CHAPTER 2

LITERATURE REVIW AND SCOPE OF THE PRESENT STUDY

2.1 INTRODUCTION

This chapter provides the background information for this thesis. It covers extensive literature collection on secondary metabolites production from microbes, advantages of natural biosynthesis from microbes over chemical synthesis, how marine *actinobacteria* act as a source of bioactive secondary metabolites and significance of optimization of fermentation environmental parameters for secondary metabolites production from *actinomycetes*. This chapter also discusses in detail about the importance of extraction methods influencing the metabolites production are discussed. Secondary metabolites biosynthesis from *actinomycetes* especially from *Streptomyces* sp. is proposed and the studies on reported works have been presented.

2.2 ANTIBIOTIC RESISTANCE - NECESSITATE UNIVERSAL SOLUTIONS

Infectious diseases growing throughout history have included some of the major deadly plagues and cholera of the past (Morse & Schluederberg 1990, Morse 1993). The emerging infections are newly appeared in the inhabitants are quickly increasing in frequency of geographic range. The changes in the ecology occur due to the rise of infectious diseases, including

the development in economy and agriculture are among the most commonly recognized issues in emergence. The increasing proximity and changing conditions support an increased population of the microbe or its natural host (Morse 1991).

Around the twentieth century, impetus increase in infectious diseases being brought under control. This was mainly due to the widen development of novel antibiotics, vaccines, improvements in hygiene, cleanliness and improved nutrition. Earlier, human beings have come under attack from previously unfamiliar infectious diseases. Extensive research on infection control with the aid of potent antibiotics is the basic principle in order to prevent the avoidable infections and control the present existing antibiotics (Zarb & Goossens 2012).

Nowadays, the effectiveness of the antibiotics is decreasing in treating common infections with the discovery of untreatable strains. As the consequence of mutations in microbes and the assortment pressure from the antibiotics provides the competitive gain for mutated strains is the primary reason for the decrease in the effectiveness of the antibiotics. Sub optimum antibiotic doses help stepwise selection of resistance (Laxminarayan & Heymann 2012). Before the arrival of penicillin, there are several resistant strains of bacteria noticed. The use of millions of tonnes of antibiotics over the past 75 years has made approximately agaist all disease causing bacteria resistant to antibiotics which are commonly used to treat the respective pathogenic strains (Abraham & Chain 1988). The load of resistance in antibiotics possibly concentrated into three main categories; firstly prolong extent of illness, secondly, the superior rates of mortality in humans with upcoming challenging infections due to pathogens and the growing expenditures of treatment for resistant infections and its failure to carry out measures which rely on competent antibiotics to prevent infection.

With the scarcity of novel antibiotics, scientific blocks in the research and technology necessitate to be overcome with the acknowledgment of promising guide and crossing from basic and translational research to improvement of clinical trials (Davies & Davies 2010). Several antibiotic scaffolds derived from microbial secondary metabolism were chemically modified to get better efficiency and circumvent opposition direct to regular ease of use of abundant potent medicines to address invading challenges from growing multi drug resistant pathogens. With rising alarm over the ineffective antibiotic drug discovery pipeline and to investigate the alternatives to small molecule drugs is a great deal of interest. The products derived from the natural marine sources have had outstanding effect on modern medicine this class of bioactive therapeutically expensive compounds act as a feasible substitute to antibiotics (Fernebro 2011). A new sustainable global model for the detection, growth and division of antibiotics from new microbial species needs be developed in which the private and public sectors work in partnership. Intensive investigation attempts need to be focussed to keep hold of the efficacy of our existing antibiotics by substantial investment in antibiotic option from natural origin. Besides antibiotic research, the increased possessions require to be made in alternative to antibiotics. The goal is to persuade researchers and biotechnologists to reassess the reliance on antibiotics and to explore other means of controlling infections (Zarb & Goossens 2012).

2.3 ADVANTAGE OF NATURAL BIOSYNTHESIS PRODUCTS AND CHEMICAL SYNTHESIS

In the year 2007, the consequence of a seven-year antibacterial drug discovery program runs at GlaxoSmithKline. A genomics imitative, target-based screening plan of synthetic compound libraries aspiring at new classes of antibiotics with desirably novel modes of action. About 300 genes with

potential new antibacterial targets were assessed and further examined in the screening programme. The production of the antibacterial screening program of the individual screening targets was unsatisfactory with the indefensible low success rates of chemical synthetic library and the biological targets were scarce and not potent for the discovery of novel antibacterial leads (Payne et al 2007).

