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Unveiling Novel Bioactivities of Endophytic Fungi from Acorus calamus: Antimicrobial, Antioxidant, and Cytotoxic Properties

Endophytic fungi associated with medicinal plants are a rich source of bioactive compounds exhibiting diverse biological activities. This study investigated the antimicrobial, antibiofilm, antioxidant, and cytotoxic properties of ethyl acetate crude extracts (ECEs) from two endophytic fungi—*Plectosphaerella sp.* (ACR-12) and *Stilbella aciculosa* (ACR-15)—isolated from the rhizomes of *Acorus calamus*. The identity of these fungi was confirmed through morphological, microscopic, and molecular analyses (including ITS sequencing), and their sequences have been deposited in GenBank (PV082262 for *S. aciculosa*; PV082167 for *Plectosphaerella sp.*). GC-MS analysis revealed a range of bioactive metabolites, including erythro-9,10-dibromopentacosane from *Plectosphaerella sp.* and 1-nonadecene from *S. aciculosa*. Antimicrobial activity assessed via disc diffusion and microdilution assays showed significant inhibition against *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633), *Aspergillus niger* (ATCC 16404), *Fusarium oxysporum* (ATCC 48112), and *Candida albicans* (ATCC 10231) with MIC values ranging from 3.125 to 50 µg/mL. Both ECEs exhibited antibiofilm activity against *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853), with *S. aciculosa* showing the highest inhibition (up to 67% at half the MIC). The DPPH assay indicated notable antioxidant activity for *Plectosphaerella sp.* (IC_{50} = 59.37 µg/mL). Cytotoxicity testing against MCF-7 breast cancer cell lines (24-hour exposure) revealed IC_{50} values of 95.39 µg/mL for ACR-12 and 32.58 µg/mL for ACR-15. These findings highlight the potential of *A. calamus* endophytes, particularly *Plectosphaerella sp.* and *S. aciculosa*, as promising sources of novel antimicrobial, antibiofilm, antioxidant, and cytotoxic agents, warranting further pharmaceutical exploration.

Keywords : Endophytes, *Acorus calamus*, *Plectospharella*, *Stilbela aciculosa*, antimicrobial, antibiofilm.

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