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Original Article

SPECTROSCOPIC ANALYSIS OF BIOACTIVE COMPOUNDS FROM *STREPTOMYCES CAVOURESIS* KUV39: EVALUATION OF ANTIOXIDANT AND CYTOTOXICITY ACTIVITY

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ABSTRACT

Objective: To assess the antioxidant and anticancer activity of crude ethyl acetate extract from *Streptomyces cavouresis* KU-V39 isolated from vermicompost.

Methods: To determine its antioxidant activity by total phenol and flavonoid content, Hydrogen peroxide radical scavenging activity and Ferrous reducing power assay. To determine *in vitro* cytotoxicity, various concentrations of extract was tested on HeLa cell line by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Fourier transform infrared spectroscopy (FT-IR) and Gas chromatography–mass spectroscopy (GC-MS) have been carried out to investigate the bioactive compound of crude extract.

Results: The Phenol and flavonoid content of crude extract was found to be 20.24 and 26.60 mg /g metabolite. Isolate KU-V39 exhibited H_2O_2 scavenging activity at IC_{50} value is 42.35 ± 0.75 µg/ml. The ferrous reducing power assay has higher absorbance value which indicates the high antioxidant capacity of the extracts. The analysis of FT-IR spectroscopy indicate presents of Phenol, carboxylic acid, alkanes, nitro and aliphatic amines functional groups. The GC – MS analysis revealed that presence of Pentanoic acid, L-Proline, Lysine, Erucic acid, isosteviol, Pentadecanoic acid, Phthalic acid, Hexadecanoic acid, Octadecenoic acid, Dichloroacetic acid, Cyclohexanecarboxylic acid and Pyrrolo pyrazine derivative.

Conclusion: This spectral study clearly proved that the vermicompost derived *Streptomyces* with extra cellular metabolism showed promising antioxidant and anticancer activity.

Keywords: Anticancer, Antioxidant, Bioactive compound, *Streptomyces cavouresis* KU-V39, FT-IR and GC-MS.

INTRODUCTION

In recent years more attention has been given to natural antioxidants and their association with health benefits [1]. Oxidative stress caused by reactive oxygen species plays an important role in the development of various diseases, such as the Alzheimer and Parkinson diseases. It has been the major area of focus for many researchers particularly in the field of drug discovery [5]. The main characteristic of an antioxidant is its ability to trap free radicals which oxidize nucleic acids, proteins, lipids or DNA.

The search of novel drug is still a priority goal for cancer therapy, due to the rapid development of resistance to multiple chemotherapeutical drugs. In addition, the high toxicity usually associated with cancer chemotherapeutical drug and their undesirable side effects increase the demand for novel antitumor drug active against untreatable tumors, with fewer side effects and with greater therapeutic efficiency [2]. Anticancer drug focuses on inhibition of tumor cell growth and induction of apoptosis in the malignant cell population. It is characterized by the ability of a cell to undergo a self-suicide program without affecting neighbouring or adjoining cells [6]. Members of the actinomycetes genus especially *Streptomyces* sp. have been recognized as prolific producer of useful bioactive metabolite with broad spectrum of activities. Streptomyces have many vital bioactive compounds with high commercial values and are able to produce wide variety of antibiotics and extra cellular enzymes. The bioactive molecules have many clinical valuable drugs such as vancomycin and erythromycin (antibacterial), cyclosporin (immune suppressant) and antitumor drug such as antracyclines, aureolic acids, enediynes and other [10].

We have previously reported that the isolation of actinomycetes from vermicompost were characterized and identified as *Streptomyces cavouresis.* 16 s RNA gene sequence is submitted in the gene bank (NCBI, USA) with accession number KF974778. The extracellular compound of this strain has antimicrobial activity against pathogenic bacteria (*E.coli, Bacillus, Pseudomonas, Streptococcus* and *Proteus*) and fungi (*C.albicans*) [9]. The objective of this research was spectroscopic analysis of bioactive compound produced by *Streptomyces cavouresis* KUV39 and to evaluate the antioxidant and anticancer activity of the metabolite.

MATERIALS AND METHODS

Vermicompost samples were collected from vermin compost units, Department of Biotechnology, Karpagam University, Coimbatore, Tamil Nadu, India. All chemicals were purchased from Hi Media, Mumbai.

