ABSTRACT

Soil samples collected from different locations of malnad region which is part of western ghats of Shimoga district were screened for microorganisms with antibiotic production potential. Totally 112 bacteria and 119 fungal strains with potential antibiotic production were isolated. Based on zone of inhibition exhibited on target bacteria, the best two isolates were selected for further antimicrobial screening. The two bacteria were identified as Bacillus cereus and Bacillus pumilus. The metabolites of the two bacteria were subjected to various solvent extractions like petroleum ether, ethyl acetate and methanol, and test samples were prepared. The test samples of Bacillus cereus (BC) were labeled as BC-1, BC-2 and BC-3 respectively. The test samples of Bacillus pumilus (BP) were labeled as BP-1, BP-2 and BP-3 respectively. The test samples were tested for antibacterial and antifungal activity against human pathogens. The ethyl acetate extracts of both the organisms showed good antibacterial activity but insignificant antifungal activity. The Minimum Inhibitory Concentration (MIC) studies proved the potency of the isolates for antibiotic production. During TLC-bioautographic studies, the bands obtained at Rf values 0.87 and 0.85 showed components with antibacterial properties for Bacillus cereus and Bacillus pumilus respectively.

The metabolites of both bacteria were evaluated for antioxidant potential. The metabolites of both bacteria exhibited significant antioxidant activity by exhibiting 97% and 88.5% radical scavenging by ABTS method for B. pumilus and B. cereus with IC$_{50}$ values at 16.2±1.17µg/ml and 55.12±2.51µg/ml respectively. They showed comparatively less radical scavenging activity by DPPH assay.

The metabolites of both bacteria were evaluated for cytotoxicity and anticancer activity. The metabolites were tested for cytotoxicity against normal human liver cell lines and two cancer cell lines by MTT assay. The two cancer cell lines used were human cancerous liver cells and human laryngeal epithelial carcinoma cells. The test samples that exhibited significant cytotoxicity were fractions obtained from Bacillus cereus. The petroleum ether and ethyl acetate fractions were further evaluated for anticancer activity by nuclear staining studies and DNA fragmentation analysis. Both the fractions showed significant anticancer activity by membrane blebbing during nuclear staining and damaged DNA patterns during DNA fragmentation analysis. The metabolites of Bacillus pumilus did not exhibit anticancer activity.

The metabolites of both bacteria were evaluated for analgesic activity. The analgesic activity was performed by tail warm water immersion method and Eddy’s Hot-plate method using Swiss albino mice. The methanol extract of B. cereus and ethyl acetate extract of B. pumilus showed significant analgesic activity with a reaction time of 33.63 ± 0.61 seconds and 34.63 ± 1.24 seconds at a dose of 50 mg/Kg body weight when compared to standard with a reaction time of 15.43 ± 0.41 seconds.

The metabolites of both bacteria were evaluated for CNS depressant activity and skeletal muscle relaxant activity. The CNS depressant activity was performed using photoactometer, skeletal muscle relaxant activity by rotarod method and anticonvulsant activity by maximal electric-shock (MES) method. The methanolic extract of both bacteria exhibited significant CNS depressant activity in mice with 58.10% and 54.54% change in locomotor activity in comparison with control. The methanolic extracts of both bacteria also exhibited skeletal muscle relaxation properties with a 52.90% and 53.64% in comparison with control. However, none of the sample extracts showed any significant anticonvulsant activity. The metabolites of both bacteria were evaluated for anthelmintic activity. The solvent extracts were tested for anthelmintic activity against Pherephila posthuma at 20 mg/ml concentration. The time of paralysis and time of death of the worms was determined for all the extracts. Albendazole was taken as a standard reference and sterile water as a control. All the sample extracts showed significant anthelmintic activity in paralyzing the worms comparable with that of the standard drug. The time of death exhibited by B. pumilus metabolites was close to the time exhibited by standard. However, the extracts of B. cereus took more time for death of worms than B. pumilus.