Screening Of Antibacterial And Antioxidant Activities Of *Streptomyces* Species Isolated From Western Ghats Region Of Karnataka

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ABSTRACT
Soil *Actinomycetes'* secondary metabolites possess wide range of biologically active compounds and are the potential source of novel bioactive metabolites. Currently *Actinomycetes* emerged as an important source for bioactive natural products with chemical diversity. In this study, *Actinomycetes* strain was isolated from the Western Ghats region of Karnataka and characterized by the 16S rRNA gene sequence. The strain was identified as *Streptomyces* species. The strain was characterized for antioxidant and antibacterial activity. The isolated strain exhibited broad spectrum of antibacterial activity against Gram positive bacteria (*S. aureus* and *B. subtilis*) and Gram negative bacteria (*P. aeruginosa* and *E. coli*). The ethyl acetate extract showed 86.6 % and 98 % of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2´-azinobis-(3-ethylbenzothizoline-6-sulfonic acid) radical scavenging assay at 11 mg/ml and 8.25 mg/ml respectively. However, purification and further characterization of bioactive metabolites from *Streptomyces* species is required for their optimum utilization towards antibacterial and antioxidant purposes.

INTRODUCTION
Actinomycetes are a group of prokaryotic organisms belonging to subdivision of the Gram-positive bacteria phylum. Most of them are grouped under subclass Actinobcteridae, order Actinomycetales. The members of this order are characterized by the high G+C content which accounts about >55 mol% in their DNA (Stackebrandt *et al*, 1997). These are one of the riches source of important natural products especially antibiotics. So far, approximately 10,000 antibiotics were reported, and almost half of them are produced by soil actinomycetes *Streptomyces* (Lazzarini *et al*, 2000). Bioactive metabolites produced from Streptomyces have high commercial value and important applications in human as well as livestock medicine and in agriculture (Watve *et al*, 2001). The biological active compounds produced by actinomycetes are being used as antibiotics, immunosuppressant, extracellular hydrolytic enzymes, plant growth promoters, lytic enzymes, herbicides, insecticides, antitumor agents and siderophores. Approximately World's 80% antibiotics are obtained by actinomycetes, majorly from genus Streptomyces and Micromonospora (Pandey *et al*, 2004).
Chemotherapy using antimicrobial agents has been a leading cause for the rise of average life expectancy in the past Century. However, infectious agents that have become resistant to antibiotic drug therapy are an increasing public health problem. Currently, about 70% of the bacteria which cause infections to humans in hospitals are resistant to at least one of the drugs most commonly used for treatment of infection caused by them. Certain organisms are resistant to all approved antibiotics and infections caused by these organisms can only be treated with experimental and potentially toxic drugs. The increase in antibiotic resistance of bacterial will cause community acquired infections and also causes morbidity (Bisht *et al*, 2009). Development of antibiotic resistance in bacteria, as well as economic incentives has resulted in identification new antibiotic strains of actinomycetes in order to maintain a pool of effective drugs at all times (Stephen & Kennedy, 2011).
Free radicals and oxidants play a dual role as both toxic and beneficial compounds, since they can be either harmful or helpful to the body. It has been implicated in the development of many human diseases. A few of them include arthritis, inflammatory diseases, kidney diseases, cataracts, inflammatory bowel disease, colitis, lung dysfunction, pancreatitis, drug reactions, skin lesions and aging (Lakhtakia *et al*, 2011). They are produced either from normal cell metabolism *in situ* or from external sources (like pollution, cigarette smoke, radiation and medication). When an overload of free radicals cannot gradually be destroyed, their accumulation in the body generates a phenomenon called oxidative stress. This process plays a major part in the development of several chronic and degenerative illnesses (Pham-Huy et al., 2008).
Moreover, it has been shown that antioxidants and free radical scavengers are crucial in the prevention of pathologies, in which reactive oxygen species (ROS) or free radicals are implicated (Rathna Kala and Chandrika, 1993). Synthetic antioxidants have been used in stabilization of foods (Hajji *et al*, 2010). But their use is being restricted nowadays because of their toxic and carcinogenic effects (Kekuda *et al*, 2010). Thus, interest in finding natural antioxidants, without any undesirable effects has increased greatly (Rechner *et al*, 2002).Oxidative stress is ultimately involved in endothelial dysfunction, a condition which is evident in adults suffering from various cardiovascular diseases including thalassemia (Shinar and Rachmilewitz, 1990; Hebbel, 1990; Grinberg et al, 1995). Antioxidant and other supportive therapies protect red blood cells against oxidant damage (Filburn, 2007; Kukongviriyapan et al, 2008). It is well known that the generation of free radicals happens because of microbial infection which leads to DNA damage (Maeda and Akaike, 1998). In the present study, we point out biological activities of secondary metabolites produced by actinomycetes in effort to combat infectious diseases.