Ethyl acetate extraction of *streptomyces cavouresis* KU-V39

KU-V39 isolate was taken in 500 ml Erlenmeyer flasks containing 100 mL of yeast malt extract broth (4 g/L yeast extract, 10 g/L malt extract, 4 g/L dextrose) media and incubated at 28 ^oC in a shaker (180 rpm) for 7 days. After 7th day the culture broth was filtrated to remove the biomass. Equal volumes of ethyl acetate were added to culture broth and kept in shaker for 2 hours. The organic solvent layer was transferred to a clean and sterile flask. The extracts were stored in -4 ^oC and it was used for further analysis.

Antioxidant activity

Several methods have been developed in which the antioxidant activities were assessed by scavenging of synthetic radical in polar organic solvent. Common synthetic radical used in the present study included total phenol content, total flavonoid content, hydrogen peroxide radical scavenging and ferric reducing power.

Determination of total phenols and flavonoids contents

Total phenols were determined by using Folin - Ciocalteu method [17]. Analyses were performed by visible spectrophotometry at 750 nm after reaction with Folin Ciocalteu's reagent. In brief, an amount of 0.1 ml extract samples with different dilution were mixed with 2 ml of 20 mg/ml Na_2CO_3 for 2 min, and then 0.9 ml of Folin - Ciocalteu's reagent (previously diluted 2-fold with distilled water) was added. The absorbance of reaction was measured at 750 nm by using the Shimadzu – 1601 UV-VIS spectrophometer, after 30 min of

incubation at room temperature. Total phenol contents of the metabolite were expressed in term of milligram of gallic acid (mg) per gram of extract. Total flavonoids were determined by using spectrophotometrical methods [13]. To 0.1 ml of extract samples with different dilution, 2 ml of distilled water was mixed with 0.1 ml of 5% $NaNO_2$ for 6 min, and then 0.2 ml of 10% $AlCl_3$ were added and mixed for 5 min. The total volume was made up to 3 ml with distilled water. The absorbance of reaction was measured at 420 nm against the blank spectrophotometrically. Results were expressed as mg /g metabolite.

Hydrogen peroxide radical scavenging activity

The hydrogen peroxide radical scavenging activity was assessed by Ruch *et al* method using ethyl acetate extract of *S. cavouresis* KUV39 and aliquots of 50 – 250 µg/ml were added to a 0.6 ml hydrogen peroxide (40 mM) with the already prepared phosphate buffer (pH 7.4). The reaction mixtures were incubated at room temperature for 10 mins. After incubation, the reaction mixture was read at 230 nm against the blank solution with phosphate buffer (pH 7.4).

The percentage of inhibition was calculated based on the formula: % of inhibition = $(A_1-A_2)\ /A_1$ X 100

Where A_1 -absorbance of reaction mixture without extract and A_2 - absorbance of the reaction mixture with extract

Ferrous reducing power assay

The reducing ability of ethyl acetate extract of *S. cavouresis* KUV39 was assessed according to the method of Oyaizu, 1986 [14]. Various concentrations (50-250 µg /ml) of the ethyl acetate extracts (0.250ml) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. with TCA (10%: 2.5 ml). Then mixture was centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (1%) and the absorbance was measured at 700 nm. The increasing absorbance with increasing concentration of the reaction mixture indicated greater reducing power. The reducing power of *S. cavouresis* KUV39 ethyl acetate extract was compared with standard antioxidant L-ascorbic acid (mg/ml).

Cytotoxic activity MTT (3(4, 5-dimethylthazol-2-yl)-2-5-diphenyl tetrazolium-bromide)

The human cervical cancer cell line (HeLa) was obtained from National centre for cell science (NCCS) pune, India. Cells were grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS) and NIH 3T3 Fibroblasts were grown in Dulbecco's Modified Eagles Medium (DMEM) containing 10% FBS. For screening experiment, the cells were seeded into 96 well plates in 100µl of respective medium containing 10% FBS at plating density of 10,000 cells/well and incubated at 37, 5% CO_2, 95% air and 100% relative humidity for 24 hours prior to addition of extract. The extract was solubilised in Dimethyl Sulfoxide and diluted in respective serum free medium. After 24 hours, 100ml of the medium containing the extract at various concentrations (4.12, 8.25, 16.5, 32.1, 64.5, 125, 150, 500, 750, 1000, 2000 µg/ml) was added and incubated at 37°C, 5% CO_2,95% Air and 100% relative humidity for 48 hours. Triplicate was maintained and medium containing without extract were served as control. After 48 hours, 15µl of MTT (5mg/ml) in PBS was added to each well and incubated at 37C for 4 hours. The medium with MTT was flicked off and the formed formazan crystals were solubilised in 100µl of DMSO and then measured the absorbance at 570nm using micro plate reader. The % cell inhibition was determined using following formula.

