

Pharmacological Activities of *Streptomyces* Species PO-178 Isolated from Rhizosphere Soil of Agumbe, Karnataka, India

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Abstract

In the present study, pharmacological activities namely anti-inflammatory, analgesic, antipyretic and CNS depressant activity of crude extract of *Streptomyces* species PO-178 isolated from Western Ghats soil of Agumbe, Karnataka, India have been studied. The isolate PO-178 was grown in starch casein nitrate broth medium. The culture filtrate was condensed to obtain crude extract. Anti-inflammatory, analgesic, antipyretic and CNS depressant activities of the crude extract were determined by Carrageenan-induced paw edema, tail flick, yeast induced pyrexia and spontaneous locomotor activity respectively. The extract exhibited dose dependent anti-inflammatory, analgesic, antipyretic and CNS depressant activity, however, the activities observed were lesser than standard drugs. Further studies are under progress to recover pharmacologically active principles from the extract of *Streptomyces* species PO-178 and to determine their activities.

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INTRODUCTION

Rhizosphere, the soil adjacent to and influenced by plant root exudates, is a unique habitat with a variety of microflora comprising bacteria, fungi, protozoa and algae. The input of organic materials derived from the plant roots and root exudates nutritionally favours the microbial community in rhizosphere. The number and the activity of microorganisms are generally higher in rhizosphere (Gonzalez-Franco *et al.*, 2009). Actinomycetes are one of the major microbial populations present in soil. They represent an extensive and diverse group of Gram-positive, aerobic, mycelial bacteria capable of playing significant ecological roles in soil nutrient cycling by actively participating in biogeochemical cycles. Apart from this, these organisms are also considered as novel sources antibiotics, enzymes, vitamins etc (Gonzalez-Franco *et al.*, 2009; Franco-Correa *et al.*, 2010). Among soil actinomycetes, the genus *Streptomyces* is represented in nature by the largest number of species and varieties and differs in morphology, physiology and biochemical characteristics. Species of *Streptomyces* are dominating in soil in terms of number and the

bioactive compounds what they produce. These bacteria are considered as important sources of bioactive agents having pharmaceutical and agricultural importance. They have provided 2/3rd of naturally occurring antibiotics discovered so far (Narayana *et al.*, 2007; Ramakrishnan *et al.*, 2009; Ripa *et al.*, 2009; Gopal *et al.*, 2010; Alimuddin *et al.*, 2011; Rahman *et al.*, 2012). Western Ghats are one of the 34 biodiversity hotspots in the world and known for their diverse flora and fauna. However, a very few microbiological studies have been carried out on the soil of this region. The studies which have been carried out on the actinomycetes of Western Ghats of Karnataka revealed biological activities *viz.*, antimicrobial, antioxidant, enzyme inhibitory, cytotoxic, anthelmintic, insecticidal analgesic, anti-inflammatory and antipyretic activity (Kekuda *et al.*, 2010a; Kekuda *et al.*, 2010b; Shobha and Onkarappa, 2011; Kekuda *et al.*, 2011; Kekuda *et al.*, 2012; Manasa *et al.*, 2012; Gautham *et al.*, 2012; Gautham *et al.*, 2013a; Gautham *et al.*, 2013b). In an earlier study, Gautham and Onkarappa (2013b) showed anti-inflammatory,

antipyretic and analgesic activity of *Streptomyces fradiae* GOS 1 isolated from a soil of Agumbe, Karnataka, India. In this study, we have screened the pharmacological activities namely anti-inflammatory, analgesic, antipyretic and CNS depressant activity of crude extract of *Streptomyces* species PO-178 recovered from the soil of Agumbe, Karnataka, India.

MATERIALS AND METHODS

Isolation of actinomycete isolate PO-178

The isolate PO-178 employed in this study was isolated from a rhizosphere soil of Agumbe rain forest, Western Ghats, Karnataka, India during June 2009. The surface soil was removed and the soil sample was collected from a depth of 10cm in a sterile polythene bag. The soil sample was brought to laboratory and dried aseptically at 40°C. The soil was serially diluted (in 0.85% sterile saline), plated on sterile Starch casein nitrate (SCN) agar and incubated at 30°C for up to 7 days. The isolate PO-178 was subcultured on sterile SCN agar slant and stored in the refrigerator. SCN agar, used for isolation and subculturing, was amended with antifungal antibiotic fluconazole in order to prevent the growth of fungal contaminants (Kekuda *et al.*, 2012).

