Indian Journal of Experimental Biology
Vol. 60, February 2022, pp. 81-90

Antimicrobial, antioxidant and anticancer potential of *Streptomyces* species isolated from the rhizosphere of *Avicennia marina* (Forssk.) Vierh.

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Received 10 June 2020; *revised 27 September 2020*

Free radicals produced through biochemical processes cause dangerous health problems due to their oxidative effect on cellular proteins and lipids. There is an urgent need for natural antioxidants to be used as therapeutic agents. *Streptomyces* are known as producers for antioxidants, in this study, two *Streptomyces* species were isolated from the rhizosphere soil of mangrove tree *Avicennia marina* (Forssk.) Vierh. The isolates were identified by conventional as well as molecular methods as *Streptomyces atrovirens* (MS5) and *S. labedae* (MR15). The ethyl acetate extracts of cell free production broth medium of the two isolates demonstrated potent biological activities against Gram positive and Gram negative bacteria and *Candida albicans*. Moreover, a radical scavenging activity in DPPH assay with significant inhibition percentage of 62 and 78%, respectively, was recorded. The IC_{50} values were 3000 and 241 µg/mL (P <0.05) for *S.atrovirens* (MS5) and *S. labedae* (MR15), respectively. *Streptomyces atrovirens* extract showed anticancer activity against hepatocellular carcinoma cells (HepG-2) and colon carcinoma cells (HCT-116) cell lines with 61 and 50.6%, respectively, while *S. labedae* (MR15) showed anticancer activity against all the tested cell lines with 92.9 and 85.89% against (HepG-2) and (HCT-116) compared to the control cells and showed selective cytotoxicity. LC-MS/MS analyses revealed the presence of compounds with known antioxidant and anticancer activities such as Gamma Aminobutyric acid (GABA) and Indole-3-carboxyaldehyde, linoleic acid and phenyl chromen-4-one derivative with various intensities.

Keywords: Actinobacteria, Antibacterial, Anticancer, Antimicrobial, Antioxidant, Grey or White mangrove

Actinobacteria are considered the reservoir of many medically important bioactive compounds. The search for potent actinobacterial species can lead to the discovery of new therapeutic agents[1,2]. Bioactive compounds produced by actinobacteria have diverse clinical use as antibacterials, antifungals, antioxidants, antitumors, and antiparasitics[3,4]. Actinobacteria produce about 65% of the current used antibiotics, over 10,000 of them are produced by *Streptomyces* species[1].

Mangrove forests are rich ecosystem of bioactive metabolite producers due to their various properties of high salinity, strong winds, extreme tides, high temperature, anaerobic soils, and muddinesswhich induce the establishment of specific microbial community[3,5,6]. *Avicennia marina* (Forssk.) Vierh., commonly called grey or white mangrove, has a wide range of salinity tolerant microorganisms in their rhizospheric region that adapt to environmental fluctuations. The secondary metabolites of *Streptomyces* spp. play role in elimination of plant pathogens through antibiotic production and other volatile bioactive compound production[8] Streptomycetes from mangrove soil act as a source of antioxidant and anticancer compounds[5] *viz*. melanin[9], phenazine[10] and other agents[11]. Doxorubicin produced from *Streptomyces*[7] is used as chemotherapeutic agent against various lymphoma; epirubicin exhibits anticancer activity against breast cancer, ovarian cancer, lung cancer and leukemia[12]. Indolocarbazoles and streptocarbazoles A and B isolated from mangrove soil *Streptomyces* sp. have also demonstrated antitumor properties[13].

Synthetic antioxidants used for medication such as butylated hydroxytoluene(BHT) and propyl gallate (PG)[9] have side effects; short term exposure to BHT has a toxic effect on the liver; high level exposure to PG has a minor systemic toxicity and low amounts can be absorbed by skin[14]. On the other hand, Streptomyces spp. Are known as a rich resource of bioactive compounds. Hence, in this study, we investigated the antioxidant and anticancer potential of actinobacterial isolates *Streptomyces* species from Egyptian mangrove.

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Materials and Methods

Soil samples

Six soil samples were collected from the rhizosphere area of grey or white mangrove trees *Avicennia marina*, 35 km south of MarsaAlam City, Egypt (24°43`N, 35° 9`E). These trees, 2-3 m in height, are located at the coast line of Wadi El Gemal. Soil samples were collected in sterile plastic bags, mixed, air dried, ground with mortar and pestle, and sieved. The dry soil were packed in clean plastic bags and kept at –20ºC.

