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Bioactivities of *Streptomyces* species from soils of Western Ghats of Karnataka, India

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ABSTRACT

The present study was carried out to screen insecticidal, anthelmintic, pancreatic amylase and pancreatic lipase inhibitory activity of 17 Streptomyces species (PO-01 to PO-16 and PO-18) isolated from soils of different places of Western Ghats of Shivamogga district, Karnataka, India. Insecticidal activity was assessed in terms of larvicidal effect against 2nd and 3rd instar larvae of Aedes aegypti. 2nd instar larvae were more susceptible to extracts when compared to 3rd instar larvae. Isolates PO-02, PO-08 and PO-11 showed marked larvicidal efficacy when compared to other isolates. Anthelmintic activity, in terms of time taken for paralysis and death of worms, was performed using adult Indian earthworm model. Two isolate namely PO-02 and PO-13 showed marked anthelmintic efficacy. Amylase inhibitory effect of solvent extracts was tested against pancreatic amylase. Isolates PO-13 and PO-14 displayed marked inhibitory effect when compared to other isolates. Inhibitory activity of solvent extracts against lipase activity was tested against lipase from chicken pancreas. Among isolates, two isolates namely PO-16 and PO-01 showed high and least inhibitory activity against lipase activity respectively. In conclusion, Streptomyces species of Western Ghats of Shivamogga district are promising resources for development of insecticidal and anthelmintic agents and enzyme inhibitors. Further studies are to be carried out to isolate active principles from solvent extracts and to determine their bioactivities.

Key words: *Streptomyces*, Western Ghats, Larvicidal, Anthelmintic, Amylase, Lipase

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INTRODUCTION

Soil is one of the richest reservoirs of several kinds of microorganisms. The rhizosphere region of soil is shown to contain huge number of microbes owing to the secretion of exudates by plant roots. Soil microbes are considered as one of the important sources of natural products having many beneficial effects. Actinomycetes (order Actinomycetales) are gram positive, filamentous prokaryotes characterized by high GC content in their genome. Typically, actinomycetes produce two types of mycelia viz., substrate (growing on or within substratum) and aerial mycelium (grow erect and produces spores). They are considered as biotechnologically valuable prokaryotes due to their capacity to produce a large number of bioactive metabolites having agricultural as well as veterinary use, mainly antibiotics. They are the dominant producers of bioactive metabolites among microbes. Actinomycetes are among the most important soil microbiota. Soil actinomycetes live primarily as saprophytes deriving their nutrition from organic matter present in soil. In soil, they contribute to significant turnover of complex biopolymers such as cellulose, pectin and lignin. Among various actinomycetes genera, the genus *Streptomyces* is known to be the dominant genera, especially in soil. They have provided a vast majority of bioactive compounds having commercial importance **[1-7]**.

Western Ghats of India covers an area of 1,80,000 km^2 which is just under 6% of the land area of India. Being one of the global biodiversity hotspots, Western Ghats harbor >30% of all plant, fish, herpeto-fauna, birds, and mammal species found in India. The hill ranges of Western Ghats runs through states viz., Gujarat, Maharashtra, Goa,

Karnataka and Kerala **[8,9]**. Several studies have been carried out on biological activities of actinomycetes from different places of Western Ghats of Karnataka such as Agumbe, Talakaveri, Thirthahalli, Kodachadri, Dandeli and Kudremukh. Bioactivities such as antimicrobial, antioxidant, enzyme inhibitory, insecticidal, cytotoxic, anthelmintic, analgesic, anti-inflammatory, CNS depressant and antipyretic activities have been exhibited by actinomycetes isolated from soils of Western Ghats of Karnataka **[10-20]**. In our previous study, we reported antimicrobial and cytotoxic potential of solvent extracts of 17 *Streptomyces* species recovered from different rhizosphere soils of Western Ghats of Shivamogga district, Karnataka, India. These isolates were shown to exhibit antimicrobial, antioxidant and cytotoxic activity **[21]**. In the present study, we report antioxidant, insecticidal, anthelmintic and amylase and lipase inhibitory activity of solvent extracts of these 17 bioactive *Streptomyces* species.

EXPERIMENTAL SECTION

Streptomyces species used in this study

The bioactive *Streptomyces* species selected for this study were recovered from rhizosphere soil samples collected at different places of Western Ghats of Shivamogga district, Karnataka, India. Isolation and identification of isolates and solvent extraction was described in our earlier study. Solvent extracts showing marked antimicrobial activity were selected for this study **[21]**.

Insecticidal activity of solvent extracts

Insecticidal activity of different concentrations of solvent extracts was tested against 2nd and 3rd instar larvae of *Aedes aegypti* mosquito. In brief, 20 larvae were transferred into beakers containing different concentrations of solvent extracts (0.25, 0.50, 1.0 and 2.0mg/ml). A control was kept without adding extract. The larvicidal effect of extracts was determined by counting the number of dead larvae after 24 hours **[14]**.

Anthelmintic activity of solvent extracts

The anthelmintic activity of solvent extracts was performed using adult Indian earthworm (*Pheretima posthuma*) model. The worms of equal size were selected and were washed using normal saline (0.85%) to remove extraneous matter. Six worms were transferred into normal saline containing different concentrations of solvent extracts (0.25, 0.50, 1.0 and 2.0mg/ml). The time taken for paralysis and death of worms was noted. Paralysis was said to occur when no movement of worms was observed except when the worms were shaken vigorously. The death time was recorded when worms did not show movement on shaking vigorously and when dipped in slight hot water (50°C). Normal saline served as control **[22]**.