Identification of Isolate PO-178

Microscopic characteristics

Cover slip method was employed to study the characteristic spore arrangement of isolate PO-178. Here, thin blocks of sterile SCN agar were cut with the help of sterile blade and placed on sterilized glass slides. The culture of isolate PO-178 was inoculated on the agar block by streaking over the surface and a cover slip was placed over the block. The glass slides with inoculated agar blocks were placed in sterile moist chamber and incubated until appreciable growth was observed. Later, the cover slip was removed, placed on a drop of dilute crystal violet stain taken on a clean glass slide and the slide was observed under oil immersion objective (total magnification 2000X) in order to study the characteristic arrangement of spores (Kekuda *et al.*, 2012).

Staining and Biochemical Characteristics

Staining techniques *viz.*, Gram's and Acid-fast staining and biochemical tests *viz.*, starch hydrolysis, gelatin liquefaction, casein hydrolysis, catalase test, oxidase test, citrate test, cellulose hydrolysis, nitrate reduction test, hydrogen sulfide (H₂S) production test and sugar fermentation tests were performed (Shirling and Gottlieb, 1966; Aneja, 1996; Cappuccino and Sherman, 1999; Augustine *et al.*, 2004; Kekuda *et al.*, 2010; Florencio *et al.*, 2012).

Fermentation

Mass cultivation of isolate PO-178 was carried out in SCN broth by stationary culture system. In brief, loop-full inocula of the well sporulated culture of isolate PO-178 was inoculated into flasks containing sterile SCN broth aseptically. The inoculated flasks were incubated at 28°C for 10 days aerobically. After incubation, the contents of the flasks were aseptically filtered through sterile Whatman filter paper No. 1 (Kekuda *et al.*, 2012). The culture filtrate was concentrated in vacuum under reduced pressure. The crude extract thus obtained was subjected to pharmacological activities.

Animals

Swiss Albino mice of both sex (weighing 25-30g) and male Wistar rats (weighing 200-250g) were used to evaluate pharmacological activities. The animals were kept in standard environmental conditions (12 hours light/dark cycle, temperature 25±0.2°C) and animals had free access to standard food and water *ad libitum* and fasted 12 hours prior to use. Only water was supplied during the period of fasting. The studies were conducted as per the study protocol, relevant standard operating procedures of the testing facility, committee for the purpose of control and supervision on experiments on animals (CPCSEA) guidelines and Institutional animal ethics committee guidelines (HSKCOP/IAEC, CLEAR/2010-11/1-11).

Acute Toxicity

Acute oral toxicity of crude extract was determined in Swiss Albino mice of either sex. Changes in autonomic and behavioural responses of animals were recorded up to 4 hours. The animals were observed for 7 days and gross effect and mortality was observed during this period (Rizwani *et al.*, 2012). No adverse effect of the crude extract was observed at the dose of 2000 mg/kg body weight (b.w). Extract concentrations 200 and 100 mg/kg b.w was administered into animals.

Antiinflammatory Activity

The antiinflammatory efficacy of crude extract was evaluated by Carrageenan-induced paw edema model in male wistar rats (Taufiq-Ur-Rahman *et al.*, 2005). Administration of 0.1ml of freshly prepared suspension of carrageenan (1%) into the subplantar region of the right hind paw of each rat of four groups (n=6) produced edema in situ due to localized inflammation. One hour prior to the administration of carrageenan, the test groups, standard group and control group rats were administered orally with extract (100 and 200 mg/kg

b.w), diclofenac (100 mg/kg b.w) and normal saline (2 ml/kg b.w) respectively. Paw volume was measured immediately after injection of carrageenan and after 1, 3 and 5 hours using Plethysmometer. The inhibition of edema formation (%) was calculated using the formula: % inhibition = $(C - T / C) \times 100$, where C is the average paw volume of control animals and T is the average paw volume of treated animals.

Analgesic Activity

Tail flick method was employed to determine analgesic activity of crude extract in Swiss Albino mice (Rizwani *et al.*, 2012). The distal portion (3cm) of tail of mice of all groups (n = 6) was immersed in water bath maintained at 50 ± 0.5 °C. The time taken by the mice to withdraw their tail from hot water was noted and considered as basal time. Animals of control group, standard group and test groups received normal saline (2 ml/kg b.w), aspirin (200 mg/kg b.w) and extract (100 and 200 mg/kg b.w) respectively. The tail flick latency was determined at intervals of 30, 60, 90, 120, 150 and 180 minutes after administration of standard and extract.