Isolation of actinobacteria

Soil sample were heated at 60ºC for one hour, 10 g of heat treated soil was mixed with 100 mL sterilized distilled water, Ten-fold dilutions of soil sample were prepared. Three different isolation media used for selective isolation of actinobacterial isolates excluding other microbial species were RASS agar[15], starch nitrate agar (ISP4)[16], M1 agar medium[17]. The isolates were maintained on the same isolated medium, cultures, stored in 20% v/v glycerol at –20°C. Actinobacterial isolates were screened for their antimicrobial activity using disc agar diffusion method according to Kirby–Bauer test[18]]. Each isolate was sub-cultured on three different media have enriched components to optimize the growth conditions forantimicrobial agents;starch casein agar (SC)[19], oatmeal agar media (ISP3)[20], and glycerol asparagine (ISP5)[21]. Three discs were cut from each plate and put onto Muller Hinton plates inoculated with 100 µL of 10^6CFU mL^{-1} of five different tested microorganisms *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus* and *Candida albicans.* The tested microorganisms were clinical isolates donated by Microbiology Department research laboratory, Ain Shams University. The plates were incubated at 37°C for 24 h and 30°C for *Candida albicans*. Three independent replicates were carried out; inhibition zone around each disc was measured in mm. The potent isolates were detected according to the best antagonistic activity.

Identification and phylogenetic analysis of potent isolates

The selected potent isolates were cultivated on four different media to characterize the different cultural aspects based on the colour of aerial and substrate mycelium, texture and detection of soluble pigments[16] on starch nitrate agar (ISP4)[16], starch casein agar (SC)[19], glycerol asparagine agar (ISP5)[21] and oatmeal agar (ISP3)[20]. The plates were incubated for 7 days at 30°C. The culture characteristics of aerial and substrate mycelia were recorded as well as pigment production. Scanning electron microscopy was carried out using electron microscope (JSM 1500 LV, Japan) at the regional center for mycology and biotechnology in Al Azahr University. Physiological characteristics such as optimum growth temperature, pH and NaCl tolerance, starch hydrolysis, proteolytic activity, H_2S production, catalase production and melanin pigment production were carried out according to Williams *et al*.[22,23].

Identification for the selected isolates was carried out by amplification of partial 16S rRNA gene.DNA was extracted using colony PCR method[24]. PCR was performed in a total volume of 50 µL. Each reaction mixture contained the following: 25 µl of master mix (ThermoScientific), 2 µL of 10 µM of each primer-PA (5'-AGAGTTTGATCCTGGCTCAG-3') and 517R (5'-ATTACCGCGGCTGCTGG-3' (Thermoscientific)[25], 3 µL of 20 ng DNA template. PCR protocol was as follows: initial denaturation at 94°C for 5 min then 35 cycles of 40 s each at 94°C, 55°C, and at 72°C and a final step of 10 min at 72°C. PCR products were analyzed by 1% agarose gel electrophoresis using TAE buffer, agarose gel was examined under UV transillumination. Qiagen extraction kit was used to purify the PCR products before DNA sequencing. The nucleotide sequence was assembled using BioEdit V7.0.5. Multiple alignments were carried out using Clustal W and Neighbor-joining phylogenetic tree was constructed using Phylip version 4.0. Evolutionary distances for the neighbor-joining algorithm were computed using Kimura's two-parameter model[26]. Nucleotide sequences were submitted in GenBank.

Extraction of bioactive compounds

Potent actinobacterial isolates that showed the highest antimicrobial activity were inoculated in starch casein broth and incubated at 28°C in a rotary shaker at 160 rpm for 7 days. After incubation period, the cultures were filtered through Whatman filter paper No.1. The cell free filtrates were extracted by ethyl acetate at a ratio of 1:1 then the organic ethyl acetate layer was collected and evaporated in rotary evaporator then stored at 4ºC[27].

