

## Screening Biological Activities of a *Streptomyces* Species Isolated from soil of Agumbe, Karnataka, India

Prashith Kekuda TR <sup>1\*</sup>, Shobha KS <sup>1</sup>, Onkarappa R <sup>1</sup>, Goutham SA <sup>1</sup>, Raghavendra HL <sup>2</sup>

<sup>1</sup>Department of Studies and Research in Microbiology, Sahyadri Science College (Autonomous), Shivamogga-577203, Karnataka, India.

<sup>2</sup>Faculty of Medical Sciences, Wollega University, Post Box No: 395, Nekemte, Ethiopia.

### Abstract

The present study was undertaken to screen biological activities namely antimicrobial, insecticidal, pancreatic lipase inhibitory and cytotoxic potential of crude extract of a *Streptomyces* species isolated from soil of Agumbe, Karnataka, India. Primary and Secondary screening for antimicrobial activity was determined by Cross streak method and Agar well diffusion method respectively. Insecticidal efficacy of extract was tested against 3<sup>rd</sup> instar larvae of *Aedes aegypti*. Pancreatic lipase inhibitory activity was determined against chicken pancreatic lipase. The brine shrimp lethality test was conducted to determine cytotoxic nature of crude extract. The actinomycete isolate was identified as a species of the genus *Streptomyces* on the basis of microscopic, cultural and biochemical characteristics. In both primary and secondary screening, Gram positive bacteria were found most sensitive. *Candida albicans* has shown high sensitivity when compared to *Cryptococcus neoformans*. The mortality of the 3<sup>rd</sup> instar larvae of *A. aegypti* was found to be concentration dependent and the mortality was 100% at extract concentration 1.5mg/ml and higher. The inhibition of lipase was found to be dose dependent and an inhibition of over 50% was observed at extract concentration 50mg/ml. In cytotoxic assay, the degree of lethality of extract was directly proportional to the concentration. The LC<sub>50</sub> of extract was found to be 21.95µg/ml and hence the extract is toxic. The soils of Western ghats are rich sources for microorganisms with potent biological activities and screening programs are to be conducted to reveal the presence of pharmacologically active actinomycete isolates. Further studies are needed to be carried out to isolate and characterize the active principles present in the crude extract and determine their biological activity.

\*Corresponding author, Mailing address:

**Prashith Kekuda. T.R**

Email: [p.kekuda@gmail.com](mailto:p.kekuda@gmail.com)

### Key words:

*Streptomyces*, Western ghats, Agar well diffusion, Pancreatic lipase, Brine shrimp, Larvicidal activity

### How to Cite this Paper:

**Prashith Kekuda T R\***, **Shobha K S**, **Onkarappa R**, **Goutham S A**, **Raghavendra H L** "Screening Biological Activities of a *Streptomyces* Species Isolated from soil of Agumbe, Karnataka, India" Int. J. Drug Dev. & Res., July-September 2012, 4(3): 104-114

### Copyright © 2012 IJDDR, Prashith Kekuda T R et al.

This is an open access paper distributed under the copyright agreement with Serials Publication, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### Article History:-----

**Date of Submission: 13-05-2011**

**Date of Acceptance: 23-06-2011**

**Conflict of Interest: NIL**

**Source of Support: NONE**

### INTRODUCTION

Microbes produce some of the most important medicines. They are the sources of drugs for bacterial and fungal infections, transplant rejection, high cholesterol and others. Microbial natural products

are notable not only for their potent therapeutic activities but also for the fact that they frequently possess the desirable pharmacokinetic properties required for clinical development [1]. Actinomycetes are the most widely distributed groups of microbes in nature. These are Gram positive bacteria frequently filamentous and sporulating organisms with DNA rich in G+C from 57-75%. They have showed marked chemical and morphological diversity but form a distinct evolutionary line of organisms. Majority of the Actinomycetes are free living, saprophytic bacteria found widely distributed in soil, water and colonizing plants. These organisms are one of the major groups of soil population. Soil Actinomycetes are prokaryotes with extremely various metabolic possibilities. These are prolific producers of various bioactive compounds, and have provided over two third of naturally occurring antibiotics discovered and continue to be major source of novel and useful compounds such as antibiotics, enzymes, pigments, immunomodulators etc [2-6]. The present study was undertaken to screen biological activities namely antimicrobial, insecticidal, pancreatic lipase inhibitory and cytotoxic potential of crude extract of a *Streptomyces* species isolated from soil of Agumbe, Karnataka, India.

## MATERIALS AND METHODS

### Isolation and Identification of Actinomycete Isolate

The actinomycete species of this study was isolated from a soil sample of Agumbe, Karnataka, India. The soil sample was collected from a depth of 5cm in sterile pouch. The soil sample was dried, serially diluted and plated on Starch casein agar (HiMedia, Mumbai). The inoculated plates were incubated at 30±2°C for a week aerobically. The isolate was sub-cultured on fresh medium and identified to genus level based on colony morphology (colony color, distinctive reverse color of vegetative mycelium, production of diffusible pigment, and surface colony

appearance), microscopy (cover slip method), staining (Gram's and Acid fast staining) and biochemical reactions (Starch hydrolysis, gelatin liquefaction, casein hydrolysis, sugar fermentation, hydrogen sulfide production) [7-10]. The isolate was maintained on Starch casein agar slant.

