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LC-ESI-MS/MS Analysis of Bioactive Metabolites from *Streptomyces rochei* with Antimicrobial, Antioxidant, and Cytotoxic Properties

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ABSTRACT

Actinomyces, particularly *Streptomyces*, have a major impact on protecting plant roots by inhibiting the growth of fungal species based on their capacity to release antifungal antibiotics. Herein, FT-IR spectra for *Streptomyces rochei* extract revealed the varied patterns of functional group absorption of the bioactive compounds (BACs). Furthermore, using LC-ESI-MS/MS analysis, 21 novel diverse secondary metabolite compounds from *S. rochei* extract were found: nine flavonoids, one flavonoid derivative, one phytohormone, eight phenolic acids, one benzoic acid esters and one triterpenoid. Likewise, a DPPH radical scavenging capacity experiment was conducted to assess the antioxidant properties of four different concentrations (50, 100, 150 and 200 µg/ml) of *S. rochei* extract. In addition, BACs from *S. rochei* exhibited significant antibacterial activity against dangerous human pathogens *Bacillus cereus*, *Staphylococcus aureus* and gram-negative bacteria *Salmonella typhimurium*. Also, antimycotic activity against *Candida albicans* was measured. *S. rochei* extract showed a moderate level of cytotoxicity against lung cancer cell lines (A-549) with low cytotoxic effects on lung normal cell lines (WI-38). The biological activities of *streptomyces* extract might be attributed to the presence of a variety of metabolites, and hence, *S. rochei* can be used as an excellent source of bioactive metabolites.

Keywords: *Streptomyces rochei*, LC-ESI-MS/MS, FT-IR, antimicrobial, antioxidant and cytotoxicity activity.

1. INTRODUCTION

Actinomycetes are gram-positive bacteria that form spores that can be found in in nature, at both terrestrial and aquatic environment, they are recognizing for producing wide varieties of significant bioactive metabolites [1]. The diversity and activity of terrestrial Actinobacteria have been estimated on different ecosystems. Many significant secondary metabolites, including antibiotics like streptomycin, actinomycin and tetracycline, have been successfully produced by actinomycetes. Numerous insecticides, aliphatic and aromatic chemicals, hydrocarbons, and other substances are degraded by actinomycetes. According to Salari *et al.* [2], they carry out microbial conversions of organic molecules, with significant commercial value.

The genus *Streptomyces* spp. was demonstrated by Niyomvong *et al.* [3] as the most common actinomycetes capable of secreting a diverse of valuable natural products with significant biological activities in the fields of medicine, environment, food industries and agronomy sectors. In the last 20^{y}, according to Adeyemo *et al.* [4], the utilization of secondary metabolites from Streptomyces has been suggested as a different approach for the biocontrol of plant diseases and to lessen the usage of chemical agents. *Streptomyces* sp. was proved to be the most prevalent supplier of bioactive metabolites with a broad range of beneficial traits. These metabolites are known to possess antibacterial, antifungal, antioxidant, cytotoxicity, anti-algal, anti-helminthic, anti-malarial, anti-inflammatory and plant growth-promoting properties [5]-[11].

Streptomyces species have been considered bio-factories of a diverse range of secondary metabolites that produce around 100,000 antibiotic compounds, which account for 70–80% of all natural bioactive products with pharmacological or agrochemical applications [12]. Alenazi *et al.* [13] reported that *Streptomyces* spp. produce specialized metabolite compounds that enhance nutrient uptake, stimulate plant growth, induce resistance, and lessen or prevent pest or pathogen invasion. These metabolites are beneficial to plant health. Similar to this, Sarika *et al.* [14] demonstrated that *S. felleus* BHPL-KSKU5 contained bioactive compounds with anticandidal activity. These investigations support the findings of [15] which discovered that *S. olivaceus* secretes 28 secondary metabolites. El Hussein *et al.* [16] reported that *S. rochei* R92 was highly effective in vitro at inhibiting the growth of *D. halodes*, *A. alternata*, *A. sesami*, *and M. phaseolina*, also found to be effective in preventing the occurrence and progression of leaf spot diseases on tomato and sorghum that are caused by *A. alternata* and *D. halodes*, respectively. *S. rochei* ACTAI55I has been shown by Kanini *et al.* [17] to be able to shield tomato seeds from *F. oxysporium* pathogen effects. In addition, *S. rochei* AMET 311 strain exhibited *in vitro* activity against *R. solani* [18].