Screening for antimicrobial activity of cell free extracts

The antimicrobial activity of the selected actinobacterial ethyl acetate extracts was carried out by agar well diffusion method against five test

microorganisms. Aliquots of 100 µL of adjusted microbial inoculum (0.5 McFarland) was inoculated onto the surface of Mueller-Hinton agar plates then wells were made using sterile cork borer (8 mm) and inhibition zones were measured in mm. Two fold dilutions of the crude ethyl acetate extracts of the selected isolates was made using 10% DMSO, 100 µL was inoculated in each well. The plates were incubated at 37°C for 24 h and 30°C for *Candida albicans*[28]. Three replicates were made for each experiment; the diameter of inhibition zone was measured in mm for determination of MIC**.**

Free radical scavenging activity

Ethyl acetate extracts were tested for their free radical scavenging activity by DPPH assay. Each extract was dissolved in 10 mL methanol and different concentrations (50, 100, 500, 1000, 2000 and 5000 µg/mL) were prepared. Ascorbic acid was used as a standard where different concentrations of ascorbic acid (50, 100, 200, 400, 800, 1000, 2000 and 5000 µg/mL) were prepared. From each concentration (extract or standard), 2 mL were added to 2 mL of 0.002% freshly prepared methanolic DPPH and the reaction was incubated at 25°C for 30 min in dark, the DPPH solution with methanol only was used as a control. The OD was measured using spectrophotometer (UNIC 7200) at 515 nm, where the experiment was carried out in triplicates and methanol was used as a blank[29,30]. The percentage of radical scavenging was measured by the following formula :

% Radical scavenging activity = [(control OD – sample OD) / control OD] × 100.

Determination of cytotoxicity using MTT assay

Four human cancer cell lines were used: HepG-2 (Hepatocellular carcinoma cells), MCF7 (Breast carcinoma cells), A-549 (Lung carcinoma cells) and HCT-116 (colon carcinoma cells), in addition to control cells MRC-5 (Normal human lung fibroblast cells). Cell lines were maintained in RPMI (Roswell Park Memorial Institute) -1640 (Gibco) supplemented with 10% fetal bovine serum and 1X antibiotic-antimycotic (Gibco) at 37ºC humidified incubator containing 5% CO_2 and 95% air and seeded into a sterile flat bottom 96-well plate at a density of 5×10^4 cells/well and allowed to adhere overnight. Twenty microliter of each ethyl acetate extract with 0.5% (v/v) DMSO was added into each well with final concentration ranging from 3.9-500 µg/mL. DMSO was used as negative control. The Cells were further incubated with the extract for 24 h before performing MTT assay. About 10 µL of 12 mM MTT stock solution were added to 100 µL of fresh culture RPMI 1640 medium without phenol red in each well including the untreated controls. The 96-well plates were then incubated at 37°C and 5% CO_2 for 4 h. Aliquots of 85 µL of the media was removed from the wells, and 50 µL of DMSO was added to each well, mixed thoroughly and incubated at 37°C for 10 min[31]. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc., USA) to determine the number of viable cells. The percentage of viability was calculated as (sample OD/control OD) × 100%. The concentration required to cause toxic effects in 50% of intact cells (IC_{50}) was estimated from graphic plots of the dose response curve for each concentration using Graphpad Prism 8.

Liquid chromatography mass spectrometry (LC-MS/MS) analysis

To characterize the bioactive compounds, LC-MS/MS analysis was conducted. An Exionl C Sciex, Triple TOF 5600+ Sciex Mass Spectrometer was used. Mass spectrometer operating in electrospray ionization (ESI) positive mode with Analyst TF 1.7.1 system was applied.

The setting for electrospray ionization voltage was set to 4500 kV. Mass range for MS/MS scan was adjusted from $5x10^5$ to $1x10^7$ Da. Columns were a PhenomenexSynergi Fusion RP (3.5 µm, 2.1×50 mm internal column diameter), 3 µL particle size, Phenomenex, CA, USA) was used to obtain separation. The mobile phase was made up of DI-Water contains 0.1% formic acid (solvent A) and acetonitrile100% (solvent B). The compounds were separated with the following linear-programmed solvent gradient: 0 min (10% B), 21 min (90% B), 25 min (90% B) then equilibrating back to 10% B for 3 min. The flow rate for the column was set at 0.3 mL/min while the column temperature was set at 40°C and injection volume at 25 µL. Peak view 2.2 software was used for identification of the compounds.

Statistical analysis

Data of antimicrobial activity were expressed as mean ± standard deviation (SD) of three independent replicates. Differences were considered significant when *p* value was less than 0.05. All statistical tests were performed using SPSS for Windows version 18.0 (SPSS, USA).

Results and Discussion

Isolation of actinobacterial species

Isolation of actinobacteria from six soil samples from MarsaAlam coast line were carried out using three isolation media RASS agar, starch nitrate agar (ISP4), M1 agar medium. Thirty isolates were recovered from the plates; the isolates were checked and grouped according to their culture characteristics.

