CHAPTER 5

SUMMARY AND CONCLUSION

5.1 CONCLUSION

Secondary metabolites are being used and right away indispensable to contradict and turn round the increase of antibiotic resistant pathogens and to combat severe chronic diseases as concern. Production of secondary metabolites is considered as classy due to several factors such as cost of the fermentation medium, maintenance, separation, purification and marketing. This study was developed with the objective of extraction of secondary metabolites from marine microbe by incorporating the improvements in production through optimization of environmental fermentation parameters and its biological applications.

Production of secondary metabolites has been examined extensively in the past decade by many researchers using different sources. The major disadvantages are side effects caused by the product and stability. In this present study, the marine environment was selected for the isolation of secondary metabolites producing actinomycetes as they are non-pathogens. The present study indicated that marine environment can afford a well-off source of such microorganisms. Out of diverse microorganisms isolated from the marine environment, *Streptomyces cirratus SRP11* was found to be more potent for the production of secondary metabolites. The active strain isolated from Manora Fort, Thanjavur, Tamilnadu, India 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 and antifungal activities to its respective pathogenic strains.

Identification of the marine isolate was done by Morphological, physiological, biochemical and 16S rRNA analysis. The aerial mass colour with small, round and velvety in nature and gray aerial mycelium and spore morphology of the marine isolate was 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. In 16srRNA analysis, a total of 1477 nucleotide base pairs for the marine isolate was published in NCBI and received the accession number as KJ469763. The sequence of the marine isolate demonstrated closest match of 96% similarity with *Streptomyces cirratus* strain 7g7 16S ribosomal RNA gene, partial sequence obtained from the BLAST search. The phylogenetic tree constructed by neighbour joining analysis and maximum likelihood analysis using bootstrap consensus test with 1000 replications in MEGA 6 software.

Amongst four different liquid media tested, MYEB media supported good growth for secondary metabolites production of *S. cirratus* SRP11 than the other media. 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.

Furthermore, optimization of the fermentation environmental parameters for secondary metabolites production in marine isolate were analyzed using RSM (Design Expert 7.0). The greater yield of secondary

metabolites was attained 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) were done and the results showed that pH 7, temperature 28 °C, incubation period 4 days, agitation 200 rpm and salinity 8% (w/v) was obtained optimum for maximum yield of dried weight of crude secondary metabolites of *S. cirratus* SRP11.

The yield of the secondary metabolites was found to be suitable and elevated in chloroform extraction compared to ethyl acetate. The crude chloroform extract containing secondary metabolites was analysed by UV, FTIR and GC-MS.

Moreover, TLC- Contact bioautography results confirmed the presence of antibacterial and antifungal compounds in the crude extract. The viability percentage of NIH 3T3 cells was found be increased with the increase in the concentration of crude chloroform extract up to 650 µg/ml of the isolate. The chloroform extract showed anticancer activity and inhibitory concentration value 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. LDH cytotoxicity assay revealed that the treatment of HT-29 and HeLa cancer cells with crude extract of the marine isolate caused the decrease in the LDH release. As the concentration of crude extract of *S. cirratus* SRP11 was elevated and the release of LDH decreased in a concentration dependent manner in cancer cell lines. The induction of caspase-3 in cancer cell lines was found to be increase in the

concentration of *S. cirratus* crude chloroform extract. Further, 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 reduction of the transcription factor reached up to 40% in HT-29 cancer cells.

DPPH scavenging activity of the crude extract of marine was found up to 72.88% and total antioxidant activity was ranged up to 81.39%. Chloroform extract of culture filtrate of marine isolate (1139.5±62.3 µmol Fe (II)/mg extract) exhibited higher ferric reducing activity in FRAP assay. The metal chelating capacity of the extract ws found to be increased with the increase in the concentration of the extract up to 86.22%. ABTS radical scavenging activity exposed that the extract of the isolate scavenged the ABTS in the concentration dependent manner up to 75.3%. Superoxide radical scavenging activity and nitric oxide-scavenging activity of the extract was observed to be up to 79.44% and 79.11%. From these results, the antioxidant potency of the crude chloroform extract of *S. cirratus* SRP11 was greatly apparent. The stability of the crude secondary metabolites was found to stable till 60 °C, pH 9 and shelf life 4 months.

5.2 SCOPE FOR THE FUTURE WORK

In the present work, marine *actinomycetes* was used as a source for the production of secondary metabolites. Optimization of environmental parameters using RSM and solvent extraction have been implemented for enhanced secondary metabolites production. The proposed technique may be improved further by carrying out the following related works and analyzing the results obtained.

- Renovation of new and additional advancement of existing isolation and cultivation techniques.

- Genome-based bioprospecting should be developed in equivalent. Development of bioinformatic analysis of the genomes allowing identification of unique biosynthetic gene clusters, chemoinformatic software for prediction of structural descriptions based on gene analysis and additional development of host/vector systems for heterologous expression of gene clusters.

- Increase in production of secondary metabolites can be accomplished by expression of the recently engineered pathways into industrially optimized strains.

- Deletion and overexpression of genes directed to up or down regulate the expression of secondary metabolite gene clusters, or to make ineffective blocks in the biosynthesis of these compounds.

- Advances in developing DNA manipulation tools and augmentation in genome sequencing technologies.

- Incorporation of genome shuffling has been described and demonstrated as a new method for rapid enhancement of secondary metabolite production.