MATERIALS AND METHODS

Soil sampling and pretreatment: Soil samples were collected from forest areas of Western Ghat region of Karnataka and Kerala. Each collection will be made from 10-15 cm depth of the soil. These were air-dried for 1 week, crushed and sieved. The sieved soils were then used for actinomycetes isolation.

Isolation of Actinomycetes: Each sample were air-dried at room temperature (about 25 ± 2 °C) for 5 to 7 days and then ground in a mortar and used for further study. Dilution technique was used to isolate actinomycetes from the soil samples. Soil samples were serially diluted with sterile 0.9% (w/v) saline solution to give final concentrations of 10^{-2} and 10^{-3}. Using a sterile glass rod, the soil suspensions were spread onto sterile Glycerol Asparagine Agar (GAA). All plates were incubated at 28 °C for 7days.

Characterization of actinomycetes: The isolated actinomycetes were characterized by morphological and biochemical methods. Morphological characterization was done by macroscopic and microscopic methods. Microscopic characterization was carried out by cover slip culture method (Kawato and Sinobu, 1979) by observing mycelium structure, color and arrangement of conidiospore and arthospore on the mycelium through the oil immersion (100X). The observed morphological characters were compared with Bergey's manual of determinative bacteriology and the isolate was characterized.

Screening for antimicrobial activity: Antimicrobial activities of isolates were tested preliminarily by cross streak method on Nutrient Agar plates (Egorov, 1985). Actinomycetes isolates were streaked across diameter on nutrient agar plates. After incubation at 28 °C for 6 days, 24 hrs cultures of test organisms were streaked perpendicular to the central strip of actinomycetes culture. All plates were again incubated at 37 °C for 24 hrs and zone of inhibition was measured.

Extraction of antimicrobial compounds: The antibacterial compounds were isolated from the actinomycetes isolates by following Westley *et al,* 1979 and Liu *et al,* 1986 method with slight modifications. The selected antagonistic actinomycete isolates were inoculated into Yeast Extract Malt Extract (YEME) broth, and incubated at 28 °C in a shaker (200-250 rpm) for seven days. After incubation the broths were filtered through Whatman No.1 filter paper. To the culture filtrate, equal volume ethyl acetate was added and shaken vigorously for 1 hr for the extraction of antimicrobial compound. The ethyl acetate extract was evaporated to dryness in rotary flash evaporator. The extracts were tested for their antimicrobial activity by well diffusion method (Sen *et al,* 1995) against the test pathogens. The antimicrobial efficacy was assessed by measuring the zone of inhibition after 24 hrs of incubation.

DETERMINATION OF ANTIOXIDANT ACTIVITY:

DPPH scavenging activity: DPPH scavenging activity of the EA extract was determined by the method of Manjunatha *et al,* 2013 with slight modifications. 100 µM methanol DPPH solutions were mixed with different concentrations of EA extract. Ascorbic acid (standard) was used as positive control. The tubes were incubated at room temperature in dark for 30 min and the optical density was measured at 517 nm. The absorbance of the control, DPPH• alone (containing no sample), was also noted (Khalaf NA, 2008). The percentage of radical scavenging activity of the extract against the stable DPPH• was calculated using following equation:

% DPPH• Scavenging activity = $[A_{control} - A_{sample}]/ A_{control}$ X 100

ABTS scavenging activity: To generate ABTS radical cation, 50ml of 2mM ABTS and 0.3mL of 17mM Potassium persulfate were mixed together and incubated in the dark for 12-16 h to develop Prussian blue colored $ABTS^{\cdot+}$ solution which has an absorption maxima at 734nm [Re et al., 1999]**.** To determine scavenging activity of extracts of different concentrations was added to 1.6ml of $ABTS^{\cdot+}$ solution. The absorbance was measured at 734nm after 20 minutes at room temperature. All readings were performed in triplicates and the free radical scavenging activity was calculated from equation:

% ABTS Scavenging activity = $[A_{control} - A_{sample}]/ A_{control}$ X 100

Molecular characterization of actinomycetes strain by 16S rDNA sequence: The actinomycetes strain was grown at 30 ˚C for 5 days in shake flasks, containing 100 ml of ISP 2 medium (4 g/l yeast extract, 10 g/l malt extract and 4 g/l glucose). Mycelium was obtained by centrifugation and washed twice with bi-distilled water. Approximately, 200 mg of mycelium were used for genomic DNA extraction as follows: the sample was dispersed in 800 ml of the lysis solution (100mM Tris-HCl, pH 7.4, 20mM EDTA; 250mM NaCl; 2% SDS; 1 mg/ml; lysozyme; 100 ml H_20), added with5 ml of RNase (50 mg/ml) and incubated at 37 ˚C for 60 min. Then 10 ml of proteinase K solution (20 mg/ml) were added, and the lysis solution was reincubated at 65 ˚C for 30 min. The lysate was extracted two times with an equal volume of phenol, centrifuged and then re-extracted with chloroform (v/v) to remove residual phenol. DNA was precipitated by adding NaCl (at a final concentration of 150 mM) and 2 volumes of 95%cool ethanol. After centrifugation, the DNA was cleaned with 50 ml of ethanol 70%, centrifuged, and then re-suspended in 50 ml of TE buffer (10mM Tris-HCl, pH 7.4; 1mM EDTA, pH 8.0). The DNA purity and quantity were checked by spectrophotometer at 260 and 280 nm.

PCR amplification of the 16S rDNA of actinomycetes strain was performed using two primers: 27f (50-AGAGTTTGATCCTGGCTCAG-30) and 1492r (50-GGTTACCTTGTTACGACTT-30). The 16S rDNA was amplified by PCR using Promega kit. The final volume of reaction mixture of 50 ml contained 1X PCR buffer (10mM Tris-HCl, 50mM KCl, pH 9.0 at 25 ˚C), 1.5mM $MgCl_2$, 200 mM of each dNTP, 1mM of each primer, 1.25 U of Taq DNA polymerase and 500ng of template DNA. The amplification was performed according to the following profile: an initial denaturation step at 98 ˚C for 3 min, after which *Taq DNA polymerase* was added, followed by 30 amplification cycles of 94 ˚C for 1 min, 52 ˚C for 1 min and 72 ˚C for 2 min, and a final extension step of 72 ˚C for 10 min. The PCR product was detected by agarose gel electrophoresis and was visualized by UV fluorescence after ethidium bromide staining.

The PCR products obtained were submitted to Genome Express for sequence determination. The same primers as above and an automated sequencer were used for this purpose. The sequence determined was compared for similarity level with the reference species of bacteria contained in genomic database banks, using the NCBI Blast available at the ncbi-nlm-nih.gov Web site.

RESULTS

Cultural characteristics and Microscopic study:

The actinomycetes isolate was named as SBR1 (1). The colony characteristics of the isolate were studied on the GAA, Inorganic salt-starch agar (ISSA) and Oat Meal Agar (OMA) media. The growth was good on GAA and ISSA whereas moderate growth was observed on OMA. The color of substrate and aerial mycelium varied in different media. The organism produced colonies of 3 mm diameter, white colony with secondary black metabolites, blue pigmentation, greys at centre and hard on GAA with inhibition of neighboured colony, on oat meal agar it produced colony with poor growth, 3 mm diameter, cream colored spores with entire and umbonate margin, on ISSA colonies were light cream colored entire, elevated margin, good growth, outer periphery shows ring of spores. The organism was gram positive and positive for starch hydrolysis, casein hydrolysis and lecithinase and lipase production, negative for gelatine hydrolysis, citrate utilization. Based on morphological biochemical and on characterization of the organism it was shown to be *Streptomyces* sp.

Table 1: Cultural characteristics of SBR 1(1)

Media	Growth	Substrate mycelium	Aerial mycelium	Diffusible pigment
GAA	Good	Grey	White	Blue
OMA	Moderate	Grey	Cream	---
ISSA	Good	Grey	White	---

Preliminary antibacterial activity and MIC: A total of 7 isolates were recovered from the soil samples. All the isolates were subjected for cross streak method in order to assess antagonistic property against gram negative and gram positive bacteria. Presence of clear zone or reduced growth of test bacteria near the growth of actinomycetes was considered as positive for antagonistic activity. All the isolates were potent enough to inhibit at least one of the test bacteria. SBR 1(1) showed prominent inhibition of test bacteria in cross streak technique, so it was selected for further study.

The antibacterial efficacy of the ethyl acetate extract of SBR 1(1) is studied using 3 Gram positive and 2 Gram negative bacteria. It was observed that extract was having broad spectrum antibacterial activity inhibiting both Gram positive and Gram negative bacteria. The study of MIC has shown that MIC of ethyl acetate extract was <25 µg for Gram positive bacteria (*B. subtilis* and *S. aureus*) and 75 µg for Gram negative bacteria (*E. coli*) (Table 2 & 3; Figure 1).