Pancreatic lipase inhibitory activity of solvent extracts

The inhibitory activity of different concentrations of solvent extracts (10-500µg/ml) 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 pancreatic lipase for an hour in rotary shaker. The reaction was stopped by the addition of 1.5ml of 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 100µl 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) × 100, where A is lipase activity, B is activity of lipase when incubated with the extract **[14]**. IC_{50} value was calculated by Origin 6 software. It denotes the concentration of extract required to produce 50% inhibition of enzyme activity.

Pancreatic amylase inhibitory activity of solvent extracts

The inhibitory activity of different concentrations of solvent extracts (10-500µg/ml) was determined against pancreatic amylase (porcine) by following the method Jayasri *et al*. **[23]** with minor modifications. The enzyme (0.5%) was prepared in phosphate buffer (pH 6.8). in brief, 500µl of different concentrations of solvent extracts and 500µl of 0.1M phosphate buffer (pH 6.8) containing amylase were incubated at 25°C for 10min. After preincubation, 500µl of a 1% starch solution in 0.1M phosphate buffer (pH 6.8) was added to each tube and the tubes were further incubated at 25°C for 10 min. The reaction was stopped by addition of 1ml of dinitrosalicylic acid reagent. The same was performed for control where extract was replaced with buffer. The test tubes were placed in a boiling water bath for 10min and cooled. To each tube, 10ml of distilled water was added and the absorbance (A) was measured at 540 nm. The inhibition of amylase activity (%) was calculated using formula:

Amylase inhibition (%) = (A_{540}Control - A_{540}Extract / A_{540}Control] x100.

IC_{50} value was calculated by Origin 6 software. It denotes the concentration of extract required to produce 50% inhibition of enzyme activity.

RESULTS

Insecticidal activity of solvent extracts

Result of insecticidal activity of solvent extracts in terms of larvicidal activity against II and III instar larvae of *A. aegypti* is shown in Figure 1 and 2. Extracts of 7 out of 17 isolates were not effective in causing mortality of 2nd instar larvae. Extract of isolate PO-02 and PO-08 caused >50% mortality of 2nd instar larvae at 2mg/ml concentration. Extracts of 8 isolates were not effective in causing mortality of 3rd instar larvae. Remaining extracts caused larvicidal effect in a dose dependent manner. 2nd instar larvae were more susceptible to extracts. At concentration 0.25 and 1.0mg/ml, none of the extracts displayed larvicidal activity. Overall, when compared to other isolates, isolate PO-02, PO-08 and PO-11 exhibited marked insecticidal activity against 2nd and 3rd instar larvae.