On the other hand, the natural products range provides phytochemical assets with multiple targets were hardly found in molecules plagiaristic from combinatorial synthesis (Baltz 2006). In addition, natural product based drug finding is a complex, time-consuming and expensive effort. Since metabolic products of living microorganisms have the authentic benefits of evolving to be energetic in target cells *in vivo*, abandoning a chief barrier of target based approaches (Nussbaum et al 2006)

2.4 GROWING INTEREST ON SECONDARY METABOLITES FROM MICROBES AGAINST INFECTIOUS DISEASES

The increase in the necessity for drugs makes proficient to supervise new illnesses or resistant strains of microorganisms inspired to look for unconventional new sources of bioactive natural products from microorganisms. Even though widespread progress is being made within the fields of engineered biosynthesis and chemical synthesis of antimicrobial compounds but nature at a halt remains the richest and the most important inventive basis for newer secondary metabolites (Koehn & Carter 2005, Baltz 2006, Peláez 2006). The thrust of finding newer antibiotics or metabolites turned out to be a prominent field of ocean. Since then, massive attempts have been made on the isolation of new metabolites from marine organisms.

On contrary, secondary metabolites isolated from marine microorganisms is a rapid promising field that can be observed from several

reported reviews contributed to this concern (Liberra & Lindquist 1995, Kobayashi & Ishibashi 1993, Davidson 1995, Pietra 1997, Jensen & Fenical 1994, 1996, 2000, Bernan et al 1997, Fenical 1993, Faulkner et al 2000). The significance of terrestrial bacteria and fungi as sources of expensive bioactive metabolites has been very eminent for more than half a century. Bacteria and fungi strains revealed from soil and water samples led to a notable evidence of antibacterial, antifungal and cytotoxic agents. A fungal metabolite, Penicillin was approved for the beginning of antibiotics golden age and it paves the way for several investigations.

From this investigation onwards, the microbial metabolites were focussed for the profound application in industry, agricultural and forest applications (Scholar & Pratt 2000, Demain 1999). The screening of the microorganisms for the secondary metabolites production of antibiotics is the basis in the research programme. Fungi and *actinomycetes* produce natural products with broad ranges of chemical structures. More than 400 metabolites with antimicrobial activities were discovered from bacteria, fungi, plants, insects and vertebrates. Amoxycillin, cephalosporin and griseofulvin are the important antibiotics recognized as an important source of antibiotic compounds by micro fungi (Deacon 1997). Australian fungi with greater variety are largely endemic in nature and as a result of its diversity many novel metabolites from under explored environment was found (Chapman, 2009).

Many of the macro fungi were reported for the significant pharmacological activity including antibacterial, antifungal, antiviral, cytotoxic, antiallergenic, anti-inflammatory, anti atherogenic, hypoglycaemic, hepatoprotective, immunomodulatory and hallucinogenic activity (Suay et al 2000, Lindequist et al 2005). It was reported that from a total of 258 reported substances 79 (31%) have shown some biological activity, 47 metabolites

were isolated from bacteria and 32 from fungi. The numbers of antitumor and antibacterial compound from bacteria stay the same but fungi are added competent sources of anti-cancer (57%) rather than antibacterial (22%) compounds.

2.5 MARINE ACTINOBACTERIA – A PRIME CONCERN SOURCE FOR POTENTIAL PRODUCER OF BIOACTIVE SECONDARY METABOLITES

The *actinomycetes*, best known for their ability to produce biologically active secondary metabolites. *Actinomycetes* are prokaryotes with enormously various metabolic possibilities and produce numerous substances essential for health such as antibiotics (Berdy 1995, Gocheva & Ilieva 1983, Omura 1979, Okamoto et al 1980, Tamamura 1985). Secondary metabolites produced by the marine *actinobacteria* own extensive ranges of biological activities (Mann 2001, Berdy 2005, Oldfield et al 1998, Manivasagan et al 2013). Each strain of *actinomycetes* is likely to have the genetic prospective set up for the production of 10-20 secondary metabolites (Bentley et al 2002, Lam 2006). Till date, around 23,000 antibiotics have been discovered from microorganisms. Approximately it was estimated that about 10,000 of them have been isolated from *actinomycetes*. Comparing with other genus of *actinobacteria*, mainly the genus *Streptomyces*, have the skill in the production of wide variety of secondary metabolites as bioactive compounds.