Antimicrobial activity of the potent isolates

The isolates were investigated for production of antimicrobial agents using disc diffusion method. Only 11 (36.6%) exhibited potential activity against the tested microorganisms. Isolates showed the best antimicrobial activity on starch casein agar media (SC). Isolate MS5 showed antimicrobial activity against *Escherichia coli*, *Bacillus cereus* and *Candida albicans* with inhibition zones 15, 19 and 15 mm, respectively while MR15 showed antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* with inhibition zones 2.5 and 13 mm, respectively. The two isolates were selected for further investigation.

Identification of selected isolates MS5 and MR15

Phenotypic characteristics of the isolates MS5 and MR15 such as the gross morphology and cultural characteristics were investigated. MS5 isolate showed gray, yellow and white aerial mycelium while MR15 isolate showed gray, yellow, buff and brown colours. The substrate mycelium of MS5 isolate had yellow colour while substrate mycelium of MR15 isolate had gray, brown and yellow colours. Growth on different media and scanning electron microscopy revealed that MS5 has long spiral spore chains with heavy spins on spore surface, while MR15 has spiral spore chains with spiny spore surfaceas shown in Fig. 1. Physiological characteristics showed the ability of the two isolates to produce the melanin and to hydrolyze the starch and casein. Optimum growth temperature for the two isolates as at 37ºC, the two isolates had a tolerance to NaCl up to 2% and a PH tolerance up to 9. The characterization of the two isolates is summarized in Table 1. The phenotypic results of MS5 and MR15 showed that they were belonging to *Streptomyces* species.

Nucleotide sequences of partial 16S rRNA gene of isolates MS5, MR15 were compared with related sequences on the data base (ncbi.nlm.nih.gov/blast). Isolate MS5 was similar to *Streptomyces atrovirens* with blast identity 99%, while isolate MR15 was 99% identical to *S. labedae*. Nucleotide sequences were submitted in GenBank under accession numbers MK15617**2**, MK15617**0** for isolates MS5 and MR15, respectively. Neighbor-joining phylogenetic tree revealed the close relatedness between isolate MS5 and *S. atrovirens* with bootstrap value 58 while MR15 grouped with *S. labedae* with boot strap value 80 as shown in Fig. 2.

Antimicrobial activity of cell free ethyl acetate extracts

The selected potent isolates were inoculated in starch casein broth as a production media that showed the best antagonistic activity against

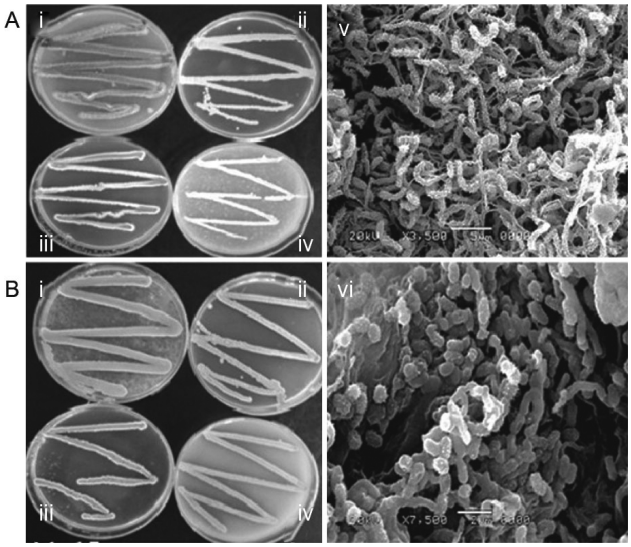

Fig. 1 — Growth of isolates (A) MS5; and (B) MR15 on four culture agar media: (i) Starch nitrate, (ii) glycerol asparagine, (iii) starch casein, and (iv) Oatmeal; Scanning electron micrograph of isolates spore chain of (v) MS5, 3500X, and (vi) MR15, ×7500X.

Table 1 — Antimicrobial activity of the actinobacterial isolates that grown on three different media against five tested microorganisms using Kirby-Bauer method: diameter of inhibition zone in (mm).