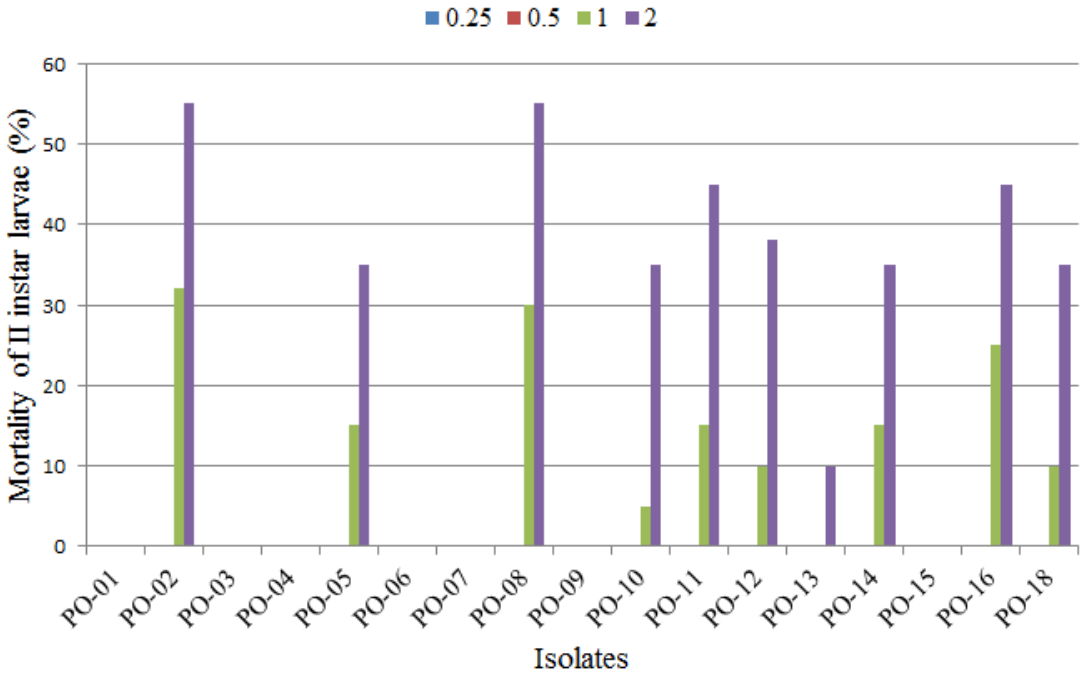

Figure 1: Mortality of 2nd instar larvae by solvent extracts

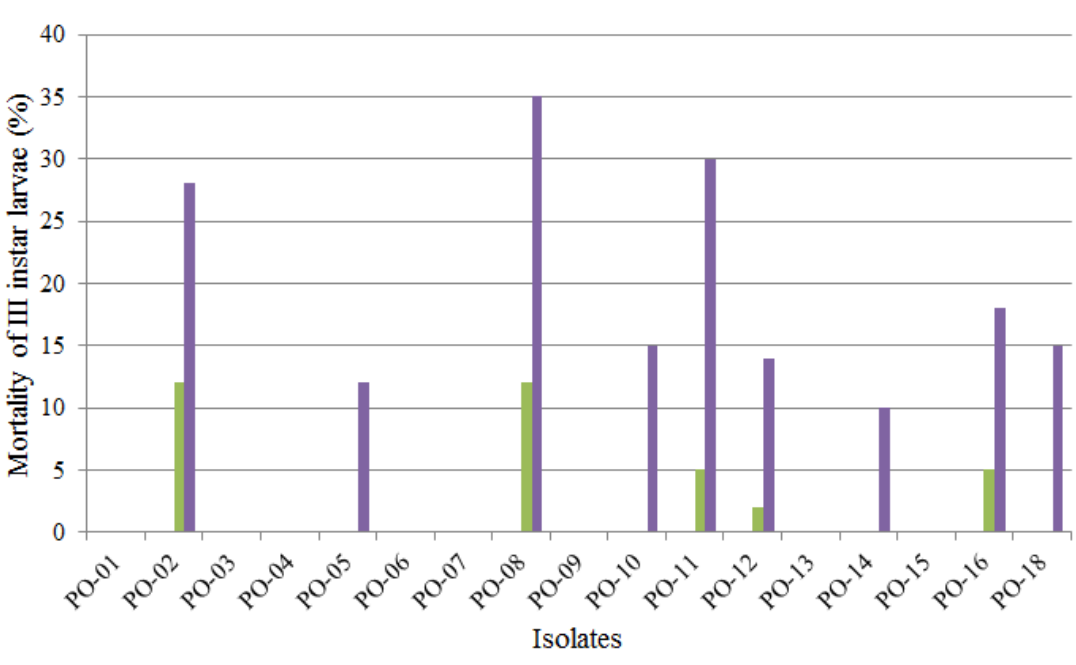

Figure 2: Mortality of 3rd instar larvae by solvent extracts

Anthelmintic activity of solvent extracts of selected actinomycetes

Table 1 shows the result of anthelmintic activity of solvent extracts in terms of time taken to cause paralysis and death of adult earthworms. Out of 17 isolates, 6 isolates were totally lacking anthelmintic effect. Other extracts showed dose dependent anthelmintic activity. None of the isolates caused paralysis and death of worms at extract concentration 0.25mg/ml. At 0.5mg/ml, effect was observed only in case of PO-02. Among extracts, marked anthelmintic effect was observed in case of extract of isolate PO-02 and PO-13.

Table 1: Anthelmintic activity of solvent extracts of selected actinomycetes

Isolates	Time taken (minutes) by different concentrations of extracts (mg/ml) to cause paralysis (P) and death (D) of worms							
	0.25		0.5		1.0		2.0	
	P	D	P	D	P	D	P	D
PO-01	-	-	-	-	-	-	185.00	233.00
PO-02	-	-	235.00	295.00	185.00	208.00	135.00	168.00
PO-03	-	-	-	-	-	-	205.00	285.00
PO-04	-	-	-	-	-	-	-	-
PO-05	-	-	-	-	265.00	335.00	195.00	248.00
PO-06	-	-	-	-	-	-	-	-
PO-07	-	-	-	-	-	-	-	-
PO-08	-	-	-	-	310.00	365.00	232.00	268.00
PO-09	-	-	-	-	-	-	-	-
PO-10	-	-	-	-	205.00	278.00	168.00	195.00
PO-11	-	-	-	-	233.00	282.00	181.00	202.00
PO-12	-	-	-	-	305.00	392.00	276.00	321.00
PO-13	-	-	-	-	105.00	178.00	85.00	143.00
PO-14	-	-	-	-	-	-	-	-
PO-15	-	-	-	-	-	-	-	-
PO-16	-	-	-	-	-	-	375.00	446.00
PO-18	-	-	-	-	303.00	356.00	231.00	268.00