There are several reports available in the genus *Streptomyces* alone for the production of greater number of bioactive molecules. This may be due to the biosynthetic potential of the genus which remains confront as the efficient competitor among other microbial community. Moreover the possibility of isolation of a novel *Streptomyces* from terrestrial region has undermined. There are several reports in the enormous number of *Streptomyces* sp. from the soil sediments (Watve et al 2001). The resistance of

the pathogenic strains to the active antibiotics is the foremost reason for discovering novel secondary metabolites from *actinomycetes* (Lam 2006, *Uchechi & Erinma* 2007). Meanwhile, the increase in the number of deaths due to these robust pathogenic organisms is on the growth. The isolation of secondary metabolites from marine *actinomycetes* outline the basis for the synthesis of novel therapeutic drugs in the medical field which acts a effective drugs to combat numerous resistant microbes and about 10% of *actinomycetes* colonizing in the marine aggregates isolated from marine sediments (Fenical & Jensen 2006, Ward & Bora 2006).

2.6 MARINE *STREPTOMYCES* - A FRONTIER AND NOTABLE *ACTINOMYCETES* IN THE HISTORY OF BIOACTIVE METABOLITES PRODUCTION

In the search of finding newer competent drug molecules by the massive international soil screening projects in Europe, North America and Japan leads to the discovery of most outstanding groups of microbial secondary metabolite producers belong to the common soil *actinobacteria* of the genus "*Streptomyces*". Till today, *Streptomyces* derived metabolites uphold to maintain a central role in the medical treatments. For several decades *Streptomyces* have been subjected to strong genetic research study for the secondary metabolism pathways. Slowly, the research has enlarged in the route of protein level study to reveal molecular mechanisms of the *Streptomyces* behind the biosynthetic diversity and to understand the limits and potential of diverse technologies in propose of novel compounds.

From the several literature reports it was cited that marine *actinobacteria* were found to be the most tremendous basis of secondary metabolites and huge number of the compounds were derived from the single genus *Streptomyces* which are diversified broadly in the marine and terrestrial habitats (Berdy 2005, Pathom-Aree et al 2006) The profitable interest of

marine *actinomycetes* is due to their unique capacity to produce novel metabolites. About ¾ of all the recognized medicinal, agricultural and commercial useful antibiotics are derived from the single genus *Streptomyces* which are known as the commercially and medicinally useful antibiotics (Cundlife 1989, Kieser et al 2000, Okami & Hotta 1988). It was obvious that *Streptomyces* sp. are broadly based on its distribution and they own abundant spores that are voluntarily scattered (Antony-Babu et al 2008) these filamentous bacteria are well adapted in the marine environment and can break multifaceted complex biological polymers (Anderson & Wellington 2001). Peptide-type compounds, macrocyclic lactones and various quinine derivatives are the three chief classes of compounds with vast structural and functional diversity found in the *Streptomyces* and the biosynthetic capacity of the species is even remain without rival in the microbial world (Watve et al 2001).

It was reported that approximately 60% of the antibiotics discovered for the agricultural purposes are from the genus *Streptomyces* (Tanaka & Omura 1993). *Streptomyces* have been revealed to have the capability to produce insecticidal (Pimentel-Elardo et al 2010), anti-inflammatory (Patzer & Braun 2010, Renner et al 1999), anti-parasitic (Pimentel-Elardo et al 2010), anti-fungal (Shah 1998, Prabavathy et al 2006, Zarandi et al 2009, Ramesh & Mathivanan 2009, Berdy 2005, Prapagdee et al 2008), immunosuppressant (Sehgal 2003, Saunders et al 2001, Vezina et al 1975) antibacterial (McGuire et al 1952, Ramesh & Mathivanan 2009, Berdy 2005), antiviral (Khan 2011, Sacramento et al 2004), antitumor (Skovsgaard & Nissen 1975, Lam 2006, Hong et al 2009, Berdy 2005), antifouling (Xu et al 2010), anti-infective (Rahman et al 2010) and plant growth promoting compounds (Sousa et al 2008) as well as many other agents such as enzyme inhibitors (Hong et al 2009) and vitamins (Atta 2007) because of these characteristic descriptions they are well identified as industrially essential

microorganisms (Tamehiro et al 2003, Higginbotham & Murphy 2010, Williams et al 1983).