$$\% \text{ Cell inhibition} = \frac{\text{Mean absorbance of Sample}}{\text{Mean absorbance of control}} \times 100$$

Spectroscopic analysis of crude sample

The functional groups of crude compound were analyzed by using Fourier trans-form infrared spectroscopy (Perkin-Elmer 1725x). The infrared spectra of the samples were recorded using KBr pellet technique in the region 4000–400 cm^{-1}. The compounds of ethyl acetate crude extract were analyzed by Gas Chromatograph – Mass Spectrometry (SHIMADZU). For GC-MS analysis, a 30m×0.25 mm TR-35MS capillary column with a film thickness of 0.25 µm was used. The carrier gas was helium maintained at a column flow of 1.51 mL/min (at a pressure of 105 kPa). A 1.0 µL sample of the extract was injected and the column temperature was maintained at 70°C for 2 min followed by temperature programming at 10 °C/min to 200 °C for 2 min. This was raised to 240 °C at a rate of 5 °C/min for 2 min, and finally to 300°C at a rate of 35 °C/min for 2 min (Scan range: 40 – 1000 m/z).

Identification of compounds

Interpretation of mass spectrum of GC-MS was made using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULT AND DISCUSSION

Total Phenolic and flavonoid content

Phenolic have been considered as the major antioxidant in scavenge reactive oxygen species. It is due to their redox properties, electron donating properties and singlet oxygen quenching properties [3]. In the present study the total phenolic content was found to be 20.24 mg gallic acid equivalents/g of metabolite for gallic acid. The flavonoids show antioxidant activity and their effect on human health is considerable. The mechanisms of flavonoids are through scavenging or chelating process [7]. In the present study the flavonoid content of metabolism was 26.60 mg /g metabolite.

Hydrogen peroxide radical scavenging activity

The scavenging ability of the ethyl acetate extract of *S. cavouresis* KUV39 are shown in Figure.1. The L-ascorbic acid was used as standard antioxidant and IC_{50} value was 42.35 ± 0.75 µg/ml. The presence of H_2O_2 in the cell culture may lead to the oxidative DNA damage. Hence removing hydrogen peroxide is very essential for antioxidant defense in cells.

Ferrous reducing power assay

In order to examine the ferrous reducing power of extracts, the reduction of Fe^{3+} to Fe^{2+} was investigated in the presence of extracts. The tested samples would result in the reduction of Fe3+/ferricyanide complex to the ferrous form (Fe^{2+}). The reducing power of ethyl acetate extract of *S. cavouresis* KUV39 has been revealed and shown in Figure 2. The higher absorbance value indicated high antioxidant capacity of the extracts. It specifies the electron donating and reducing capacity of the extracts.

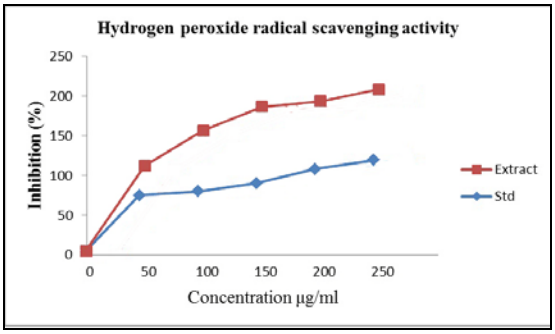

Fig. 1: Estimation of hydrogen peroxide radical scavenging activity on ethyl acetate extract of *S. cavouresis* KUV39

Assessment of cytotoxicity using MTT assay

The cytotoxicity assay of the crude antibiotic was performed against HeLa line by MTT assay. The IC_{50} of crude compound against HeLa

cells was found to be 63.9 µg ml^{-1} (Figure 3). These results suggest that crude compound from *Streptomyces cavouresis* KUV39 was toxic to HeLa cells. The cytotoxic activity of *Streptomyces cavouresis* could be clinically important and need to be investigated for the further anticancer properties. This result is very close to the criteria of cytotoxicity activity for the crude extract, as established by the American National cancer institute (NCI) is IC_{50} < 30 µg ml^{-1}.