### Primary Screening for Antimicrobial Activity of the Isolate

Preliminary screening for inhibitory metabolite producing ability of the isolate was tested by Cross-streak method against two Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), two Gram negative bacteria (*Proteus vulgaris* and *Pseudomonas aeruginosa*) and two human pathogenic fungi (*Candida albicans* and *Cryptococcus neoformans*). The isolate was inoculated as a single streak in the centre of the petridish containing media and incubated at 30±2°C for 3-4 days to permit growth and antibiotic production. Later the test bacteria and fungi were inoculated by streaking perpendicular to the growth of isolate. The plates were incubated for 24-48 hours at 37°C in case of bacteria, 72 hours at room temperature in case of fungi. After incubation, inhibition of test bacteria and fungi around the growth of isolate was taken as positive for inhibitory activity [11,12].

### Fermentation and Extraction

Loop-full culture of the isolate was inoculated into bottles containing sterile Starch casein broth medium aseptically. The inoculated bottles were incubated at 28°C for 12 days aerobically. After incubation, the contents of the bottles were filtered through sterile Whatmann filter paper No. 1 aseptically. The filtered broths were concentrated to pasty mass by keeping in hot air oven at 40°C [13]. The crude extract was subjected to antimicrobial, insecticidal, pancreatic lipase inhibitory and cytotoxic activity.

### UV/Visible absorption of extract

The UV-Visible absorption spectra of the extract was tested by using SHIMADZU UV-2550 spectrophotometer at 200-400nm to determine the  $\lambda$  maximum of the band [14].

### Antibacterial Activity of Extract

The antibacterial efficacy of extract was tested by Agar-well-diffusion method [15] against two Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram negative bacteria (*Proteus vulgaris* and *Pseudomonas aeruginosa*). Briefly, 24 hours old Nutrient broth (HiMedia, Mumbai) cultures of test bacteria were swabbed uniformly on solidified sterile Nutrient agar (HiMedia, Mumbai) plates using sterile cotton swab. Then, wells of 6mm diameter were bored in the inoculated plates with the help of sterile cork borer and the extract (20mg/ml of sterile water) and Standard (Chloramphenicol, 1mg/ml of sterile water) were added separately into respectively labeled wells. The inoculated plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition formed around the well was measured. The experiment was carried in triplicates to get average reading.

### Antifungal Activity of Extract

The antifungal efficacy of extract was tested by Agar-well-diffusion method against two human pathogenic fungi namely *Candida albicans* and *Cryptococcus neoformans* [15]. Briefly, 48 hours old Sabouraud dextrose broth (HiMedia, Mumbai) cultures of test fungi were swabbed uniformly on solidified sterile Sabouraud dextrose agar (HiMedia, Mumbai) plates using sterile cotton swab. Then, wells of 6mm diameter were bored in the inoculated plates with the help of sterile cork borer and the extract (20mg/ml of sterile water) and Standard (Amphotericin B, 1mg/ml of sterile water) were added separately into respectively labeled wells. The inoculated plates were

incubated at 37°C for 24 hours in upright position and the zone of inhibition formed around the well was measured with a ruler. The experiment was carried in triplicates to get average reading.

### Insecticidal Activity of Extract

The insecticidal efficacy of different concentrations of extract was tested against 3<sup>rd</sup> instar larvae of *Aedes aegypti*. Twenty larvae were placed separately into beakers containing different concentrations of extract. The larvicidal effect of the extract was determined by counting the number of dead larvae after 24 hours. Dead larvae were identified when they failed to move after probing with a needle in siphon or cervical region. The test was repeated thrice and the percentage of larval mortality for each concentration of extract was calculated [16].

### Pancreatic Lipase Inhibitory Activity of Extract

The inhibitory activity of crude extract was tested against lipase extracted from the chicken pancreas. The activity of lipase was determined by incubating an emulsion containing 8ml of olive oil, 0.4ml of phosphate buffer and 1ml of chicken pancreatic lipase for an hour in rotary shaker, followed by stopping the reaction by addition of 1.5ml of a mixture solution containing acetone and 95% ethanol (1:1). The liberated fatty acids were determined by titrating the solution against 0.02M NaOH using phenolphthalein as an indicator. Lipase inhibitory activity of different concentrations of extract was tested by mixing 100 $\mu$ l of each concentration of extract, 8ml of oil emulsion and 1ml of chicken pancreatic lipase followed by incubation of 60 minutes. The reaction was stopped by adding 1.5 ml of a mixture solution containing acetone and 95% ethanol (1:1). The liberated fatty acids were determined by titrating the solution against 0.02M NaOH. The inhibition of lipase activity (%) was calculated using the formula: Lipase inhibition = A-

$B/A \times 100$ , where A is lipase activity, B is activity of lipase when incubated with the extract [17].

