering the rapid development of bacterial resistance against the antibiotics thereby depleting the antibiotic pipeline. To address this issue of bacterial resistance and to find antibiotic potentiating plant metabolites, the present study was carried out under four objectives.

High throughput method was employed for screening of 180 extracts in different solvents obtained from 30 plants. These extracts were screened for β-lactamase inhibition and β-lactam potentiation ability against the MDR bacterial isolates (*Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Bacillus cereus*). Among the extracts, acetone extract of *Ficus religiosa* and hexane extract of *Acorus calamus* was found to be the most effective in terms of β-lactamase inhibition and ampicillin potentiation.

The metabolites present in F. religiosa were identified using HR-LCMS. Further, to understand the binding of the metabolites with Class A β-lactamases (TEM 1, SHV1, KPC-2 and 6D17) molecular docking and MD simulation studies were performed. This revealed that taxifolin and miquelianin showed the best binding affinity with all the four class A β-lactamases. Hydrogen bond occupancy of >200 % was seen with Glu166, Glu240, Asn132, etc. which are located in the enzyme active site. Additionally, the ADMET profile of the metabolites showed their drug likeliness and non-toxic nature.

Fractionation of bioactive compounds from *A. calamus* rhizome hexane (AC-R-H) extract was performed and its MIC, FIC index and mode of action was determined. AC-R-H bioactive fraction was found to reduce the MIC of ampicillin against *E. coli* (100 mg/mL to 25 mg/mL), *P. aeruginosa* (15 mg/mL to 3.25 mg/mL), *A. baumannii* (12.5 mg/mL to 1.56 mg/ml), and *B. cereus* (10 mg/mL to 1.25 mg/mL). Further it recorded synergistic activity with ampicillin against *B. cereus* (FICI = 0.365), *P. aeruginosa* (FICI = 0.456), and *A. baumannii* (FICI = 0.364). This activity can be attributed to its ability to alter the fatty acid composition of the bacterial cell membranes, hinder the membrane integrity, permeability, and cause cell membrane damage. It also showed good antibiofilm activity against *B. cereus* and a moderate β-lactamase inhibition ($IC_{50}$ = 6.2 mg/mL). Characterization of AC-R-H bioactive fraction through UV-Vis, FT-IR, and GC-MS revealed Asarone as the major compound present in the bioactive fraction.

Finally, PLGA based formulation was prepared by encapsulating the clarified crude of *A. calamus*. The encapsulated particles exhibited size (300-500 nm), PDI (0.3-0.4) and zeta potential (-25 to -15 mV). The loading capacity and encapsulation efficiency of the particles were (40 to 60 %) and *in vitro* release profile showed burst release with ~70 % of cumulative release in 24 h. The encapsulated particles showed antibacterial efficacy and ampicillin potentiation against *A. baumannii*. Further, the cell toxicity studies of clarified crude and encapsulated particles (20 µg/ml) on Vero cells showed ~20 % inhibition. Overall, the study identifies two plants *F. religiosa* and *A. calamus* and their metabolites as potential candidates for countering multidrug resistance in bacteria. Also, the development of PLGA based formulation encapsulating *A. calamus* clarified crude showcases the feasibility to develop biocompatible formulation with multiple compounds.