'-' No paralysis or death even after 8 hours

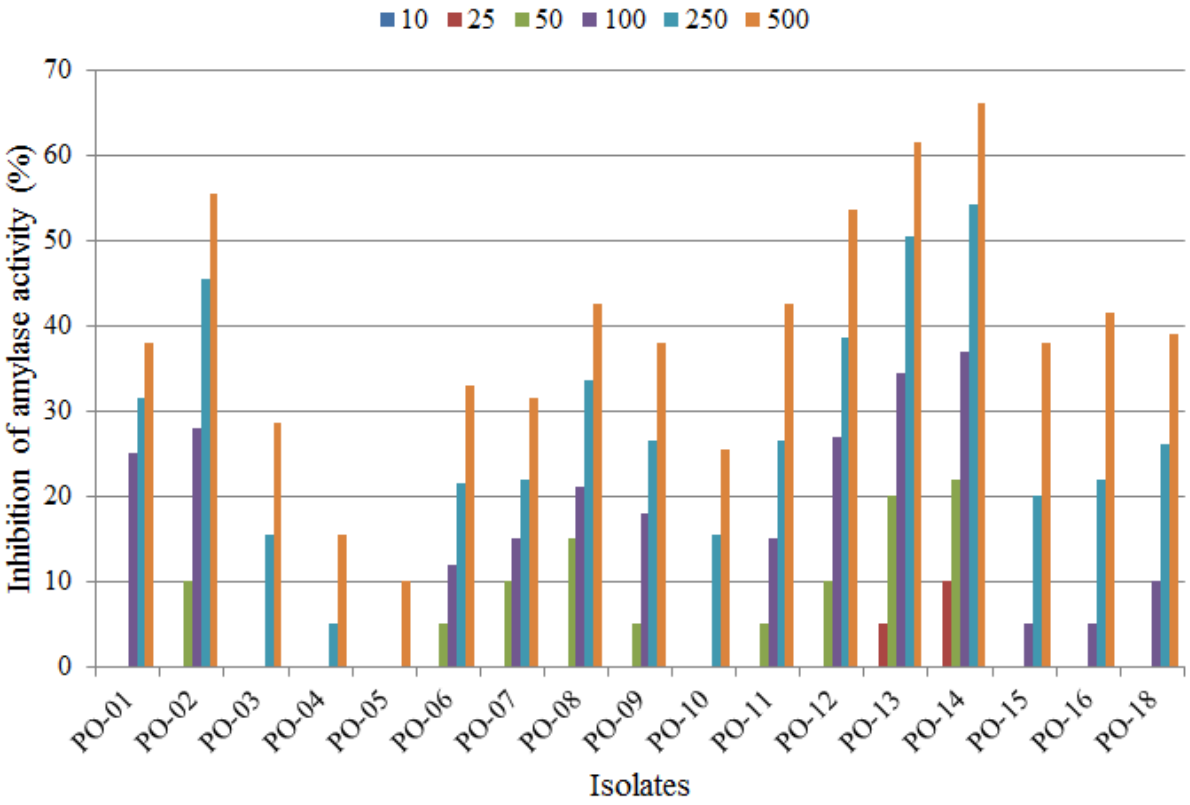

Figure 3: Inhibition of Amylase activity by solvent extracts

Pancreatic amylase inhibitory activity of solvent extracts

The result of effect of extracts against activity of pancreatic amylase is shown in Table 2 and Figure 3. The extracts exhibited inhibitory activity against amylase activity in a dose dependent manner. The inhibition of amylase activity ranged from 10 to 66% at extract concentration of 500µg/ml. The IC_{50} values ranged from 213.33 to 1690.03µg/ml.

Marked and least inhibitory activity was observed in case of extract of isolate PO-13 and PO-14 and PO-05 respectively.

Table 2: IC_{50} values (amylase inhibition) of solvent extracts

Extract	IC_{50} (µg/ml)	Extract	IC_{50} (µg/ml)
PO-01	887.74	PO-10	1099.32
PO-02	392.64	PO-11	581.83
PO-03	943.67	PO-12	431.74
PO-04	1690.03	PO-13	231.66
PO-05	-	PO-14	213.33
PO-06	769.81	PO-15	591.72
PO-07	903.07	PO-16	591.43
PO-08	599.49	PO-18	642.13
PO-09	658.4		

Pancreatic lipase inhibitory activity of solvent extracts of selected actinomycetes

Figure 4 and Table 3 shows the effect of solvent extracts to inhibit activity of chicken pancreatic lipase. The activity of lipase was affected when incubated with extracts. The extracts exhibited dose dependent inhibitory activity against lipase. At concentration 500µg/ml, inhibition of enzyme ranged between 12 to 58.50%. The IC_{50} values for extracts ranged from 384.71 to 2555.1µg/ml. Among extracts, potent and least inhibitory activity was observed in case of PO-16 and PO-01 respectively.

