Piloquinone, potent cytotoxic compound from Egyptian *Streptomyces pilosus* SBG-NRC-216

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Background and objective
Piloquinone shows biological activity as an anticancer and antitrypanosomal agent, and it acts as an inhibitor for monoamine oxidase, the main causative factor of Alzheimer's disease. The aim of this study was to produce Piloquinone as a bioactive compound from a local *Streptomyces* isolate for many medical purposes.
Materials and methods
Fifty *Streptomyces* isolates for their capacity of Piloquinone production were tested. The potent Piloquinone producer, SBG-NRC-216 isolate, was identified by conventional and genetic methods. The produced compound was purified and identified on the basis of the spectroscopic analysis. A series of experiments were conducted to investigate efficacy of the compound.
Results and conclusion
The producer isolate was identified by phenotypic and genotypic methods. These methods confirmed that the strain name was *Streptomyces pilosus* SBG-NRC-216. The produced compound was purified and identified as Piloquinone. It showed a potent anticancer activity against five different human tumor cell lines: breast cancer cell line MCF-7, human liver cancer cell line HepG2, human lung cancer cell line A549 and human colon cancer cell lines Caco-2 and HCT-116. The results also indicated that Piloquinone had a weak antiviral activity against the H5N1 virus. The results proved that Piloquinone can be used as a bioactive compound that has many industrial and medical purposes.

Keywords:
anticancer, antimicrobial activity, antioxidant, antiviral, Piloquinone, *Streptomyces pilosus*

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Introduction

Natural products that had been isolated in the past 60 years number about 28 000. More than 10 000 of these products are biologically active; however, antibiotic and anticancer agents represent more than 8000 of these compounds. The microbial products which have been clinically used as antibiotics, antitumor drugs, and agrochemicals exceeded more than 100 products [1–3].

Natural products compete, with the other methods for drug discovery, showing a fair share of new clinical drugs. Some of these compounds have already proceeded further onto the market, especially as anticancer, immunosuppressant, antihypertensive, and neurological disease drugs [4].

The development of instruments such as nuclear magnetic resonance (NMR), mass spectroscopy (MS) service to clearly identify natural products and the unceasingly updated chemical databases (AntiBase, Le-Petit-Quevilly, France; DNP, Florida, USA) prevent replication of known compounds, and also by conducting the screening analysis of natural products in combination with high-performance liquid chromatography (HPLC), ultraviolet (UV), HPLC-DAAD, HPLC-CD, HPLC-MS, HPLC-NMR-MS or GC-MS systems [5] participate in elucidating structure of unknown compounds accurately.

Since the 1950s, natural products were recognized to be anticancer agents by US National Cancer Institute (NCI) [6]. Moreover, it was reported that natural products represent about 60% of the currently used anticancer drugs [7].

Cancer is considered the major cause of death in the world. As reported by the WHO, cancer was the cause of 8.2 million cases of death in 2012 [8]. It was informed that cancer cases increased 11% to reach 14.1 million cases in 2012, and the most predominant diagnosed cancers were the lung (1.8 million cases, 13.0% of the total), breast (1.7 million, 11.9%), and large bowel (1.4 million, 9.7%).

However, the most common causes of cancer-related death were due to lung (1.6 million, 19.4% of the total), liver (0.8 million, 9.1%), and stomach (0.7 million, 8.8%) cancers. In general, cancer cases are predicted to reach 25 million cases over the next two decades [9].

Piloquinone is produced biologically by *Streptomyces* spp. and is chemically defined as 1, 8-dihydroxy-2-methyl-3-(4-methyl-1-oxopentyl)-9, 10-phenanthrenquinone [10]. Piloquinone is a compound that has gained some attention by the researchers due to its rarity in nature [11]. Since the discovery of Piloquinone in 1963 [12], only few researches were accomplished on this compound; most of them focused on chemical synthesis of the compound to prove its elucidated structure, and the others deal with biological activity such as the potency of Piloquinone as an anticancer and antitrypanosomal agent [13] and as an inhibitor for monoamine oxidase, the main causative factor of Alzheimer's disease [10].

Materials and methods

Isolation and maintenance technique

Soil samples were collected in sterile containers from ten Egyptian locations; samples were collected using sterile spatula by digging about 10 cm deep into the soil to avoid surface contamination. Samples were delivered to the lab within 24 h and preserved in the refrigerator until use.