Tested micro-organsims	*Staphylococcus aureus*			*Escherichia coli*			*Bacillus cereus*			*Pseudomonas aeruginosa*			*Candida albicans*		
Isolate code	Starch casein	Glycerol asparagin	Oat meal	Starch casein	Glycerol asparagin	Oat meal	Starch casein	Glycerol asparagin	Oat meal	Starch casein	Glycerol asparagin	Oat meal	Starch casein	Glycerol asparagin	Oat meal
MR15	25± 1.2	-	14± 0.4	-	-	-	-	-	-	-	-	-	13± 1.2	10± 0.8	-
MS5	-	-l	-	15± 1.6	15± 1.2	-	19± 0.8	20± 1.2	15 ±1.6	-	-	-	15± 0.8	15± 1.6	-

Fig. 2 — Neighbor-joining phylogenetic tree based on 16S rRNA sequences of the two isolates MS5 and MR15 and other related taxa. [Bootstrap values> 50% based on 1000 re-sampled datasets are shown at branch nodes. Scale Bar, 10% substitutions per site]

the tested microorganisms in primary scanning as shown in Table 2. Ethyl acetate extracts of *Streptomyces atrovirens* (MS5) and *S. labedae* (MR15) showed antimicrobial activities against four of the test microorganisms, and *S. atrovirens* (MS5) showed antibacterial activity against three microorganisms, *E. coli* with MIC value 3 mg/mL, *B. cereus* with MIC value 5 mg/mL and *C. albicans* with MIC value 10 mg/mL while the ethyl acetate extract of *S. labedae* (MR15) showed antimicrobial activity against *Staphylococcus aureus* with MIC at 500 µg/mL and against *C albicans* with MIC at 500 µg/mL.

Radical scavenging activity using DPPH assay

DPPH assay is reliable widely used assay to detect the antioxidant activity through discolouration of DPPH molecules from purple colour into yellow coloured diphenylpicrylhydrazine. In this study, the colour change of DPPH molecules indicate to the antioxidant activity of the ethyl acetate extracts of the *Streptomyces atrovirens* and *S. labedae*.

The ethyl acetate crude extract of the *S. atrovirens* (MS5) and *S. labedae* (MR15) showed the highest inhibition of DPPH free radicals activity at 5 and 2 mg/mL with radical scavenging activity by 62 and

Table 2 — Cultural and biochemical characteristics of the two selected isolates *S. atrovirens* (MS5) and *S. labedae* (MR15)

Culture media	Tested character	MS5	MR15
Starch nitrate agar	Aerial mycelium	gray	gray
	Substrate mycelium	yellow	gray
	Texture	powder	powder
	Soluble pigment	none	none
Glycerol aspara-gines agar	Aerial mycelium	yellow	Yellowish gray
	Substrate mycelium	yellow	brown
	Texture	leathery	Leathery
	Soluble pigment	none	brown
Oatmeal agar	Aerial mycelium	White	Buff
	Substrate mycelium	yellow	brown
	Texture	Powder	leathery
	Soluble pigment	none	none
Starch casein agar	Aerial mycelium	gray	gray
	Substrate mycelium	Yellow to brown	yellow
	Texture	powder	powder
	Soluble pigment	none	none
Bio-chemical reactions	Temperature		
	30	+++	+++
	37	++	++
	50	-	-
	55	-	-
	NaCl tolerance		
	0.5%	++	++
	1%	+	++
	1.5%	+	++
	2%	+	+
	PH tolerance		
	4	-	-
	7	+++	+++
	8	+	++
	9	+	+
	Melanin production	+	+
	Casein hydrolysis	+	+
	Catalase test	±	±
	Starch hydrolysis	+	+
	H_2S production	-	-

78%, respectively (P <0.05), compared with the reference compound ascorbic acid which had scavenging activity of 85% at the same concentration. IC_{50} values were 3000 and 241 µg/mL (P <0.05) for *S. atrovirens* and *S. labedae*, respectively as shown in Fig. 3.

The radical scavenging activity percentage of *S. labedae* (MR15) and *S. atrovirens* (MS5) was about Ten- and five-folds, respectively higher than the scavenging activity percentage reported by Tan *et al*. exerted by Streptomyces MUM256 at the same concentration at (4, 5 mg/mL), respectively[4]. The percentages of the radical scavenging activity of *S. atrovirens* (MS5) were close also to that exerted by *Streptomyces* sp. strain MUSC 14 while *S. labedae* (MR15) had 1.2 fold higher radical

