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ORIGINAL ARTICLE

Cytotoxicity and antioxidant activity of 5-(2,4-dimethylbenzyl)pyrrolidin-2-one extracted from marine *Streptomyces* VITSVK5 spp.

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Cytotoxic activity;
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Total antioxidant activity

Abstract The aim of the present study was to evaluate the cytotoxicity and antioxidant activity of 5-(2,4-dimethylbenzyl)pyrrolidin-2-one (DMBPO) extracted from marine *Streptomyces* VITSVK5 spp. The strain was isolated from sediment samples collected at the Marakkanam coast of Bay of Bengal, India. Systematic screening of isolates for anti-*Aspergillus* activity resulted in the identification of *Streptomyces* species designated as *Streptomyces* VITSVK5 spp. Bioactivity guided extraction and purification yielded a compound 5-(2,4-dimethylbenzyl)pyrrolidin-2-one (DMBPO) and was tested for cytotoxicity and antioxidant activity. The structure of the extracted compound was established by spectroscopic studies and identified as 5-(2,4-dimethylbenzyl)pyrrolidin-2-one (DMBPO). DMBPO exhibited cytotoxic activity on HEP 2 and Hep G2 cell lines with the IC_{50} value of 2.8 μg/ml and 8.3 μg/ml, respectively, as compared to Vero cell line (22.6). DMBPO showed the hemolytic EC_{50} value of 288 μg/ml on human erythrocytes. DMBPO treatment showed fewer (31.7%) aberrations, gaps and chromatid breaks as compared to untreated controls (27.8%) of human chromosomes. DMBPO also exhibited significant (44.13% at 5 μg/ml DMBPO) DPPH radical scavenging activity and total antioxidant activity (50.10% at 5 μg/ml DMBPO). The results of this study showed that DMBPO is cytotoxic to cancer cells and possesses antioxidant property.

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1. Introduction

Over the past 75 years, natural product derived compounds have led to the discovery of many drugs to treat numerous human diseases (Grabley and Thiericke, 1999). Natural products are chemical compounds derived from living organisms e.g. plants, animals and microorganisms. They can be defined as chemical compounds isolated or derived from organisms as primary or secondary metabolites. By employing sophisticated techniques under various screening programs, the rate of discovery of natural compounds exceeded 1 million so

far (Pimentel-Elardo et al., 2010). Out of which 22,500 biologically active compounds that have been extracted are from microbes, 45% are produced by actinobacteria, 38% by fungi and 17% by unicellular bacteria (Demain and Sánchez, 2009). The oceans cover more than 70% of earth surface and little is known about the microbial diversity of marine sediments, which is an inexhaustible resource that has not been fully exploited. Marine extremophiles serve as valuable natural resource for novel products such as antibiotics, antitumor agents and other therapeutic substances (Amador et al., 2003). Microbial secondary metabolites have been known as one of the immense reservoir of natural chemical diversity with potent biological activity (Bush and Macielag, 2000). Most bacterial secondary metabolites are generated through a unique, multi-step biosynthetic process with specific enzymes for each complex structure formation. Their encoding genes are normally clustered within the genome of the organism and the precursors for the biosynthesis are derived from primary metabolites. Marine actinomycetes are potential providers of novel bioactive metabolites and have been currently emerging as an important source for natural products with unique chemical diversity. Members of the class actinobacteria especially *Streptomyces* spp. have long been recognized as prolific sources of useful bioactive metabolites, providing more than 85% of naturally occurring antibiotics discovered to date and continuing as a rich source of new bioactive metabolites (Berdy, 2005).