Isolation procedures were started within a week from the collection date. One gram from each sample was weighed and added to 10 ml of sterile 0.9% saline solution, and a serial dilution was performed; 0.1 ml of each dilution was inoculated on a plate of starch casein agar medium with the following composition (g/l): Soluble starch, 10; Casein, 0.3; KNO_3, 2.0; NaCl, 2.0; $MgSO_4$, 0.05; K_2HPO_4, 2.0; $CaCO_3$, 0.02; $FeSO_4 7H_2O$, 0.01; Agar, 20.0; pH 7.2. This was followed by incubation in inverted position at 28°C in an incubator for 7 days [14]. Separate colonies were picked up and reinoculated on plates of the same agar medium to ensure purity of the selected colonies. Pure isolates were inoculated on agar slants of starch casein medium; colonies were labeled with a code referring to the source of collection, that is, the place where the sample was collected.

Propagation of the selected isolates

Because of the inability to form a homogenous spore suspension of *Streptomyces* sp., two plaques (15 mm) of 7-day-old cultures were used to inoculate a 250 ml Erlenmeyer flask containing 50 ml of seed medium composed of g/l: soy meal, 2.5; dextrin, 10.0; glucose, 1.0; yeast extract, 5.0; casein hydrolysate, 7.0; K_2HPO_4, 0.2; NaCl, 0.5; $MnCl_2$ $4H_2O$, 0.005; $FeSO_4$ $7H_2O$, 0.025; $ZnSO_4$ $7H_2O$, 0.001; $MgSO_4$ $7H_2O$, 0.005; and $CaCl_2$, 0.02; pH 7.0. The inoculated flasks were incubated for 2 days at 28°C and 250 rpm on rotary shaker (New Brunswick Innova 43, New Jersey, USA). Ten percent of seed medium was used to inoculate a 250 ml Erlenmeyer flask containing 50 ml of fermentation medium composed of g/l: dextrin, 90; glucose, 5.0; soy meal, 10; peptone, 10; glycerol, 10; l-lysine, 2.5; K_2HPO_4, 1.0; $CaCO_3$, 1.5; polyethylene glycol 6000, 1.0; pH 6.5. The flasks were incubated for 7 days at 28°C and 250 rpm [15].

Identification of the promising isolate

The selected isolate was characterized morphologically and physiologically following the directions given by the International *Streptomyces* project (ISP) [16] and Bergey's Manual of Systematic Bacteriology [3].

Morphological, physiological and biochemical characterization

Cultural characteristics of the pure isolate in various media were recorded after incubation for 7, 14 and 21 days at 28°C. Morphological observations were made with light microscope by using the method described by Shirling and Gottlieb [16]. Spore chains were detected by taking electronic micrographs of spores using a copper grid coated carbon and observed by transmission electron microscopy (TEM) (JEM-2100 JEOL, Ltd., Tokyo, Japan). The targeted isolate was identified up to the species level, as described in Bergey's manual [3], on the basis of morphological characteristics of spore chains, by using the cover-slip method [17]. Spores' color (aerial and substrate mycelia) was visually estimated by using a stamp color key [18]. Carbon utilization was also determined using ISP basal medium 9 containing 1.0% of different carbon sources [19]. The medium was incubated at 28°C, and growth was noticed after 7, 14 and 21 days, using glucose as positive control [16] and medium without any carbon source as negative control.

Molecular characterization

Extraction of genomic DNA was conducted by using lysozyme (20 mg/ml) and proteinase k (1 mg/ml) and purified using isopropanol buffer [20]. Amplification of 16S rRNA was carried out using the two primers, F (5′-AGAGTTTGATCCTGGCTCAG-3′) and R (5′-TACGGTTACCTTGTTACGACTT-3′). The PCR amplification included initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 30 s, at 55°C for 30 s, and at 72°C for 45 s for complete extension [21]. The PCR product was purified by QIAquick Gel Extraction Kit (QIAGEN, Maryland,

USA) and run on agarose gel for sequencing. Identification was achieved using the BLAST program (National Centre for Biotechnology Information).

The sequences were aligned using Jukes Cantor Model. The phylogenetic reconstruction was carried out using the neighbor-joining (NJ) algorithm with 500 bootstrap values and submitted to the Gene Bank.

Extraction of bioactive ingredients

During the extraction process, both intercellular and extracellular metabolites were pursued by centrifuging the culture medium at 5000 rpm for 5 min and extracting the intercellular metabolites by soaking the cells (precipitate) in acetone 100% within an ultrasonic water bath for 2 h. Extracellular metabolites (filtrate) were extracted using ethyl acetate. Acetone and ethyl acetate extracts were collected and concentrated using rotary evaporator [22].