They are familiar for their ability of producing a variety of extracellular hydrolytic enzymes including ribonucleases (Nicieza et al 1999, Ramesh et al 2009, Hong et al 2009, Ramesh & Mathivanan 2009, Cal et al 1995, Brunakova et al 2004). These qualities make this genus a unique microbe in the field of research area was under discussion from the academic and manufacturing viewpoint (Tanaka & Omura 1993). Table 2.1 shows the literature collection on production of secondary metabolites from marine *Streptomyces* sp. and its biological activity.

Table 2.1 Secondary metabolites from marine *Streptomyces* sp. and its biological activities

Bioactive metabolite	Source	Biological activity	Reference
2-Allyloxyphenol	*Streptomyces* sp.	Antimicrobial; food preservative; oral disinfectant	Arumugam et al (2009)
Albidopyrone	*Streptomyces* sp.	Cytotoxic	Hohmann et al (2009a)
Aureolic acid	*Streptomyces* sp.	Antitumor	Lu et al (2012)
Aureoverticillactam	*Streptomyces aureoverticillatus*	Antitumor	Mitchell et al (2004)
Avermectins	*Streptomyces avermitilis*	Anti- parasitic	Burg et al (1979)
Bisanthraquinone	*Streptomyces* sp.	Antifungal and anticaner	Socha et al (2006)
Bonactin	*Streptomyces* sp.	Antifungal	Schumacher et al (2003)
Caboxamycin	*Streptomyces* sp.	Antibacterial; anticancer	Hohmann et al (2009b)
Chinikomycins	*Streptomyces* sp	. Anticancer	Li et al (2005)

Table 2.1 (Continued)

Bioactive metabolite	Source	Biological activity	Reference
Chalcomycin	*Streptomyces* sp.	Antitumor	Wu et al (2007)
Chloro-dihydroquinones	*Streptomyces* sp.	Anticancer	Soria-Mercado et al (2005)
Cyclomarins	*Streptomyces* sp.	Anti-inflammatory	Renner et al (1999)
Daryamides	*Streptomyces* sp.	Antitumor	Asolkar et al (2006)
Essramycin	*Streptomyces* sp.	Anti-inflammatory	El-Gendy et al (2008b)
Elaiomycins B and C–	*Streptomyces* sp.	Phycotoxicity	Helaly et al (2011)
Frigocyclinone	*Streptomyces griseus*	Antibacterial	Bruntner et al (2005)
Glaciapyrroles	*Streptomyces* sp.	Antibacterial	Macherla et al (2005)
Gutingimycin	*Streptomyces* sp.	Antifungal and anticancer	Maskey et al (2004)
Lajollamycin	*Streptomyces nodosus*	Antibacterial	Manam et al (2005)
Lincomycin	*Streptomyces lincolnensis*	Antifungal and anticancer	Peschke et al (2006)
Marinopyrroles	*Streptomyces* sp.	Cytotoxic	Hughes et al (2008)
Mansouramycin C	*Streptomyces* sp.	Cytotoxic	Hawas et al (2009)
Mitomycin C	*Streptomyces lavendulae*	Antibacterial	Mao et al (1999)
Manumycins	*Streptomyces* sp.	Cytotoxic	Chauhan et al (2005)
Nonactin	*Streptomyces* sp.	Phycotoxicity	Jeong et al (2006)
Piperazimycins	*Streptomyces* sp.	Cytotoxic	Miller et al (2007)
Resistoflavin methyl ether	*Streptomyces* sp	Antibacterial; anti-oxidative	Kock et al (2005)
Salinamides A and B	*Streptomyces* sp.	Anti-inflammatory	Moore et al (1999)

Table 2.1 (Continued)

Bioactive metabolite	Source	Biological activity	Reference
Staurosporinone	*Streptomyces* sp.	Antitumor; phycotoxicity	Wu et al (2006)
Staurosporinone	*Streptomyces* sp.	Phycotoxicity	Wu et al (2006)
Streptokordin	*Streptomyces* sp.	Phycotoxicity	Jeong et al (2006)
Sesquiterpene	*Streptomyces* sp.	Unknown	Wu et al (2006)
1,8-Dihydroxy-2-ethyl-3-methylanthraquinone	*Streptomyces* sp.	Antitumor	Huang et al (2006)
Daryamides	*Streptomyces* sp.	Antifungal; anticancer	Asolkar et al (2006)
Himalomycins	*Streptomyces* sp.	Cytotoxic	Maskey et al (2003)
Piericidins	*Streptomyces* sp.	Antitumor	Hayakawa et al (2007)
Streptokordin	*Streptomyces* sp.	Antitumor	Jeong et al (2006)
Streptopyrrolidine	*Streptomyces* sp.	Antimicrobial	Shin et al (2008)
Tirandamycins	*Streptomyces* sp.	Antibacterial	Carlson et al (2009)
Usabamycins	*Streptomyces* sp.	Cytotoxic	Sato et al (2011)
1,4-Dihydroxy-2-(3-hydroxybutyl)-9,10-anthraquinone 9,10-anthrac	*Streptomyces* sp.	Antifungal and anticancer	Ravikumar et al (2012b)
1,8-Dihydroxy-2-ethyl-3-methylanthraquinone	*Streptomyces* sp.	Antitumor	Huang et al (2006)
1-Hydroxy-1-norresistomycin	*Streptomyces chinaensis*	Antibacterial	Gorajana et al (2005) and Kock et al (2005)
2-Allyloxyphenol	*Streptomyces* sp.	Antimicrobial	Arumugam et al (2010)