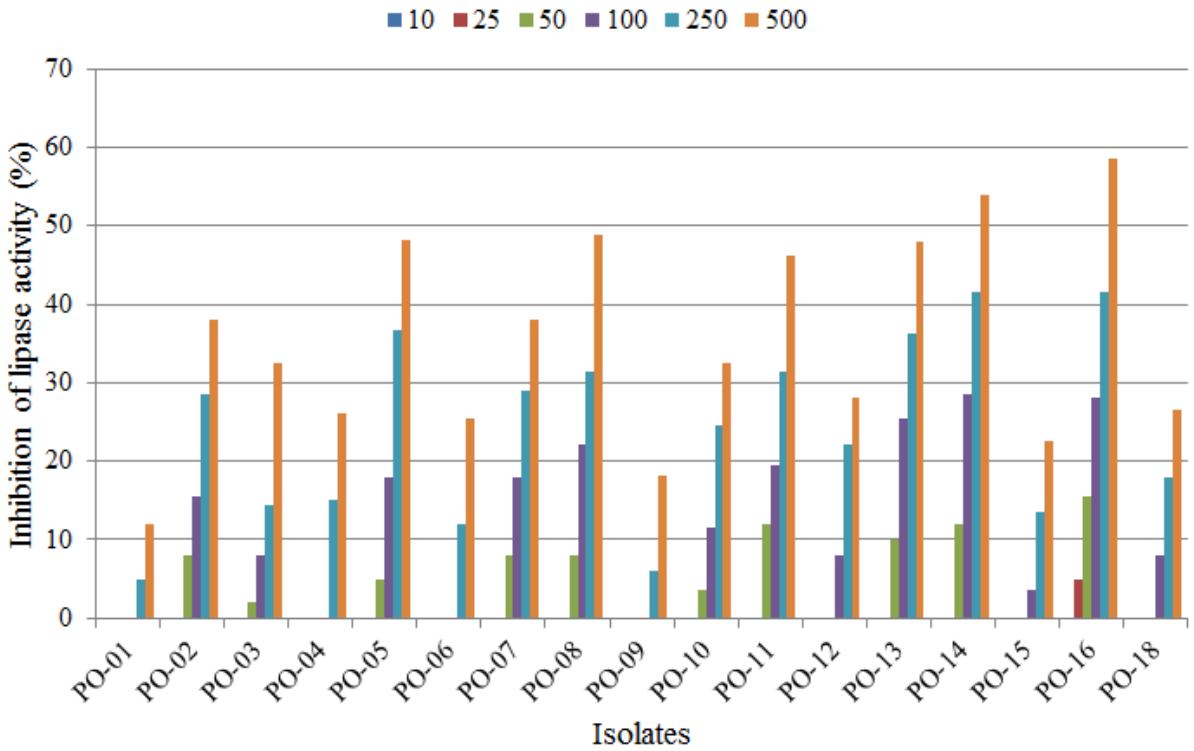

Figure 4: Inhibition of lipase activity by solvent extracts

Table 3: IC_{50} values (lipase inhibition) of solvent extracts

Extract	IC_{50} (µg/ml)	Extract	IC_{50} (µg/ml)
PO-01	2555.1	PO-10	750.43
PO-02	664.65	PO-11	536.3
PO-03	786.75	PO-12	936.49
PO-04	1112.8	PO-13	499.05
PO-05	481.38	PO-14	419.16
PO-06	1080.13	PO-15	1986.09
PO-07	665.11	PO-16	384.71
PO-08	498.32	PO-18	1005.55
PO-09	1472.12		

DISCUSSION

Insecticidal activity of extracts of *Streptomyces* species

Mosquitoes transmit more diseases than any other group of arthropods. The species of *Anopheles*, *Culex*, *Aedes* etc., are known to transmit several dreadful diseases. These diseases affect millions of people all over the world, especially in developing and under developing countries. These mosquito-borne diseases are prevalent in many countries and India being a country with high incidence of mosquito borne diseases. Mosquitoes transmit life threatening diseases such as malaria, yellow fever, dengue fever, chikungunya ferver, filariasis and West Nile virus infection. Hence, mosquito control is extremely essential so as to prevent mosquito borne diseases and to improve quality of environment and public health. The use of synthetic insecticides such as organochlorine and organophosphate compounds is a widely used approach for controlling mosquitoes. However, the use of these insecticides is accompanied with certain drawbacks such as high cost, harmful effect on human and other non-target populations, residual problem and resistance development in mosquitoes. Therefore, search for alternate, ecofriendly, cost-effective, target specific agents to control mosquito vectors **[24-28]**.

The crude extracts and purified metabolites of several actinomycetes have shown to exhibit potent insecticidal activity against various kinds of insect pests **[29-32]**. In the present study, we evaluated insecticidal activity of extracts of *Streptomyces* species in terms of larvicidal effect against 2^{nd} and 3^{rd} instar larvae of *A. aegypti*. Susceptibility of larvae to solvent extracts varied according to the developmental stage i.e., 2^{nd} instar larvae were shown to be more sensitive to solvent extracts than 3^{rd} instar larvae. Solvent extracts exhibited dose dependent larvicidal effect. Similar dose dependent larvicidal efficacy was observed in case of extracts obtained from *Streptomyces* species isolated Western Ghats of Karnataka against different larval development stage of mosquito *A. aegypti* **[14,15,31]**. Several researchers have shown the efficacy of actinomycetes to exhibit insecticidal activity in terms of larvicidal effect against larvae of several mosquitoes. The study of Mishra *et al*. **[30]** showed potent insecticidal activity of several genera of actinomycetes against larvae of *A. aegypti* mosquito. El-Khawagh *et al*. **[33]** showed the efficacy of seven species of *Streptomyces* isolated from Egypt to cause 100% mortality of 3^{rd} instar larvae of *Culex pipiens*. The study of Vijayakumar *et al*. **[34]** revealed the marked potential of *Streptomyces* species and *Streptosporangium* species to cause mortality of larvae of *Anopheles*. Anwar *et al*. **[28]** showed 100% mortality of 3^{rd} instar larvae of *C. quinquefasciatus* by three *Streptomyces* species isolated from salt range, Pakistan. More recently, Shukla *et al*. **[35]** observed larvicidal effect of soil actinomycetes against 4^{th} instar larvae of *A. aegypti*.

Anthelmintic activity of *Streptomyces* species

Helminthic infections are considered to be one of the most common infections affecting human population, crops and livestock all over the world. Parasitoses have been of concern for centuries. These infections are more common in developing countries. These infections results in malnutrition, anemia, eosinophilia, and pneumonia. The principal mode for control of these parasitic helminths is based on the use of commercial anthelmintics. However, indiscriminate use of these drugs resulted in several drawbacks including development of resistance. These alarming situations i.e., increase in development of anthelmintic resistance and toxicity have triggered immense research on searching alternatives against gastrointestinal nematodes **[10,15,36,37]**. Crude extracts and purified compounds from actinomycetes are shown to exhibit anthelmintic activity **[10,15,38-42]**.