Flavonoids constitute a large group of biological products which are responsible for the colors of flowers and aromas of plants and protect them against microbial pathogen infections. Numerous flavonoid compounds are

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known to be beneficial for both human and animal nutrition due to their abundance in fruits, vegetables, and nuts. Some of these compounds are also used in cosmetic products and pharmacological activities, e.g., quercetin and kaempferol that have, respectively, antioxidant and antitumor activities. An earlier study by Martín and Liras [19] found naringenin is formed by actinobacterium *Streptomyces* clavuligerus, a well-known microbe used in the industrial production of clavulanic acid. Several strains of *Streptomyces* can promote plant growth and biocontrol pests, diseases, weeds and phytopathogenic microorganisms by producing phytohormones (IAA and abscisic acid). Abscisic acid is important to alleviate abiotic stresses, such as salinity, drought, and organic and inorganic contaminants in soil. Herence, *Streptomyces* spp., is a promising way to promote sustainable agriculture by improving crop productivity and reducing the use of agrochemicals, evidencing an eco-friendly approach for agricultural purposes [20].

The current investigation aims to identify and characterize strong bioactive metabolites of *S. rochei* AC143 strain and its antioxidants, antimicrobials and cytotoxic activities.

2. MATERIAL AND METHOD

2.1. Preparation of crude extract

The *S. rochei* strain (Genbank; OQ519886, AC143), previously isolated and identified by the authors, was cultivated on solidified starch nitrate agar (SNA) medium [1], which contained (g/l): soluble starch (20); KNO_3 (2); NaCl (0.5); K_2HPO_4 (1); $MgSO_4.7H_2O$ (0.5); $CaCO_3$ (3); $FeSO_4.7H_2O$ (0.001); (20) agar; pH 7.0 ± 2, and incubated at 28 ± 2°C for 8 days. After incubation, the broth was centrifuged (at 4000 rpm. for 20 min.) and filtered through Whatman No. 1 filter paper, followed by a 0.45 µm vacuum membrane filtration set. The filtrates were transferred aseptically into conical flasks. Then, in order to achieve full extraction, ethyl acetate was added to the filtrates in a ratio of 1:4 (v/v). The filtrate in this ratio was then moved to a separator funnel and left overnight. A C_{18} column (SEP.PAK, USA, 1 g) was used to separate the ethyl acetate phase containing the bioactive compounds from the aqueous phase. Following the elution process, the extract was dried at 45 °C in a rotary evaporator [21].

2.2. Fourier transforms infrared spectroscopy (FT-IR) analysis

The significant functional groups pattern of bioactive metabolites (BAMs) was identified using FT-IR (Nicolet 380 FT-IR, Thermo Scientific, USA). Potassium bromide powder was crushed with the dried extract before it was compressed into a disk with a diameter of 10 mm. Based on a resolution of 4/cm and 2 scans, the FT-IR measurements ranged from 4000 to 500 cm [22].

2.3. Liquid Chromatography-Mass Spectrometry (LC-ESI-MS/MS) analysis

This analysis was carried out to separate and distinguish the BACs that the *S. rochei* strains produced. The procedure for separation was carried out as previously mentioned in Section (2.1.) where the confirmation of metabolite compound identification was achieved through the use of tandem mass spectrometry (LC-MS/MS) for separation and electrospray ionization (ESI)-equipped MS/MS system for detection [23], [24].

Negative ionization mode: The separation was performed with a Ascentis® Express 90 Å C_{18} Column (2.1×150 mm, 2.7 µm). The mobile phases were comprised of two eluents A: 5 mM ammonium formate pH 8 and B: acetonitrile (LC grade). The mobile phase gradient was programmed as follows: 5% B at 0-1 min, 5-100% B from 1-20 min, 100% B from 20-25 min, 5% at 25.01 and 5 % from 25.01-30 min. The flow rate was 0.3 ml/min and the injection volume was 5 µl. For MS/MS analysis, negative ionization mode was utilized with a scan (EMS-IDA-EPI) from 100 to 1000 Da for MS1 with the following parameters: curtain gas: 25 psi; IonSpray voltage: -4500; source temperature: 500 °C; ion source gas 1 & 2 were 45 psi and from 50 to 1000 Da for MS2 with a declustering potential: -80 vol.; collision energy: -35 vol. Compounds' identification was performed using MS-DIAL using Respect library.

2.4. Bio-applications of the produced bioactive metabolites (BAMs)

2.4.1. Antioxidant activity (free radical-scavenging activity)

The antioxidant activities of the BAMs were measured using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method [25] to determine the free radical-scavenging potential of BAMs sample from *S. rochei* strain. The mixture, which comprised 2 ml of the BAMs at multiple concentrations (50, 100, 150 and 200 µg/ml) and 2 ml of DPPH (0.2 mM in methanol), were vigorously shaken and kept in darkness for 30 min. The UV-VIS Shimadzu spectrophotometer 2401PC (Shimadzu, Japan) was then used to measure the absorbance at 517 nm. Three separate experiments were used to calculate the mean values. The following equation was used to express the antioxidant activity (also known as radical scavenging activity) of BAMs as a percentage of inactivated DPPH reagents:

% DPPH = [Abs. 517 of control – Abs. 517 of sample / Abs. 517 of control] ×100.