Actinomycetes represent one of the most studied and exploited classes of bacteria for their ability to make a wide range of biologically active metabolites (Ikeda et al., 2003). The actinobacteria plays a very important role among the marine bacterial communities, because of its diversity and ability to produce novel chemical compounds of high commercial value (Hopwood, 2007; Amador et al., 2003). The compounds isolated from marine *Streptomyces*, 2-allyoxyphenol and streptopyyrolidine have been reported to possess antioxidant and no cytotoxic activity (Arumugam et al., 2010; Shin et al., 2008). The studies on marine actinomycetes with respect to antioxidant and cytotoxic activity are very limited in the Indian sub-continent and most of the actinomycetes isolated were yet to be screened for bioactive secondary metabolites. Hence a study was carried out to extract the active compound from marine *Streptomyces* VITSVK5 spp. and to study its hemolytic activity on human red blood cells, cytotoxic effect on normal and selected cancer cells, DPPH free radical scavenging, and total antioxidant activity. In this study the cytotoxicity and antioxidant activity of a novel compound 5-(2,4-dimethylbenzyl)pyrrolidin-2-one extracted from *Streptomyces* VITSVK5 spp. was reported.

2. Materials and methods

2.1. Strain

The strain *Streptomyces* VITSVK5 spp. was isolated from the salt pans of Marrakanam coast of Bay of Bengal, India. The strain was selectively isolated using Starch casein agar, ISP No. 1 medium and the nutritional and cultural conditions for the growth were optimized. Isolation and characterization of the strain was previously reported (Saurav and Kannabiran, 2010).

2.2. Extraction and purification of the compound

Well grown slant culture of the potential isolate was used for the preparation of seed culture. The seed culture was inoculated in 50 ml medium containing the optimized production medium prepared with sea water 50%, distilled water 50%, pH 8.2 and incubated for 2 days in a rotary shaker (200 rpm) at 30 °C. The inoculums (10%) were transferred into 200 ml production medium in 1 l Erlenmeyer flasks and kept for fermentation for a week. After fermentation, the broth was centrifuged at 4000 rpm for 10 min at 10 °C and the supernatant was separated and filtered in 0.2 μm membrane filter. The supernatant was extracted twice with *n*-butanol (400 ml) and washed with 500 ml water. After separation, the organic phase was dried over Na_2SO_4 (anhydrous). The extract was then concentrated in a rotary vacuum and lyophilized using a freeze drier (Thermo, USA) at 5 °C for 5 h. The crude extracts were stored at −20 °C. The butanol layer was concentrated and the residual suspension (750 mg) was chromatographed over silica gel column and eluted with chloroform:MeOH (10:0, 9.5:0.5, 9:1, 8.5:1.5, 8:2, 7.5:2.5, 7:3). The active fractions were collected, concentrated and further separated by preparative TLC on silica gel with chloroform:MeOH (8:2) and the purity of the compound was analyzed.

2.3. Structure elucidation

The UV spectra of the compound were measured using UV–Visible spectrophotometer (Techcomp, Hong Kong). In order to investigate the presence of various functional groups in bioactive compound, the sample was lyophilized and mixed with KBr (1:20; 0.02 g of sample with KBr at a final weight of 0.4 g) and then grounded, desorbed at 60 °C for 24 h and pressed to obtain IR-transparent pellets. Infrared spectra of the compound were obtained using a Fourier Transform Infrared Spectrometer (FT/IR-AVATAR 330). The spectra were collected within a scanning range of 400–4000/cm. The FT-IR was first calibrated for background signal scanning with a control sample of pure KBr, and then the experimental sample was scanned. The spectra obtained was analyzed for various functional groups.

The proton NMR (^{1}H NMR) and carbon NMR (^{13}C NMR, V Bruker Avance III 500 MHz (AV 500)) spectra of the compound were obtained by using a dimethyl sulfoxide d6 (DMSO-d6) as solvent. It was further evaluated with DEPT-135. It was further confirmed by mass spectroscopy (HR-MS, Jeol GCMATE II). The structure of the compound was established with the help of spectral data obtained from spectroscopic techniques. The 3D structure of the compound was obtained by using chemdraw software (Ultra 8.0).

2.4. Assay of hemolytic activity

Hemolytic effect of the lead compound on human erythrocytes was evaluated by using washed human erythrocytes (RBCs). For the preparation of human erythrocytes the method of Malagoli (2007) was followed. The human erythrocyte was obtained from the peripheral blood (B+) of a healthy volunteer. The blood was used within 24 h after bleeding. The erythrocyte fraction was washed thrice with saline and resuspended in 10 ml PBS. The hemolytic activity of the compound was tested as reported earlier under *in vitro* conditions in 96-well