UNVEILING THE BIOACTIVE POTENTIAL OF SECONDARY METABOLITES PURIFIED FROM ENDOPHYTIC FUNGI OF SOLANUM SURATTENSE THROUGH INTEGRATED INSILICO AND INVITRO APPROACHES

ABSTRACT

The emergence of antimicrobial resistance necessitates the discovery of new bioactive small molecules from underexplored microbial sources. In this study, endophytic fungi associated with *Solanum surattense* were investigated for their potential to produce antimicrobial secondary metabolites. Endophytic strains were isolated from surface-sterilized stem and fruit tissues plated on potato dextrose agar (PDA) and incubated at 20–28 °C for 7–21 days. Emerging fungal colonies were purified and screened for antimicrobial activity using bacterial lawn assays against *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi.* The purified colonies were also tested for antifungal activities *against Aspergillus niger* and *Aspergillus flavus* The most promising isolate, based on bioactivity, was identified as *Fusarium solani* using morphological characteristics, light microscopy and internal transcribed spacer (ITS) sequencing followed by phylogenetic analysis.

The active isolate was cultured in Czapek Yeast Broth (supplemented with 2% glucose and 3% starch) at 26–28 °C with shaking at 150 rpm for 12–14 days. The culture broth was acidified and extracted with ethyl acetate, followed by brine washing, drying over anhydrous sodium sulfate, and solvent evaporation at temperatures below 45 °C. Crude extracts were subjected to silica gel column chromatography using a gradient of n-hexane to ethyl acetate, and further purified by preparative thin-layer chromatography (TLC), guided by UV and iodine staining. This process yielded two major bioactive metabolites, designated as compound 270 and compound 430, based on their nominal masses. Both compounds were structurally characterized using a combination of gas chromatography–mass spectrometry (GC–MS), Fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy. GC–MS of compound 270 revealed a single dominant peak (retention time ~16–19 min) with a molecular ion at m/z 270 and characteristic fragmentation of aryl–alkyl esters. FTIR analysis showed prominent bands corresponding to hydroxyl (-OH), aliphatic C–H, ester C=O, and C–O functionalities. NMR data supported a para-disubstituted phenyl ring, an ester-linked aliphatic chain, and de-shielded methylene protons consistent with halogen substitution. These results confirmed the structure of compound 270 as 4-hydroxyphenyl 8-chlorooctanoate. For compound 430, GC–MS indicated a single peak with m/z ~430, suggesting a larger and more oxygenated scaffold. FTIR bands indicated the presence of hydroxyl, ester, and conjugated double bonds. NMR analysis revealed a complex structure with aromatic, olefinic, ester, and methylene features. The combined data support its assignment as a highly oxygenated aryl–alkyl ester bearing additional

heteroatom-containing substituents. The chemical structures of both compounds were confirmed and presented in the thesis.

To predict potential biological targets, both compounds were subjected to in silico molecular docking studies. Protein structures were retrieved from the Protein Data Bank (PDB) and prepared in UCSF Chimera v1.17. Docking simulations were performed using AutoDock Vina v4.1 via PyRx, targeting active sites of relevant bacterial and fungal proteins. Top-ranked docking poses were further analyzed for interaction profiles. Compound 270 exhibited strong binding affinities with fungal lanosterol 14α-demethylase (CYP51; PDB ID: 5FRB, −7.4 kcal/mol) and bacterial ClpP protease (3V5I, −7.1 kcal/mol). Compound 430 showed even stronger predicted binding with Saccharomyces cerevisiae CYP51 (4LXJ, −11.4 kcal/mol) and moderate affinity with *Candida glabrata* CYP51 (8R6N, −7.01 kcal/mol). To assess the stability of these protein–ligand complexes, molecular dynamics (MD) simulations were carried out using Desmond with the force field (FF19SB) and TIP3P water model under standard temperature and pressure for 100 ns. Analyses of root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration, solvent-accessible surface area (SASA), and interaction timelines confirmed stable binding of both compounds. Notably, compound 430 exhibited consistent interaction patterns and compact structural retention within fungal enzyme pockets. MM/GBSA binding energy calculations further validated these observations with values of −58.95 kcal/mol (4LXJ) and −57.86 kcal/mol (8R6N), indicating favorable energetics.

Laboratory assays were performed to validate the predicted antimicrobial effects. Antibacterial activity of compound 270 was assessed using disc and well diffusion methods on Mueller–Hinton agar against *S. aureus* and *E. coli* at 500, 1000, and 1500 µg/mL. Dose-dependent zones of inhibition were recorded, reaching 26 mm for *S. aureus*, 22 mm for *E. coli* For *P. vulgaris* 17 mm, for *B. subtilis* 22 mm and for *S. typhi* 19 mm at the highest dose. Antifungal activity of compound 430 was tested against *Aspergillus fumigatus* using the poisoned food technique at 500 and 1000 µg/mL. The compound inhibited fungal growth by approximately 50% and 76%, respectively, comparable to the inhibition observed with a standard azole control. Collectively, the combination of chemical characterization, computational modeling and biological assays provides strong evidence that both 4-hydroxyphenyl 8-chlorooctanoate and 4-(1-amino-2-(((1E,5E)-8-(hydroxymethyl)-9-methyl-7-oxodeca-1,5,8-trien-3-yn-1-yl)oxy)-2-(4-oxo-4H-imidazol-5-yl)ethyl)benzonitrile possess notable antimicrobial properties. These compounds demonstrated clear in vitro antibacterial and antifungal activities, supported by robust in silico predictions of target engagement and binding stability. The findings support their potential as promising leads for further investigation, including minimum inhibitory concentration (MIC), bactericidal/fungicidal profiling, mechanism-of-action studies, cytotoxicity screening and in vivo validation.