Purification of the targeted compound

A glass column of normal-phase silica gel (230–400 mesh) was prepared in a 3×35 cm dimension. A gradient elution of the extract was carried out using [(hexane : ethyl acetate), 2 : 1, 1 : 1, and 1 : 2 v/v, respectively]. Fractions eluted with hexane : ethyl acetate (2 : 1) were collected, concentrated, and purified using polystyrene divinylbenzene polymer column. A column of 1×15 cm dimension was prepared and equilibrated with a 40% acetone solution for about 10-column volume, loaded with the sample and washed with 10-column volume of 40% acetone solution. Elution was then started using 53% acetone solution to produce a semipure fraction [23,24]. This fraction was filtered using 0.2 µm syringe filter and subjected to HPLC purification using an analytical column (Zorbax C18 250×4.6 mm, 5 µ). The injection volume was 100 µl with the UV-spectrophotometer (Agilent UV-1260, California, USA) and Agilent chromatographic software [Chem Station Rev. B. 04.03 (16), Agilent, California, USA] for the evaluation of the chromatograms. Elution was carried out using Isocratic elution system: 60% acetonitrile: 40% water with a flow rate of 1 ml/min; the column compartment temperature was adjusted to 60°C, and the UV detector was adjusted to measure at multichannel wavelengths 214, 220, and 254 nm. Injection was performed several times to produce piloquinone (3.0 mg).

FTIR spectra measurements

Fourier transform infrared spectrum was recorded by forming a thin film of the dried purified compound mixed with potassium bromide (KBr) and measured in the mid region spectrum (4000/cm^{-1} and 400/cm^{-1}) at 4/cm^{-1} spectral resolution with the JASCO FT/IR-6100 (controlled by Spectra Manager II cross-platform software, JASCO, Maryland, USA).

UV spectra measurements

UV spectra were recorded using Shimadzu UV 2401 PC double-beam spectrophotometer in 1.0 cm quartz cells. The purified compound was diluted and measured in methanol (HPLC grade) in a range of 200–900 nm.

Liquid chromatography-mass spectroscopy

Liquid chromatography-mass spectroscopy (LCMS) instrument is high-pressure liquid chromatography (HPLC) coupled with MS, and it is considered as a powerful method for detection of the purity of samples with expected molecular weight of compounds from its mass spectrum, and could be used for separation of complex crude mixtures. Mass spectrum was recorded using LCMS/MS (Mass Thermo/Finnigan NCQ advanced Max ion trap, Agilent, California, USA).

NMR spectral analysis of purified compound

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 600 or JEOL ECX-400 NMR spectrometer in $CDCl_3$. Chemical shifts were referenced to the residual solvent peaks δH 7.26 and δC 77.0 for $CDCl_3$.

Biological activity of piloquinone

In-vitro cytotoxicity assessment

MTT cytotoxicity assay (IC_{50}): screening for anticancer activity of piloquinone was implemented by growing of five different human tumor cell lines including human liver cancer cell line HepG2, breast cancer cell line MCF-7, human lung cancer cell line A549 and human colon cancer cell lines Caco-2 and HCT-116 (obtained from the American Type Culture Collection, Rockville, Maryland, USA) on Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37°C in a humidified atmosphere containing 5% CO_2. The cells were prepared in a concentration of 0.5×10^5 cells per well and supplemented with DMEM culture medium in a 96-well plate and incubated for 24 h at 37°C in a humidified atmosphere containing 5% CO_2, followed by inoculating the wells with different concentrations of the tested compound in triplicates, and the plate was incubated for another 24 h; after that, the cells were washed several times with PBS (0.20 M, pH 7.4), and 40 µl MTT solution/well was added,

followed by incubation for an additional 4 h. The formed MTT crystals were solubilized by 10% DMSO, and the plate was shaken gently for 10 min at room temperature. The absorbance was determined spectrophotometrically at 570 nm using microplate ELISA reader (Microplates Reader; Asys Hitech, Austria), wherein the optical density was directly proportion to the number of living cells in the culture. The results were normalized to the control value and expressed as percentage of control. The concentration, which gives 50% growth inhibition, is referred to as the IC_{50} [25].

Selectivity index

Because the key feature of an effective anticancer drug is its ability to kill cancer cells selectively and not its ability to kill cancer cells at low concentrations [26]. Piloquinone was experimented for its selectivity to cancer cells, by exposing normal (nonmalignant) cells of primary peripheral blood mononuclear cells (PBMC) to different concentrations of piloquinone, using MTT cytotoxicity assay [7]. Selectivity index was calculated by dividing IC_{50} of piloquinone on cancer cells by IC_{50} of piloquinone on normal cells. The higher (>2) the selectivity index, the better the potency achieved [26].