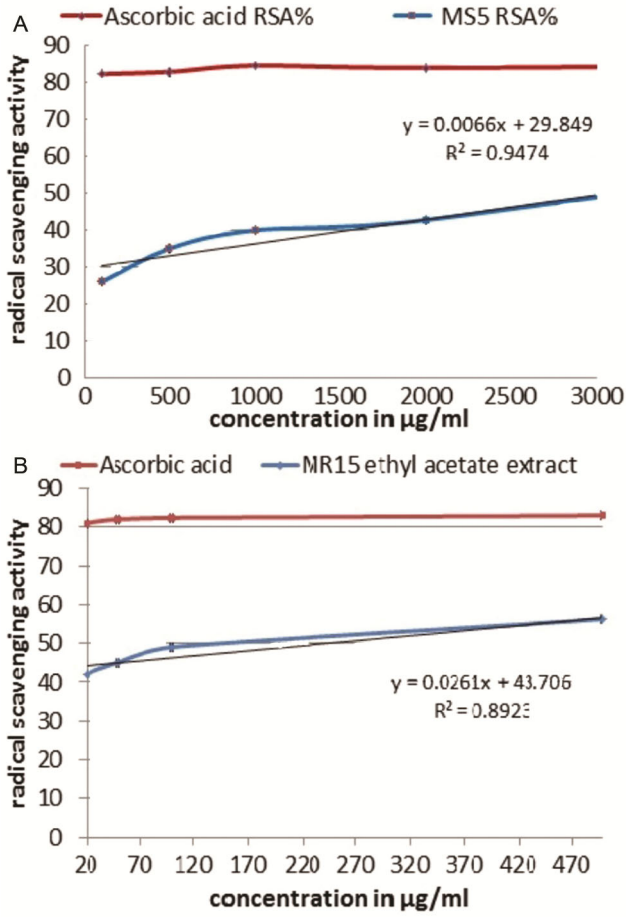

Fig. 3 — Radical scavenging activity percentage exerted by EA acetate extract of potent isolates (A) *Streptomyces atrovirens* MS5; and (B) *S. labedae* MR15 compared to ascorbic acid using DPPH assay.

scavenging activity percentage than exerted by *Streptomyces* sp. strain MUSC 14[32].These results indicated to presence of potential compounds with antioxidant activity in the ethyl acetate extract of the *S. atrovirens* (MS5) and *S. labedae* (MR15).

Antioxidants have the ability to scavenge the free radicals, and hence these molecules have potential to inhibit the signaling pathways that are essential for the remaining cancer cells[33]. These results demonstrate that the ethyl acetate extracts of *Streptomyces atrovirens* (MS5) and *S. labedae* (MR15) had antimicrobial activity against various microorganisms and radical scavenging activity with significant inhibition percentage.

Cytotoxicity assay

The ethyl acetate extract of *Streptomyces atrovirens*(MS5) showed cytotoxic activity by 61% and 50.6% (P <0.05) against HepG-2 and HCT-116 cell lines, respectively, at concentration of 500 µg/mL compared to the reference MRC-5 cells with minimum cytotoxic activity percentage by 21.74±1.72% (P <0.05),The cytotoxic effect of ethyl acetate extract of *Streptomyces atrovirens*(MS5) on HepG-2 cell line and HCT-116 cell was more than the control cells by 2.8 ad 2.3 fold stronger cytotoxic effect (P <0.05) at the highest used concentration 500 µg/mL. This cytotoxic effect ratio indicates to the selective cytotoxicity to cancerous cells at the highest used concentration (500 µg/mL). IC_{50} values of the MS5 extract against HepG-2 cell line and HCT-116 cell line were 392±9.4 and 494±16.7 µg/mL, respectively (P <0.05), while IC_{50} in control cells (MRC-7) was >500 µg/mL as shown in Fig. (4A).

