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Isolation of Endophytic Fungi from *Vitex trifolia* and Evaluation of Their Antimicrobial Activity

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Structured Abstract

Background: The rapid rise of multidrug-resistant bacteria has highlighted the urgent need for the discovery of novel antimicrobial agents. *Vitex trifolia* is a well-known medicinal plant reported to produce bioactive compounds with activity against common pathogens. However, excessive exploitation of this plant may lead to potential extinction. Consequently, endophytic fungi residing within the plant have emerged as an alternative source of bioactive compounds, as they can produce phytochemicals that mimic those of their host. Therefore, this study aims to isolate endophytic fungi from *V. trifolia* and evaluate their antibacterial activity.

Methods: Endophytic fungi from *V. trifolia* leaves were isolated using two culture media, namely potato dextrose agar (PDA) and potato plant extract (PPE). Prior to isolation, a surface imprint test was conducted to confirm the effectiveness of surface sterilization. The antimicrobial potential of the isolated endophytes was initially screened using an agar plug assay. Selected isolates were subsequently subjected to fermentation, followed by solvent–solvent extraction using ethyl acetate. The resulting extracts were concentrated using a rotary evaporator to obtain crude extracts. Antimicrobial activity was further evaluated using the disc diffusion method, and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined.

Results: A total of seven endophytic fungi were isolated from *V. trifolia* leaves, with most isolates obtained from PPE medium. In the agar plug assay, four endophytes demonstrated inhibitory activity against the tested bacteria, indicating the presence of antimicrobial properties. Disc diffusion assays further confirmed that the crude extracts exhibited inhibitory effects against both Gram-positive and Gram-negative bacteria, suggesting broad-spectrum antimicrobial activity. Among the isolates, extracts from MPPE2 and MPDA showed the most pronounced antimicrobial effects. The MIC and MBC values for all tested bacteria ranged from 3.125 to >50 mg/mL, indicating significant antimicrobial activity.

Conclusion: In conclusion, endophytic fungi isolated from *V. trifolia* demonstrated significant antibacterial activity against the tested bacteria. Among the isolates, MPPE2 and MPDA consistently exhibited inhibitory effects against all tested bacteria, indicating strong and broad-spectrum antibacterial potential. These findings highlight the promise of *V. trifolia* endophytes as potential sources of novel antimicrobial agents.

Keywords: Endophytic fungi, Bioactive compound, *Vitex trifolia*, Multi-drug resistant bacteria, Antibacterial activity.

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