The effective concentration (EC_{50}) was considered as the BAMs concentration (µg/ml) at which the DPPH absorbance was reduced by 50 %.

2.4.2. Antimicrobial activity

According to Bauer *et al.* [26], the disc-diffusion experiment was used to assess the antimicrobial properties of the produced BAMs. 10 µl of the BAMs samples (100 mg/ml solubilized in DMSO) were saturated in sterile paper discs and placed into the inoculated agar medium with test organisms, including gram-positive bacteria (*Bacillus cereus* ATCC 33018, *Staphylococcus aureus* MRSA ATCC 43300, and *Listeria monocytogenes* ATCC 7644), gram-negative bacteria (*Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9072, and *Escherichia coli* ATCC 25922) on Mueller-Hinton agar medium at 30-37 °C for 24 h, and fungal strains (*Candida albicans* ATCC 10231) on Sabouraud dextrose agar medium at 25 °C for 24-72 h. Subsequently, the antimicrobial activity was evaluated by measuring the inhibition zone diameter (mm) around the BAMs saturated discs. Discs loaded with penicillin G (10 µg), ampicillin (10 µg), and nystatin (100 units) were served as positive standard antimicrobials for Gram-positive bacteria, Gram-negative bacteria, and fungi, respectively.

2.4.3. Antitumor activity (cytotoxicity)

a. Cell lines maintenance

The used cell cultures were human lung cancer (A-549) and human lung normal (WI-38). Cell lines were measured using the MTT assay in Roswell Park Memorial Institute (RPMI) medium with 2% serum (maintenance medium). These cell lines were obtained from the Tissue Culture Unit, Holding Company for Science Way for Scientific Researches and Consultations (Mokatam, Cairo, Egypt) [22].

b. MTT assay

Cells were cultivated in individual 96-well plates containing Dulbecco's Modified Eagle's medium (DMEM) complemented with 2% (v/v) fetal bovine serum (FBS) at a density of 105 cells/ml (100µg/well). Prior to performing the MTT assay, adherent cells were incubated at 37 ˚C for 24 h. Afterward, they were treated with 100µl of the BAMs solution at six different concentrations (31.25, 62.5, 125, 250, 500 and 1000µg/ml in DMSO) for 48 h. DMEM was used as a negative control, while doxorubicin (100 µg/ml) was used as a positive control (giving 100% inhibition). The supernatant of each well was replaced by 100µl of fresh medium (without FBS) containing 20µl of 5mg/mL of MTT (Sigma) solution (5 mg/ml in PBS) (BIO BASIC CANADA INC) at separate wells and incubated at 37 ˚C in a humid atmosphere with 5% CO_2 and 95% air for 4h, then 200 µL of DMSO was added to dissolve the formazan crystals. The amount of formazan product was determined spectrophotometrically. The optical density (OD) of the well contents was read at 560 and 620 nm with an ELISA plate reader (Mindray MR-96A Instrument, CHINA) [22]. The experiments were triplicated on normal cells as the control where the growth inhibition rates were calculated using the following formula:

Growth inhibition rate (%) = [A-B/A] x 100.

Where: A and B are the absorbance of the supernatant of untreated and treated cell cultures, respectively. Then, the half-maximum inhibitory concentration (IC_{50}) was estimated from the plotted graph.

2.5. Statistical analysis

All experiments were repeated three times independently, and the summary statistics are expressed as mean ± standard deviation (SD). Data were analyzed by one-way ANOVA with LSD test (using XLSTAT 2019.1.2.56963 - ANOVA - Microsoft Excel 15.04420 software). Differences between samples mean values of $p < 0.05$ were considered to be significant [27].

3. RESULTS AND DISCUSSION

3.1.1. Characterization of the bioactive compounds (BACs)

3.1.2. *FT-IR Characterization of the BACs*

The IR spectra of biosynthesized BACs are shown in Fig. (1). A broad, intense band at 4000 to 500 cm^{-1} with a resolution of 4 cm^{-1}. The range of absorbance values at EthAS extract of *S. rochei* strain is within the range of 3460 to 610 cm^{-1} for AC143.The functional groups include N-H, C-H, C=O, C-O-H, C-O stretch, C-C, and (C-Cl, C-Br, C-I). These functional groups indicated that the compounds can be classified into Vmax (cm^{-1}): secondary amine (3460), alkanes (2964), ketones (2632 and 1724), carbohydrate ether (1376), alkyl or aryl, ether and secondary alcohol (1246), alkanes, alkyl chains and ester (1050 and 944) and halogen compounds.

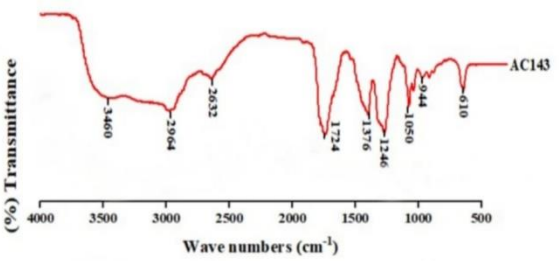

Fig. 1. FT-IR analysis of bioactive compounds produced by *S. rochei* (AC143).

