

PUBLICATIONS

LC-MS/MS-BASED MOLECULAR NETWORKING OF THE RESIDUAL METABOLOME OF *Rhizopus arrhizus* 8.2 FROM *Ziziphora pedicellata* PAZIJ ET VVED.

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Endophytic fungi associated with medicinal plants are renewable sources of chemically diverse natural products. *Ziziphora pedicellata* Pazij et Vved. is native to Uzbekistan, but its fungal endophytes remain insufficiently characterized by high-resolution metabolomics. This study aimed to profile the residual metabolome of the kojic-acid-producing endophyte *Rhizopus arrhizus* 8.2 after removal of the dominant crystalline metabolite.

R. arrhizus 8.2 was cultivated in potato dextrose broth for 14 days at 28 °C and 150 rpm. The culture broth was extracted with ethyl acetate, and kojic acid was isolated from the crude extract. The residual fraction was analyzed using an Orbitrap Exploris 120 LC-MS/MS system with an ACE Excel Super C18 column (250 x 4.6 mm, 5 µm). Mobile phases were 0.1% formic acid in water and methanol; data were acquired in positive H-ESI mode over m/z 100-1600. Raw files were converted to mzML, processed in MZmine 3, submitted to GNPS for feature-based molecular networking, and visualized in Cytoscape.

The LC-MS/MS profile demonstrated that the post-kojic acid fraction is a chemically complex metabolite pool rather than an inactive by-product. Putatively annotated metabolite families included furocoumarins, phenolic and phenylpropanoid derivatives, tyrosine-derived alkaloid-like compounds, oxidized fatty-acid metabolites, oxygenated heterocycles, and indole-containing pyrrolinone derivatives. Selected metabolites of interest were oxypeucedanin methanolate, hordenine, 5-allyloxypsoralen, heraclenin, salicin, 3,4-dihydroxy-beta-phenylpropionic acid, 5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid, and pyrrolinone derivatives. Molecular networking grouped related MS/MS features into metabolite families and improved interpretation of minor compounds that could be overlooked by conventional targeted analysis.

Because authentic standards and NMR validation were not used at this stage, all annotations should be considered tentative, corresponding to MSI level 2/3 confidence. Ochratoxin A-like and organophosphorus-like matches should be interpreted as safety-relevant alerts, not definitive identifications, and require targeted confirmation. Overall, *R. arrhizus* 8.2 represents a promising object for metabolomics-guided natural product discovery, while rigorous structural validation and safety assessment are necessary before pharmaceutical or biotechnological evaluation.

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