2.7 IMPORTANCE OF FERMENTATION PROCESS FOR SECONDARY METABOLITES PRODUCTION

Optimization is the prime importance in fermentation conditions (Sanchez & Demain 2002, Martin & Demain 1980, Sujatha et al 2005) since it can boost the production of secondary metabolites to several folds (Sujatha et al 2005, Bode et al 2002, Fourati et al 2005). The synthesis of secondary metabolites begins in the microorganism during the slowdown of growth or completely stopped (Augustine et al 2004, Demain et al 1983, Sanchez & Demain 2002, Doull & Vining 1990).

The enhancement and reduction in the production of secondary metabolites are mainly influenced by means of nutrition conditions, environmental parameters and its cultivation but the capacity of the *Streptomyces* sp. to produce the secondary metabolites is not a predetermined possession of the microbe (Waksman 1961). Therefore the fermentation process parameters collectively with the metabolic competence of the microbe of interest greatly influence bioactive metabolite biosynthesis. Modification in the nature and type of media composition or nutritional requirements profoundly influences the secondary metabolites and its biosynthesis in *Streptomyces* sp. (Abbanat 1999). Moreover the secondary metabolites production is affected with the incubation of the culture broth for a longer time in higher temperature (Chen et al 2008, Higashide 1984). During the slower lag phase of growth of microorganism and the regulatory proteins are synthesized on the basis of new information. The production of the secondary metabolites is quite less and slow in nature during the exponential growth or log phase of the microbe. The growth of the microbe is intensive (Martin & Demain 1980) during the log phase of growth of the microbe.

Moreover, during the production phase of the microbe, when the growth rate is diminished, the dry weight of the secondary metabolites was found to be steady and stable. On the other hand, to achieve an elevated yield of secondary metabolites production, an adequate biomass yield in a short period (e.g., short log phase) is essential (Bibb 2005). It is essential to optimize the nutrient and environmental conditions of the producer strains for accomplish the maximum production of secondary metabolite of interest or antibiotics (Raytapadar & Paul 2001). Changes in the parameters such as aeration (gas flow), agitation system, stirrer speed showed a vast impact in the secondary metabolites production yields of the strains investigated with the 20 fold increase in the productivity was observed by the controlled excess oxygen compared to the optimal fermentation conditions of *Streptomyces* strains (Pfefferle et al 2000).

Process control is frequently employed method to augment the fermentation process and improve the productivity of secondary metabolites to maximum extent (Stafford & Stephanopoulos 2001). There are quite a few number of reports have been available describing the optimization of pH, temperature, regulation of agitation and aeration conditions for the production of bioactive products (Shipanova et al 1995, Slininger Shioya et al 1999, Slininger & Shea-Wilbur 1995). The production of secondary metabolites in *actinomycetes* is greatly influenced by various fermentation parameters such as pH (Atta 2015, Sharon et al 2014, Sujatha et al 2005, Nomila Merlin et al 2013, Singh & Rai 2012), temperature (Uddin et al 2013, Sharon et al 2014, Singh & Rai 2012, Merlin et al 2013, da Silva et al 2012, Sujatha et al 2005), agitation (da Silva et al 2012, Merlin et al 2013, Bode et al 2002), incubation period (Merlin et al 2013, Bashir et al 2012, Sharon et al 2014, Vastrad &

Neelagund 2011), salinity (Sharon et al 2014, Merlin et al 2013, Ripa et al 2009).