A corresponding result was witnessed in the existence of functional groups of compounds by Sholkamy *et al.* [28], who confirmed the FT-IR spectrum of ethyl acetate extracts of *S. cuspidosporus SA4.* In general, functional groups such as alcohols, phenols, alkanes, aldehydes, aromatic compounds, secondary alcohol, aromatic amines, and halogen compounds are detected from FT-IR analysis [29]. Similar results were also reported by Retnowati *et al.* [30] in regard to the discovery of some functional groups in this study. The outcome of the obtained result is consistent with the results published by Chakraborty *et al.* [31], wherein FTIR spectroscopy analysis from *S. violacceusniger* KS20 revealed the presence of different kinds of functional groups such as alcohols, esters and carboxylic acids.

3.1.3. LC-MS/MS analysis of BACs

The BACs composition that was produced by *S. rochei* (AC143) was characterized using LC-MS/MS analysis of the extract, as shown in Fig. (2). The obtained results of chromatograms exhibited the discover of 21 compounds in Table (1) as the following: Diosmetin or Chryseriol, Apigenin, Naringenin, Querecetin, Apigenin C-hexoside (Isovitexin), Luteolin, Vitexin, Taxifolin, Tetramethyl-O-scutellarin, Methylsudachitin, Abscisic acid, 3,3′-di-O-methyl ellagic acid, Caffeic acid, 3.4-Dihydroxybenzoic acid, P-Coumaric acid, Vanillin, Ferulic acid, Liquiritin apioside, Protocatechualdehyde, Di-n-butyl phthalate and Ursolic acid or Oleanolic acid.

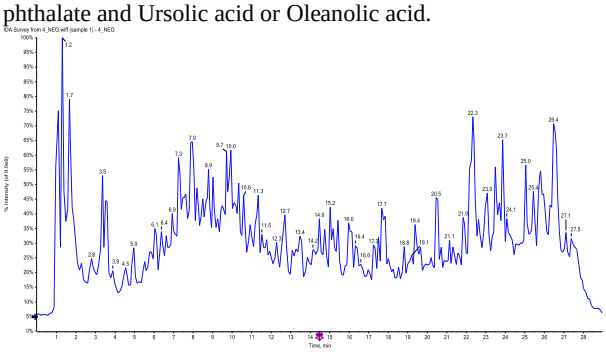

Fig. 2. LC-MS/MS analysis of bioactive metabolites produced by *S. rochei* strain.

Phenolic compounds like 3,3′-di-O-methyl ellagic acid, caffeic acid, 3.4-dihydroxybenzoic acid, P-coumaric acid, vanillin, ferulic acid, liquiritin apioside and protocatechualdehyde were detected and showed antioxidant, antimicrobial, and antifungal activity. This finding was similarly reported by Passari *et al.* [32], who demonstrated that ferulic acid, an anticancer compound, was reported in the isolate *Streptomyces* sp. strain BPSAC121. This finding was in agreement with results

reported by Almuhayawi *et al.* [33], who indicated that actinomyces isolate 21 contained the highest concentrations of ferulic, protocatechuic, galic, p-coumaric, chlorogenic, sinapic, ellagic acids, catechin, resorcinol, quercetin, isoquercetin, rutin, velutin, naringenin, genistein, fisetin and O-hydroxydaidzein, whereas isolate 19 had the highest ratios of caffeic acid, apigenin and daidzein. Naringenin is a valuable chemical for industry due to its several therapeutic characteristics and essential role in the biosynthesis of flavonoids.

Table 1. Metabolites compounds from *S. rochei* identified using LC-ESI-MS/MS analysis in negative ionization mode.