The ethyl acetate extract of *S. labedae* (MR15) showed cytotoxic activity by 92.9 and 85.89% against HepG-2 and HCT-116 cell lines, respectively. The MR15 extract showed a selective cytotoxic effect against all types of the cell lines used with minimal cytotoxic effect on MRC-5 with 26.63±3.49% (P <0.05) inhibition percentage up to 62.5 µg/mL. HepG-2 cancer cell line was the most sensitive to MR15 EA extract with IC_{50} = 25.8±0.90 µg/mL (P <0.05) followed by A549 cancer cell line with IC_{50} = 27.1±1.56 µg/mL (P <0.05) followed by MCF-7 cell line with IC_{50} = 59.8±2.68 µg/ml (P <0.05) then HCT-116 cancer cell line with 60.5±2.71 µg/mL (P <0.05) and Selective cytotoxic index values at the IC_{50} concentration against HepG-2, A549, MCF-7 and HCT-116 were 4.5, 4.2, 1.92 and 1.9, respectively, as shown in Fig. 4. The hepatocellular carcinoma cells (HePG-2) were the most sensitive cells against the ethyl acetate extract treatment of *S. atrovirens* (MS5) and *S. labedae* (MR15). Selective index (SI) is the ratio expresses the level of cytotoxic effect selectivity of the sample extracts, and calculated as follows: CC_{50} in normal cells/IC_{50} in tumor cells. Higher (SI) values indicate high selective cytotoxicity[4]. These results were similar to previous studies by Ahmed *et al*.[34] These results are in line with the results of the study on *Streptomyces* strain MUM256 that reported a five-fold higher IC_{50} value against HCT-116 cancer cell line showing *S. labedae* (MR15) against the same cancer cell line[4]. *S. labedae* (MR15) had IC_{50} values against the tested cancer cell lines that were also in a line with reported about *Streptomyces bingchenggensis* ULS14[35]. *Streptomyces labedae* (MR15) have showed a high cytotoxic effect against cancer cell lines in addition to a high selective index

values >1 that demonstrate high selective cytotoxicity. *Streptomyces atrovirens* (MS5) and *S. labedae* (MR15)showed a cytotoxic effect against the tested cancer cell lines with IC_{50} values ranges from 25.8 to 494 µg/mL and showed different levels of selective cytotoxicity.

The morphological changes were observed using inverted phase-contrast microscopy. The control MRC-5, HepG2, (treated only with DMSO) showed the normal morphology of cobblestone-like appearance with strong cell-cell adhesion, monotonous spindle-shaped cells containing single round nuclei with flattened cytoplasm, and epithelioid amnion cells that grow in a closely apposed monolayer, respectively. However, MRC-5, HepG2 cells treated with the streptomycetes extract showed shrinkages, dispersing, and irregularity in shape and complete detachments of cells from the surface and loss of cytoplasmic vacuole as shown at Fig. 5. These observed morphological changes indicate to the effect of the *S. labedae* (MR15) against HepG2 cell line compared to MRC-5 cells at different concentrations.

Liquid Chromatography-Mass Spectrophotometry (LC-MS/MS) analysis

Liquid chromatography-Mass Spectrophotometry data revealed the detection of high intensities of Gamma Aminobutyric acid (GABA), Indole-3-carboxyaldehyde and DL-Pipecolinic acid in ethyl acetate extract of *S. atrovirens* (MS5), while high intensities of 4-hydroxy-3-(3-methyl-2-butenyl) acetophenone, linoleic acid and phenyl chromene-4-dervitative in the ethyl acetate extract of *S. labedae* (MR15) (Table 3). Previous results reported the bioactivity of the identified compounds; the γ-

Table 3 — Bioactive compounds identified by LC MS/MS in ethyl acetate extracts of the two isolates*S. atrovirens* (MS5) and *S. labedae* (MR15)

Isolates	Chemical compounds	Retention time (min)	Mol. wt.	Mol. formula
MS5 EA extract	gamma-aminobutyric acid (GABA)	0.54	104.07061	$C_4H_9NO_2$
	DL-Pipecolic acid	1.68	130.08626	$C_6H_{11}NO_2$
	Proline	2.94	116.1	$C_5H_9NO_2$
	Indole-3-carboxyaldehyde	3.67	146.0594	C_9H_7NO
	4-methylumbelliferone	9.2	177.05462	$C_{10}H_8O_3$
MR 15 EA extract	4-hydroxy-3-(3-methyl-2-butenyl) acetophenone	14.54	205.0853	$C_{13}H_{16}O_2$
	5-[4,5-dihydroxy-6-(hydroxymethyl)-3-(3,4,5-trihydroxyoxan-2-yl)oxyoxan-2-yl]oxy-7,8-dimethoxy-3-(4-methoxyphenyl)chromen-4-one	15.55	623.3035	$C_{29}H_{34}O_{15}$
	Linoleic acid	19	281.247	$C_{18}H_{32}O_2$

Fig. 4 — Inhibition percentage of growth of control cells (MRC-5) and four cancer cell lines (HepG-2, A-549, HCT-116 and MCF-7) exerted by ethyl extract of (A) *Streptomyces atrovirens* MS5; and (B) *S. labedae*MR15.

