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Original Article

CYTOTOXICITY AND ANTIOXIDANT ACTIVITY OF A *STREPTOMYCES* SP. FROM MANGROVE SEDIMENTS OF DAR ES SALAAM, TANZANIA

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ABSTRACT

Objective: The present study aimed at screening for cytotoxicity and antioxidant properties of extract from *Streptomyces* sp. isolated from Dar es Salaam mangrove sediments.

Methods: The *Streptomyces* sp. (isolated in a previous study), was cultivated in broth and the non polar fractions of its extracellular metabolites were harvested via ethyl acetate (liquid-liquid) extraction. The obtained extract was first screened for cytotoxicity activity using brine shrimp lethality bioassay. Antioxidancy was assessed on the basis of scavenging ability of the stable 1, 1 Diphenyl-2-picry Hydrazyl (DPPH).

Results: The extract was found to possess cytotoxicity activity with the LC_{50} value of 236.86µg/ml. On screening for antioxidant activity this study found that, the *Streptomyces* sp. crude extract had Antioxidancy scavenging ability which ranged between 94.31% - 95.69% at the concentration from 4200 µg/ml to 14000µg/ml. These findings proved that the strain had potent antioxidant activity as the Ascorbic Acid which is the known antioxidant.

Conclusion: The findings indicate that, the marine *Streptomyces* sp. could be a promising source for novel drug discovery in the pharmaceutical industry.

Keywords: Mangrove, Sediments, *Streptomyces* sp., Cytotoxicity, Antioxidant activity.

INTRODUCTION

The tremendous biochemical diversity of marine microorganism and their biochemical potential is becoming more and recognized not only by microbiologists but also by the pharmaceutical industry [1]. This is why these microbes are regarded as the most prized microbes in the pharmaceutical field [2]. Marine Actinomycetes which are the gram positive and filamentous bacteria have been the best source for novel drug's discovery. *Streptomyces,* a genus in Actinomycetes, is popular in producing numerous substances essential for health such as antioxidants, antibiotics, enzymes, and immunomodulators [3].

Oxidative stress is the consequence of a disproportion of pro-oxidants (free radicals) and antioxidants in support of pro-oxidants. Oxidative stress pilots to widespread harm in cell structures in humans and this is measured as a major explanation for the pathogenesis of several chronic diseases such as diabetes mellitus, cancer, inflammation and neurodegenerative diseases [4]. In this regard, free radical scavengers possessed by our body which constitute the repair systems for biomolecules are damaged by pro-oxidants or free radicals [5, 6].

The main characteristic of an antioxidant is its ability to trap free radicals which oxidize nucleic acids, proteins, lipids or DNA. All the organisms are protected from the attack of free radical generation which is highly prevented by defence mechanism called antioxidant system [7]. In 2007, [8] reported that, though almost all humans are well protected against free-radical damaged by enzymes (dismutase and catalase) or by compounds (ascorbic acid, tocopherols and glutathione), but, these structures are inadequate to stop damage completely. Usually, there is a sense of balance among the amount of free radicals created in human being's body and the antioxidants that scavenge the free radicals [9]. When it happens, the balance is moved in the direction of free radicals fabrication, and transformed into oxidative stress which ends up into aging and diseases such as cancer and diabetes [7].

The primary sources of naturally occurring antioxidants are whole grain, fruits, and vegetables [10, 11]. But they can also be extracted naturally from microorganisms [3]. 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. Synthetic antioxidants have been used in stabilization of foods. But their use is being restricted nowadays because of their toxic and carcinogenic effects. Thus, interest in finding natural antioxidants, without side effects, has greatly increased [12].

Though there have been reports in Tanzania on antimicrobial and antimalarial activities from Marine *Streptomyces* [13, 14], reports on their antioxidants properties are lacking. The aim of this research was to investigate the cytotoxicity and antioxidant properties of marine *Streptomyces* isolated from mangrove sediments collected in Dar es Salaam, Tanzania.