No.	Proposed Compound	Rt (min.)	$[M-H]^-$ (m/z)	MS/MS fragments (m/z)	M.F.	M.W.	References
				Flavonoid			
1	Diosmetin or Chryseriol	9.69	299	299**, 284**, 256, 255	$C_{16}H_{12}O_6$	300	Antibacterial & anti-inflammatory & antioxidant activity [38].
2	Apigenin	9.39	269	269, 225, 151, 149, 139, **117**	$C_{15}H_{10}O_5$	270	Antifungal activity [39].
3	Naringenin	8.70	271	271, 167, 151, **119**	$C_{15}H_{12}O_5$	272	
4	Querecetin	9.05	301	301, 151, **134**	$C_{15}H_{10}O_7$	302	
5	Apigenin C-hexoside **(**Isovitexin)	5.22	431	431, 415, 339, 313**, 311,** 283, 193, 174	$C_{21}H_{20}O_{10}$	432	Antimicrobial Activity [40].
6	Luteolin	8.91	285	285, 267, **241**, 155, 133	$C_{15}H_{10}O_6$	286	Antifungal activity [41].
7	Vitexin	5.22	431	431,415, 339, 341, **311**, 193, 174	$C_{21}H_{20}O_{10}$	432	Antifungal activity [42].
8	Taxifolin	18.26	303	**303**, 285, 271, 259, 241, 205, 177, 161, 151	$C_{15}H_{12}O_7$	304	Antifungal, antimicrobial and antioxidant activity [43].
9	Tetramethyl-O-scutellarin	8.71	341	341**,** 326**, 311**, 285, 283	$C_{19}H_{18}O_6$	342	Antioxidant & antimicrobial and cytotoxic activity [44].
				Flavonoid derivatives			
10	Methylsudachitin	4.85	373	373, 358, 343, **340**, 328, 283, 269, 147, 121	$C_{19}H_{18}O_8$	374	Antimicrobial activity [45].
				Phytohormone			
11	Abscisic acid	3.95	263	219, 204, 203, 201, 176, 175, 163, 151, **148**	$C_{15}H_{20}O_4$	264	**Massbank** Antifungal activity [46].
				Phenolic acid			
12	3,3′-di-O-methyl ellagic acid	6.60	329	**329**, 311, 249, 197	$C_{16}H_{10}O_8$	330	Antimicrobial and antioxidant activities [47].
13	Caffeic acid	6.18	179	179, 135, 133, 125, **107**	$C_9H_8O_4$	180	Antifungal activity [39].
14	3.4 Dihydroxy benzoic acid	1.23	153	153, 111, **109**, 108	$C_7H_6O_4$	154	Antioxidant activity [48].
15	P-Coumaric acid	1.51	163	163**, 119**, 135, 145	$C_9H_8O_3$	164	Antioxidant and Cytotoxic potential [39].
16	Vanillin	4.01	151	151, 136, 124, **108**	$C_8H_8O_3$	152	Antioxidant, Antimicrobial and antifungal activity [50], [39].
17	Ferulic acid	11.83	193	**193**, 178, 177, 149, 134	$C_{10}H_{10}O_4$	194	Antifungal activity [39].
18	Liquiritin apioside	22.44	549	549, **255**	$C_{26}H_{30}O_{13}$	550	Antifungal and antioxidant activity [51].
19	Protocatechualdehyde	2.63	137	137, **136,** 109	$C_7H_6O_3$	138	Antioxidant, cytotoxic, and antimicrobial activity [52].
				Benzoic acid esters			
20	Di-n-butyl phthalate	10.69	277	277, 233, 231**, 205,** 203, 191, 127, 125, 121	$C_{16}H_{22}O_4$	278	Massbank Antifungal, antimicrobial, antioxidant, and Cytotoxic Activities [53].
				Triterpenoid			
21	Ursolic acid or Oleanolic acid	20.57	455	**455**	$C_{30}H_{48}O_3$	456	Antifungal activity [54].

The results obtained by Ye *et al.* [34] exhibited that *S. albidoflavus* J1074 as a microbial cell factory for production of naringenin. An additional investigation disclosed that S. albus and S. coelicolor exhibit de-novo biosynthesis of the pharmaceutical flavonoids myricetin, kaempferol, and quercetin [35]. Li *et al.* [36] successfully isolated *S. pactum* Act12 that enhanced wheat's resistance to drought, the osmotic adjustment and antioxidant capacity of plants through induction of abscisic acid accumulation and up-regulation of drought resistance-related gene expression. In addition, the obtained results of the current study are in agreement with the results reported by Abdl Aziz *et al.* [37] observed a comparable result when they used the LC-ESI-MS/MS technology to examine 59 metabolites in *B. madagascariensis* and 66 compounds in *B. purpurea* methanol extracts, which identified these metabolic extracts as flavonoids (such as naringenin, apigenin, luteolin, quercetin, and hesperetin), phenolics (like caffeic acid, 3,4-dihydroxy benzoic acid and rosmarinic acid), carboxylic and fatty acids, coumarins, stilbenes and acyclic diterpenoids. Additionally, Awla *et al.* [21] used LC-MS analysis to identify a range of antifungal compounds secreted by *Streptomyces* sp. UPMRS4. Among these are ergotamine, amicoumacin, fungichromin, rapamycin and N-acetyl-D,L-phenylalanine. Also, Chanthasena *et al.* [24] revealed the production of two active substances, actinomycin D and dihomo-γ-linolenic acid (DGLA), from *S. actinomycinicus* PJ85.

3.2. Biological activities of BACs

3.2.1. a. Antioxidant activity

Living microorganisms undergo harm to their cells due to the action of free radicals, particularly reactive oxygen species (ROS). Therefore, it is critical to inhibit these substances in order to preserve cell viability. BAMs are the most effective compounds in this regard due to the inhibiting activity of them oxidative reactions as well as their low toxicity [25]. Within this current investigation, the BAMs produced by the *S. rochei* strain showed concentration-dependent DPPH scavenging ability ranging from 50 to 200 µg/ml.