Antipyretic Activity

Yeast induced pyrexia was performed in male Wistar rats to evaluate antipyretic activity of extract (Reanmongkol and Wattanapiromsakul, 2008). Pyrexia was induced in rats by injecting subcutaneously brewer's yeast suspension (20% in normal saline, 10 ml/kg b.w) into all groups of rats (n = 6). 18 hours after injection, the rectal temperature of rats was recorded using digital thermometer and rats showing an increase in temperature of at least 1°C were selected. Extract (100 and 200 mg/kg b.w), standard (Paracetamol, 150 mg/kg b.w) and control (normal saline, 2ml/kg b.w) were administered orally to respective groups. The rectal temperature of rats was measured at 1, 3 and 5 hours after administration of standard/extract.

CNS Depressant Activity

Spontaneous locomotor activity of Swiss Albino mice was studied using Actophotometer in order to evaluate CNS depressant activity of extract (Raju *et al.*, 2011). The test groups, standard group and control group animals were administered with extract (100 and 200 mg/kg body weight), diazepam (5 mg/kg b.w) and normal saline (2 ml/kg b.w) respectively. Spontaneous motor activity was monitored in Actophotometer after interval of 45, 90 and 135 minutes by placing mice in Actophotometer for a period of 5 minutes. Basal activity score was recorded for all mice before administering extract/standard. A reduction in the locomotor activity in test groups shows CNS depressant activity.

Statistical Analysis

All the grouped data were statistically evaluated with Prism software 5.0. The data were expressed as mean \pm SD. The significance of differences among the groups was assessed using one-way analysis of variance (ANOVA) test followed by Dunnett's multiple comparison test. The values of $p < 0.05$ were considered as significant.

RESULTS

The actinomycete isolate PO-178 of this study was identified as a species of *Streptomyces* based on cultural and micromorphological characteristics. Table 1 represents the cultural, staining, biochemical and micromorphological characteristics of the isolate.

Table 1: Characteristics of *Streptomyces* species PO-178.

Colony	Velvety
Substrate mycelium	Dark brown
Aerial mycelium	Grey
Spore arrangement	Retinaculum apertum (RA)
Diffusible pigment	Brown
Gram's staining	Gram positive
Acid fast staining	Non-acid fast
Starch hydrolysis	Positive
Casein hydrolysis	Positive
Cellulose hydrolysis	Positive
Carbohydrate fermentation (glucose)	Acid production; no gas production
Hydrogen sulphide production	Negative
Catalase test	Positive
Gelatin hydrolysis	Negative
Citrase test	Positive
Nitrate reduction	Positive

Anti-inflammatory Activity

Table 2 depicts anti-inflammatory effect of crude extract and standard drug in carrageenan induced paw edema in male wistar rats. Carrageenan produced acute inflammation and the extent of paw edema (ml) increased with time in control group. Standard drug Diclofenac showed significant ($p < 0.001$) inhibition when compared to crude extract. Oral administration of crude extract showed a dose dependent inhibition of edema formation and 200 mg/kg b.w administration of crude extract showed significant ($p < 0.001$) anti-inflammatory effect. However, the anti-inflammatory potency of extract was lesser than that of standard.

Table 2: Anti-inflammatory activity of crude extract and standard.

Treatment	Paw volume (ml±SD) at different intervals (hours)			
	0 hr	1 hr	3 hr	5 hr
Normal (saline)	0.6633±0.01528 ^{ns}	0.6267±0.0116	0.6533±0.0058	0.6533±0.0058
Control (Carrageenan)	0.6367±0.01528	1.247±0.0451	1.353±0.0351	1.350±0.0100
Standard (Diclofenac, 100 mg/kg b.w)	0.6733±0.02082 ^{ns}	0.3667±0.0153 ^{***}	0.3633±0.0153 ^{***}	0.3733±0.0058 ^{***}
Extract (100mg/kg b.w)	0.6233±0.02517 ^{ns}	1.213±0.0351 ^{ns}	1.167±0.0153 ^{***}	1.247±0.0153 ^{***}
Extract (200mg/kg b.w)	0.6700±0.01732 ^{ns}	1.107±0.0208 ^{***}	1.043±0.0208 ^{***}	1.183±0.0252 ^{***}