In the present study, we evaluated anthelmintic activity of solvent extracts of selected *Streptomyces* species using adult Indian earthworms due to anatomical and physiological resemblance with the human intestinal roundworm parasites **[43,44]**. Studies have shown the anthelmintic potential of *Streptomyces* species isolated from Western Ghats of Karnataka. In an earlier study, we showed dose dependent anthelmintic activity of butanol extract of two *Streptomyces* species isolated from Agumbe **[10]**. In another study, Kekuda *et al*. **[15]** showed anthelmintic efficacy of ethyl acetate extract of *Streptomyces* species SRDP-07 isolated from a soil sample of Thirthahalli. The study of Al-Doori *et al*. **[40]** revealed the inhibitory effect of *Streptomyces* strain S-70 against dog round worm *Toxocara canis*. Culture filtrates of *Streptomyces avermitilis* isolates significantly inhibited egg hatching of *Meloidogyne incognita* **[41]**. Novel macrocyclic lactones isolated from *Streptomyces avermitilis* NEAU1069 caused nematocidal activity against *Caenorhabditis elegans* **[45]**. Fervenulin and isocoumarin, isolated from the culture filtrate of *Streptomyces* sp. CMU-MH021 were shown to possess inhibitory activity against egg hatching and juvenile stage of the root-knot nematode *M. incognita* **[42]**.

Pancreatic lipase inhibitory activity of *Streptomyces* species

Dietary lipids are the major source of unwanted calories. Obesity or hyperlipidemia is developed when the balance between energy intake and expenditure is lost. Obesity is a worldwide health problem that has increased at an alarming rate. Obesity is associated with a series of severe diseases such as atherosclerosis, hypertension, diabetes, and dysfunction of certain organs. Often, obesity is shown to be a strong risk factor for type 2 diabetes. The use of drugs (mainly inhibitors of nutrient absorption) to control lipid metabolism offers a possible way to prevent or treat

obesity. Targeting enzymes involved in lipid metabolism is one of the widely used approaches for treatment of obesity. Pancreatic lipase is the main lipid-digesting enzyme which acts on dietary triglycerides and yields the lipolytic products. Inhibition of pancreatic lipase is an attractive targeted approach for the treatment of obesity. Orlistat is one of the clinically approved drugs for treatment of obesity treatment and is shown to inhibit pancreatic lipase. Globally, it is one of the best-selling drugs, however, it causes some side effects such as oily stools, oily spotting, and flatulence. Hence, interest in searching lipase inhibitors lacking the side effects is much more focused. Natural products from plants, animals and microbes present an exciting opportunity for the discovery and development of newer anti-obesity agents **[14,46,47,48]**.

Researchers have shown that crude extracts and purified compounds from actinomycetes possess inhibitory activity against lipase **[14,49,50]**. In the present study, we determined inhibitory efficacy of extracts of *Streptomyces* species against chicken pancreatic lipase. The extracts displayed concentration dependent inhibition of enzyme activity. Only 2 isolates displayed >50% inhibition of enzyme activity at extract concentration 500µg/ml. At concentration 500µg/ml, inhibition of enzyme ranged between 12 to 58.50%. The IC_{50} values for extracts ranged from 384.71 to 2555.1µg/ml. Among extracts, potent inhibitory activity was observed in case of PO-16. Similar dose dependent inhibition of chicken pancreatic lipase activity by extracts of *Streptomyces* species isolated from Agumbe was observed in our previous studies **[14,51,52]**. Novel pancreatic lipase inhibitors Panclicins A, B, C, D and E from *Streptomyces* sp. NR 0619 were shown to inhibit porcine pancreatic lipase **[49]**. (E)-4-Aminostyryl acetate, isolated from *Streptomyces* sp. MTCC 5219, showed marked inhibition of bacterial lipase and porcine pancreatic lipase **[50]**.

Amylase inhibitory activity of solvent extracts

An estimate reveals that 285 million people, worldwide, have diabetes and there will be a 54% increase by 2030. Various approaches have been used to treat diabetes. One such approach is to reduce the post prandial glycemia. The retardation of absorption of glucose can be achieved by inhibiting enzymes amylase and glucosidase which hydrolyze carbohydrate. In small intestine, the enzyme pancreatic α-amylase hydrolyzes the starch to oligosaccharides and maltose. Another enzyme α-glucosidase (membrane bound) hydrolyzes di- and oligosaccharides to glucose. Inhibition of these enzymes reduces the rate of starch digestion and hence, decreased post-prandial blood glucose levels especially in diabetic patients. Acarbose is one of the well known drugs which acts by inhibiting α-glucosidase pancreatic α-amylase and is widely used to treat type-2 diabetes. However, Acarbose and related drugs do have some side effects such as abdominal pain, flatulence and diarrhea. This has necessitated search for new amylase inhibitors from natural sources possibly lack side effects **[53-56]**.