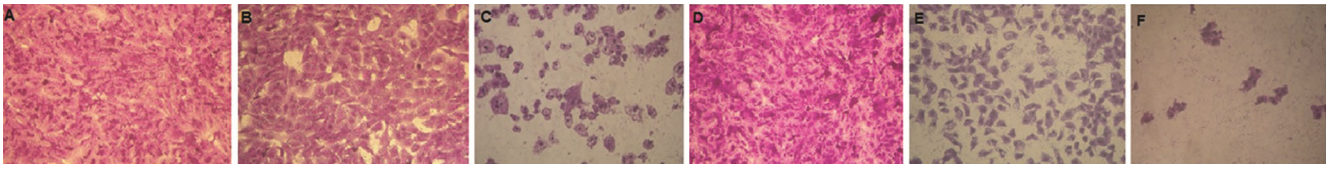

Fig. 5 — Effect of treatment of MRC-5 cells and HepG-2 cells with extract of *Streptomyces labedae* (MR15) at two different concentrations under microscope (40X): MRC-5 cells as: (A) control; (B and C) treated with 62.5 and 500 µg/mL of extract, respectively; HepG-2 cells as (D): control; (E and F) treated with 62.5 and 500 µg/mL of extract, respectively

aminobutyric acid GABA has a major role in pharmaceutical industry due to its therapeutic role in improvement proteins biosynthesis in brain[36] and inhibition of cancer cells proliferation[37] and a major inhibitory neurotransmitter in mammalian brain[38]. Indoles with 3-carbon substitution are considered as a nucleus of many bioactive compounds and have a clinical importance as antimicrobials, antioxidants and antitumors[39]. Detection of these compounds with known bioactivity can relate the demonstrated antimicrobial activity of *S. atrovirens* (MS5) against three tested microorganisms of different groups G +ve, G –ve and *Candida albicans* in addition to scavenging radical activity of DPPH molecules with inhibition percentage up to 62% and antitumor activity against (HePG-2) and HCT-116 cell lines.

Streptomyces labedae (MR15) produce DL-pipecolic acid which is known as a precursor for rapamycin or polyrapamycin[40]. In addition, it produces4-hydroxy-3-(3-methyl-2-butenyl) acetophenone, which has antifungal activity against *C. albicans* in addition to the reported selective cytotoxicity against breast carcinoma[41,42], these compounds could be the bioactive compounds that responsible for the antifungal activity of *S. labedae* (MR15) against the tested *Candida albicans* and the demonstrated antitumor activity against the four tested cancer cell lines

Phenyl derivatives of Chromen-4-one belong to isoflavones which are known as antioxidant, antimicrobial and anticancer. Linoleic acid (LA) can synthesize all dietary ω-6s. Studies reported that the high dose of LA can suppress the colon cancer proliferation[43]. Presence of acetophenone, Chromen-4-one derivative compound and linoleic acid that have known antimicrobial, antioxidant and antitumor activities give an indication that they could be the bioactive compounds responsible for the demonstrated antimicrobial, antioxidant and antitumor activities of *S. labedae* (MR15).

Detection of these compounds is compatible with the reported diversity of chemical structure of bioactive compounds produced by actinobacteria including macrolides, lactones and recently 5,7-dimethoxy-4-phenylcoumarin and 5,7-dimethoxy-4-p-methoxylphenylcoumarin and rapamycin derivative[44]. Isolates have a wide range of bioactivity as antimicrobial, antioxidant and antitumor activities could be a nucleus for medical applications[45,46].

Conclusion

This study demonstrate that the ethyl acetate extracts of *Streptomyces atrovirens* (MS5) and *S. labedae* (MR15) had antimicrobial activity against various microorganisms and radical scavenging activity with significant inhibition percentage.The ethyl acetate extracts of the two species showed antioxidant potential with radical scavenging activity of 62 and 78%, respectively as well as cytotoxic activity against cancer cell lines in comparison to the control cells. LC-MS/MS analyses confirmed the presence of some compounds with known antioxidant and anticancer activities such as Gamma Aminobutyric acid (GABA) and Indole-3-carboxyaldehyde in the ethyl acetate extract of *Streptomyces atrovirens* (MS5), linoleic acid and phenyl chromen-4-one derivative in the ethyl acetate extract of *Streptomyces labedae* (MR15). According to these results the two isolates *S. atrovirens* (MS5) and *S. labedae* (MR15) could be promising for therapeutic applications as antioxidant and anticancer agents.

Conflict of interest

Authors declare no competing interests.

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