Table 2: Antibacterial activity of ethyl acetate extract of isolate SBR 1(1)

Test Bacteria	Zone of inhibition in cm		
	SBR 1(1)	Standard (Streptomycin 10 µg))	DMSO
B. cereus	2.6	2.0	0.0
E. coli	2.0	1.1	0.0
B. subtilis	2.7	1.9	0.0
S. aureus	2.5	2.4	0.0
P. putida	1.0	1.3	0.0

Table 3: Minimum Inhibitory Concentration (MIC) of ethyl acetate extract of isolate RHC-1

Test bacteria	MIC for ethyl acetate extract of isolate SBR 1(1) Zone of Inhibition in cm				
Concentration	Standard 10 µg	25 µg	50 µg	75 µg	100µg
E. coli	2.5	-	-	1.1	1.3
B. subtilis	1.2	1.5	1.7	1.8	1.9
S. aureus	1.8	1.2	1.4	1.5	1.7

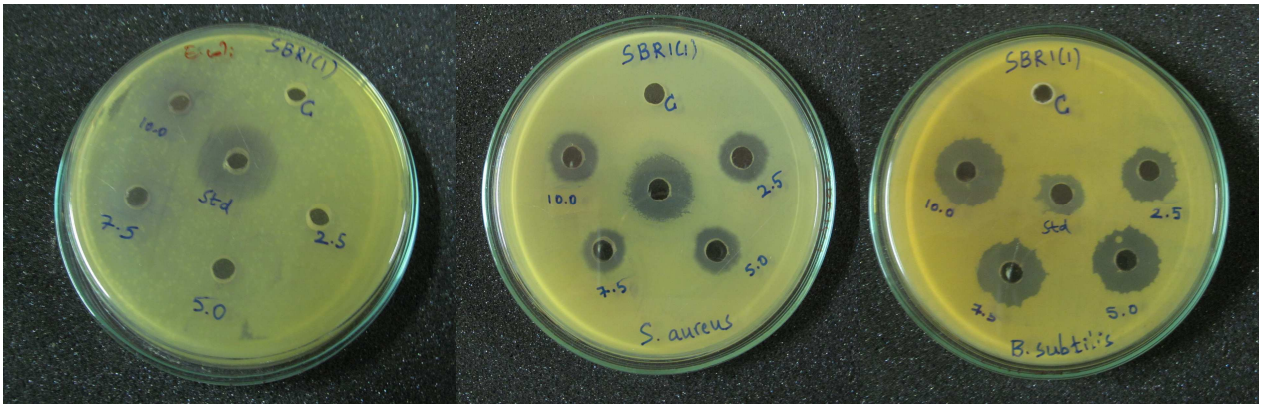

Figure 1: MIC for ethyl acetate extract of RHC-1

Screening of antioxidant activity:

DPPH Radical Scavenging Assay: Study of DPPH scavenging ability of the extract showed that ethyl acetate extract has free radical scavenging ability but the activity of extract is less when compared with the ascorbic acid standard. DPPH scavenging studies have revealed that the extract possesses 86.6% scavenging ability at a concentration of 11 mg/ml and the DPPH free radical scavenging ability of the extract was dose dependent (Figure. 2).