RSM set of empirical techniques dedicated to the assessment of association's of active connecting a collection of controlled experimental factors and its exact responses, according to one or more selected criteria (Azanza Teruel et al 1997, Lee & Chen 1997, Silva and Roberto, 2001). RSM is a process development methodology which occupies an important use in the optimization of fermentation process parameters at manufacturing industrial level, among which CCD methodology provides the interaction effects among the variables (Han et al 2008). To achieve the maximum production of secondary metabolites using microorganisms of interest, the optimization of fermentation conditions through experimental factorial design (Fannin et al 1981) and RSM has been successfully applied in lab scale as well as industrial scale (Haltrich et al 1994, Houng et al 1989, Liu et al 1999, Prapulla et al 1992, Ramanamurthy et al 1999, Shirai et al 2001). It was already reported in literature that the production of neomycin was increased in the study of core and interaction effects of factors on the production of neomycin. A 2^n factorial central composite design and response surface methodology were used in the study of production of neomycin (Box et al 1978, Akhnazarova & Kafarov 1982, Yee & Blanch 1993, Mak et al 1995, Khuri & Cornell 1987). Since optimization of medium by the classical method engage in varying one independent variable (nutrient, pH, temperature, etc.) while fixing all others at a fixed level but advantage of RSM in particularly scores in time-consuming and performed for large number of variables with their interaction effects (Adinarayana et al 2003).

To attain a realistic model, a prior understanding of the process and process variables are crucial for achieving an additional realistic model with

enhanced product yields, compact process variability, closer authentication of the output response to nominal, objective requirements and reduced development time and overall costs (Akhnazarova & Kefarov 1982). The drawback of the conventional practice is that time consuming, unreliable and it does not depict the combined effect of all the factors involved in the process. These limitations of a single factor optimisation process factors can be eliminated by means of optimizing all the affecting parameters collectively by statistical experimental design using RSM. RSM estimate the relative consequence of numerous affecting factors even in the presence of multifaceted interactions (Hounsa et al 1996, Jagannadha Rao et al 2000, Dey et al 2001, Hamseveni et al 2001). Since RSM eliminates the disadvantage of conventional classical methods and has established to be reliable and useful for the optimization of the targeted secondary metabolites production with relative outcome of several variables concurrently (Deepak et al 2008, Li et al 2008, Liu & Wang 2007, Sayyad et al 2007).

It was reported earlier that medium resulted in a 3-fold increase in the level of xylanase (27.77 UA/ml) production contrast to the initial preliminary level (8.30 UA/ml) after 120 h of fermentation and optimization of fermentation medium for the biosynthesis of xylanases by *Streptomyces* sp. P12-137 using RSM and CCD (Coman & Bahrim 2011). Similarly, a report was made on *Streptomyces alboflavus* 313 on the production of novel cyclic hexapeptide antibiotic with wider applications, including antimicrobial activity and antitumor agents. The medium explained 2.73-fold increase in the antibiotic production using predicted model built in the experiments of RSM and CCD (Guo et al 2012). Similarly, a literature stated that RSM was used for optimization of fermentation medium for antibacterial agent cyclo (tyr-pro) production by *Streptomyces* sp. A11 by applying the CCD statistical

design. It was observed that the differences of experiment with expectation response value were 2.91%. With the use of the optimized parameters, the studies confirmed an increase in the cyclo (tyr-pro) was 2.5 fold from 20 mg L-1 to 50 mg L^{-1} (Sunaryanto 2012).

2.8 EXTRACTION AND CHARACTERIZATION OF SECONDARY METABOLITES

The fermentation products (antimicrobial compounds) in the culture broth medium was extracted by liquid-liquid extraction method using a solvent was reported by Wardani et al in (2013). The effective separation of bioactive compounds from the culture broth of alkaliphilic *Streptomyces werraensis* is based on choosing a suitable solvent for the extraction. Sanghvi et al in (2014) projected that a crude antimicrobial compound produced in culture was effectively extracted from the culture broth using equal volume of chloroform in ratio of 1:1 (v/v).

A polyene natured antibiotic isolated from the fermentation broth of a *Streptomyces* strain AZ-55 yielded an active metabolite using chloroform (1:1, v/v) at pH 7.0. The active antibiotic metabolite ingredient was further purified by means of thin layer chromatography (Atta et al 2012). Similarly, a report was made in the synthesis of antimicrobial potential of *Streptomyces* sp. JRG-04 from marine origin was effectively extracted twice with equal volume of chloroform and concentrated (Govindarajan et al 2014). Arasu et al in (2009) reported that antimicrobial compound present in the supernatant was extracted using equal volume of different solvents such as hexane, chloroform, ethyl acetate and ether. Among the four extracts used, chloroform extract and ethyl acetate extract showed promising activity against Gram positive and Gram-negative bacteria. Similarly, a report made by Shiyamala devi et al in (2014) reveals that chloroform was found to be the suitable

solvent for antibacterial metabolites from the culture broth medium of *Kocuria* sp. SRS88.

