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Research Article

BIOACTIVE COMPOUNDS AND ACTIVITIES OF *STREPTOMYCES TUIRUS* CRUDE EXTRACTS FROM SANKARABARANI RIVER ESTUARY, PUDUCHERRY

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ABSTRACT

The study was carried out to evaluate the biological activity (Anti-inflammatory, antioxidant and anti-cancer) of crude extract from *S.tuirus* isolated from Sankarabarani river estuary, Ariyankuppam–Puducherry. The active fraction of *S.tuirus* was obtained by solvent extraction method and was further confirmed by GC-MS. Free radical scavenging assay and the anti-inflammatory activity of crude extracts of *S.tuirus* were also assessed. *In vitro* anticancer activity was examined by MTT assay. GC-MS analysis revealed that the fractions contained totally 25 bioactive compounds which included thiophene, 2-propyl and 2-acetoxy-5-nitrobenzaldehyde represented by a unique spectra. *In vitro* free radical scavenging activities of DPPH, FRAP (91%), NO (87%) and H_2O_2 (87%) by the crude extract were of *S. tuirus* were confirmed. In anti-inflammatory activity assay, 99.08% of inhibition action was observed and the anticancer activity with an IC_{50} value of 222.30 µg/ml denoted that the active fraction contained a spectrum of biological activity.Isolation and characterization of the potential *S.tuirus* from the study place was endowed with enormous biological applications on an remarkable scale.

INTRODUCTION

Marine organisms are still chiefly unexploited resources and embrace led a remarkable potential in the exploration and unearthing of novel natural bioactive compounds. Owing to the enormous biodiversity in the ocean, it is all the time more acknowledged that numerous new-fangled chemical compounds subsist in the oceans. It has been accounted that merely 1% of the marine microorganisms have been investigated. Marine actinobacteria are a practically indefinite source of new chemical structures with lots of prospective therapeutic applications in recent treads in biological sciences. The characteristic of actinobacteria, which most kindles the concern of biotechnologists and the microbiologists, is their capability to generate an assorted range of metabolic products, a number of which encompass vital functions in the field of medicine.

Actinobacteria are broadly distributed in terrestrial, freshwater and marine environments (Kuster, 1968). They actively participate in the decomposition of organic matter and supply nutrients to soil. Marine sources included enormous numbers of actinobacteria, among which *Streptomyces* species occupied more space and had vital role in biological applications. Almost 70% of naturally synthesized antibiotics have been isolated from various species of actinobacteria (Jensen *et al*., 2007). Among all those, *Streptomyces* is the largest genus recognized for the production of numerous secondary metabolites.

Recent research is focused on natural antioxidants from plants and microorganisms, which serve as safe therapeutics (Suriyavathana and Nandhini, 2010). *Streptomyces* sp. having metabolites are useful as antibiotic and anti-inflammatory agents (Moore *et al*., 1999). In medical field, antibiotics produced by *Streptomyces* are used as drugs for tumor (cytotoxic/anticancer antibiotics) that reduce and fight with developing tumors (Azambuja *et al*., 2005). Some other antitumor compounds are marinomycin A, daryamide C, lucentamycins (A, B), mansouramycins (Bentley *et al*., 2002) and tatrolons, which were isolated from the marine actinobacterial strains (Bhatnagar *et al*., 2010). In the present study, an attempt has been made to screen the novel S.tuirus for bioactive compounds with potent biological applications.

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MATERIALS AND METHODS

The gas liquid chromatography mass spectra of the crude extract

Identification of the crude extract's bioactive constituents of the *Streptomyces* isolate was done using the Trace GC Ultra and DSQII model MS from Thermofisher Scientific Ltd and National Institute Standard and Technology (NIST4) and WILEY9

DPPH radical scavenging activity

The free radical scavenging activity of crude extract of *S.tuirus* was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as described by Braca *et al.,* (2001).

Ferric reducing/antioxidant power (FRAP) assay

The antioxidant capacity of crude extract samples were estimated according to the procedure previously described by Benzie and Strain (1996) and as modified by (Pulido *et al*,. 2000).