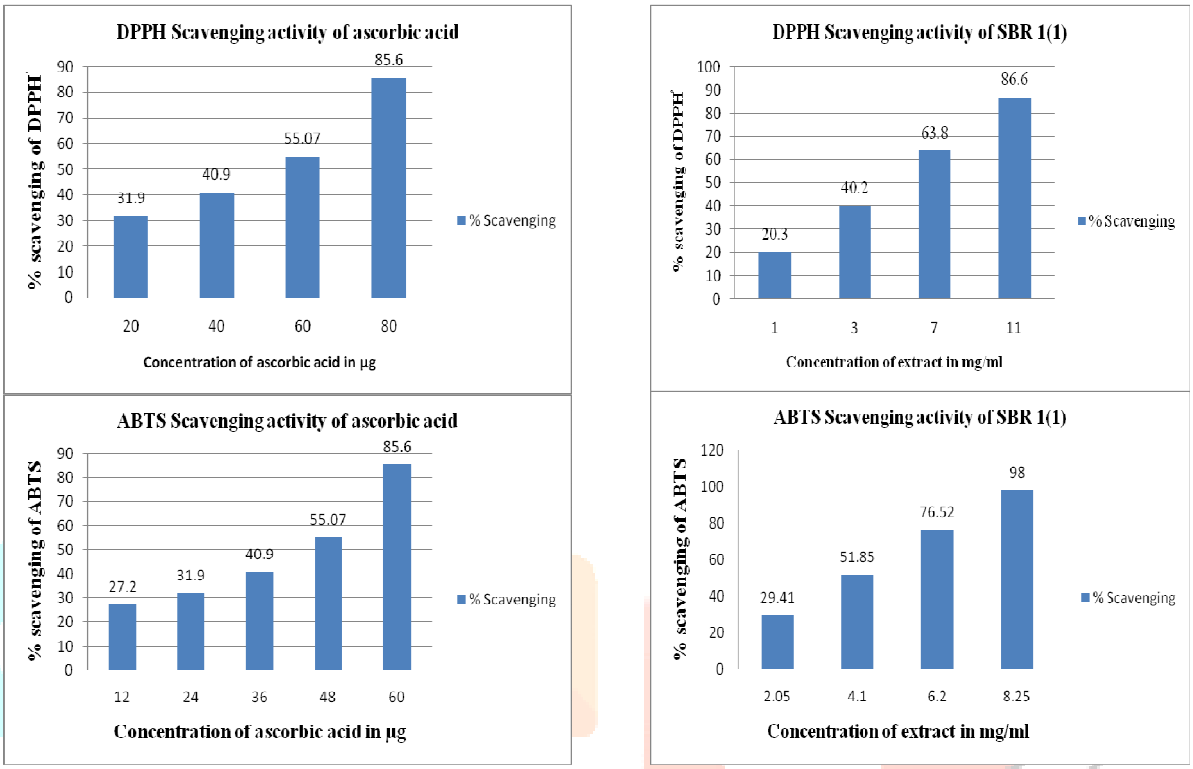

Figure 2: Antioxidant activity of ethyl acetate extract of SBR 1(1)

Assay of ABTS scavenging activity: ABTS scavenging studies have revealed that the ethyl acetate extract of SBR 1(1) is capable of scavenging the ABTS radical efficiently. But ability of the extract is less when compared with ascorbic acid standard. The ethyl acetate extract had 98% scavenging activity at a concentration of 8.25 mg/ml. Even ABTS free radical scavenging ability of the extract is also dose dependent (Figure 2).

Molecular characterization by 16S rDNA sequencing: Through 16S rDNA sequence analysis, an amplified fragment of 747 bp was obtained and compared with sequences of the reference species of bacteria contained in genomic database banks. The similarity level ranged from 96.3% to 97.8% with Streptomyces species 13636G having the closest match. The phylogenetic tree obtained by applying the neighbor joining method is illustrated in Fig. 8. The sequence results for SBR 1(1) as follows:

GGCGTTTTTTCGCTCTCAGCGTCAGTAATGGCCCAGAGATCCGCCTTCGC
CACCGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATT
CCGATCTCCCCTACCACACTCTAGCTAGCCCGTATCGAATGCAGACCCGG
GGTTAAGCCCCGGGCTTTCACATCCGACGTGACAAGCCGCCTACGAGCTC
TTTACGCCCAATAATTCCGGACAACGCTTGCGCCCTACGTATTACCGCGG
CTGCTGGCACGTAGTTAGCCGGCGCTTCTTCTGCAGGTACCGTCACTTGC
GCTTCTTCCCTGCTGAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCA
CGCGGCGTCGCTGCATCAGGCTTTCGCCCATTGTGCAATATTCCCCACTG
CTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGGTC
GCCCTCTCAGGCCGGCTACCCGTCGTCGCCTTGGTAGGCCATTACCCCAC
CAACAAGCTGATAGGCCGCGGGCTCATCCTTCACCGCCGGAGCTTTCAAC
CCCGTCCCATGCGGAACAGAGTATTATCCGGTATTAGACCCCGTTTCCAG
GGCTTGTCCCAGAGTGAAGGGCAGATTGCCCACGTGTTACTCACCCGTTC
GCCACTAATCCACCACCGAAGCGGCTTCATCGTTCGACTTGCATGTGTTA
AGCACGCCGCCAGCGTTCGTCCTGAGCTGTTTTAAAAACTTAAAAAC.FASTA