Reasonable antioxidant activity from 62.7 to 70% with EC_{50} 39.8 µg/ml was recorded for the strain AC143 as shown in Fig. (3) is comparable with ascorbic acid as positive control with 96.82% to 97.83% at 50 to 200 µg/ml with EC_{50} 25.8 µg/ml. This result is in harmony with Bodhaguru *et al.* [55] and Ashok *et al.* [25], when comparable antioxidant activities of 91 ± 0.16% at 800 µg/ml extract were produced by *S. rochei*, but increasing concentrations of crude extract recorded a substantial increase in radical scavenging activity as follows: 26 ± 0.568 < 33 ± 0.41 < 55 ± 0.10 < 74.0.13 % at 50–400 µg/ml concentrations, respectively.

In the same way, it has been observed that *Streptomyces* spp. has significant antioxidant activity in terms of DPPH, ABTS, superoxide radical scavenging, and also metal-chelating activity [56]. Researchers have recorded several organic extracts and compounds, such as alkylated phenol and its derivatives, terpenes, long-chain fatty acids and alkaloids, from actinomycetes that are thought to possess remarkable antioxidant qualities.

3.2.2. Antimicrobial activity

Antifungal activity of BACs from *S. rochei* AC143 against *C. albicans* showed the highest significant, subsequently *S. aureus* and the methicillin-resistant strain (MRSA) as Gram-positive bacteria, followed by *B. cereus* and Gram-negative bacteria *S. typhimurium* among the tested microorganisms. These findings were reported in the disc-diffusion assay results in Table 2 and Fig. (4) these results showed that *S. rochei* was more potent against tested microorganisms. The negative control, DMSO, did not affect on the test strains. The findings from this research are exciting because this special strain of *S. aureus* is a serious public health threat worldwide [57]. Antibacterial studies were conducted by Sapkota *et al.* [58] on two isolates of *Streptomyces* strains C2 and H16, which demonstrated notable effectiveness against *S. aureus*. As well, *B. cereus* is another harmful organism to humans that causes foodborne illness. In contrast, the strains that possess an extensive range of activities may be attributed to their secretion of different substances, which may account for the observed variance in the test organisms' susceptibility to Gram-positive and Gram-negative bacteria [59], [60]. The results of this study were in line with previous publications by Adeyemo *et al.* [4], which demonstrated the isolated actinobacteria strains' antibacterial efficacy against various test strains of bacteria. The AFCs against *Candida* were discovered by Sarika *et al.* [14], which produced from *S. felleus* BHPL-KSKU5 as a potent isolate for anticandidal properties. The BACs inhibiting activity against Gram-positive bacteria could be attributed to the differences in the cell wall constituents. According to Budhathoki and Anima [61], actinomycetes from the soil sample that were isolated from Nepal showed strong antibacterial activity against both gram-positive and gram-negative bacteria. As it relates, the *Streptomyces* genus has a great track record for BACs discovery [62].

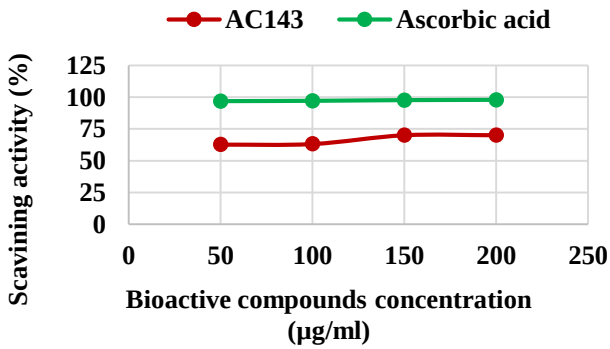

Fig. 3. Antioxidant scavenging activity of BACs produced by *S. rochei* (AC143) strain.

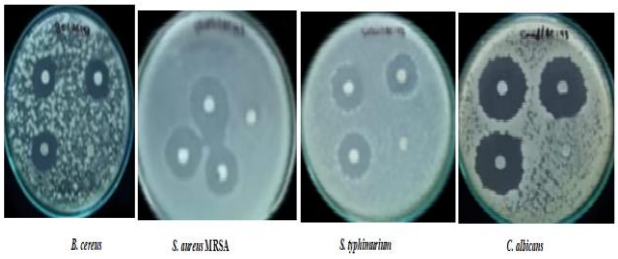

Fig. 4 . Antimicrobial activity of an antifungal compounds produced by *S. rochei* (AC143).

Table 2. Antimicrobial activity of antifungal compound produced by *S. rochei*.