Nitric oxide (NO) scavenging activity

NO scavenging activity of the sample was determined (Garrat., 1964) by adding 400 µL of 100 mM sodium nitroprusside, 100 µL of PBS (pH - 7.4) and 100 µL of different concentrations of crude extract of *S.tuirus*.

Hydrogen peroxide scavenging activity (H_2O_2)

Hydrogen peroxide scavenging activity of crude extract of *S.tuirus* was evaluated by the method of (Ruchet *et al*., 1989).

Anti-inflammatory activity

Inhibition of protein denaturation

Inhibition of protein denaturation of the test extract was evaluated by the method of Mizushima and Kobayashi (1968) and Sakat *et al*. (2010) with slight modification.

In vitro anticancer activity

Cell line

The human breast cancer cell line (MCF- 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^{0}C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylene diamine tetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with the medium containing 5% FBS to give a final density of 1x10^5 cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at the plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^{o}C, 5% CO_2, 95% air and 100% relative humidity was evaluated as recommended by Mosmann (1983).

MTT assay

After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^{o}C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solublized in 100µl of DMSO and then the absorbance were measured at 570 nm using microplate reader. (Kumar and Kannabiran, 2012)

RESULTS AND DISCUSSION

The GC-MS analysis of the crude extract from S.*tuirus* revealed that the fractions contained 25 bioactive compounds (Table.1) among which, thiophene, 2-propyl and 2-acetoxy-5-nitrobenzaldehyde had a sharp and unique spectra (Fig.1).

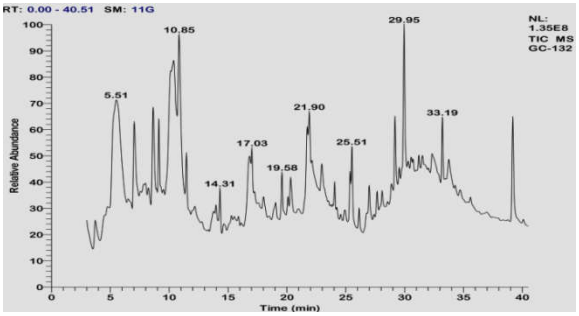


Fig 1 GC-MS spectra of the crude extract of *Streptomyces tuirus*

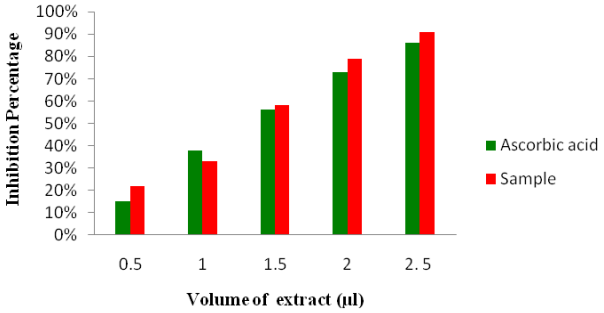


Fig 2 DPPH scavenging activity of crude extract of *S. tuirus*

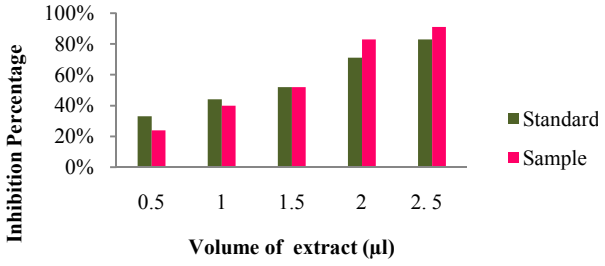


Fig 3 FRAP antioxidant activity of crude extract of *S.tuirus*

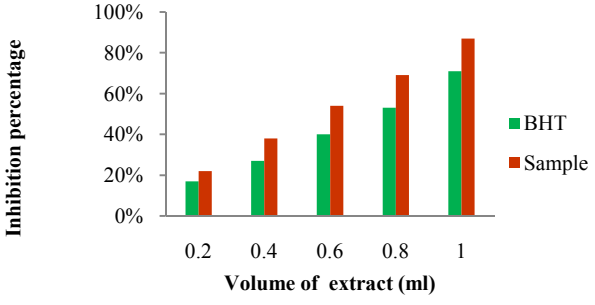


Fig 4 NO scavenging activity of crude extract of of *S.tuirus*

Table 1 Bioactive compounds of the crude extract from *Streptomyces tuirus* revealed by GC-MS analysis