MATERIALS AND METHODS

Production and extraction of secondary metabolites

Streptomyces sp. test culture was provided by Molecular Biology and Biotechnology department culture reserve. Colonies of *Streptomyces* sp. were inoculated in four 500 ml conical flasks each containing 250 ml Starch casein medium. The conical flasks were kept on a shaker (150 rpm) at 37°C for overnight and then incubated at 37°C for 7 days. After incubation, the broth was aseptically filtered through Whatman No.1 filter paper. The culture broths of *Streptomyces* sp*.* were subjected for solvent extraction method to recover metabolites. Ethyl acetate was added to the filtrate in the ratio of 1:1(v/v) and shaken vigorously for 1 h for complete extraction. The extract was concentrated to dryness by using a rotary evaporator under vacuum at 40°C [13].

Cytotoxicity assay of *Streptomyces sp.* crude extracts

Brine shrimp (*Artemia salina*) lethality test was done according to [15] and [16]. To begin the bioassay, 0.5 g of brine shrimp eggs (Great Salt Lake, USA) was hatched in 300ml of filtered sea water in a conical flask and allowed to shake for 48 hours with illumination using an electric bulb. Thereafter, ten shrimp larvae were selected and transferred into wells of the microtiter plate by means of 100µl

micropipette and the final volume of each vial was adjusted to 200µl using natural sea water. The extract (stock solution) was introduced into the wells by pipetting volumes of 15µl, 6µl, 5µl, 4µl, 2µl and 1.5µl to make concentrations of 240µg/ml, 120µg/ml, 100µg/ml, 80µg/ml, 40µg/ml and 24µg/ml respectively. The negative control sample was DMSO which was not treated with extract. The microtiter plate was maintained under illumination. The number of the brine shrimp nauplii that died after 24 hours was counted with the aid of a stereo microscope and the mortality of larvae at each dose was determined. The concentration that killed 50% of the brine shrimp nauplii (LC_{50} in µg/ml and Confidence Intervals 95%) was determined using probit analysis [17, 18] and ®PoloPlus version 1.0 software [19]. The findings were presented graphically by plotting log of concentrations versus percentage of mortality of brine shrimp nauplii from which LC_{50} was determined by extrapolation. The assay was performed in duplicate and the result was calculated as an average of two determinations.

Antioxidant activity

Preparation of Test Solution

Preparation of test and standard solutions was done as by [20]. An amount of 70mg of dried *Streptomyces* sp. crude extract was dissolved in 5ml of Dimethylsulfoxide (DMSO) solvent. From this stock solution of different concentrations ranging from 4200 µg/ml to 14000µg/ml (4200, 5600, 7000, 8400, 9800, 11200, and 12600 and 14000µg/ml) were prepared.

Preparation of Standard Solution

25 mg of Ascorbic acid was weighed and dissolved in 1ml of Dimethyl sulfoxide (DMSO) to obtain a concentration of 25 mg/ml (25000µg/ml). From this stock solution different concentrations ranging from 25000µg/ml to 100µg/ml were prepared (2500, 5000, 7500, 10000, 12500, 15000, 20000 and 25000µg/ml).

Procedure

Antioxidant activity was done using the Diphenyl Picryl Hydrazil (DPPH) radical scavenging method. 4mg of DPPH was dissolved in 100ml of ethanol and kept in darkness overnight for generation of DPPH radicals. The scavenging activity for DPPH free radicals was measured according to the procedure described by [20]. An aliquot of 3ml of DPPH solution in ethanol and 0.1ml of crude extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 minutes. Decolonization of DPPH was determined by measuring the absorbance at 517 nm by using visible scanning spectrophotometer. A control was prepared using 0.1ml of respective vehicle in ethyl acetate extract or ascorbic acid.