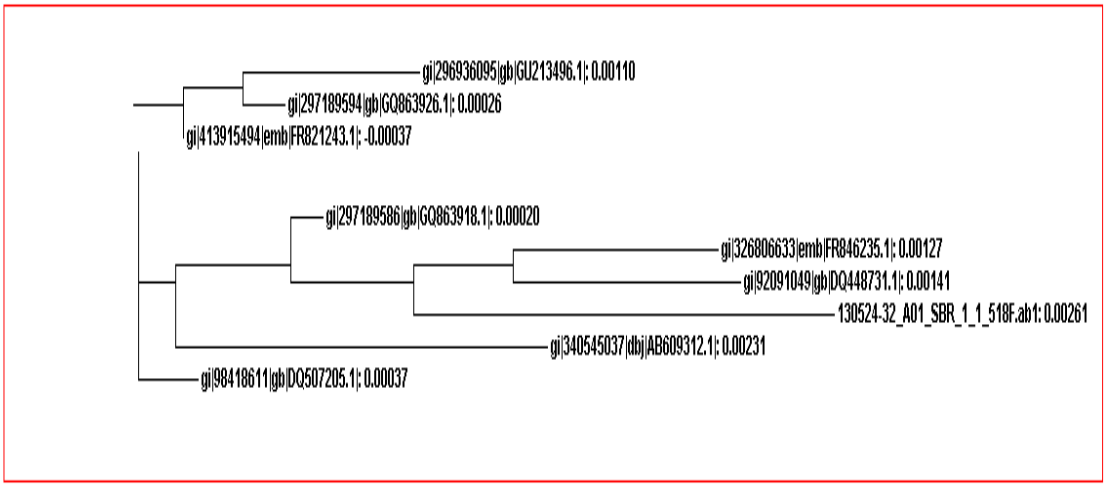

Figure 3:

Phylogram based on 16S rDNA sequences showing the relations between strain SBR 1(1) and type species of the genus *Streptomyces*

DISCUSSION

Western Ghats of India are the less explored regions for actinomycetes diversity and this study has shown that Western Ghats contain diverse species producing the antibiotic. The plant root primarily determines the nature and abundance of the rhizosphere soil microflora, when conditions affect root growth or metabolism, it will be reflected in quantitative and qualitative changes in microbial populations of rhizosphere. Conversely, microbial communities can affect rooting patterns, stimulate and promote plant root growth (e.g., release of hormones, neutralizing toxic substances, etc.), and influence the supply of available nutrients for plant uptake. Microbial turnover of rhizo-deposits plays an important role in carbon flow through soils [Rathna Kala and Chandrika, 1993; Rechner et al, 2002; Behal, 2003; Kekuda et al., 2010]. Actinomycetes are an important class of bacteria and constitute one of the important groups of the rhizosphere microflora. Members of *Streptomyces* are most abundant in soil and accounts for about 90% of actinomycetes isolated from soil [Shinar and Rachmilewitz, 1990; Rathna Kala and Chandrika, 1993; Khamna et al., 2009;]. In the present study, we have recovered 07 actinomycetes isolates from a rhizosphere soil collected at Western Ghats of Karnataka. All the isolates were subjected to primary screening for antibacterial activity by cross streak method. This dual culture method is widely used to screen the ability of actinomycetes strains to produce antimicrobial metabolites [Shinar and Rachmilewitz, 1990, Hebbel et al., 1990; Kekuda et al., 2012]. Out of 07 isolates, all the 7 isolates have shown inhibition of all test bacteria. We have selected a potent isolate SBR 1(1) which inhibited both gram positive and Gram negative bacteria. The characterization of SBR 1(1) revealed that the isolate is a *Streptomyces* species. The life cycle of Streptomycetes provides 3 distinct features for microscopic characterization namely vegetative mycelium, aerial mycelium baring chains of spores and the characteristic arrangement of spores and the spore ornamentation. The latter two features produce most diagnostic information [Kukongviriyapan et al., 2008, Filburn et al., 2007]. Details on cultural and microscopic characteristics together with biochemical properties assisted the researchers to classify actinomycetes as members of the genus *Streptomyces*. Many studies have been carried out where the actinomycetes isolates were identified as species of *Streptomyces* based these properties or characteristics (Ceylan *et al*, 2008; Pham-Huy *et al*, 2008; Grinberg, 1995; Maeda and Akaike, 1998; Jeffrey *et al*, 2007; Sahin and Ugur, 2003). In the present study, the cultural and microscopic characteristics of the isolate SBR1(1) were consistent with its classification as a member of the genus *Streptomyces*.

The members of *Streptomyces* can be distinguished from other sporing actinomycetes based on morphology and hence morphology plays an important role in the Antimicrobial agents play an indispensable role in decreasing morbidity and mortality associated with infectious diseases caused by bacteria, fungi, viruses and parasites. However, selective pressure exerted by the use of antimicrobial drug became the major driving force behind the emergence and spread of drug-resistance pathogens. In addition, resistance has been developed in pathogens after discovery of major class of antimicrobial drugs, varying in time from as short as 1 year in case of penicillin to >10 years in case of Vancomycin (Jeffrey *et al*, 2007). This alarming situation necessitated search of new bioactive compounds capable of acting against pathogens in particular drug resistant pathogens. It is well known that microorganisms, in particular bacteria and fungi are an unexhaustible source of natural compounds having several therapeutic applications. In the present study, it was found that both Gram positive bacteria and Gram negative bacteria were susceptible to high extent (Singh *et al*, 2006; Pandey *et al*, 2004).