All the values are mean±SD, One way analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparison Test. ^{*} $p<0.05$, ^{**} $p<0.01$, and ^{***} $p<0.001$ as comparison to control group (Carrageenan). ns= not significant ($p>0.05$)

Analgesic Activity

Tail flick latency method was employed to assess analgesic potential of crude extract and standard drug and the results are shown in Table 3. Extract has shown a dose dependent analgesic effect. Tail flick latency increased till 60 minutes and further it decreased. Aspirin exhibited significant ($p<0.001$)

analgesic efficacy when compared to crude extract. Time taken by the animal to withdraw tail from hot water increased till 120 minutes. Analgesic effect of crude extract was lesser than that of standard drug aspirin and crude extract showed significant ($p<0.001$) analgesic activity at 30th, 60th and 90th min.

Table 3: Analgesic activity of crude extract and standard.

Treatment	Tail flick latency (Sec±SD) after different intervals (min)						
	0	30	60	90	120	150	180
Normal (saline)	3.013±0.0351	3.217±0.0289	3.167±0.0289	3.053±0.0451	3.117±0.0289	3.233±0.02887	3.250±0.0500
Standard (Aspirin, 200mg/kg b.w)	3.283±0.0289	3.653±0.0351 ^{***}	4.577±0.2040 ^{***}	5.510±0.0656 ^{***}	5.880±0.1253 ^{***}	5.193±0.1721 ^{***}	5.093±0.0902 ^{***}
Extract (100mg/kg b.w)	3.020±0.0265	3.293±0.0404 ^{ns}	3.430±0.0265 [*]	3.203±0.0503 [*]	3.133±0.02887 ^{ns}	3.067±0.02887 ^{ns}	3.033±0.0289 ^{**}
Extract (200mg/kg b.w)	3.233±0.0764	3.633±0.02889 ^{***}	3.767±0.0288 ^{***}	3.533±0.0764 ^{***}	3.427±0.0252 ^{**}	3.227±0.0462 ^{ns}	3.203±0.0551 ^{ns}

All the values are mean±SD, One way analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparison Test. ^{*} $p<0.05$, ^{**} $p<0.01$, and ^{***} $p<0.001$ as comparison to Normal group (Saline). ns= not significant ($p>0.05$)

Antipyretic activity

Table 4 shows results of the antipyretic effect of the crude extract. Eighteen hours after subcutaneous injection of brewer's yeast, a significant increase in rectal temperature was observed in control animals. Treatment with standard drug Aspirin significantly ($p<0.001$) reduced fever induced by brewer's yeast right from the 1 hour after oral administration and decreased

the rectal temperature of rats at all time tested. Administration of extract showed dose dependent reduction in the rectal temperature but the effect was very negligible on comparing with standard drug. Administration of crude extract (100 and 200 mg/kg b.w) showed significant ($p<0.001$) antipyretic activity at 3rd and 5th hour.

Table 4: Antipyretic activity of crude extract and standard.

Treatment	Rectal temperature at different intervals (mean±SD)			
	-18 hr	1 hr	3 hr	5 hr
Control (saline, 2ml/kg b.w)	36.86±0.06558	37.57±0.0874	37.82±0.02517	37.63±0.02517
Standard (Aspirin, 200mg/kg b.w)	36.82±0.06429 ^{ns}	37.06±0.0361 ^{***}	36.80±0.0500 ^{***}	36.62±0.1253 ^{***}
Extract (100mg/kg b.w)	36.90±0.05000 ^{ns}	37.63±0.0252 ^{ns}	37.51±0.0656 ^{***}	37.21±0.07095 ^{***}
Extract (200mg/kg b.w)	36.77±0.07211 ^{ns}	37.52±0.0251 ^{ns}	37.25±0.0551 ^{***}	37.20±0.04509 ^{***}

All the values are mean±SD, One way analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparison Test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$ as comparison to Normal group (Saline). ns= not significant ($p>0.05$)

CNS depressant activity

The CNS depressant activity of different concentrations of crude extract was evaluated by spontaneous locomotor activity in digital Actophotometer. Crude extract has shown a dose dependent reduction in locomotor score revealing CNS depressant activity. However, the depressant

activity of crude extract was lesser when compared with standard drug diazepam ($p<0.001$) which showed a marked reduction in locomotor score (Table 5). Administration of crude extract (200 mg/kg b.w) showed significant ($p<0.01$) CNS depressant activity at 135 minutes.