The mixture was then shaken vigorously at room temperature in a dark room. Absorbance was read immediately at 0 minute using a spectrophotometer at 517nm and thereafter after 30min of reaction. Ascorbic acid was used as a standard control. The scavenging effect on of the DPPH radical was calculated using the following equation:

$$\text{Scavenging effect}\,(\%) = \left(1 - \frac{\text{Absorbance of sample at 517nm}}{\text{Absorbance of control at 517nm}}\right) * 100$$

EC_{50} value was determined from plotted graph of scavenging activity against the concentration of extracts, which is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%. Duplicate measurements were carried out and their average scavenging effect was calculated based on the percentage of DPPH scavenged.

RESULTS

Cytotoxicity assay of Marine S*treptomyces* sp. extract

Crude extract from *Streptomyces* sp. was found to have lethal concentrations (LC_{50}) of 23686 µg/ml (Table 1). The control and the S*treptomyces* sp. extract exhibited LC_{50} values greater than100 µg/ml.

Antioxidants Activity

All the 70 mg of the crude extract were dissolved in DMSO and preparation of various concentrations from this stock solution was done. The highest percentage of antioxidant inhibition was 95.69% at 8400 µg/ml and 9800 µg /ml concentrations. The results are shown below.

Table 1: Cytotoxicity of *Streptomyces* sp. extracts to Brine shrimp larvae and it's LC_{50} at 95% Confidence Interval (CI); the extract results are average of two replicates.

S.No.	Isolate	LC_{50}µg/ml	Lower 95% CI
1	*Streptomyces* sp.	236.86	147.32
2	Control	199.35	95.38

Table 2: Mortality of brine shrimps after 24 hours of exposure to different concentrations of the *Streptomyces* sp. extract

S. No.	Concn µg/ml	No. exposed	No responded	% Mortality
MS1	24	10	0	0
	40	10	1	10
	80	10	1	10
	100	10	2	20
	120	10	4	40
	240	10	5	50
Control	24	10	0	0
	40	10	0	0
	80	10	0	0
	100	10	0	0
	120	10	0	0
	240	10	1	10

DISCUSSION

In the brine shrimp lethality bioassay, the crude extract exhibited significant cytotoxic activities of LC_{50}>100 µg/ml (Fig. 1). According to [21], [22] and [23] the brine shrimp results are interpreted as follows: LC_{50}<1.0µg/ml highly toxic; LC_{50} 1.0 - 10.0µg/ml - toxic; LC_{50} 10.0 - 30.0µg/ml - moderately toxic; LC_{50}>30 <100µg/ml - mildly toxic, and > 100µg/ml as non-toxic. In this regard then, crude extract of this study is considered to be safe and can be used for other bioactivities. The same observations were reported by [23], whereby, LC_{50} values of >100µg/ml was obtained in a BST bioassay indicating that, they have cytotoxic potential.

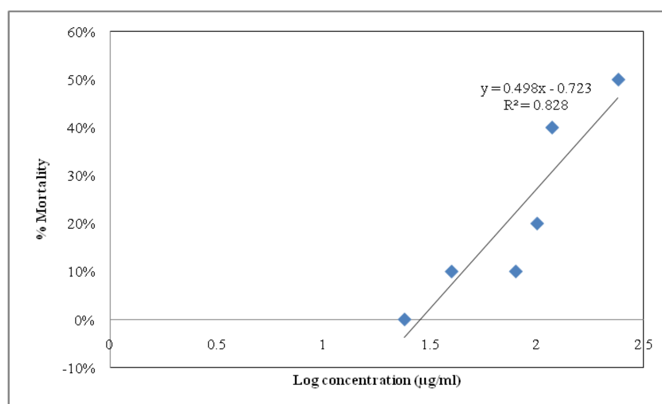

Fig. 1: A graph showing activity of *Streptomyces* sp. extract against brine shrimps.