### Cytotoxic Activity of Extract

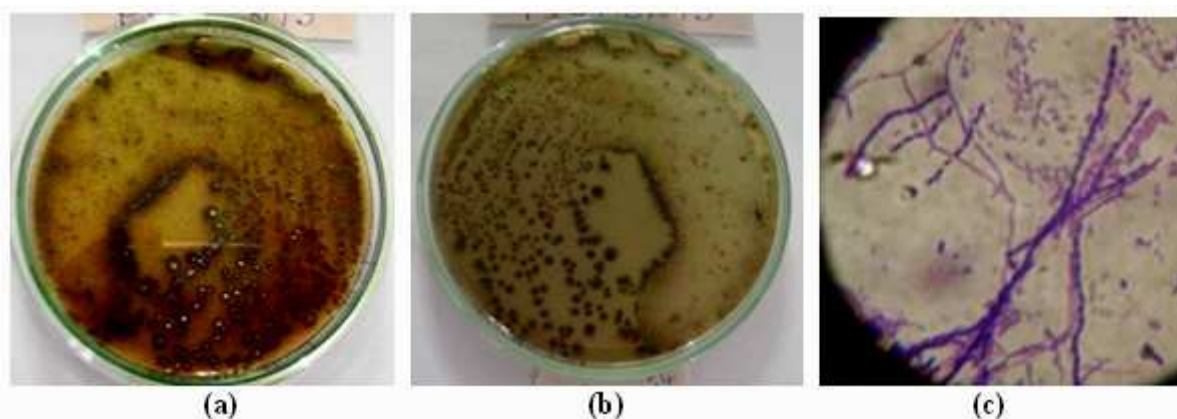
The brine shrimp lethality test was conducted according to the method of Kekuda *et al.* [18] to determine cytotoxic nature of crude extract. Brine shrimp *Artemia* nauplii eggs (Nihon Animal Pharmaceutical Inc., Tokyo, Japan) were hatched in a container filled with air-bubbled artificial sea water which was prepared with 10g of a commercial salt mixture (GEX Inc., Osaka, Japan) and 500 ml of distilled water. After 36-48 hours, the phototropic shrimps were collected by pipette for bioassay. The different concentrations of extract (10-1000µg/ml) were tested in vials containing 5ml of brine and 25 shrimp in each of three replicates. The vials were

incubated at 25°C and surviving shrimps were counted after 24 hours. LC<sub>50</sub> value was calculated by regression analysis.

## RESULTS

### Identification of Actinomycete Isolate

The actinomycete isolate was identified as a species of the genus *Streptomyces* on the basis of microscopic, cultural and biochemical characteristics. The colony was brown in color with white centre. Spores were arranged in long chains (Figure 1). The isolate was Gram positive and non-acid fast. The isolate was found to produce starch hydrolysis and gelatin liquefaction and utilized glycerol. Casein was not hydrolyzed, sucrose and maltose were not utilized and hydrogen sulfide was not produced. The isolate produced brown pigment.



**Figure 1:** a) front view b) reverse view c) Spore arrangement of *Streptomyces* species

### UV-Visible Absorption Maxima of Solvent Extract

The absorption maxima ( $\lambda_{max}$ ) of the extract of *Streptomyces* isolate was found to be 283.5nm.

### Primary Screening for Antimicrobial Activity of The Isolate

The result of screening for antimicrobial activity of the isolate by cross-streak method is depicted in Table 1. Result was recorded as the absence of growth of test microbes around the growth of isolate and was taken as '+'. All the tested bacteria and fungi were

shown to be susceptible as their growth around the isolate was inhibited.

**Table 1:** Primary screening of *Streptomyces* species for antimicrobial activity.

Test bacteria	Result
<i>S. aureus</i>	++
<i>B. subtilis</i>	+++
<i>P. aeruginosa</i>	+
<i>P. vulgaris</i>	+
<i>C. albicans</i>	++
<i>C. neoformans</i>	+

### Antibacterial Activity of Extract

The antibacterial activity of extract was investigated against four bacteria by agar well diffusion method. Results were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of bacterial growth and is reported as positive and the absence of zone as negative. It was found that the extract caused marked inhibition of Gram positive bacteria when compared to Gram negative bacteria. Among bacteria, *B. subtilis* was inhibited to high extent followed by *S. aureus*, *P. aeruginosa* and *P. vulgaris*. Inhibition caused by standard antibiotic was higher than that of extract (Table 2)

**Table 2:** Antibacterial activity of extract of *Streptomyces* species.

Test bacteria	Zone of inhibition in mm	
	Extract	Standard
<i>S. aureus</i>	19	28
<i>B. subtilis</i>	22	29
<i>P. aeruginosa</i>	16	23
<i>P. vulgaris</i>	14	21

### Antifungal Activity of Extract

The antifungal activity of extract was tested by agar well diffusion method. Among fungi, *C. albicans* was inhibited to higher extent when compared to *C. neoformans*. Here also, inhibition caused by standard antibiotic was higher than that of extract (Table 3).