S.NO	RT	Name of the Compound	Molecular Formula	MW	Biological activity
1	3.73	2-Furanmethanol	$C_5H_6O_2$	98	Antiviral ,Antioxidant and Antimicrobial activities
2	4.47	o-Acetyl-L-serine	$C_5H_9NO_4$	147	Affinity inhibitors and antioxidant activity
3	5.48	Glyceraldehyde	$C_3H_6O_3$	90	Anti-inflammatory
4	7.03	4,5,6,8-PTetramethoxy-2,3-dihydroindeno[1,2,3-ij]isoquinolin-9-ol	$C_{19}H_{19}NO_5$	341	Anti hypertension antifungal and antiseptic activies
5	7.99	Isovaleric acid, 3-methylbutyl-2 ester	$C_{10}H_{20}O_2$	200	On-central analgesic, Antipyretic ,anti-inflammatory agents, anti rheumatic agents; Non-steroidal
6	8.63	l-Alanyl-l-alanine ethylamide	$C_8H_{17}N_3O_2$	187	Affinity inhibitors and antioxidant activity
7	10.11	D-Mannopyranose	$C_6H_{12}O_6$	180	Antifouling, Antimicrobial and Anti-inflammatory activity
8	10.85	Thiophene, 2-propyl	$C_7H_{10}S$	126	Antimicrobial activity
9	11.46	CYSTINE, TBS 2X			Affinity inhibitors and antioxidant
10	13.97	QUERCETIN 7,3',4'-TRIMETHOXY	$C_{18}H_{16}O_7$	344	Antioxidant activity
11	14.31	1,3-Bis(4-chlorobenzyl)-5,6-dihydrobenzo[f]quinazoline	$C_{26}H_{20}C_{l2}N_2$	430	Anti-apoptotic
12	15.28	d-Xylose	$C_5H_{10}O_5$	150	Animal medicine
13	16.80	1H-Purin-6-amine, [(2-fluorophenyl)methyl]	$C_{12}H_{10}FN_5$	243	Anticancer activity
14	18.02	à-D-Glucopyranoside, O-à-D-glucopyranosyl-(1.fwdarw.3)-á-D-fructofuranosyl	$C_{18}H_{32}O_{16}$	504	Antioxidant and Preservative
15	19.04	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis	$C_{19}H_{36}O_3$	312	Anticarcinogenic, Antimalarials, Antineoplastics, Treatment of cancer, Antipruritic, Antihypercholesteolemic, Antiprotozoal, Antineurohenic, Antiinflammatory, Antiviral, Antiseborrheic, Menopausal disorders treatment, Transplant rejection treatment, Gyneco logical disorders treatment, Alzheimers's disease treatment, Anticholinergic agent, Therapeutic agent.
16	20.32	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	Potential biofuels can be prepared. Also used in cigarettes to increase nicotine delivery in smoke and binding of nicotine to neural receptors
17	22.96	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	Antioxidant activity
18	25.51	Methyl stearate	$C_{19}H_{38}O_2$	298	Anti-inflammatory, Anti-diarrheal, cytotoxic and anti proliferative activity.
19	27.00	l-Leucine, N-cyclopropylcarbonyl-, hexadecyl ester	$C_{26}H_{49}NO_3$	423	Affinity inhibitors , antioxidant
20	29.18	1,2-O-Isopropylidene-á,L-threose	$C_7H_{12}O_4$	C7H12O4	Antifouling , Anti-inflammatory, Antimicrobial activity
21	29.95	2-Acetoxy-5-nitrobenzaldehyde	$C_9H_7NO_5$	209	Anti-inflammatory, Anti-diabetic, anti-neoplastic,and anti-hypertensive.
22	32.34	à-Amyrin	$C_{30}H_{50}O$	426	Ant proliferative, useful in treating neurodegenerative disease such as Alzheimer's and Parkinson's diseases.
23	33.19	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	Anti-inflammatory, Antimicrobial and Antihistamine activities.
24	33.76	20-Methyl-5-pregnene-3,20-diol	$C_{22}H_{36}O_2$	332	Anti-inflammatory, used to treat a number of bone-degenerative diseases, , Mainly used as a female hormones
25	39.18	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337	Treating Epstein Barr virus infection , Insulin depended Diabetes, Prevent night time hypoglycemia