Screening for antiviral activity

In order to perform the antiviral experiment, Piloquinone compound should be tested for cytotoxic effect against Madin Darby Canine kidney (MDCK) cells, following the procedures mentioned previously in MTT cytotoxicity assay passage (except for adding 200 µl of acidified isopropanol (0.04 M HCl in absolute isopropanol=0.073 ml HCL in 50 ml isopropanol) to dissolve the formed MTT and, subsequently, the safe concentration of the compound (i.e. noncytotoxic) will be submitted to the antiviral test.

Plaque reduction assay

Antiviral activity was conducted by preparing 10^5cells/ml of 24 h-old culture of MDCK cells and 10^4 PFU/well of A/CHICKEN/M7217B/1/2013 (H5N1) virus. The test was implemented by mixing the safe concentration of the tested active compound with the desired count of influenza virus; after incubation for 1 h at 37°C, the mixture was added to the cells of MDCK, and supplemented with 3 ml of DMEM (contain 2% agarose), followed by pouring the medium in the cell monolayer; the plates were then incubated for 4 days at 37°C. After 4 days, formalin (10%) was added for 2 h, followed by staining the plate with 0.1% crystal violet [27]. Finally, the plaques were counted, and the percentage of plaque reduction, in comparison with control wells, was recorded as follows:

$$\% \text{ inhibition} = [\text{viral count(untreated)} - \text{viral count(treated)}/\text{viral count(untreated)}] \times 100$$

Determination of antibiotic activity using paper disc agar diffusion method

Twenty milliliters of bioassay medium (nutrient agar or potato dextrose agar medium) was seeded with 100 µl of cell suspension (containing 1.5×10^8 CFU/ml) of test organisms [*Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 278223, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 19430, *Staphylococcus aureus* (MRSA) ATCC 43300 *Candida albicans* ATCC 10231, and *Aspergillus niger* ATCC 16404] individually using the appropriate type of medium. A paper disc of 6 mm (Whatman no. 3) was loaded with 5 µl of 60 µg/ml of piloquinone dissolved in methanol and carefully placed onto the surface of the assay medium. After diffusion at 4°C for 1 h, the plate was incubated at 37°C for 24 h. Diameter of the inhibition zone was determined to identify the antimicrobial activity of Piloquinone [28].

Antioxidant capacity of piloquinone using 2, 2-diphenyl-1-picryl-hydrazyl radical scavenging activity

Antioxidant capacity was determined by preparing 1 mmol/l of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) stock solution (22 mg of DPPH in 50 ml methanol) and storing it at −20°C until use. The working solution (0.12 mmol/l) was prepared by mixing 6 ml of stock solution with 100 ml of methanol to obtain an absorbance value of 0.2±0.02 at 515 nm. A total volume of 0.1 ml of Piloquinone solution was vortexed for 30 s and mixed with 3.9 ml of DPPH working solution. After 30 min of incubation at room temperature in the dark, the absorbance was recorded at 515 nm. DPPH solution without the sample was used as a control. The percentage of DPPH radical scavenging activity was calculated as follows: $[(A_{control}-A_{sample})/A_{control}]\times100$ [29].

Results

Identification of producer organism

Morphological, physiological, and biochemical characterization

Many parameters are put into consideration when classifying new *Streptomyces* spp. It depends mainly on soluble pigment production, aerial, substrate mycelia color, spore shape, and ornamentation of spore surface. Table 1 illustrates that the aerial mass color varied from grayish to gray; therefore, it could be

Table 1 Morphological, physiological, and biochemical characterization of the isolated organism

Characters	Results
Morphological characteristic	
Spore chains	Spirals
Spore surface	Hairy
Color of aerial mycelium	Gray
Physiological characteristics	
Hydrolysis of starch	Positive
Action of milk	No coagulation in 14 days
Nitrate reduction	Negative
Gelatin liquefaction	None
Melanin production	Positive
Utilization of C-source	
D-glucose	+
D-xylose	++
L-arabinose	++
L-rhamnose	++
D-fructose	++
D-galactose	+++
Raffinose	++
D-mannitol	++
Mesoinositol	+
Salicin	++
Sucrose	++
Cellulose	++
N-source	
DL-methionine	No growth
DL-Iso-leucine	No growth
L-arginine	Good growth
L-lysine	Weak growth
L-glutamic	Good growth
L-histidine	Good growth
pH-alanine	No growth
L-asparagine	Good growth
L-valine	Weak growth
L-cysteine	Weak growth
Glycine	Good growth
Proline	Weak growth
Ornithine	Good growth
Tyrosine	No growth
DL-serine	Weak growth
Cell wall hydrolysis	
LL diaminopimelic acid	Positive
Sugar pattern	ND