**Table 3:** Antifungal activity of extract of *Streptomyces* species.

Test fungi	Zone of inhibition in mm	
	Extract	Standard
<i>C. albicans</i>	15	21
<i>C. neoformans</i>	13	19

### Insecticidal Activity of Extract

The insecticidal efficacy of different concentrations of extract was evaluated against 3<sup>rd</sup> instar larvae of *A. aegypti*. The mortality of the 3<sup>rd</sup> instar larvae of *A. aegypti* was

found to be concentration dependent. The mortality was 100% at extract concentration 1.5mg/ml and higher. There was no mortality of larvae in control beakers i.e., beakers containing no extract (Table 4).

**Table 4:** Insecticidal activity of extract.

Concentration (mg/ml)	Mortality of larvae (%)
0.0	0.0
0.5	20.0
1.0	65.0
1.5	100.0
2.0	100.0
2.5	100.0

### Pancreatic Lipase Inhibitory Activity of Extract

Inhibitory activity of different concentrations of extract was tested against chicken pancreatic lipase using olive oil as the substrate. It was observed that the activity of lipase was affected when incubated with the extract. The inhibition of enzyme was found to be dose dependent i.e., inhibition of enzyme was increased on increasing the concentration of extract. An inhibition of >50% was observed at extract concentration 50mg/ml whereas lipase inhibition was not observed at extract concentration 2.5mg/ml (Table 5).

**Table 5:** Pancreatic lipase inhibitory activity of extract.

Concentration (mg/ml)	Inhibition of lipase activity (%)
0.0	0.0
2.5	0.0
5.0	03.86
10.0	13.14
25.0	29.64
50.0	51.69

### Cytotoxic Activity of Extract (Brine Shrimp Lethality)

The cytotoxic activity of different concentrations of extract, in terms of mortality of brine shrimps, is presented in Table 6. The degree of lethality of extract was directly proportional to the concentration

of the extract i.e., higher the concentration, mortality was higher. Highest lethal effect was observed at 1000µg/ml extract concentration at which the mortality was 100%. LC<sub>50</sub> values greater than 1000µg/ml were considered inactive (non-toxic). The LC<sub>50</sub> of extract was found to be 21.95µg/ml and hence the extract is toxic. Lethal effect was lesser (33%) in case of 10µg/ml extract concentration.

**Table 6:** Brine shrimp lethality of extract.

Concentration (µg/ml)	Mortality of shrimps (%)
0.0	0.0
10.0	33.0
100.0	80.0
1000.0	100.0

## DISCUSSION

Actinomycetes are the abundant organisms that form thread-like filaments in the soil. They form a large group of Gram positive bacteria that grow as hyphae like fungi responsible for the characteristically “earthy” smell of soil. Actinomycetes are belonging to the order Actinomycetales, characterized by the formation of substrate and aerial mycelium on solid media, presence of spores and a high GC content of the DNA. The majority of soil actinomycetes form a very important class of bacteria since they produce numerous natural products such as antibiotics and enzymes. More than 50% of the known natural antibiotics produced are from actinomycetes. Actinomycetes involve in important ecological processes in a wide range of habitats such as degradation of plant and animal in soils, degradation of high molecular weight compounds like hydrocarbons in the polluted soil, biological control of soil environments by nitrogen fixation and production of various secondary metabolites [19-24].

Actinomycetes are very well known and successfully exploited as a source of secondary metabolites. Out of the approximately 10,000 known antibiotics, 45-55 % are produced by streptomycetes. Various

antimicrobial substances from actinomycetes have been isolated and characterized including aminoglycosides, anthracyclines, glycopeptides, beta-lactams, macrolides, nucleosides, peptides, polyenes, polyester, polyketides, actinomycins and tetracyclines. Most of the antibiotics are extracellular metabolites which are normally secreted in culture media and have been used as herbicides, anticancer agents, drugs, immunoregulators and antiparasitic drugs [25]. A number of literatures are available on antibacterial and antifungal activity of actinomycete metabolites. A polypeptide antibiotic Chandramycin, isolated from *Streptomyces lydicus*, showed activity against *Clostridium perfringens* and *Clostridium septicum* [26]. A lactone group of antibiotics Okilactomycin from *Streptomyces griseoflavus* strain showed weak activity against *S. aureus* and *S. pyogenes* [27]. Frigocyclinone, a new angucyclinone antibiotic isolated from *Streptomyces griseus* NTK 97 exhibited antibacterial activities against Gram positive bacteria [28]. An octaketide antibiotic Fogacin isolated from *Streptomyces* strain TU-6319 showed moderate activity against *B. subtilis* [29]. P3-1 partially purified antibacterial substance, from *Streptomyces* sp. No 87 was found to possess antibacterial activity [30]. Antifungal activity of actinomycetes isolated from cultivated field soil, garden soil, compost, decaying organic matter and stored agricultural products was evaluated by Jain and Jain [31]. Out of 287 isolates, a total of 166 isolates were found antagonistic to *C. albicans*, while 164, 134 and 132 actinomycetes showed antagonistic properties against *A. niger*, *M. gypseum* and *Trichophyton* sp., respectively. Jain and Jain [32] determined antifungal activity of heptane group of antibiotics from *Streptomyces sampsonii* against four fungi namely *Candida albicans*, *Aspergillus niger*, *Microsporium gypseum* and *Trichophyton* sp. In our study, it was found that the extract caused marked inhibition of test bacteria and fungi.