It has been shown experimentally that *Streptomyces* species possess amylase inhibitory activity. Bioactive metabolites from actinomycetes have shown promising in terms of inhibition of amylase activity. Haim, a proteinaceous α-amylase inhibitor, isolated from the culture filtrate of *S. griseosporeus* YM-25 inhibited α-amylases of animal origin **[57]**. Similarly, Acarviostatins isolated from *Streptomyces* strain ZG0656 were shown to inhibit porcine pancreatic α-amylase **[53]**. In the present study, we evaluated amylase inhibitory efficacy of solvent extracts of *Streptomyces* species to inhibit pancreatic amylase. The extracts exhibited concentration dependent inhibition of amylase activity. Among isolates, potent inhibitory activity was shown by PO-14 while isolates PO-04 and PO-05 revealed least inhibitory activity. It has been shown that solvent extracts of *Streptomyces* species exhibit amylase inhibitory efficacy. In a study, ethyl acetate extract of *Streptomyces* sp. VITPK9 and *Streptomyces* sp. VITSTK7 exhibited dose dependent inhibitory activity against α-amylase **[55]**. In another study, ethyl acetate extract of *Streptomyces* sp. VITMSS05 was shown to exhibit concentration dependent inhibition of amylase activity **[56]**.

CONCLUSION

In conclusion, the *Streptomyces* species of Western Ghats of Shivamogga district appears to be promising resources for development of insecticidal and anthelmintic agents and enzyme inhibitors. Further studies on recovery of active compounds from the solvent extracts and their bioactivity determinations are to be carried out.

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REFERENCES

[1] I Saadoun; F Al-Momani. *World Journal of Microbiology and Biotechnology*, **2000**, 16, 139-142.
[2] CL Doumbou; MKH Salove; DL Crawford; C Beaulieu. *Phytoprotection*, **2001**, 82(3), 85-102.
[3] J Berdy. *Journal of Antibiotics*, **2005**, 58(1), 1-26.

[4] PTR Kekuda; KS Shobha; R Onkarappa. *Journal of Pharmacy Research*, **2010**, 3(2), 250-256.
[5] R Ghorbani-Nasrabadi; R Greiner; HA Alikhani; J Hamedi; B Yakhchali. *Journal of Soil Science and Plant Nutrition*, **2013**, 13(1), 223-236.
[6] HS Chaudhary; J Yadav; AR Shrivastava; S Singh; AK Singh; N Gopalan. *Journal of Advanced Pharmaceutical Technology and Research*, **2013**, 4(2), 118-123.
[7] M Sharma. *International Journal of Current Microbiology and Applied Sciences*, **2014**, 3(2), 801-832.
[8] SA Gautham. Molecular characterization and pharmacological activities of metabolites from *Streptomyces* spp. Ph.D thesis. Kuvempu University, Karnataka, India, **2012**.
[9] MK Nampoothiri; B Ramkumar; A Pandey. *Journal of Scientific and Industrial Research*, **2013**, 72, 617-23.
[10] PTR Kekuda; KS Shobha; R Onkarappa. *Journal of Pharmacy Research*, **2010**, 3(1), 26-29.
[11] KS Shobha; R Onkarappa. *Indian Journal of Microbiology*, **2011**, 51(4), 445-449.
[12] SA Gautham; KS Shobha; R Onkarappa; PTR Kekuda. *Research Journal of Pharmacy and Technology*, **2012**, 5(2), 233-238.
[13] M Manasa; G Poornima; V Abhipsa; C Rekha; PTR Kekuda; R Onkarappa; S Mukunda. *Science, Technology and Arts Research Journal*, **2012**, 1(3), 39-44.
[14] PTR Kekuda; KS Shobha; R Onkarappa; SA Gautham; HL Raghavendra. *International Journal of Drug Development and Research*, **2012**, 4(3), 104-114.
[15] PTR Kekuda; N Dileep; S Junaid; KN Rakesh; SC Mesta; R Onkarappa. *International Journal of Drug Development and Research*, **2013**, 5(3), 268-285.
[16] PTR Kekuda; R Onkarappa; HL Raghavendra. *Science, Technology and Arts Research Journal*, **2013**, 2(2), 83-91.
[17] MD Akshatha; BK Manjunatha; R Pooja; TM Umesh; R Sreevijeth. *Paripex- Indian Journal of Research*, **2013**, 2(3), 11-13.
[18] SA Gautham; R Onkarappa. *International Journal of Chemical Sciences*, **2013**, 11(1), 583-590.
[19] S Junaid; N Dileep; KN Rakesh; PTR Kekuda. *Pharmanest*, **2013**, 4(4), 736-750.
[20] PTR Kekuda; R Onkarappa. Biological activities of *Streptomyces variabilis* strain PO-178 isolated from Western Ghat soil of Agumbe, Karnataka, India. In: AM Deshmukh; JD Jawalikar; PS Wakte (Editors). Current Research in Biochemistry and Microbiology, Oxford Book Company, Delhi, **2015**; 166-193.
[21] PTR Kekuda; R Onkarappa; SA Gautham; SC Mesta; HL Raghavendra. *Science, Technology and Arts Research Journal*, **2015**, 4(2), 164-180.
[22] AS Grime; RD Bhalke; PB Ghogare; VD Tambe; RS Jadhav; SA Nirmal. *Dhaka University Journal of Pharmaceutical Sciences*, **2006**, 5(1-2), 5-7.
[23] MA Jayasri; A Radha; TL Mathew. *Journal of Herbal Medicine and Toxicology*, **2009**, 3(1), 91-94.
[24] S Cheng; C Huang; W Chen; Y Kuo; S Chang. *Bioresource Technology*, **2008**, 99, 3617-3622.
[25] R Kaushik; P Saini. *Journal of Vector Borne Diseases*, **2009**, 46, 244-246.
[26] KS Vinayaka; SP Swarnalatha; HR Preethi; KS Surabhi; PTR Kekuda; SJ Sudharshan. *African Journal of Basic and Applied Sciences*, **2009**, 1(5-6), 110-116.
[27] A Ghosh; N Chowdhury; G Chandra. *Indian Journal of Medical Research*, **2012**, 135, 581-598.
[28] S Anwar; B Ali; F Qamar; I Sajid. *Pakistan Journal of Zoology*, **2014**, 46(1), 83-92.
[29] T Ikemoto; T Katayama; A Shiraishi; T Haneishi. *Journal of Antibiotics*, **1983**, 36(9), 1097-1100.
[30] SK Mishra; JE Keller; JR Miller; RM Heisey; MG Nair; AR Putnam. *Journal of Industrial Microbiology*, **1987**, 2(5), 267-276.
[31] PTR Kekuda; KS Shobha; R Onkarappa. *Journal of Natural Pharmaceuticals*, **2010**, 1(1), 30-32.
[32] HB Chen; L Ma; JC Han; HP Liu; YP Yan. *Ying Yong Sheng Tai Xue Bao*, **2011**, 22(9), 2419-23.
[33] MA El-Khawagh; KS Hamadah; TM El-Sheikh. *Egypt Acad Journal of Biological Sciences*, **2001**, 4(1), 102-113.
[34] R Vijayakumar; S Murugesan; A Cholarajan; V Sakthi. *International Journal of Microbiological Research*, **2010**, 1(3), 179-183.
[35] RK Shukla; P Tripathi; S Kumar. *Indian Research Journal of Genetics and Biotechnology*, **2015**, 7(2), 248-254.
[36] RCS Nunomura; ECC daSilva; DF Oliverira; AM Garcia; JN Boeloni; SM Nunomura; AM Pohlit. *Acta Amazonica*, **2006**, 36, 327-330.
[37] PSV Kumar; PTR Kekuda; KS Vinayaka; SJ Sudharshan. *Pharmacognosy Journal*, **2009**, 1(4), 238-242.
[38] G Cassinelli; E Cotta; G D'Amico; DC Bruna; A Grein; R Mazzoleni; ML Ricciardi; R Tintinelli. *Archives of Microbiology*, **1970**, 70(3), 197-210.
[39] RW Burg; BM Miller; EE Baker; J Birnbaum; SA Currie; R Hartman; YL Kong; RL Monaghan; G Olson; I Putter; JB Tunac; H Wallick; EO Stapley; R Oiwa; S Omura. *Antimicrobial Agents and Chemotherapy*, **1979**, 15(3), 361-367.
[40] M Al-Doori; AA Al-Tae; S Jalil; SA Hassan. *Folia Parasitologica*, **1991**, 38(4), 379-382.
[41] J Jayakumar. *Karnataka Journal of Agricultural Sciences*, **2009**, 22(3S), 567-571.