assigned to the gray series. Furthermore, the isolate has a substrate mycelium color range from beige to brown; hence, it could be categorized as brown series. Considering the microscopic examination, the results showed that the photos picked by TEM revealed that the isolate has cylindrical hairy spores as shown in Fig. 1a. However, the aerial hyphae bears spiral spores (Fig. 1b).

Identification of new isolates depends on many physiological parameters such as nitrate reduction, gelatin liquefaction, melanin production and milk coagulation. Table 1 represents the results of physiological parameters. Biochemical characterization depends mainly on carbon source utilization pattern.

Phylogenetic analysis of the producer microorganism

The 16S rRNA sequence was aligned with representative sequences of related type strains of the genus Streptomyces in the NCBI databases using BLAST. The phylogenetic tree (Fig. 2) was constructed with the neighbor-joining algorithm and maximum-likelihood method. The 16S rRNA gene sequence reported in this paper was deposited in the NCBI GenBank nucleotide sequence databases with accession number MG383403. The isolate was called *S. pilosus* SBG-NRC-216.

Characterization and structure elucidation of the purified compound

By careful comparison of NMR spectral data and mass spectrum of isolated compound, as shown in supplementary material (Table S1 and Fig. S1-S10), with that of known compounds isolated from *S. pilosus*, it was found to be piloquinone (Fig. 3).

Estimation of some Piloquinone biological activities

In-vitro cytotoxicity assessment

Piloquinone was screened for antitumor activity utilizing five different human tumor cell lines including human liver cancer cell line HepG2, breast cancer cell line MCF-7, human lung cancer cell line A549 and human colon cancer cell lines Caco-2 and HCT-116, obtained from the American Type Culture Collection (Rockville, MD, USA). As indicated in Table 2 and Fig. 4, the results revealed the potency of Piloquinone as anticancer, especially for MCF-7, HepG2, and A549.

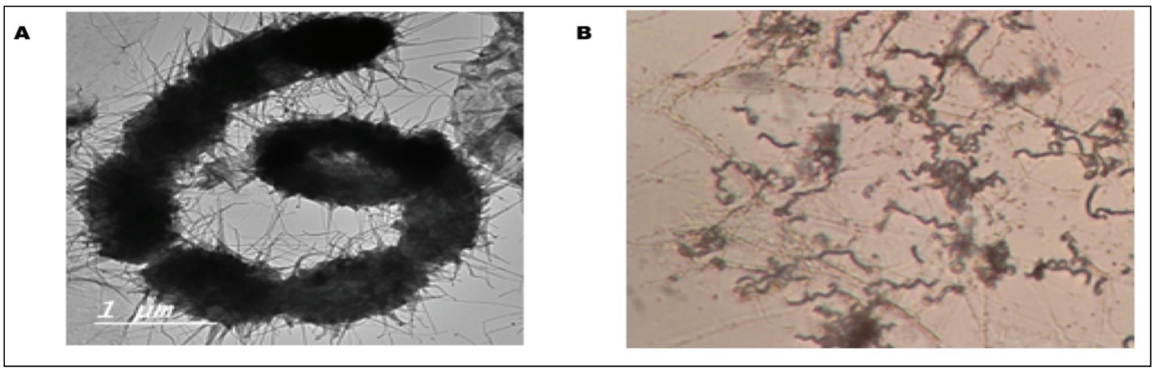
However, the results showed a moderate effect against Caco-2 and HCT-116; the results were compared with the effect of piloquinone on normal cells (PBMC), as illustrated in Fig. 5, and selectivity index was calculated as mentioned in the materials and methods section.

Antiviral activity of piloquinone

In order to study the antiviral activity of piloquinone, cytotoxic activity on Madin Darby Canine kidney (MDCK) cells should be determined first. The results showed that TC_{50} of piloquinone was equal to 2.3 μg/ml (Table 3).

