ABSTRACT

Production of various valuable added products especially primary and secondary metabolites has been so far reported out using different sources such as plants, animals and microbes. Among them, secondary metabolites from microbes are one which possesses significant medical applications for the treatment of several diseases caused by the existing pathogenic strains and mutations in cells. Although the secondary metabolites have been so far reported by various sources, the necessity of secondary metabolites which would not cause any side effects is getting prompted now-a-days. Hence a potent marine microorganism has been used as a source of secondary metabolites production in this study.

Potent secondary metabolites producing *actinomycetes* isolated from the marine soil sediment samples of Manora Fort, Thanjavur, Tamilnadu, India. The active strain was screened for primary screening against bacterial pathogenic strains such as *E. coli, B. subtilis, S. aureus, S. epidermidis, P. aeruginosa* and *V. cholerae* and fungal pathogenic strains such as *A. niger, A. flavus, A. fumigatus* and *C. albicans*. The marine isolate was found to possess the antibacterial activity of 26±0.46 mm, 23±0.39 mm, 18±0.20 mm, 20±0.25 mm, 12±0.82 mm and 16±0.05 mm against the respective bacterial strains through zone of inhibition using cross streak technique. Similarly, it showed the antifungal activity of 17±0.78 mm, 20±0.10 mm, 24±0.29 mm and 19±0.90 mm against its respective fungal pathogens.

The marine isolate was identified for aerial mass colour with small, round and velvety in nature and gray color aerial mycelium. The spore

morphology of the marine isolate was smooth walled spores with spirales in nature analyzed by light microscopy and SEM. The isolate showed positive results for Gram's staining, MR-VP test, Urease, H_2S production, Oxidase, Catalase, Nitrate reduction, Hypoxanthine production, Gelatin/Casein/ Cellulose/ Starch/ Tween 20/Tween 80 hydrolysis analyzed by standard physiological and biochemical tests. Further the isolate was identified by 16S rRNA sequence analysis and phylogenetic tree construction. The gene sequence obtained through 16srRNA analysis generated for a total of 1477 nucleotide base pairs for the marine isolate was published in NCBI and received the accession number as KJ469763. The query sequence of the marine isolate showed closest match of 96% similarity with *Streptomyces cirratus* strain 7g7 16S ribosomal RNA gene, partial sequence obtained from the BLAST search. The phylogenetic tree was constructed by neighbour joining analysis and maximum likelihood analysis using bootstrap consensus test with 1000 replications in MEGA 6 software. The marine isolate was identified and designated as *Streptomyces cirratus* SRP11.

Among four different liquid media tested, MYEB media supported good growth for secondary metabolites production of *S. cirratus* SRP11 than the other media with respective zone of inhibition of 21±0.27 mm, 24±0.15 mm, 23±0.59 mm and 19±0.66 mm against *E. coli, B. subtilis, A. fumigatus* and *A. flavus.* The dried weight of secondary metabolites in MYEB was found to be 26±0.76 mg/50 ml. Similarly, among four different solid media tested, MYEA media supported very good growth for metabolites production of *S. cirratus* SRP11 than the other media with zone of inhibition of 20±0.54 mm, 22±0.37 mm, 22±0.11 mm and 17±0.66 mm against *E.coli*, *B.subtilis*, *A.fumigatus* and *A.flavus.* The dried weight of secondary metabolites in MYEA was found to be 25±0.99 mg/50 ml. The dried weight of the secondary metabolites with respect age of culture in MYEB medium, on the fourth day it was found to be 27±0.93 mg/50 ml with the inhibition zones of 20±0.22 mm,

19±0.77 mm, 18±0.91 mm and 16±0.34 mm against *E. coli*, *B. subtilis*, *A. fumigatus* and *A. flavus.*

Optimization of the fermentation environmental parameters has profound influence on secondary metabolites. The optimum levels of the process variables for fermentation process were analyzed using RSM (Design Expert 7.0). The model F-value 6.86 implies that the model is significant. Adeq.Precision ratio of 11.589 indicates an adequate signal. The low CV (4.06) denotes that the experiment performed is reliable. The R^2 value was found to be 0.8255 closer to 1 denotes better correlation between observed and predicted values. The maximum yield of secondary metabolites was obtained at the optimum conditions of pH 7, temperature 28 °C, incubation period 4 days, agitation 200 rpm and salinity 8% (w/v) using response optimizer tool and the yield response under this condition was observed to be 26 mg/50 ml. The validation of the CCD model using RSM was performed under optimized conditions of parameters such as pH (5-9), temperature (26-30 °C), incubation period (1-5 days), agitation (100-300 rpm) and salinity (2-10%) (w/v) and the results showed that pH 7, temperature 28 °C, incubation period 4 days, agitation 200 rpm and salinity 8% (w/v) as optimum for maximum yield of dried weight of crude secondary metabolites of *S.cirratus* SRP11. The dried weight of secondary metabolites was found to be 27 ± 0.11 mg/50 ml.