Actinomycetes play an important role in the biological control of insects through the production of insecticidally active compounds. In a study involving 41 actinomycete soil isolates to evaluate their efficiency against third larval instar of the house fly *Musca domestica*, *Streptomyces* and *Streptoverticillium* were recorded as the most effective genera [33]. A macrotetrolide antibiotic recovered from the acetone extract of *Streptomyces aureus*, isolated from Saitama Prefecture, Japan, exhibited significant insecticidal activity against *Callosobruchus chinensis* [34]. The ethyl acetate extract of *Streptomyces* sp. KN-0647 isolated from a forest soil sample of Dali Cangshan mountain, Yunnan Province, China displayed growth inhibition on the pathogenetic insects *Spodoptera exigua*, *Dendrolimus punctatus*, *Plutella xylostella*, *Aphis glycines* and *Culex pipiens*. The active compound was isolated and identified as quinomycin A [35]. An actinomycete, *Streptomyces* sp. 173, isolated from seawater and sediments, was found to have strong insecticidal activity against both brine shrimp and *H. armigera*, similar to that of avermectin B<sub>1</sub> [36]. A new member of the tartrolone series of macrodiolides, tartrolone C, was isolated from a *Streptomyces* species on the basis of its insecticidal activity [37]. In a previous study, we have reported larvicidal effect of two *Streptomyces* species isolated from the soil of Agumbe, Karnataka against second instar larvae of *Aedes aegypti* [16]. In this study, the insecticidal efficacy of different concentrations of extract was evaluated against 3<sup>rd</sup> instar larvae of *A. aegypti* and it was found that the extract caused mortality of larvae in a dose dependent manner.

One of the most important strategies in the treatment of obesity includes the development of inhibitors of nutrient digestion and absorption, in an attempt to reduce the energy intake through gastrointestinal mechanisms, without altering any central mechanisms [38,39]. Pancreatic lipase inhibition is one of the most widely studied mechanisms used to

determine the potential efficacy of natural products as antiobesity agents. Orlistat, one of the two clinically approved drugs for obesity treatment, has been shown to act by inhibiting pancreatic lipase. Although it is one of the best-selling drugs worldwide, it has certain side effects such as oily stools, oily spotting, and flatulence, among others. The success of orlistat has prompted research for the identification of inhibitors that lack some of these side effects. At present, the potential of natural products for the treatment of obesity is still largely unexplored and might be an excellent alternative strategy for the development of safe and effective antiobesity drugs [40-42]. Naturally occurring compounds present an exciting opportunity for the discovery of newer anti-obesity agents. In a previous study, we have investigated pancreatic lipase inhibitory activity of butanol extract of a *Streptomyces* species isolated from soil of Agumbe Karnataka. The inhibition of enzyme was found to be dose dependent [17]. A marked dose dependent inhibition of chicken pancreatic lipase by extract was observed in this study.

The brine shrimp lethality assay was proposed by Michael *et al.* [43], and later developed by Vanhaecke *et al.* [44], and Sleet and Brendel [45]. It is based on the ability to kill laboratory-cultured *Artemia* nauplii brine shrimp. It is considered to be very useful in determining various biological activities such as cytotoxic, phototoxic, pesticidal, trypanocidal, enzyme inhibition, and ion regulation activities [46-51]. The assay represents a rapid, inexpensive and simple bioassay for testing bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties. It is considered as useful tool for preliminary assessment of toxicity. This method needs no special equipment and no aseptic technique. It utilizes a large number of organisms for validation, relatively small amount of sample and does not require animal serum as needed for other methods of cytotoxicity testing [52-54]. A number of

studies have been carried out on cytotoxic nature of actinomycete extracts and purified metabolites on brine shrimp. Choudury *et al.* [55] showed cytotoxic activity of a brown antibiotic pigment Di- (2-ethyl hexyl)-phthalate isolated from an Actinomycetes strain. The chloroform extract as well as the isolated antibiotic showed marked cytotoxic effect against brine shrimp. From ethyl acetate extract of a *Streptomyces* strain, Sultan *et al.* [56] isolated three active metabolites from and tested their toxicity against brine shrimp. The metabolites as well as extract were found to cause a marked lethal effect on brine shrimp. Safaeian *et al.* [57] showed marked cytotoxic effect of actinomycetes isolated from Persian gulf on two *Artemia* species namely *A. urmiana* and *A. franciscana*. Manivasagan *et al.* [58] tested lethal effect of an isolated fraction BC I from the solvent extract of *Streptomyces* strain PM-32 against brine shrimp. The fraction showed strong cytotoxicity against brine shrimp. Ripa *et al.* [59] showed potent lethal effect of ethyl acetate extract and a purified compound of *Streptomyces rajshahiensis* against brine shrimp. Two compounds namely sannastatin and vicanistatin, isolated from the cultures of *Streptomyces sannanensis*, displayed significant growth inhibitory activity against brine shrimp larvae [60]. In a previous study, we have determined cytotoxic activity of extract of a *Streptomyces* species recovered from the soil of Agumbe, Karnataka. It was observed that the mortality of brine shrimp larvae by the extract was concentration dependent. Mortality of larvae was 100% at extract concentration 1000µg/ml and the LC<sub>50</sub> of solvent extract was found to be 42.11µg/ml [17]. In this study also, the extract caused dose dependent lethal effect on brine shrimp and thus the extract is toxic.