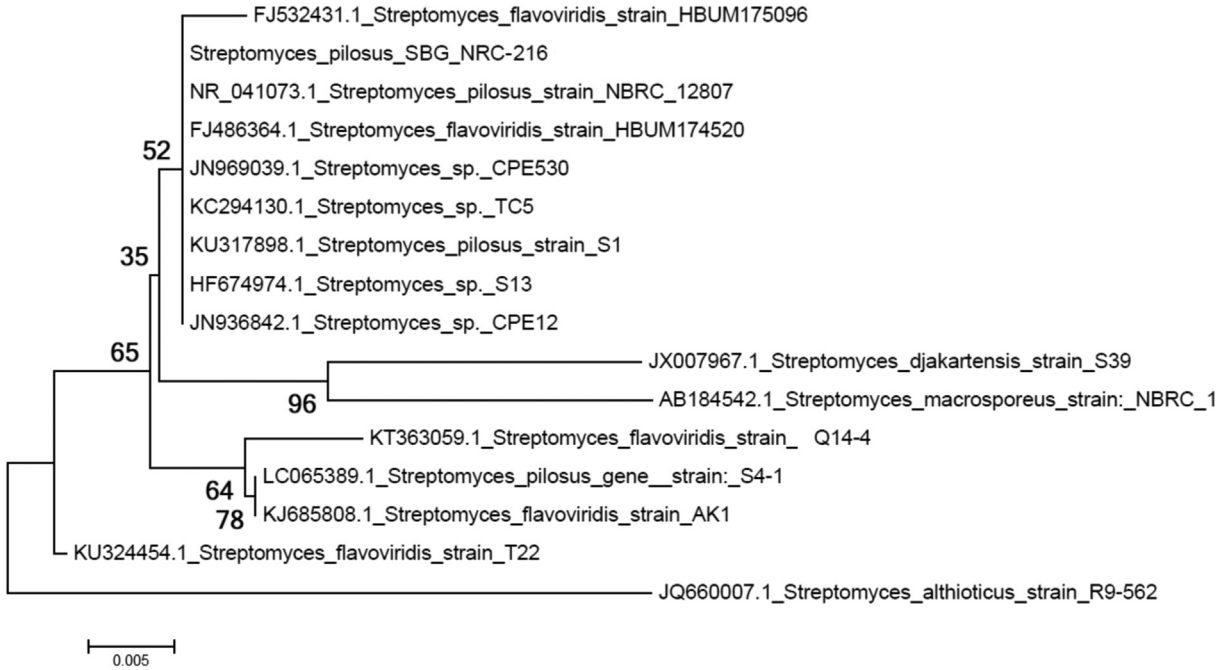
The antiviral test was conducted on two concentrations, and the results (Table 4) illustrated that Piloquinone has a weak effect on H5N1 virus.

Figure 1



Microscopic characterization of producer microorganism. (a) Transmission electron micrograph after 21 days on starch casein agar medium. (b) Light microscope showing spore-bearing aerial hyphae after 14 days cultivation on starch casein agar medium.

Figure 2



Phylogenetic tree showing the relationship of *Streptomyces pilosus* SBG-NRC-216 and other *Streptomyces* spp. based on Neighbor-joining Method.

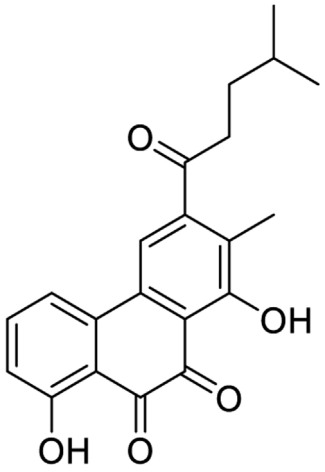
Table 2 Cytotoxic activity of Piloquinone on different human cancer cells

Compounds	MCF-7 IC_{50}µg/ml	HepG2 IC_{50}µg/ml	A549 IC_{50}µg/ml	Caco-2 IC_{50}µg/ml	HCT-116 IC_{50}µg/ml	PBMC IC_{50}µg/ml
Doxorubicin	0.44	0.977	5.842	8.508	6.87	ND
5-Fluorouracil	1.71	4.12	10.32	20.22	18.33	ND
Piloquinone	17.04	41.07	52.12	87.82	177.67	1876

Estimation of antimicrobial and antioxidant activity of piloquinone

Piloquinone was tested for antimicrobial activity against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 278223, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 19430, *Staphylococcus aureus* (MRSA) ATCC 43300, *Candida albicans* ATCC 10231, and *Aspergillus niger* ATCC 16404. The results showed that Piloquinone has no antimicrobial activity. Moreover, in order to study antioxidant activity, different concentrations (200, 100, 80, 60, 40 and 20 µg/ml) were used. The results proved that Piloquinone has no antioxidant activity at these concentrations.