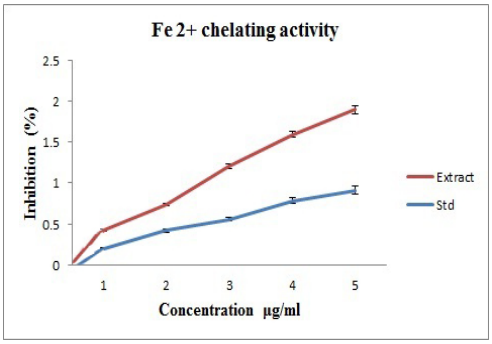

Fig. 2: Estimation of Ferrous chelating activity on ethyl acetate extract of *S. cavouresis* KUV39

Suthindhiran and Kannabiran [16] showed cytotoxicity of the crude extract on HeLa cells from *Streptomyces* strain isolated from Marakkanam coast. They concluded that crude extract has high toxicity against human cervical cancer cell line. Sudha and Masilamani selvam [15] reported that crude extract of the *Streptomyces avidinii* strain SU4 isolate exhibited IC_{50} value is 64.5 µg ml^{-1} against Hep-2 cell line, 250 µg ml^{-1} in VERO cell line.

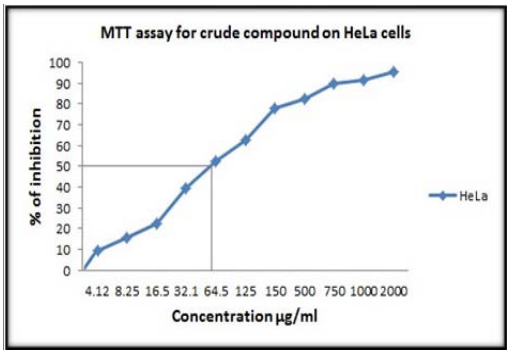

Fig. 3. Effect of crude compound on cell inhibition of HeLa cell

FTIR analysis of crude ethyl acetate of *Streptomyces cavouresis* KUV39

The IR result showed the broad spectrum range at 3379 cm^{-1} corresponding to phenol group. The band at 2962, 2931, 2870 cm^{-1} indicates the presents of alkanes, 1759 and 948 cm^{1} refers carboxylic acid, 1381 cm^{-1} which indicates nitro, 1242,1111, 1072, 1041 cm^{-1} show the presence of aliphatic amines (Figure 4 and Table 1). This study proved the presence of phenol, carboxylic acid and alkanes functional group, revealed that crude extract of *S. cavouresis* KUV39 have antimicrobial and antioxidant properties.

GC-MS analysis of crude ethyl acetate extracts of *Streptomyces cavouresis* KUV39

The ethyl acetate extract of *S. cavouresis* KUV39 was subjected to gas chromatography mass spectrometry analysis. The identification of the compound is based on the peak area, molecular weight and molecular formula. This area is directly proportional to quantity of the compound present in the extract. GC – MS analysis showed the presence of 19 compounds (Figure 5 and table 2).

The six compounds showed retention times of 14.661, 16.496, 19.546, 16.208, 16.167, and 9.937, respectively. However, based on the comparison with the standards in NIST data base it can recognize that these obtained components were 3-isobutyl hexa hrdro pyrrolo(1,2A)pyrazine-1,4-dione (24.67%), Benzene propanic acid 3,5 bis(1,1 – dimethyl ethyl) – 4 hydrxy, methyl ester (4.94%), 7,9 Di-tert-butyl-1-oxaspiro (4,5) deca-6,9 – diene-2,8-dione (4.37%), Hexadeconic acid, methyl ester (2.42), Phenol 2,4-bis(1,1-Dimethyl ester)(1.45%) and 1,2 benzene dicarboxylic acid (1.42%). Various aliphatic acids and aromatic compounds were also identified in the studied fractions. Compounds identified by GC-MS analysis possess various pharmaceutical applications. Alkylate phenol and its derivatives are known for their anti-oxidative properties and also as one of the principal antimicrobial preservative used in food and beverages.