Free radicals are chemical species containing one or more unpaired electrons that make them highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. In recent years much attention has been devoted to natural antioxidant and their association with health benefits (Ali *et al*, 2008).

DPPH is a stable, organic and nitrogen centered free radical, it has absorption maximum band around 515-528 nm (517 nm) in alcoholic solution. It accepts an electron or hydrogen atom and becomes a stable diamagnetic molecule. Though a number of *in vitro* assays have been developed to evaluate radical scavenging activity of compounds, the model of scavenging of the stable DPPH radical is one of the widely used protocols. The effect of antioxidants on scavenging DPPH radical is due to their hydrogen donating ability. In this assay, the antioxidants reduce the purple colored DPPH radical to a yellow colored compound diphenylpicrylhydrazine, and the extent of reaction will depend on the hydrogen donating ability of the antioxidants. In the

present study, a decrease in the absorption of DPPH solution in the presence of various concentrations of ethyl acetate extract was measured at 517 nm. It was observed that the radical scavenging activities of extract and ascorbic acid increased on increasing concentration. The scavenging effect of ethyl acetate extract was much lesser when compared with ascorbic acid. Although the scavenging abilities of extract was lesser, it was evident that the extracts showed hydrogen donating ability and therefore the extract could serve as free radical scavengers, acting possibly as primary antioxidants (Ali *et al*, 2008).

The ABTS radical cation decolorization assay is one of the methods for the screening of the antioxidant activity (Re *et al*, 1999). Therefore, the ABTS radical scavenging activity of the ethyl acetate extract was determined. The results indicated that the ethyl acetate extract showed a lesser tendency to decay ABTS radicals at low concentrations of reaction than at high concentrations (Fig. 6). The extract scavenged ABTS radicals in a concentration-dependent manner. The ABTS antioxidant assay, also known as the Trolox equivalent anti-oxidant capacity (TEAC) assay, assesses the total radical scavenging capacity of the plant extracts. This is determined through the ability of these extracts to scavenge the long-lived specific ABTS radical cation chromophore in relation to that of Trolox, the water-soluble analogue of vitamin E [Zhong et al., 2011].

The antioxidant activities of recognized antioxidants have been attributed to various mechanisms, including the prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of hydrogen abstraction, and radical scavenging [Diplock, 1997].

According to the results of the DPPH radical-scavenging assay and ABTS radical-scavenging assay, it was found that the isolate SBR 1(1) had antioxidant abilities.

CONCLUSION:

In this study, the results have suggested that actinomycetes isolate RHC-1 has closely related with Streptomyces spp. 13636G, having the capability to show antimicrobial and antioxidant activity and the present study highlights the necessity of further researches towards the goal of searching for novel bioactive compounds from less explored regions like Western Ghats.

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REFERENCES:

[1] Amit Pandey, Imran Ali, Kailash Singh Butola, TanushriChatterji, Vidyottma Singh. 2004. Isolation and characterisation of actinomycetes from soil and evaluation of antibacterial activities of actinomycetes against pathogens. ActaBiol Med Exper, 15:113-115

[2] Behal V. 2003. Alternative sources of biologically active substances. Folia Microbiol (Praha); 48:563-71.

[3] Bisht R, Katiy ARA, Singh R, Mittal P. 2009. Antibiotic Resistance –A Global Issue of Concern. Asian Journal of Pharmaceutical and Clinical Research. 2 (2); 34-39.

[4] Ceylan O, Okmen G and Ugur A.2008.Isolation of soil Streptomyces as source antibiotics active against antibiotic-resistant bacteria.EurAsian Journal of BioSciences.2, 73-82.

[5] Diplock, AT. 1997. Will the 'good fairies' please prove to us that vitamin E lessens human degenerative disease. Free Rad.Res.27 (5),511-532.

[6] Filburn CR, Kettenacker R, Griffin DW. 2007. Bioavailability of a silybin-phosphatidylcholine complex in dogs. J Vet PharmacolTher; 30:132-8.

[7] Grinberg LN, Rachmilewitz EA, Kitrossky N, Chevion M. 1995. Hydroxyl radical generation in ß-thalassemia red blood cells. Free Rad Biol Med; 18:611-5.

[8] Hajji M, Jarraya R, Lassoued I, Masmoudi O, Damak M, Nasri M. 2010. GC/MS and LC/MS analysis, and antioxidant and antimicrobial activities of various solvent extracts from Mirabilis jalapa tubers. Process Biochem; 45:1486-93.

[9] Hebbel RP, Leung A, Mohandas N.1990. Oxidation-induced changes in microheologic properties of the red cell membrane. Blood; 76:1015-20.