Tested microorganisms	AC143	Standard positive control*
***B. cereus* (ATCC 33018) (G+)**	12.3 ± 2.5^{c}	20
***S. aureus* MRSA (ATCC 43300) (G+)**	17.3 ± 2.5^{b}	12
***L. monocytogenes* (ATCC 7644) (G+)**	NI	19
***S. typhimurium* (ATCC 14028) (G-)**	19.3 ± 0.57^{b}	20
***P. aeruginosa* (ATCC 9072) (G-)**	NI	15
***Escherichia coli* (ATCC 25922) (G-)**	NI	18
***Candida albicans* (ATCC 10231)**	26.7 ± 2.9^{a}	15

*: penicillin G (10 µg) for G+ bacteria, ampicillin (10 µg) for G- bacteria and nystatin (100 units) for fungi; NI: No inhibition; Values are the mean ± SD; Different letters (a-e) represent significant differences between the data ($P < 0.05$).

3.2.3. Cytotoxic activity

The antitumor properties of the produced BACs from the *S. rochei* strain against human lung cancer (A-549) and human lung normal cell lines (WI-38) were *in vitro* examined with an MTT assay [22]. The data shown in Figs. (5 and 6) indicated that the various tested proportions of BAMs (31.25, 62.5, 125, 250, 500 and 1000 µg/ml) had a moderately cytotoxic effect on human lung cancer cells and less inhibition on human lung normal cells. The lowest IC_{50} value of *S. rochei* (AC143) extract was recorded for A-549 at 117.95 ± 0.9 µg/ml. Higher IC_{50} values of AC143 extract were observed for WI-38 at 301.79 ± 1.69 µg/mL. The superior result of *S. rochei* on cell viability was observed at the highest tested concentration (1000 µg/ml) and showed the strongest growth inhibition against A-549 (2.60%). This finding is in agreement with the previous report by Seenivasan *et al.* [63]. Similarly, Ibrahim *et al.* [64] suggested that the *S. tunisiensis* W4MT573222 pigment has safeguarding against normal cells and toxicity effect on three distinct cancerous cells: HepG-2 (liver cancer cell), A-549 (lung cancer cell) and PAN1 (pancreas cancer cell). In addition, an extract of *S. sennicomposti* GMY01 displayed toxicity impact on human cancer cell line A-549 and possesses cytotoxic action towards MCF-7 breast cancer cell line with IC_{50} at a value of 5.6 µg/ml [65].

In this regard, these findings suggested that *S. rochei* (AC143) extract might be able to induce cancer cell inhibition. Elucidation of the underlying mechanism of *S. rochei* extract on cancer cell lines may be a valuable area of future research, as this information could potentially assist in its development as a chemo-preventive drug.

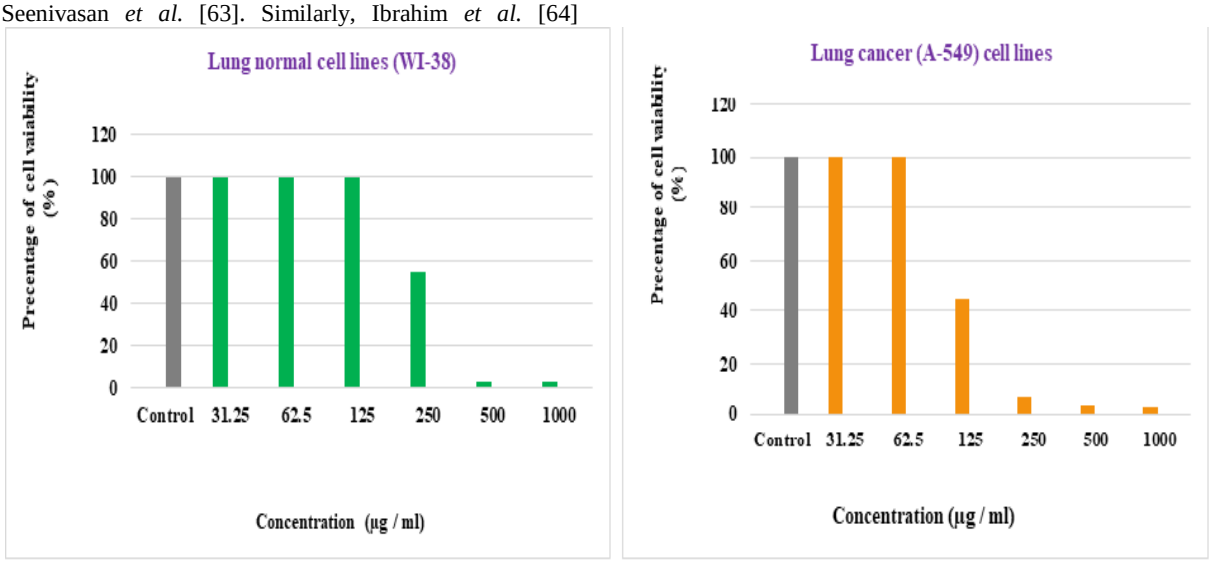

Fig. 5. Cytotoxic activity of *S.rochei* (AC143) strain against (a) human lung normal cell lines (WI-38) and (b) human lung cancer (A-549). Cell viability was measured using MTT assay.