The dried weight of the crude secondary metabolites using chloroform as a solvent was found to be 27 mg/50 ml. Similarly, the dried weight of crude secondary metabolites using ethyl acetate was found to be 21 mg/50 ml. The yield of the secondary metabolites was found to be higher in chloroform extraction compared to ethyl acetate. UV absorption spectrum was recorded a maximum absorption peak from 200 to 250 nm be a sign of production of active compounds of polyene nature and presence of peptide

antibiotic in the extract. The absorption peaks at 1560 cm^{-1} denotes the possibility of an aromatic nitro compounds or alternatively due to primary amine, N-H bend and vibrational frequency (C=C-C) indicates the aromatic ring stretch of secondary amines, N-H bend at 1458 cm^{-1} using FTIR. The compounds identified in GCMS possessing antibacterial, antifungal, antioxidant and anticancer, activities.

TLC- Contact bioautography results confirmed presence of antibacterial and antifungal compounds in the crude extract. The R_f value of antibacterial compound obtained from developed chromatogram was found to be 0.459 which possess the R_f value of reference antibacterial antibiotic Rifampicin. Similarly, R_f value of antifungal compound was found to be 0.486 which possess the R_f value of reference antifungal antibiotic Candicidin. The cotton gauze cloth finished with a dip and dry method showed effective antibactericidal and antifungicidal activity against *M. smegmatis* and *C. tropicalis.*

The greater viability percentage of NIH 3T3 cells was found up to 50.55% with the increase in the concentration up to 650 µg/ml of crude chloroform extract of the marine isolate. The chloroform extract showed anticancer activity and inhibitory concentration of (IC_{50}) 60 µg/ml against HT-29 and HeLa cancer cell lines. The anticancer activity of the extract may be due to the presence of active principle compound pyrrolo (1, 2-alpha) pyrazine 1,4 dione hexahydro 3-(2-methylpropyl) in the crude extract. Results of LDH cytotoxicity assay revealed that the treatment on HT-29 and HeLa cancer cells with crude extract of the marine isolate caused the decrease in the LDH release. When the concentration of crude extract of *S. cirratus* SRP11 was increased, the release of LDH decreased in a concentration dependent manner in HT-29 and HeLa cancer cells treated cells, from 26.16 IU/mg of protein to 9.62 IU/mg of protein in HT-29 cells and 28.93 IU/mg of protein to

9.82 IU/mg of protein in HeLa cells. The induction of caspase-3 activity was observed in HT-29 and HeLa cancer cell lines with the increase in the concentration of *S. cirratus* SRP11 crude chloroform extract. The fold increase of 2.25 and 2.32 in HT-29 and HeLa cell compared with control cells indicate the cellular damage and death of cancer cells due to the activation of caspase-3. It was found that the two cancer cell lines (HT-29 and HeLa) incubated with 250 µg/ml crude extract of *S.cirratus* SRP11 showed apoptotic DNA fragmentation profiles compared to control DNA. Meanwhile, the crude extract treatment led to a significant reduction of HIF-1α concentration in cancer cells compared with untreated control cells. The reduction of the transcription factor reached up to 40% in HT-29 cancer cells.

DPPH scavenging activity of the crude extract of marine isolate ranged from 38.24% to 72.88% and total antioxidant activity was ranged from 50.84% and 81.39%. Chloroform extract of culture filtrate (1139.5±62.3 µmol Fe (II)/mg extract) exhibited higher ferric reducing activity when compared with Ethyl acetate (1043.2±53.7 µmol Fe(II)/mg extract) and the standard butylated hydroxyanisole (814.4 µmol Fe(II)/mg extract). The metal chelating capacity of the extract was found to be increased with the increase in the concentration of the extract from 22.45% to 86.22%. Hence the ability of the extract was observed to be greater to compete with ferrozine in chelating ferrous ion. ABTS radical scavenging activity revealed that the extract of the isolate scavenged the ABTS in the concentration dependent manner from 27.6 % to 75.3%. Crude extract of *S. cirratus* exihibited superoxide radical scavenging activity and nitric oxide-scavenging activity from 24.22% to 79.44% and 22.49% to 79.11%. From these results, the antioxidant potency of the crude chloroform extract of *S. cirratus* SRP11 was highly evident from the present study. The stability of the crude secondary metabolites was found to be stable till 60 °C, pH of 9 and shelf life of 4 months.