[42] P Ruanpanun; H Laatsch; N Tangchitsomkid; S Lumyong. *World Journal of Microbiology and Biotechnology*, **2011**, 27(6), 1373-1380.
[43] ABS Kumar; K Lakshman; KN Jayaveera; R Nandeesh; B Manoj; D Ranganayakulu. *Achieves of Biological Sciences*, **2010**, 62(1), 185-189.
[44] N Muhammad; M Saeed; H Khan; M Qayum; Barkatullah; A Badshah. *African Journal of Pharmacy and Pharmacology*, **2012**, 6(10), 698-701.
[45] XJ Wang; M Wang; JD Wang; L Jiang; JJ Wang; WS Xiang. *Journal of Agricultural and Food Chemistry*, **2010**, 58(5), 2710-2714.
[46] AJ Hartz; DCJ Rupley; RD Kalkhoff; AA Rimm. *Preventive Medicine*, **1983**, 12, 351-357.
[47] C Zheng; Y Duan; J Gao; Z Ruan. *Journal of the Chinese Medical Association*, **2010**, 73(6), 319-324.
[48] A Mukherjee; S Sengupta. *Indian Journal of Biotechnology*, **2013**, 12, 32-39.
[49] M Mutoh; N Nakada; S Matsukuma; S Ohshima; K Yoshinari; J Watanabe; M Arisawa. *Journal of Antibiotics*, **1994**, 47(12), 1369-1375.
[50] P Tokdar; P Randive; M Mascarenhas; S Patil; S George. *International Conference on Life Science and Technology*, **2011**, 3, 7-10.
[51] PTR Kekuda; KS Shobha; R Onkarappa. *International Journal of Pharmaceutical & Biological Archives*, **2011**, 2(3), 932-937.
[52] PTR Kekuda; R Onkarappa. *Journal of Biological and Scientific Opinion*, **2014**, 2(2), 170-176.
[53] P Geng; G Bai; Q Shi; L Zhang; Z Gao; Q Zhang. *Journal of Applied Microbiology*, **2009**, 106, 525-533.
[54] S Karthik; KC Nandini; PTR Kekuda; KS Vinayaka; S Mukunda. *Annals of Biological Research*, **2011**, 2(4), 38-43.
[55] P Sanjenbam; M Thenmozhi; K Kannabiran. *International Journal of Pharma Research & Review*, **2013**, 2(2), 5-11.
[56] T Revathy; MA Jayasri; K Suthindhiran. *American Journal of Biochemistry and Biotechnology*, **2013**, 9(3), 282-290.
[57] S Murao; A Goto; Y Matsui; K Ohyama; M Arai. *Agricultural Biology and Chemistry*, **1981**, 45, 2599-2604.