

ABSTRACT

Hanis Naila Maytsak, 24020222120006. Bioprospecting and Secondary Metabolite Profiling of *Penicillium* sp. Isolate from the Hypersaline Area of Bledug Kuwu.

The hypersaline area of Bledug Kuwu, Grobogan Regency, Central Java, represents a potential source of halophilic fungi producing bioactive secondary metabolites. This study aimed to characterize the morphology, identify the species molecular tools, and evaluate the antibacterial, antifungal, and antioxidant activity of fungal isolate BK 3.2 from Bledug Kuwu. Morphological identification was performed using the slide culture method, while molecular identification was conducted through ITS region amplification and sequencing using ITS1 and ITS4 primers. Scale-up cultivation was carried out in 5 L PDB medium supplemented with 5% NaCl for three weeks. The extraction of the fungal broth was prepared using ethyl acetate, followed by gradient to yield 10 fractions. The fungal extract and fractions were tested against a series of pathogens, i.e. *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *E. coli* using disk diffusion, antifungal testing against *C. albicans* using disk diffusion assay; while their antioxidant were assessed using the DPPH method. Each of the active fractions was subjected to LC-MS for compound. Morphological characteristics and ITS-based phylogenetic analysis identified fungal isolate BK 3.2 as *Penicillium citrinum*. Semi-polar fractions PCB F3, F4, and F5 showed the highest antibacterial activity against *S. aureus* with inhibition zones of 17.8, 18.3, and 19.3 mm, respectively, using chloramphenicol as the positive control, while all fractions showed weak activity against Gram-negative bacteria. Antifungal activity was observed only in fraction PCB F2 with an inhibition zone of 13.1 mm against *C. albicans* using nystatin as the positive control. The highest antioxidant activity was found in fraction PCB F6 with an IC_{50} of 68.31 ppm (strong category). Metabolite profiling using LC-MS indicated the presence of tetracosanoic acid in fraction F2, deoxynivalenol or roquefortine A in fraction F4, and verruculogen or verruculotoxin in fraction F6. The diversity of these secondary metabolites suggests the potential of the halophilic fungi as a source of multifunctional bioactive compounds.

Keywords: antibacterial, antifungal, antioxidant, Bledug Kuwu, *Penicillium citrinum*