The characterization of the antimicrobial agent was recorded using UV (Ultraviolet-visible spectrophotometry), FTIR (Fourier transform Infrared spectroscopy) for the maximum peak absorption of the bioactive compound. It was found that maximum absorption peak was observed between 200 to 280 nm in UV and υ = 1570 cm^{-1} peak corresponds to primary amine in FTIR was reported by Atta et al in (2012). Atta in (2015) reported chemical structural analysis with UV, FTIR, and GC-MS (Gas chromatography–mass spectrometry) spectral analyses and confirmed that the compound produced by *Streptomyces torulosus*, T-4 is a tunicamycin antibiotic. Similarly, a report made by Atta et al in (2011) for the antimicrobial agent recorded using UV absorption peak at 230 and 270 nm and FTIR spectrum characteristic bands corresponding to 13 peaks for functional groups of antimicrobial agent was made known.

2.9 BIOMEDICAL APPLICATIONS

2.9.1 Antimicrobial Activities

The isolates from twenty different marine samples with diverse biological nature showed antimicrobial activities with variable spectrum. The main promising isolate was *Streptomyces* sp.were phenotypically characterized and identified (Rashad et al 2015). A new *actinomycetes* strain designated *Streptomyces* PAL114, producing antimicrobial compounds isolated from a Saharan soil in Ghardaïa, Algeria showed strongest activities against *Candida albicans* M3 and *Bacillus subtilis* ATCC 6633. The extracted broth of *Streptomyces* PAL114 by dichloromethane showed the antimicrobial activities (Aouiche et al 2014). *Streptomyces* sp. isolated from mangrove sediments of Andaman Island are a potent resource of novel bioactive

compounds. The antagonistic isolates in the preliminary screening showed 15 (36.58%) both antifungal and antibacterial activity, 16 (39%) isolates showed only antibacterial activity, and 10 (24.39%) isolates revealed antifungal activity. Among the 41 antagonistic isolates, nine proved high antimicrobial activity (Baskaran et al 2015). An original marine *Streptomyces coeruleorubidus* BTSS-301 isolated from marine sediment sample near Visakhapatnam coast exhibited broad spectrum of antimicrobial activity. The purified active compound responsible for the activity was identified as N-ethyl-2-(2-(3-hydroxybutyl) phenoxy) acetamide (Kandula & Terli 2013). A new *actinomycete* strain, designated US80 showed antimicrobial activities against Gram-positive and Gram-negative bacteria and fungi isolated from Tunisian oasis soil and the chemical structures of the respective three compounds responsible for antimicrobial activities was irumamycin, X-14952B and 17-hydroxy-venturicidin (Fourati-Ben Fguira et al 2005).

2.9.2 Cytotoxic Activities

A Gram positive, spore forming, filamentous, antagonistic *Streptomyces* sp. AP-123 derived from marine region of Andra Pradesh, India, showed a potent cytotoxic activity against cell lines viz. VERO and HEp2 *in vitro (*Arasu et al 2013). An *actinomycetes* isolated from marine sediment showed cytotoxic activity. The crude extract of the *actinomycetes* isolate exhibited IC_{50} value of 64.5 µg/ml against Hep2 cell line, 250 µg/ml in VERO cell line. The value obtained in the cytotoxicity of the crude extract was very close to the criteria of cytotoxicity activity for the crude extracts established by the American National Cancer Institute is in $IC_{50} < 30$ µg/mL. The GC-MS analysis explained that the active principle present in the extract such as 1, 2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (12.17%), isooctyl phthalate (15.29%) responsible for the activity eluted with the retention time 15.642 and 21.612, respectively (Sudha & Masilamani 2012).