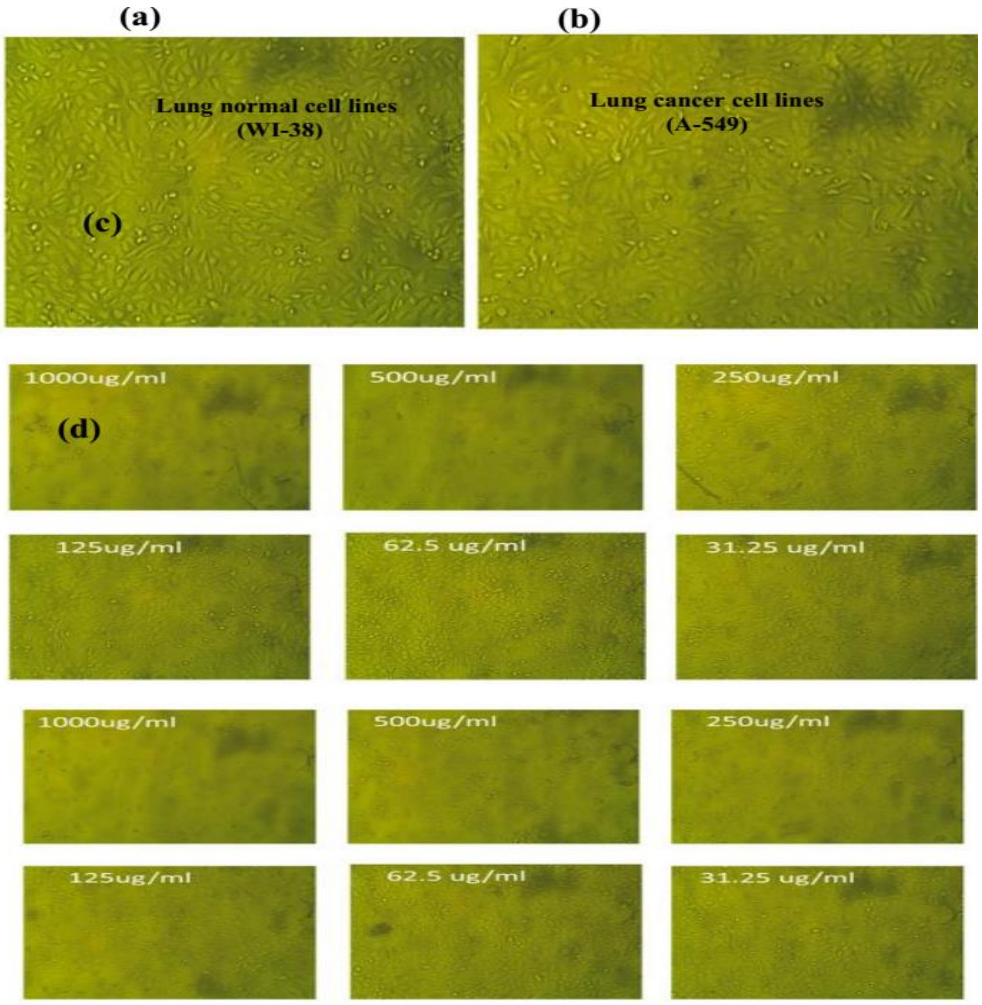

Fig. 6. Effect of *S. rochei* on (c) Lung normal cell lines (WI-38) and (d) Lung cancer (A-549) cell lines at different concentration concentration. (a) Control WI-38. (b) Control A-549. Cell viability was measured using MTT assay.

4. CONCLUSION

This study covered the identified characteristics of bioactive compounds produced by strain *S. rochei* (AC143). The functional groups analysis displays that the strain AC143 showed major profiles that characterize the BACs. Chromatographic examination of *S. rochei* using LC-ESI-MS/MS $[M-H]^-$ exhibited the discovery of 21 diverse compounds. The produced BAMs moderately exhibited antioxidant and antibacterial activity against the tested Gram-positive, Gram-negative and fungi pathogens. Importantly, the BACs from *S. rochei* showed a moderate level of cytotoxicity against lung cancer cell lines with low cytotoxic effects in lung normal cell lines.

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6. CONFLICT OF INTEREST

The authors declare that there are no conflicts or any financial support related to the current investigation that could have influenced its outcome.

7. Abbreviations:

FT-IR, fourier transform infrared; LC-MS/MS, liquid chromatography-mass spectrometry; SMs, secondary metabolites; BACs, bioactive compounds; BAMs, bioactive metabolites; DMSO, dimethyl sulfoxide.

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