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Antioxidant and Cytotoxic Activity of Compounds from *Streptomyces albidoflavus*

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Attempts were made to study the antioxidant and cytotoxic activities of compounds produced by *Streptomyces albidoflavus*. Bioactive metabolites were obtained from the crude solvent extract of culture broth collected after five days incubation of the strain. Two bioactive compounds were purified and characterized as anthracene-9,10-quinone and 8-hydroxyquinoline. Antioxidant activity of these compounds was studied by superoxide free radical scavenging activity. The compound 8-hydroxyquinoline exhibited good antioxidant properties over BHA but less activity than vitamin C. Cytotoxicity of the compounds was tested on U-937, HeLa and THP-1 cell lines. Of the two bioactive compounds isolated from strain, 8-hydroxyquinoline was found to possess significant cytotoxicity on tested cell lines.

Key words: *Streptomyces albidoflavus* Antioxidant activity, Cytotoxicity.

Actinomycetes are Gram-positive, filamentous bacteria with high G+C content. Among actinomycete population from soils, *Streptomyces* species are reported to be the most abundant forms. They continue to be rich sources of secondary metabolites with biological activities that ultimately find application as antimicrobial, anticancer agents or other pharmaceutically useful compounds (Bibb, 2005). When compared to other microbial products as evident form the report of Berdy (2005), the bioactive profile of actinomycete metabolites is very broad. Among anticancer antibiotics reported from *Streptomyces* strains, Actinomycin D, Adriamycin, Aclacinomycin, Bleomycin A_2, Chromomycin A_3, Daunomycin, Migrastatin, Mithramycin and Mitomycin C have been used as therapeutic agents for treating different cancers (Hopwood, 2007).

During the screening of actinomycetes for bioactive metabolites, a streptomycete strain was found to exhibit strong antimicrobial activities. The strain was selected and identified as *Streptomyces albidoflavus*. Taxonomy and antimicrobial spectrum of the strain were reported earlier (Narayana *et al.* 2007). In the present investigation, an attempt was made to study the antioxidant and anticancer activities of pure compounds obtained from the strain.

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MATERIAL AND METHODS

The study was carried out in Department of Microbiology, Acharya Nagarjuna University during the year 2007. The strain, *S. albidoflavus* was maintained on yeast extract-malt extract-dextrose (YMD) agar medium (Williams and Cross, 1971). Actively growing pure culture of the strain was used to inoculate 100 ml of YMD broth in 250 Erlenmeyer flasks. After 48 h incubation at 30°C, YMD culture broth (10%) was used as seed culture to inoculate 500 ml fermentation broth (4% dextrose, 0.9% protease peptone, 0.1% yeast extract, 0.6 % Ca CO_3, 0.1% K_2HPO_4, 0.1% $MgSO_4.7H_2O$, pH 7.2) in 2-litre Erlenmeyer flasks and incubated at 30°C for 5 days.

Extraction, purification and identification of bioactive compounds from the strain

After 5 days incubation of the culture, the fermentation was stopped and pH of the medium was adjusted to 3.5 with 1N HCl. Cells were removed from fermentation broth by filtration and the culture filtrate was extracted with ethyl acetate. The crude solvent extract was subjected to silica gel chromatography (22×5 cm, Silica gel 60, Merck) and eluted with gradient solvent system consisting of ethyl acetate: hexane. Elutions collected during column chromatography were concentrated and tested for their antimicrobial activity against a Gram-positive (*Bacillus subtilis* MTCC 441) and Gram-negative bacteria (*Pseudomonas aeruginosa* MTCC 424), and yeast (*Candida albicans* MTCC 183) to screen bioactive fractions. Among the different bioactive fractions of strain, two fractions were purified and identified as anthracene-9,10-quinone and 8-hydroxyquinoline respectively, by EI-MS, ^{1}H NMR and ^{13}C NMR spectral studies (Narayana *et al.* 2008).

Antioxidant activity

The antioxidant activity of the compounds was carried out by superoxide free radical scavenging activity. Superoxide radicals were generated in-vitro by non-enzymatic system and determined spectrophotometrically by following the Nitro Blue Tetrazolium (NBT) riboflavin photoreduction method of McCord and Fridovich (1969). The assay mixture contained EDTA (6.0 µM), NaCN (3µg), riboflavin (2 µM), NBT (50 µM) and various concentrations of test substances in methanol and phosphate buffer (58 mM, pH 7.8), in a final volume of 3 mL. The tubes were shaken well and the absorbance was measured at 560nm. The percentage of inhibition of superoxide radical generation was measured by comparing the mean absorbance values of control and those of the test substances. The antioxidant activity of the compounds was expressed as the 50% inhibitory concentration (IC_{50}) that was measured from the plot drawn concentration (µg) verses percentage inhibition. The antioxidant substances vitamin C and BHA were used as standards.