## CONCLUSION

The genus *Streptomyces* is the largest producer of bioactive compounds and it remains important to

discover new leader compounds from Streptomyces for drug development. The soils of Western ghats are rich sources for microorganisms with potent biological activities and screening programs are to be conducted to reveal the presence of pharmacologically active actinomycete isolates. In this context, the present study highlighted the antimicrobial, insecticidal, pancreatic lipase inhibitory and cytotoxic potential of crude extract of a *Streptomyces* species isolated from the western ghat area of Agumbe, Karnataka. The metabolites of actinomycetes from western ghat area of Karnataka may prove to be useful. Further studies are needed to be carried out to isolate and characterize the active principles present in the crude extract and determine their biological activity.

## REFERENCES

- 1) Farnet CM, Zazopoulos E. Improving drug discovery from microorganisms. In Natural products: Drug discovery and Therapeutic medicine, Edt by Zhang L, Demain AL. Humana press inc. Totowa, NJ, 2005; 95.
- 2) Lo CW, Lai NS, Cheah HY, Wong NKI and Ho CC. *Actinomycetes* isolated from Soil samples from the Crocker Range Sabah. ASEAN Review of Biodiversity and Environmental Conservation 2002; 9:1-7.
- 3) Goodfellow M and O'Donell. Search and discovery of significant Actinomycetes in microbial products. New approach. 44<sup>th</sup> symposium of the society of General Microbiology ed. Baumberg, Hunter, S and Rhodes, M. Cambridge U.K. Cambridge University Press, 1989, pp: 343-383.
- 4) Moncheva P, Tishkov S, Dimitrova N, Chipeva V, Antonova-Nikolova S and Bogatzzevska N. Characteristics of soil *Actinomycetes* from Antarctica. Journal of Culture Collections. 2000-2002; 3: 3-14.
- 5) Berdy J. The discovery of new bioactive microbial metabolites: screening and identification. In bioactive microbial metabolites, progress in



- industrial Microbiology, vol 27 (eds. M.E. Bushell and U.G. Amsterdam), Elsevier, 1989, pp 3-25.
- 6) Huck TA, Porter N and Bushell ME. Positive selection of antibiotic producing soil Actinomycetes, J. Gen Microbiol 1991; 137: 2321-2329.
  - 7) Dharwadkar A, Gupta V, Pant A. Bioprospecting the *lat* gene in soil samples. J.Biosci., 2003; 25(5): 597-604.
  - 8) Dhanashekaran D, Rajakumar G, Sivamani P, Selvamani S, Panneerselvam A, Thajuddin N. Screening of salt pans actinomycetes for antibacterial agents. Int. J. Microb., 2005; 1(2): 143-158.
  - 9) Augustine SK, Bhavsar SP, Baserisalehi M, Kapadnis BP. Isolation, Characterization and Optimization of an actinomycete of soil origin. Ind.J.Exp.Biol., 2004; 42: 928-932
  - 10) Williams TS, Sharpe EM, Holt GJ. (eds). Bergey's Manual of Systemic Bacteriology. Williams & Wilkins. Baltimore, Maryland. 1923; 2451-2508.
  - 11) Haque SK, Sen SK, Pal SC. Antimicrobial spectra and toxicity of antibiotics From *Streptomyces antibioticus* sr 15-4. Indian Journal of Microbiology 1996; 36: 113-114.
  - 12) Nanjwade B, Chandrashekhara S, Ali M, Goudanavar PS and Manvi FV. Isolation and Morphological Characterization of Antibiotic Producing Actinomycetes. Tropical Journal of Pharmaceutical Research 2010; 9 (3): 231-236.
  - 13) Gautham SA, Shobha KS, Onkarappa R and Kekuda TRP. Isolation, characterization and antimicrobial potential of *Streptomyces* species from Western ghats of Karnataka, India. Research Journal of Pharmacy and Technology 2012; 5(2): 233-238.
  - 14) Sahin N and Ugur A. Investigation of the Antimicrobial activity of some *Streptomyces* isolates. Turk.J.Biol 2003; 27: 79-84.
  - 15) Vinayaka KS, Swarnalatha SP, Preethi HR, Surabhi KS, Kekuda TRP and Sudharshan SJ. Studies on *In vitro* Antioxidant, Antibacterial and Insecticidal Activity of Methanolic Extract of *Abrus pulchellus* Wall (Fabaceae). African Journal of Basic and Applied Sciences. 2009; 1 (5-6): 110-116.
  - 16) Kekuda TRP, Shobha KS, Onkarappa R. Potent insecticidal activity of two *Streptomyces* species isolated from the soils of the Western ghats of Agumbe, Karnataka. Journal of Natural Pharmaceuticals 2010; 1(1): 30-32.
  - 17) Kekuda TRP, Shobha KS, Onkarappa R. Pancreatic lipase inhibitory and Cytotoxic potential of a *Streptomyces* species isolated from Western ghat soil, Agumbe, Karnataka, India. International Journal of Pharmaceutical & Biological Archives 2011; 2(3):932-937.
  - 18) Kekuda TRP, Raghavendra HL, Surabhi KS, Preethi HR and Swarnalatha SP. Cytotoxic activity of methanol extract of *Abrus pulchellus* Wall (Fabaceae) leaves. Biomedicine 2010; 30(3): 377-379.
  - 19) Sprusansky O, Stirrett K, Skinner D, Denota C, Westpheling J. The *bkdR* gene of *Streptomyces coelicolor* is required for morphogenesis and antibiotic production and encodes a transcriptional regulator of a branched-chain amino acid dehydrogenase complex. Journal of Bacteriology 2005; 187: 664-671.
  - 20) Lechevalier HA, Lechevalier MP. Introduction to the order *Actinomycetales*. In Star MP, Stolp H, Truper HG, Balows A, Schlegel HG (eds.), The Prokaryotes. Springer Verlag AG, Berlin, 1981, 1915-1922.
  - 21) Edwards C. Isolation, properties and potential applications of thermophilic actinomycetes. Appl. Biochem.Biotechnol. 1993; 42:161-179.
  - 22) Berdy J. New ways to obtain antibiotics. Chin. J. Antibiotics 1984; 7: 272-290.
  - 23) Miyadoh, S. Research on antibiotic screening in Japan over the last decade: a producing microorganisms approach. Actinomycetologica 1993; 7: 100-106.
  - 24) Suzuki K, Nagai K, Shimizu Y, Suzuki Y. Search for Actinomycetes in screening for new bioactive compounds. Actinomycetologica 1994; 8(2): 122-127.
  - 25) Kekuda TRP, Shobha KS, Onkarappa R. Fascinating diversity and potent biological activities of Actinomycete metabolites. Journal of Pharmacy Research 2010; 3(2): 250-256.
  - 26) Singh SK, Gurusiddaiah S. Production, purification and characterization of Chandramycin, a