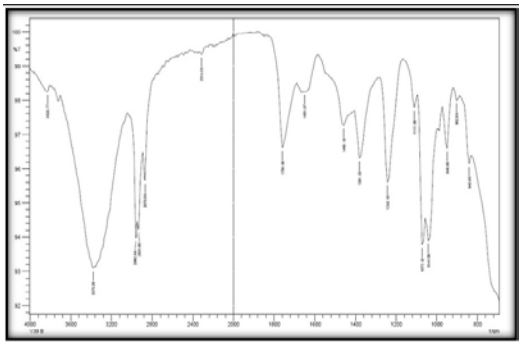

Fig. 4: FTIR analysis of ethyl acetate extract of *S. cavouresis* KUV39

Table 1: IR absorption frequencies of functional groups in *Streptomyces cavouresis* KUV39

S. No.	Characteristic Absorption (cm^{-1})	Functional group
1	3379.29	O-H stretch
2	2962.66, 2931.80, 2870.08	C-H Stretch
3.	1759.08, 948.98	H-C-H asymmetric stretch
4.	1381.03	N-O symmetric stretch
5.	1242.16,1111.00, 1072.42, 1041.56	C-N Stretch

Palmitic acid and Hexadecanoic acid possess potent antioxidant, anticancer and antimicrobial properties [14]. It could be reasonably argued that the presence of these compounds in the fractions must also be responsible for the antimicrobial, antioxidant and anticancer activity of *S. roxburghiana*. These compounds might be responsible for toxicity on cancer cell line and also have antioxidant properties.

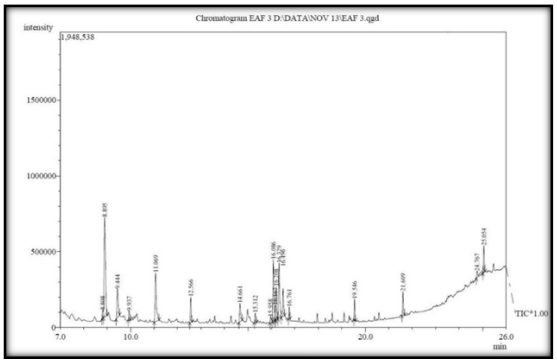

Fig. 5: GC – MS analysis of ethyl acetate extract of *S. cavouresis* KUV39

Table 2: GCMS analysis of ethyl acetate extracted compound from *S. cavouresis* KUV39

Peak	R. Time	Name
1	8.808	8,10-Undecadiene
2	8.895	3,4-Dihydro-2h-Pyran-2-Carbaldehyde
3	9.444	N-Nitroso-2-isopropyl-4,4-dimethyloxazolidine
4	9.937	Phenol, 2,4-Bis(1,1-Dimethylethyl)
5	11.069	3,6-Diisopropyl-2,5-dioxomorpholine
6	12.566	8-Heptadecene, 1-Chloro
7	14.661	3-Isobutylhexahydropyrrolo[1,2-A]Pyrazine-1,4-Dione
8	15.312	3-Keto-isosteviol
9	15.958	2,3-Dimethyl-3-decanol
10	16.089	3-Isobutylhexahydropyrrolo[1,2-A]Pyrazine-1,4-Dione
11	16.167	Hexadecanoic Acid, Methyl Ester
12	16.208	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
13	16.329	3-Isobutylhexahydropyrrolo[1,2-A]Pyrazine-1,4-Dione
14	16.496	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester
15	16.761	1,2 benzene dicarboxylic acid
16	19.546	Hexadecanoic acid, butyl ester
17	21.609	2,3-Dihydroxypropyl elaidate
18	24.767	1-heptene, 2-isohexyl-6-methyl-
19	25.054	9-octadecenoic acid (z)-, 9-hexadecenyl ester

CONCLUSION

In conclusion, vermicompost is a rich source of actinomycetes species producing the antioxidant and cytotoxic metabolites. *Streptomyces cavouresis* KUV39 showed antioxidant activity, helpful in preventing or impedance the progress of various oxidative stresses related disorders. This spectral study confirms that ethyl acetate extract of *Streptomyces cavouresis* KUV39 have an antioxidant and anticancer metabolites. It could be considered as a potential source for anti-cancer drug development. The purification and structural elucidation of the cytotoxic compound produced are under investigation.

CONFLICT OF INTERESTS

Declared None

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