Figure 3

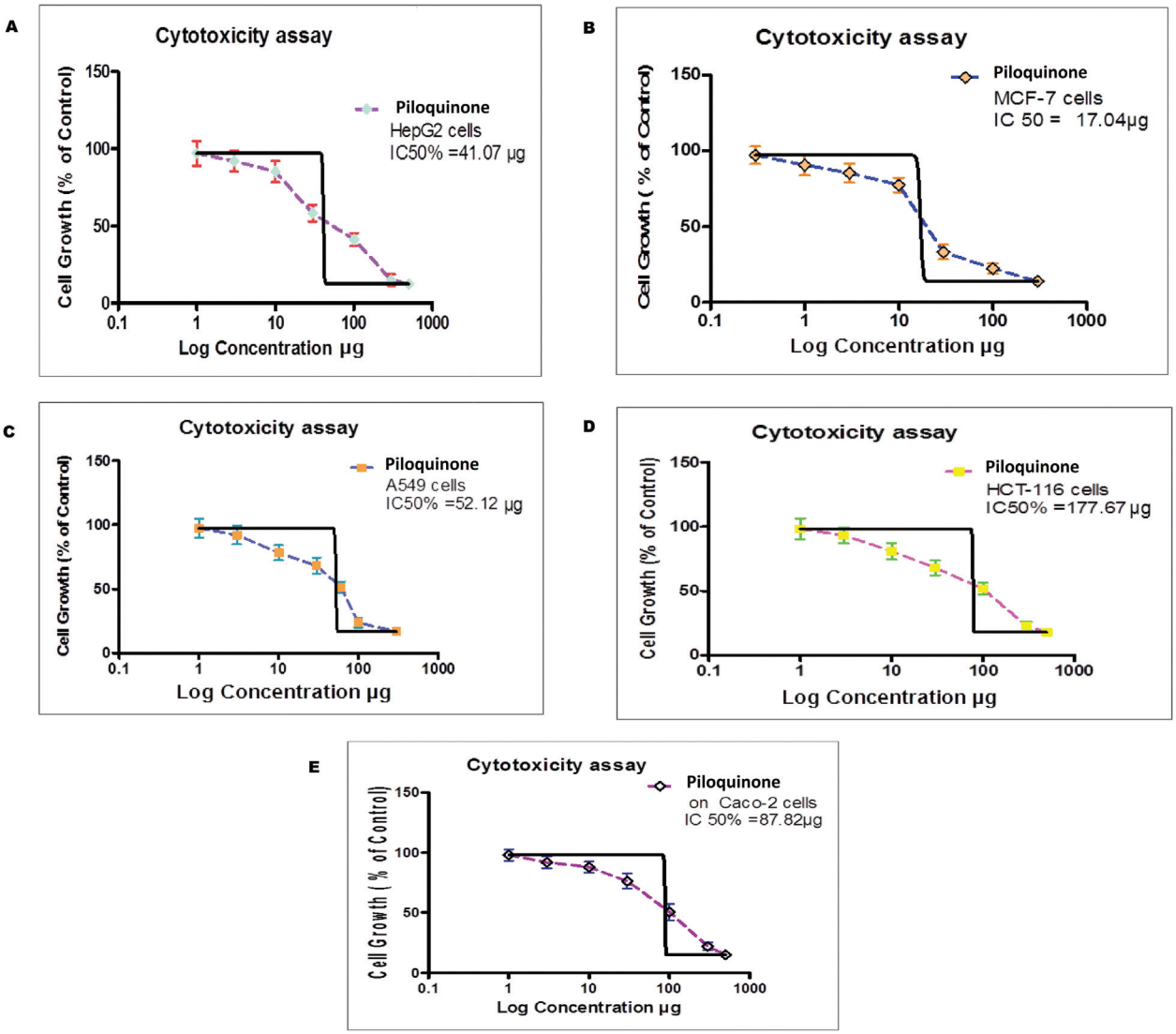


Structure of Piloquinone.