Cytotoxic activity

The cytotoxic activity of compounds produced by the strain was tested on U-937 (Human leukemic monocyte lymphoma cell line), HeLa (Human cervical cancer cell line) and THP-1 (Human acute monocytic leukemia cell line) using MTT assay (Plumb *et al*., 1989). Cell lines were obtained from National Centre for Cell

Table 1. Superoxide radical scavenging activity of purified bioactive compounds from *Streptomyces albidoflavus*

Test substance	% of inhibition at 100µg	IC_{50} (in µg)
AQ	2.58	>100
HQ	63.4	54.2
Vitamin-C	97.52	31.3
BHA	52.18	65

AQ- Anthracene-9,10-quinone
HQ- 8-hydroxyquinoline; BHA- Butylated hydroxyanisole

Table 2. Cytotoxic activity (IC_{50}) of purified bioactive compounds from *Streptomyces albidoflavus*

Cell lines	AQ	HQ	Etoposide
U-937	123.36	43.81	10.26
HeLa	155.00	99.75	13.79
THP-1	108.22	40.50	8.15

AQ- Anthracene-9,10-quinone; HQ- 8-hydroxyquinoline

Science, Pune (India) and were cultured at 37°C with 5% CO_2 using RPMI-1640 (Himedia®, India) media containing fetal bovine serum. U-937, HeLa and THP-1 (2×10^4 cells per well) were seeded in a 96-well plate containing 100 µl of RPMI medium and incubated for 24h. The cells were then treated with different concentration of bioactive compounds of the strain (0-150 µg ml^{-1}). After 48h incubation, 100µl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5,-diphenyltetrazolium bromide) reagent (Sigma Chemicals, USA) was added to each well, and the plates were incubated in a CO_2 incubator at 37°C for 4 h. Thereafter, the supernatant was removed from each well. Then 100µl DMSO was added to dissolve the colored formazan crystals produced by the MTT. Subsequently, the optical density was measured at 570nm using an ELISA reader (Molecular Devices Corp., USA).

RESULTS AND DISCUSSION

Antioxidant activity

Antioxidant activity of the two compounds viz., anthracene-9,10-quinone and 8-hydroxyquinoline extracted from the strain was studied and compared with standard antioxidants such as vitamin C and BHA. The compound 8-hydroxyquinoline exhibited good antioxidant properties over BHA but less effective than vitamin C (Table 1). Three isoflavonoids extracted from the fermentation broth of *Streptomyces* sp. were found to possess antioxidant activity (Komiyama *et al.* 1989). Hosoya *et al.* (1996) reported a novel antioxidant, diphenazithionin from *Streptomyces griseus* ISP 5236. Two antibiotics herbimycin A and dihydroherbimycin A from a *Streptomyces* strain exhibited antioxidant activities of 61% and 72% respectively at 100 µg/ml (Chang and Kim, 2007). In the present study, the compounds anthracene-9,10-quinone and 8-hydroxyquinoline isolated from culture broth of *S. albidoflavus* were found to possess antioxidant activities of 2.58 and 63.4% respectively at 100 µg/ml.

Cytotoxic activity

Cytotoxicity of pure compounds isolated from the strain was tested on different cell lines such as U-937, HeLa and THP-1 cell lines (Table 2). Significant cytotoxicity against the cell lines tested was observed only with 8-hydroxyquinoline compared to anthracene-9,10-quinone. Inhibitory activity (IC_{50}) of 8-hydroxyquinoline on U-937, HeLa and THP-1 cell lines was found at 43.81, 99.75 and 40.5 µg/ml respectively. The widely used anticancer drug, etoposide exhibited cytotoxicity activity (IC_{50}) on U-937, HeLa and THP-1 cell lines at 10.26, 13.79 and 8.15 µg/ml respectively.

Different actinomycete strains have been reported to produce anthracene-9, 10-quinone (anthraquinone) compounds. A new anthraquinone derivative has been reported from the culture broth of *Streptomyces griseorubiginosus* that inhibited the binding of activator protein-1 to the recognition sites (Goto *et al.* 1998). A new anthraquinone, Blanchaquinone along with known analogue anthraquinone reported from an Australian *Streptomyces* sp. exhibited cytotoxicity but not antibacterial activity (Clark *et al.* 2004). A new anthraquinone, 1, 8-dihydroxy-2-ethyl-3-methylanthraquinone from a marine *Streptomyces* sp. Fx-58 showed cytotoxicity against HL-60 cells (Huang *et al.* 2006).

You *et al*. (1999) described that 8-hydroxyquinoline as a potent lipophilic metal chelator whose 8-hydroxyquinoline copper chelate could be used as a fungicide in agriculture. Kenawy (2001) found that the polymers containing 8-hydroxyquinoline moiety were inhibitory to *Escherichia coli, Bacillus subtilis* and *Trichophyton rubrum.* Sugaya *et al.* (2005) reported a bioactive water soluble substance, 2-hydroxyquinoline from *Comamonas* sp. TKV 3-2-1.

In the present study, bioactive compound of the strain 8-hydroxyquinoline was found to possess promising antioxidant and anticancer properties which may be exploited as anticancer drugs for immunosuppressive patients.

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