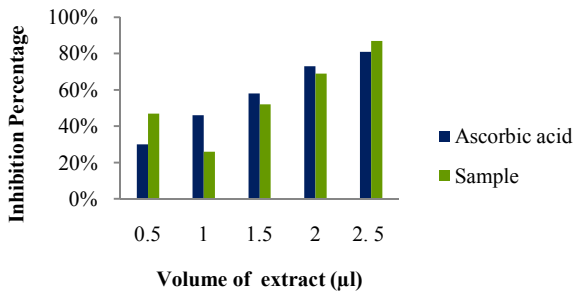


Fig 5 H_2O_2 antioxidant activity of crude extract of *S.tuirus*

DPPH is used to monitor free radical mediated chemical reactions, by donating hydrogen ions to form non radial yellow colored solution from purple coloured and indicated that DPPH scavenging activity. The results showed that the crude extract of *S.tuirus* exhibited the ability to reduce the DPPH radical in a concentration dependent manner; high concentration had a higher inhibitory action, even higher than that of ascorbic acid standard (Fig.2). Similar results were reported by Nagaseshu *et al*.,(2016). FRAP assay results indicated that the total antioxidant power of crude extract of *S.tuirus* increased with the increasing concentration of the sample (Fig.3). The NO scavenging activity of crude extract of s.tuirus with 0.5µl showing <45% inhibitory effect is shown in Fig 4. However, a higher activity was observed for 2.5µl of sample, which was even better than the standard. H_2O_2 scavenging activity also increased with the increasing concentration of the sample (Fig. 5). Standard BHT had less effect than the crude extract of *S.tuirus*. Hence, it became obvious that the isolated fraction of the crude extract of *S.tuirus* had a potential antioxidant capacity. Thenmozhi and Kannabiran (2012) reported in their findings, in Pondicherry seashore area having actinobacteria rich source of antioxidant property of. Similar finding was observed in Pondicherry costal area by Priya *et al*., (2012). The antioxidant activity of the extract was strongly correlated with cytotoxic activity (Nagaseshu *et al*., 2016).

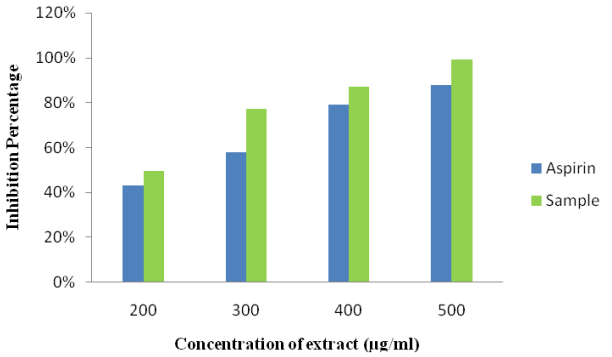


Fig.6 Anti-inflammatory activities of crude extract of *S.tuirus*

Cyclomarin A is a new cyclic heptapeptides antibiotic isolated from *Streptomyces sp*. It displayed significant anti-inflammatory activity in both *in vivo* and *in vitro* assays (Renner *et al*. 1999). Salinamides A and B are bicyclic depsipeptides, produced by *Streptomyces sp*. These metabolites are useful as antibiotic and anti-inflammatory agents (Moore *et al.*1999). Hence, the crude extract of *S.tuirus* samples at various concentrations (200, 300, 400, 500 µg/ml) showed significant protein stabilization. However, the percentage of inhibition was found to be increased with concentration. The percentage inhibition of crude extract *of Streptomyces PAS 9* at the concentration of 500 µg/mL was higher than other concentrations. The anti-inflammatory effects of crude extract of *S.tuirus* were evident at 500µl concentrations with 100% inhibition being achieved (Fig. 6).