Discussion

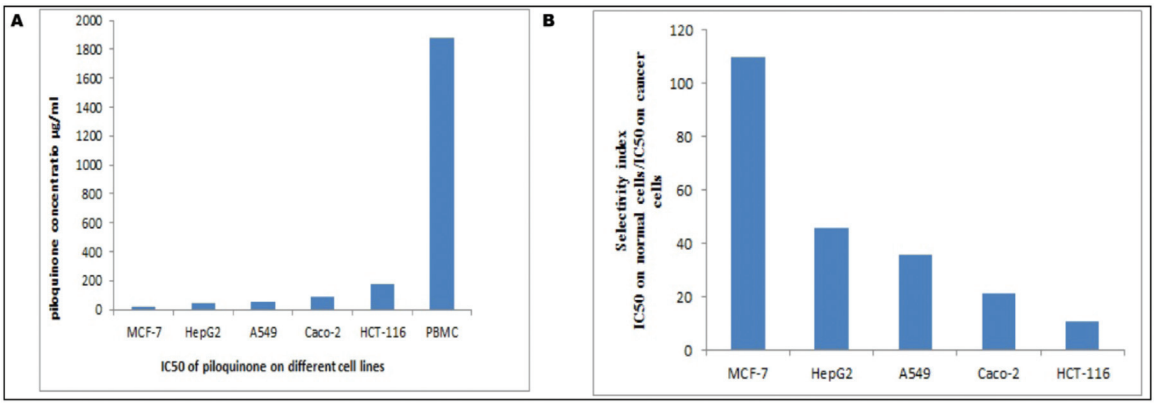
Quinones are widely distributed in nature and participate in various reactions of biological significance. Piloquinone is a product that belongs to Quinone compounds. However, because of its rarity in nature, few studies has been conducted on the biological activity of Piloquinone. Most researchers are interested in the synthesis of Piloquinone chemically. The current study focused on production of Piloquinone biologically using a bacterial strain isolated from the Egyptian soil; production of Piloquinone by the local isolated strain was confirmed by using different spectroscopic analysis, which revealed that molecular weight of the produced compound is 352; moreover, the results of UV, FTIR and NMR spectroscopic analysis agree with

Figure 4



Cytotoxic activity showing IC_{50}% of Piloquinone on (a) breast cancer cell line, (b) lung cancer cell line, (c) liver cancer cell line, (d and e) colon cancer cell lines.

Figure 5



IC_{50} of Piloquinone on different cell lines (a), selectivity index (b) (IC_{50} on normal cells/IC_{50} on cancer cells).

Table 3 Cytotoxic activity of piloquinone on Madin Darby Canine kidney cells

Concentration (µg/ml)	Cytotoxicity (%)
5	51.18852459
10	65.01639344
20	68.27868852
30	79.63114754

Table 4 Antiviral activity of piloquinone on influenza virus H5N1

Compound	Concentration (µg/ml)	Initial viral count	Viral count (PFU/ml)	% of inhibition
Piloquinone	5	80×10^3	70×10^3	12.5
	30	80×10^3	70×10^3	12.5

the published results [11,12]. Identification of the producer organism was also conducted, and the results of morphological and physiological characteristics showed how it is closely related to Bergey's description about *S. pilosus*. Classification was also confirmed by performing molecular characterization. The results showed that, the local isolate formed a distinct clade with type strain *S. pilosus* at a corresponding value of 97%, indicating the high confidence level of the association.Because of the destructive effect of cancer to economic and healthcare systems, as the number of deaths are predicted to increase and reach five million per annum by 2025; practical strategies should be applied to address cancer [30]. Surgery and radiation (therapeutic strategies) are not curative in cases of metastasis [31]. Hence, the discovery of a drug that can improve the potency and selectivity of the standard drugs would probably increase patient survival [26]. Consequently, cytotoxic activity of Piloquinone was experimented on five different cancer cell lines; the results showed that, Piloquinone is potent against MCF-7, HepG2, and A549; in contrast, Piloquinone has a moderate cytotoxic effect on Caco-2 and HCT-116. Furthermore, the results proved the high selectivity of Piloquinone to cancer cells rather than nonmalignant cells.

Investigation for other biological activities of Piloquinone was also conducted. The results showed that Piloquinone has a weak antiviral activity against H5N1 virus. Concerning the antibacterial and antifungal effects, the results showed that Piloquinone has no antimicrobial activity, at least for the tested organisms at the tested dosage, a fact that disagrees with the published data [11], which reported that Piloquinone is active against gram-positive bacteria. In contrast, the results revealed that, at concentrations of 20–200 µg/ml, Piloquinone has no antioxidant activity.

Conclusion

The producer isolate was identified as *S. pilosus* SBG-NRC-216. The obtained compound was characterized as Piloquinone. It has powerful anticancer activity against five different human tumor cell lines and a weak antiviral activity against the H5N1 virus. The results indicated that Piloquinone as a bioactive compound can be used for many industrial and pharmaceutical purposes.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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