Table 5: CNS depressant activity of crude extract and standard.

Treatment	Locomotor score (mean±SEM) at different time intervals (min)			
	0	45	90	135
Normal (saline)	419.3±5.132	419.0±3.606	418.7±3.215	418.0±2.646
Standard (Diazepam, 5mg/kg b.w)	411.0±3.606 ^{ns}	248.3±7.638 ^{***}	177.0±10.82 ^{***}	140.0±5.000 ^{***}
Extract (100mg/kg b.w)	426.4±2.517 ^{ns}	421.0±6.557 ^{ns}	410.3±5.508 ^{ns}	404.0±8.544 ^{ns}
Extract (200mg/kg b.w)	427.7±2.517 ^{ns}	410.0±5.000 ^{ns}	402.0±3.464 [*]	387.7±9.292 ^{**}

All the values are mean±SD, One way analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparison Test. * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ as comparison to Normal group (Saline). ns= not significant ($p>0.05$).

DISCUSSION

Inflammation is a biological process and is a local response of living tissues to injury. It occurs by infections, chemicals and thermal and mechanical injuries. At the site of tissue injury, an increase in blood vessel wall permeability followed by migration of leucocytes leads to edema formation during inflammation. It is a body defence response in order to eliminate or limit the spread of injurious agent. However, excessive inflammation leads to several acute and chronic human diseases (Deng *et al.*, 2011; Kaushik *et al.*, 2012; Ambedkar *et al.*, 2012). Carrageenan-induced rat paw edema model is a suitable model for determining anti-inflammatory property. It is frequently employed to screen antiedematous effect of compounds. When injected, carrageenan produces inflammation by the release

of inflammatory and pro-inflammatory mediators such as prostaglandins (PG), leucotrienes, histamine, bradykinin, TNF- α etc. The course of acute inflammation is biphasic. In the first phase, the release of histamine, serotonin and kinins occur after administration of phlogistic agent. In the second phase, release of PG like substances occurs in 2-3 hours. PG are the main mediators responsible for acute inflammation and are produced by the activity of cyclooxygenases (COX). The second phase is sensitive to clinically relevant NSAIDs such as aspirin, indomethacin, diclofenac, ibuprofen and others. These drugs inhibit the production of PG and thereby reduce inflammation (Rosenbloom and Craven, 1983; Brooks and Day,

1991; Botting, 2006; Bhukya *et al.*, 2009; Ambedkar *et al.*, 2012).

Earlier studies have shown *in vitro* and *in vivo* anti-inflammatory activity of actinomycetes. Shoshiro *et al.* (1969) isolated a new anti-inflammatory proteinase, kinonase BI from *Streptomyces kinoluteu* and studied its effect in carrageenan-induced edema. Arita *et al.* (1974) isolated L-arabinans, anti-inflammatory polysaccharides, from *Streptomyces fradiae* and found anti-carrageenan abscess activity. Lee *et al.* (1997) isolated dianemycin from *Streptomyces* species MT2705-4 and showed its anti-inflammatory activity in mouse ear edema induced by croton oil or arachidonic acid. Graziani *et al.* (2005) isolated Phaeochromycin A and C from *Streptomyces sphaeochromogenes* LL-P018 and found them as weak inhibitors of MAPKAP kinase-2. Park *et al.* (2006) isolated *Streptomyces macrosporeus* and *Streptomyces spraeocox* from marine environment and showed their potent anti-inflammatory effect in phorbol-ester-induced mouse ear edema. Taechowisan *et al.* (2007 and 2007b) showed *in vitro* anti-inflammatory activity of 4-aryl coumarins isolated from endophytic *Streptomyces aureofaciens* CMUAc130 in murine macrophage RAW264.7 cells. Taechowisan *et al.* (2009) studied *in vitro* anti-inflammatory action of lansai A-D produced from *Streptomyces* sp. SUC1 in murine macrophage RAW264.7 cells. In the present study, crude extract of isolate PO-178 has shown anti-inflammatory activity milder than that of standard drug. Similar results were observed in a previous study by Gautham and Onkarappa (2013) where the extract exhibited moderate anti-inflammatory effect when compared to standard drug.