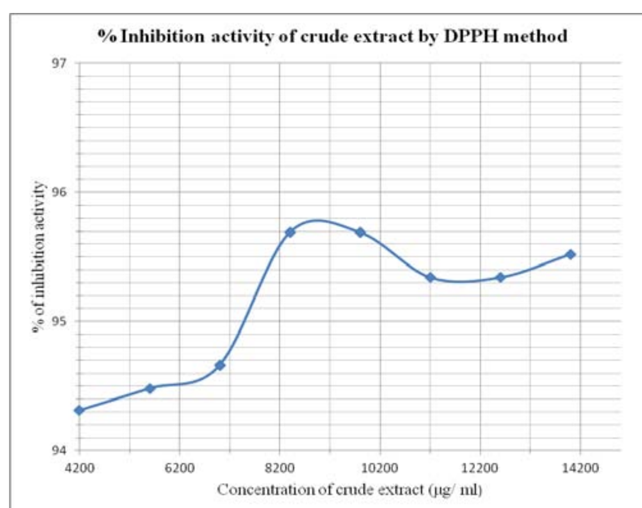

Fig. 2: Percentage (%) of scavenging abilities of crude extract

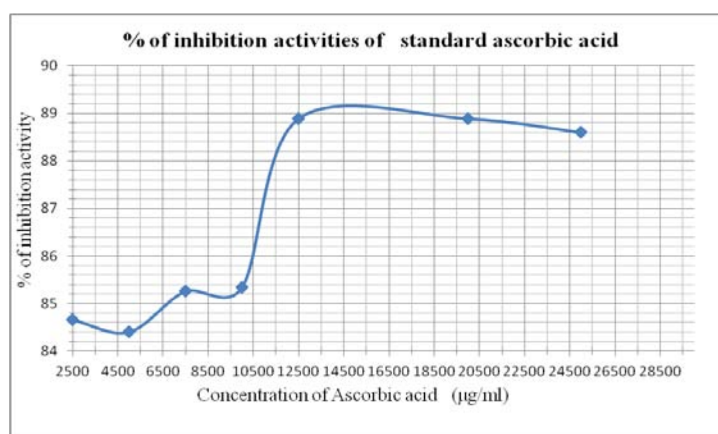

Fig. 3: Percentage(%) of scavenging abilities of Ascorbic acid

The mortality rate of the brine shrimp in the extract increased with the increase in the concentration of the sample. The same behavior was reported in a previous study by [24]. In this study, the highest mortality was observed at the concentration of 240µg/ml whereas the least mortaly was at 24µg/ml (Figure 1). This is supported by [25] where the degree of lethality in a BST was found to be proportional to the concentration of the extract. These results may suggest that, the *Streptomyces* sp. crude extract would be safer to use in pharmaceuticals and agrochemicals since the cytotoxicity levels was well above the cut off point (Tables 1 & 2).

The results of this study on the DPPH free radical scavenging activity indicated that, both the *Streptomyces* sp. crude extract and the ascorbic acid showed a good scavenging ability (Figures 2 and 3). These results support the findings of [20] whereby, the scavenging effect of crude ethyl acetate extracts of the marine *Streptomyces* isolates on DPPH radicals dose-dependently increased and was found to be 71.77% and 77.52%. Moreover, some strains of Actinomycetes isolated from marine sponges exhibited potent antioxidant activity of IC_{50} between 56.3 and 99.1µg/ml in DPPH assay [26]. In another study, a marine *Streptomyces* sp. showed

90.57% antioxidant activity at 500μg/ml [27]. But also, [28] reported a marine actinobacterial strain which revealed higher antioxidant activity, helpful in preventing or slowing down the progress of various oxidative stresses related disorders.

CONCLUSION

From the results of Brine shrimp test (BST) in this study it is clear that the *Streptomyces* sp. crude extract is well thought-out to be safe and can be used for further bioactivities. But also, the same crude extract had a noticeable effect on scavenging free radicals. The study established for the very first time in Tanzania that, the marine Actinomycetes *Streptomyces* sp. from mangrove sediments has promising pharmaceutical significance, with radical scavenging ability, and which may serve up as an excellent source of harmless natural antioxidants. This implies that the marine Actinomycetes *Streptomyces* could also be a potential source of antioxidants to complement human diet. Additional studies are recommended to identify and isolate active constituents and pharmacological usefulness of *Streptomyces* metabolites.

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