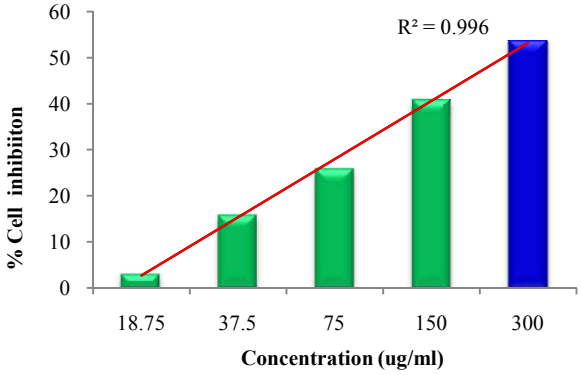
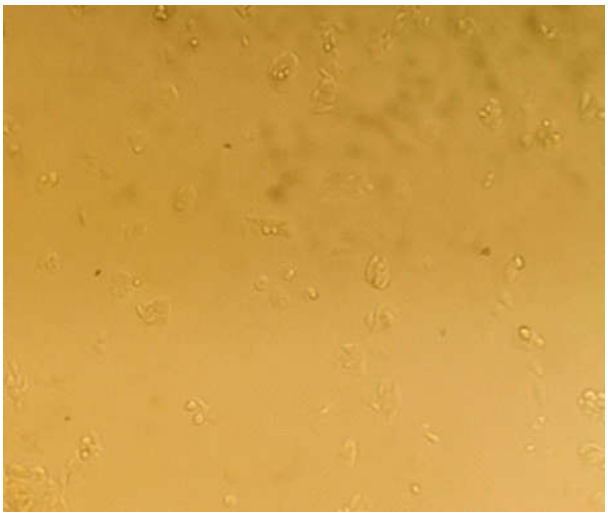


Fig 7 *In vitro* anti-cancer activity of crude extract of *S.tuirus.*

MCF cell line (control)



MCF cell line treated with 300µg of sample

Fig 8 *In vitro* anti-cancer activity of crude extract of *S.tuirus*

Actinobacteria possess new bioactive compounds such as antibiotics and enzymes (Vining, 1992 and Demain, 1995). Bioactive compounds of actinobacteria including members of the anthracycline, bleomycin, actinomycin, mitomycin and aureolic acid families had anti-tumor properties among the most important cancer chemotherapeutic agents (Rocha *et al*., 2001).

Crude extract obtained from the *S.turius* was administered to the MCF cancer cell lines. In order to conduct the *in vitro* studies, cell lines were maintained, propagated and recorded routinely and the MTT assay was carried out as described in the methodology section. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells. The percentages of growth inhibition of MCF-7 cells induced by the compounds in the crude extract of *S.tuirus* are shown in Fig.8. The bioactive metabolites from the crude extract of *S.tuirus* divulged a potential in inhibition of MCF-7 cell growth. Inhibition of cell growth also increased with the increasing concentration of the sample (Fig.7) and IC_{50} was determined to be 222.30µg/ml.

CONCLUSION

Since the past few decades, researchers are focusing on novel microorganisms producing metabolites in unexplored or less explored marine coastal areas. The current research interest on characterization of crude extract of *S.turius* from Sankarabarani river estuary (Ariyankuppam –Puducherry) was observed to produce a wide-ranging bioactive compounds for human therapeutic applications as well as various industrial applications. The crude extract of *S.turius* revealed a remarkable antioxidant, anti-inflammatory and antitumor activities.

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