It has been well established that pain cannot be directly monitored in animals and it can only be studied by examining the animal responses to certain stimuli. Pain is an unpleasant sensory and emotional experience associated with potential tissue damage. The action of chemical mediators such as PG, leucotrienes, peptides, acetylcholine, cytokines, nitric oxide, serotonin etc, produced or released during tissue injury or exogenous irritants (*viz.*, formalin, acetic acid) are able to stimulate nociceptors, induce pain and are responsible for multiplicity of events that occur during pain transmission, in both peripheral and central nervous system (Le Bars *et al.*, 2001; Bhaskar and Balakrishnan, 2009; Hossain *et al.*, 2011; Kabir *et al.*, 2012). Tail flick method is one of the widely used models to study analgesic activity of drugs. The method is distinguished by its tendency to respond to the pain stimuli conducting through neuronal pathways as tail immersion mediates a spinal reflex

to nociceptive stimuli (Chapman *et al.*, 1985; Kabir *et al.*, 2012). An analgesic is a compound which can act on central or peripheral nervous system of an individual. The agents acting on peripheral nervous system acts by blocking the generation of impulses at chemoreceptor site of pain, whereas analgesics acting on central nervous system not only rises the threshold for pain but also suppress the anxiety and apprehension (Shreedhara *et al.*, 2009; Semwal *et al.*, 2011). The crude extract of isolate PO-178 in this study displayed weak analgesic activity when compared to standard drug. Similar findings were observed in a study by Gautham and Onkarappa (2013). In an early study, Pimprinine, an extracellular alkaloid isolated from the culture filtrate of *Streptomyces* CDRIL-312 inhibited effectively tremorine-induced tremors and analgesia in mice (Naik *et al.*, 2001).

Fever is a pathophysiological condition characterized by an elevation of core body temperature above normal (37°C). It results from the interaction of central nervous and immune systems and is a result of injury, infection, tumor and inflammation. The elevation of body temperature during such conditions results from the pyrogen induced upward resetting of thermoregulatory set point. Many of the exogenous substances are known to evoke fever in animal models. These pyrogens, on injection into experimental animals, induce the production of pro-inflammatory cytokines (e.g., IL-1 β , IL-6, IFN- α and TNF) which stimulate release of local PG (produced by the activity of COX) that leads to elevation of body temperature (Kozak *et al.*, 1998; Dalal and Zhukovsky, 2006). Yeast induced pyrexia in rats is a suitable and sensitive model for evaluating antipyretic effects of compounds. Yeast induces both TNF- α and prostaglandin synthesis. Antipyretics such as acetyl salicylic acid (ASA) and other NSAID reduce fever by suppressing inflammatory messages at both peripheral sites of tissue inflammation and within central nervous system thermoregulatory sites. These drugs suppress production of pyrogenic cytokines such as TNF- α and IL-1 β , while lowering the thermoregulatory set-point by blocking COX production of PGE₂ (Kozak *et al.*, 1998; Chomchuen *et al.*, 2010). The extract administration resulted in lowering of temperature but the decrease of temperature was not comparable with standard antipyretic which showed significant antipyretic effect. Earlier study by Gautham and Onkarappa (2013) also showed similar result.

Quality of life of mankind has improved due to advances in science and technology; however, stress in modern life is responsible for onset of several psychiatric disorders. An extensive research

in psychopharmacology came up with a wide range of drugs. Benzodiazepines (e.g., diazepam, nitrazepam, lorazepam and alprazolam etc.) are the frequently prescribed synthetic drugs for anxiety, depression, epilepsy and insomnia (Garg *et al.*, 2011). Spontaneous locomotor activity is a measure of excitability of CNS which correlates well with the drug effects in an individual. Locomotor activity is considered as an index of alertness. The CNS depressant effect of compounds can be evaluated by locomotor activity in experimental animal models. A reduction in the motor activity is considered to be depressant function of the drug. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in CNS and different drugs act via GABA (Khatun *et al.*, 2011; Raju *et al.*, 2011; Sugavanam *et al.*, 2012; Kazmi *et al.*, 2012). In this study, the crude extract of isolate PO-178 showed reduction in locomotor activity but the activity was lower when compared to standard drug.

CONCLUSION

The present study showed dose dependent pharmacological activities of crude extract of *Streptomyces* species PO-178 recovered from a rhizosphere soil of Agumbe, Karnataka, India. Though, the pharmacological activities exhibited by the extract were lesser than that of reference drugs, it is clear that the extract possess components having pharmacological significance. Further studies are under progress to isolate pharmacologically active compounds from the crude extract of PO-178 and to determine their bioactivities.

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