[10] Jeffrey L.S.H., A.M. Sahilah, R. Son and S. Tosiah. 2007. Isolation and screening of actinomycetes from Malaysian soil for their enzymatic and antimicrobial activities. J. Trop. Agric. and Fd. Sc. 35(1): 159– 164.

[11] Kekuda PTR, Shobha KS, Onkarappa R, Gautham SA, Raghavendra HL. 2012. Screening biological activities of a *Streptomyces* species isolated from soil of Agumbe, Karnataka, India, International Journal of Drug Development and Research, 4(3), 104-114.

[12] Kekuda PTR, Shobha KS, Onkarappa R. 2010. Studies on antioxidant and anthelmintic activity of two *Streptomyces* species isolated from Western Ghat soil of Agumbe, Karnataka, Journal of Pharmacy Research, 3(1), 26-29.

[13] Khamna S, Yokota A, Lumyong S. 2009. Actinomycetes isolated from medicinal plant rhizosphere soils: diversity and screening of antifungal compounds, indole-3-acetic acid and siderophore production, World Journal of Microbiology and Biotechnology, 25(4), 649-655.

[14] Kukongviriyapan V, Somparn N, Senggunprai L, Prawan A, Kukongviriyapan U, Jetsrisuparb A. 2008. Endothelial Dysfunction and Oxidant Status in Pediatric Patients with Hemoglobin E-beta Thalassemia. PediatrCardiol; 29:130-5.

[15] Lakhtakia R, Ramji MT, Lavanya K, Rajesh K, Jayakumar K, Sneha C, Narayan A, Ramya B, Ramana G, Chari PVB, Chaitanya KV.2011. The role of antioxidants in human health maintenance: Small molecules with infinite functions. IJPSR; 2:1395-402.

[16] Lazzarini A, Cavaletti L, Toppo G and Marinelli F. 2000. Rare genera of actinomycetes as potential producers of new antibiotics.Antonie van Leeuwenhoek 78:399–405

[17] Maeda H, Akaike T. 1998. Nitric oxide and oxygen radicals in infection, inflammation, and cancer. Biochemistry (Moscow); 63:854-65.
[18] Manjunatha BK, Syed Murthuza, Divakara R, Archana M, Sarvani RJ, Verghees S, Paul K. 2013. Antioxidant and anti-inflammatory potency of *Mesua ferrea* L. Indian Journal of Applied Research. 3(8); 55-59.
[19] Pandey, B. Ghimire, P. Agrawal, V.P.2004. Studies on antibacterial activity of Actinomycetes isolated from the Khumbu region of Nepal. J Biol.Sci., 23**:**44-53.
[20] Pham-Huy LA, He H, Pham-Huyc C. 2008. Free radicals, antioxidants in disease and health. Int J Biomed Sci; 4:89-96.
[21] Rathna Kala R, Chandrika V.1993. Effect of different media for isolation, growth and maintenance of actinomycetes from mangrove sediments. Indian J Mar Sci; 22:297-9.
[22] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Medicine.* 26(9):1231-1237.
[23] Rechner AR, Kuhnle G, Bremmer P, Hubbard GP, Moore KP, Rice-Evans CA. 2002. The metabolic fate of dietary polyphenols in humans. Free RadicBiol Med; 33:220-35.
[24] Sahin N, Ugur A. 2003. Investigation of the antimicrobial activity of some *Streptomyces* isolates, Turkish Journal of Biology, 27, 79-84.
[25] Shinar E, Rachmilewitz EA.1990. Oxidative denaturation of red blood cells in thalassemia. SeminHematol; 27:70-82.
[26] Singh, SL., Baruha, I and Bora, TC. 2006. Actinomycetes of loktak habitat isolation and screening of antimicrobial activity. Biotech. 5 (2): 217-221.
[27] Stackebrandt E, Rainey F A and Ward-Rainey N L. 1997 Proposal for a new hierarchic classification system, Actinobacteria classis Nov. Int. J. Syst. Bacteriol, 47:479-491.
[28] Stephen T Odonkor & Kennedy K Addo. 2011. Bacteria Resistance to Antibiotics: Recent Trends and Challenges. Int J Biol Med Res. 2(4): 1204 – 1210.
[29] Watve M G, Tickoo R, Jog M, Bhole B D. 2001. How many antibiotics are produced by the genus Streptomyces? Arch. Microbiol, 176:386-390
[30] Zhong K, Gao XL, Xu ZJ, Gao H, Fan S, Yamaguchi I, Li LH and Chen RJ. 2011. Antioxidant Activity of a Novel *Streptomyces* Strain Eri12 Isolated from the Rhizosphere of *Rhizoma curcumae* Longae. *Current Research in Bacteriology, 4: 63-72.*