Streptomyces chibaensis obtained from marine sediments of Bay of Bengal showed a potent cytotoxic activity against HMO2 (Gastric adenocarcinoma cells) *in vitro* and also exhibited weak antibacterial activities against Gram-positive and Gram-negative bacteria obtained by solvent extraction followed by the chromatographic purification. The antibiotic responsible for cytotoxicity was found to be resistoflavine (Gorajana et al 2007). The cytotoxicity and antioxidant activity of DMBPO extracted from marine *Streptomyces* sp. VITSVK5 isolated from marine sediment samples from Marakkanam coast of Bay of Bengal, India. DMBPO extract revealed the cytotoxic activity on HEp2 and HepG2 cell lines with the IC_{50} value of 2.8 μg/ml and 8.3 μg/ml, respectively, as evaluated to VERO cell line (22.6 μg/ml). It also showed the hemolytic EC_{50} (concentration at half-maximal effect) value of 288 μg/ml on human erythrocytes (Saurav & Kannabiran 2012). An endophytic *Streptomyces* sp. neau50 showed cytotoxicity against human lung adenocarcinoma cell line A549 with an IC_{50} value of 29.3 μg /ml. The cytotoxicity of the strain was due to the presence of new quinoline derivative; methyl 8-(3-methoxy-3-methylbutyl)-2-methylquinoline-4-carboxylate and the respective structure was explained by extensive spectroscopic analysis (Wang et al 2011).

2.9.3 Antioxidant Activities

A Streptomyces sp. strain MJM 10778 isolated from Hambak Mountain, Korea showed good scavenging activity with the ethyl acetate extract. The IC_{50} values of the strain MJM 10778 extract on DPPH (2,2-Diphenyl-1-picrylhydrazyl), NO (Nitric acid) scavenging and ABTS (2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radicals were identified to be 92.8 μg/ml, 0.02 μg/ml, and 134.9 μg/ml respectively. Moreover, the ethyl acetate extract of the isolate MJM 10778 showed an 81.50% of cell viability at 100 μg/ml (Lee et al 2014). An active strain

of *Streptomyces* sp. from mangrove soil of Visakhapatnam region showed a potent antioxidant activity. Among the four isolates, BC 01 showed a potent antioxidant activity when compared with other isolates. The antioxidant activity was tested by using the standard methods DPPH, FRAP (Ferric antioxidant power) and total antioxidant capacity (Raghava Rao & Raghava Rao 2013). A free radical scavenging prospective of culture filtrate of *Streptomyces* sp. AM-S1 was measured by *in vitro* assays such as ferric reducing power assay, phosphomolybdenum reduction, DPPH and ABTS radical scavenging activities. The results made known that the culture filtrate of *Streptomyces* sp. AM-S1 effectively scavenged DPPH (IC_{50} 90.2 µl/ml) and ABTS (IC_{50} 13.2 µl/ml) radicals in a concentration dependent mode. The ethyl acetate extract showed higher antioxidant activity when compared with the LCF (lyophilized culture filtrate), moreover ethyl acetate extract (1123.4 µmole Fe(II)/mg extract) revealed superior ferric reducing activity than the standard BHA (Butylated hydroxyanisole) (814.4 µmole Fe(II)/mg extract) (Sowndhararajan and Kang 2013). The antioxidant activity of the marine actinobacteria extract was evaluated and the extract exhibited superior scavenging activity apart from NO scavenging activity. The IC_{50} values of marine actinobacteria extract on DPPH radical scavenging activity were found to be 41.09 µg/ml (Karthik et al 2013).

2.10 OBJECTIVE AND SCOPE OF THE PRESENT RESEARCH

The main goal of the present research work is the extraction of secondary metabolites from a novel *Streptomyces cirratus* SRP11 and its potential biological activities. To achieve the goal, the following objectives were framed steps taken for their implementations are foreseen:

- Isolation of *actinomycetes* colonies from marine sediment samples using standard soil serial dilution plate technique.

- Primary screening of potential *actinomycetes* isolates against bacterial and fungal pathogens using cross streak plate technique.
- Identification of potential *actinomycetes* isolate by morphological, physiological, biochemical and molecular techniques.
- Optimization of fermentation condition parameters such as pH, temperature, incubation period, agitation and salinity for secondary metabolites production of *Streptomyces cirratus* SRP11 using RSM and its validation.
- Extraction of extracellular secondary metabolites from *Streptomyces cirratus* SRP11 using liquid - liquid extraction technique.
- Identification and characterization of bioactive extracellular secondary metabolites using UV, FTIR and GC-MS techniques.
- Evaluation of antimicrobial, cytotoxicity and antioxidant activities of crude bioactive secondary metabolites of *Streptomyces cirratus* SRP11.
- Determination of stability of crude secondary metabolites of *Streptomyces cirratus* SRP11 with effect of pH, temperature and shelf life.