- polypeptide antibiotic from *Streptomyces lydicus*. Antimicrobial agents and Chemotherapy 1984; 26(3): 394-400.
- 27) Imai H, Suzuki K, Morioka M, Numasaki Y, Kadota S, Nagai K, Sato T. Okilactomycin, a novel antibiotic produced by a *Streptomyces* species I. Taxonomy, Fermentation, Isolation and Characterization. J. Antibiot. 1987; 60(11): 1475-1482.
- 28) Bruntner C, Binder T, Pathom-aree W, Goodfellow M, Bull AT, Potterat O, Puder C, Horer S, Schmid A, Bolek W, Wagner K, Mihm G, Fiedler H. Frigocyclinone, a novel Angucyclinone antibiotic produced by a *Streptomyces griseus* strain from Antarctica. J. Antibiot. 2005; 58(5): 346-349.
- 29) Radzom M, Zeeck A, Antal N, Fiedler H. Fogacin, a novel cyclic Octaketide produced by *Streptomyces* strain Tu6319. J. Antibiot. 2006; 59(5): 315-317.
- 30) Charoensopharat K, Thummbenjapone P, Sirithorn P, Thammasirirak S. Antibacterial substance produced by *Streptomyces* sp. No 87. Afr. J. Biotech. 2008; 7(9): 1362-1368.
- 31) Jain PK and Jain PC. Antifungal activity of some actinomycetes isolated from various habitats. Hindustan Antibiot Bull. 2003; 45-46(1-4): 5-10.
- 32) Jain PK and Jain PC. Isolation, characterization and antifungal activity of *Streptomyces sampsonii* GS 1322. Indian Journal of Experimental Biology 2007; 45: 203-206.
- 33) Ghazal SA, Bream AS, Abdel-Aziz ZK, Ibrahim SY. Preliminary studies on insecticidal activities of actinomycete strains propagated on solid and broth media using *Musca domestica* (Diptera: Muscidae). Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet 2001; 66(2b): 559-70.
- 34) Oishi H, Sugawa T, Okutomi T, Suzuki K, Hayashi T, Sawada M, Ando K. insecticidal activity of Macrotetrolide antibiotics. J. Antibiot. 1970; XXIII (2): 105-106.
- 35) Liu H, Qin S, Wang Y, Li W, Zhang J. Insecticidal action of Quinomycin A from *Streptomyces* sp. KN-0647, isolated from a forest soil. World J. Microbiol. Biotech. 2008; 24(10): 2243-2248.
- 36) Xiong L, Li J, Kong F. *Streptomyces* sp. 173, an insecticidal micro-organism from marine. Letters in Applied Microbiology 2004; 38(1): 32-37.
- 37) Lewer P, Chapin EL, Graupner PR, Gilbert JR, Peacock C. Tartrolone C: A novel insecticidal macrodiolide produced by *Streptomyces* sp. CP1130. J. Nat. Prod. 2003; 66(1): 143-145.
- 38) Strader CD, Hwa JJ, Van Heek M, Parker EM. Novel molecular targets for the treatment of obesity. Drug Discov Today 1998; 3: 250-256.
- 39) Shi Y, Burn P. Lipid metabolic enzymes: emerging drug targets for the treatment of obesity. Nat Rev Drug Discov. 2004; 3: 695-710.
- 40) Thomson ABR, De Pover A, Keelan M, Jarocka-Cyrta E, Clandinin MT. Inhibition of lipid absorption as an approach to the treatment of obesity. In: Rubin B, Dennis EA (eds). *Methods in Enzymology*. Academic Press: San Diego, CA, 1997; 3-41.
- 41) Birari RB, Bhutani KK. Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discov Today 2007; 12: 879-889.
- 42) Bhutani KK, Birari R, Kapat K. Potential antiobesity and lipid lowering natural products: a review. Nat Prod Commun. 2007; 2: 331-348.
- 43) Michael AS, Thompson CG, Abramovitz M. *Artemia salina* as a test organism for a bioassay. Science 1956; 123: 464.
- 44) Vanhaecke P, Persoone G, Claus C, Sorgeloos P. Proposal for a short-term toxicity test with *Artemia nauplii*. Ecotoxicol Env Safety 1981; 5: 382-387.
- 45) Sleet RB, Brendel K. Improved methods for harvesting and counting synchronous populations of *Artemia nauplii* for use in developmental toxicology. Ecotoxicol Env Safety 1983; 7: 435-446.
- 46) Jerry L, McLaughlin, Lingling Rogers L. The use of biological assays to evaluate botanicals. Drug Information Jour. 1998; 32: 513-524.
- 47) Anderson JE, Goetz CM, McLaughlin JL, Suffness M. A blind comparison of simple bench-top bioassays and human tumor cell cytotoxicities as antitumor prescreens. Phytochem Anal. 1991; 2: 107-111.
- 48) Fatope MO, Ibrahim H, Takeda Y. Screening of higher plants reputed as pesticides using the brine shrimp lethality assay. Int J Pharmacog. 1991; 31: 250-254.

- 49) Solis PN, Wright CW, Anderson MM, Gupta MP, Phillipson JD. A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). *Planta Med.* 1993; 59: 250–252.
- 50) Zani CL, Chaves PPG, Queiroz R, Oliveira AB, de Cardoso JE, Anjos AMG, Grandi TSM, Brine shrimp lethality assay as a prescreening system for anti-*Trypanosoma cruzi* activity. *Phytomed.* 1995; 2: 47–50.
- 51) Ozala T, Vuorela P, Kivrinta J, Vuorela H, Hiltunen R. A bioassay using *Artemia salina* for detecting phototoxicity of plant coumarins. *Planta Med.* 1999; 65: 715–718.
- 52) Hossain AM, Ferdous T, Salehuddin SM and Das AK. *In vitro* cytotoxicity (LC<sub>50</sub>) of extracts obtained from the seeds of *Zea mays*. *Asian J Food Agro-Industry* 2009; 2(3): 336–34.
- 53) McLaughlin JL. Methods in plant biochemistry. In; Hostettmann K., (Ed) Assay for bioactivity. Academic press. London, 1991; 1–32.
- 54) Luis CJ, Zairo LH, Pillar P and Naira DG. A comparison between two brine shrimp assay to detect *in vitro* cytotoxicity in marine natural products. *BMC Biotechnol.* 2002; 2(17): 1–5.
- 55) Choudury MA, Rahman MAA and Gafur MA. *In vitro* Antibacterial and Cytotoxic Activities of a Brown Antibiotic Metabolite from a Strain of *Actinomycetes*. *J Med Sci.* 2001; 1(4): 206-208.
- 56) Sultan MZ, Sathi ZS, Biswas MHU, Choudury MA, Rahman MAA and Gafur MA. *In vitro* toxicological studies of metabolites of *Streptomyces* species on brine shrimp. *J Med Sci.* 2002; 2(5-6): 240-242.
- 57) Safaeian S, Alsadat NA and Oryan S. Studies on cytotoxic activity of marine Actinomycetes from Persian gulf on *Artemia franciscana* and *Artemia urmiana*. *J Agricul Sci Nat Resour.* 2005; 11(4): 133-139.
- 58) Manivasagan P, Gnanam S, Sivakumar K, Thangaradjou T, Vijayalakshmi S and Balasubramanian T. Antimicrobial and Cytotoxic Activities of an Actinobacteria (*Streptomyces* Sp. PM-32) Isolated from an offshore Sediments of the Bay of Bengal in Tamilnadu. *Adv Biolog Res.* 2009; 3(5-6): 231-236.
- 59) Ripa FA, Nikkon F, Rahman BM and Khondkar P. *In vitro* antibacterial activity of bioactive metabolite and crude extract from a new *Streptomyces* sp. *Streptomyces rajshahiensis*. *Int J PharmTech Res.* 2010; 2(1): 644-648.
- 60) Yang S, Gao J, Zhang A and Laatsch H. Sannastatin, a novel toxic macrolactam polyketide glycoside produced by actinomycete *Streptomyces sannanensis*. *Bioorg Med Chem Lett.* 2011; 21(13): 3905-3908.

