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Isolation of Secondary Metabolites from Marine *Streptomyces sparsus* ASD203 and Evaluation its Bioactivity

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Abstract

The marine-derived actinomycetes *streptomyces* was isolated with 7 bacterial isolates from sponge *Crella cyathophora* and selected based on antimicrobial activity. Selected bacterial strain was identified morphologically and genetically by sequencing of its 16s rRNA gene as *Streptomyces sparsus.* Five compounds from ethyl acetate extract of *Streptomyces sparsus* were separated and purified using column chromatography. Identification of their structures was done using different spectroscopic analyses and by matching the data with literature reported. This is the first report of these isolated compounds from *Streptomyces sparsus* species: stearic acid , 9- heptadecenoic acid, palmitoleic acid, cedarmycin A, and phytol acetate. Antibacterial activity and minimal inhibitory concentration of compounds were measured via MTP assay against two Gram-positive and four Gram-negative bacteria isolates. Results revealed that cedarmycin A is the most potent one against all test microorganisms . Antioxidant capacity of compounds and extract were examined using DPPH radical scavenging capacity assay, cedarmycin A showed maximum activity of 71.54%. Also, biofilm inhibition activity of compounds was measured toward four pathogenic bacteria using MTP assay, showing biofilm inhibition activity. Hence, *Streptomyces sparsus* could be good source for secondary metabolites, and ethyl acetate extract may have these biological activities due to the presence of diverse metabolites.

Keywords: Marine sponge, *Streptomyces sparsus*, Bioactive compounds, Antimicrobial activity, Antioxidant activity, Antibiofilm activity.

1. Introduction

Nature has a large arsenal of structurally varied elements and pharmacologically Active chemicals that operate like extremely effective medicines or drive the creation of new medications for the fight against several diseases [1]. The oceans are considered as a unique source of a broad range of natural products, mostly from invertebrates such as tunicates, sponges, molluscs, and bryozoans, and from marine fungi, bacteria, and cyanobacteria. It's found that sponges are host organisms for various symbiotic microorganisms such as bacteria, cyanobacteria, archaea, and microalgae within their tissues where they reside in the extra- and intracellular space [2,3]. Recent investigations demonstrate that there is a relationship between the sponge-related micro-organisms and biosynthesis of marine natural products that may represent around 50 % of the bulk of a sponge [4].

Recently, great attention has been given to marine microorganisms due to their considerable biodiversity that has been found in the widespread oceans that cover over 70% of the world, and in particular, marine-derived bacteria are a source of new and natural bioactive compounds because of their secondary metabolites' chemical diversity [5]. The essential sources of natural chemicals are derived from marine microbial secondary metabolites when compared to those with potential therapeutic advantages. Many biologically active compounds have been documented to occur in marine creatures [6]. Actinobacteria, the prolific antibiotic makers and major suppliers to the pharmaceutical sector may create a range of secondary metabolites belong to the class of phenol, lactone, sterol, terpenoid, phthalate, fatty acid, a steroidal glycoside, polysaccharide-protein, etc. Actinobacteria were the most economic source of novel antibiotics and key suppliers for the pharmaceutical industry, as a large number of secondary metabolites may be produced.[7]. Many

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new advancements in this field of study over the previous five years and key results for in vitro, in vivo, and clinical bioactive chemicals for therapeutic medication have been covered by [8]. Based on the above concept actinomycetes are mainly targeted for secondary metabolites production and evaluation of compounds therapeutically.

Several kinds of research in the screening of novel antibiotics focus on the isolation of Streptomycetes from various environments. Indeed, around 75% of economically and therapeutically relevant antibiotics are produced by various Streptomyces species. More than half of the natural antibiotics identified so far are supplied and are still tested for effective molecules [9]. These organisms become more important both for their taxonomic and environmental perspectives and for the generation of new bioactive chemicals as antibiotics, antitumor agents, enzymes, immunosuppressive agents [10].

Here, we isolated and identified five compounds: fatty acids, diterpene, and butyrolactone fatty acid, from the ethyl acetate extract of *Streptomyces sparsus ASD203* isolated from the marine sponge, Hurghada, red sea, Egypt. This is the first report of these compounds from ethyl acetate extract of *Streptomyces sparsus* from the marine. Also, evaluated the antibacterial, antibiofilm, and antioxidant activities of crude extract and purified compounds, to support the possibility of its uses as a natural resource for therapeutic drug applications. The study also supports marine samples of Hurghada, red sea, Egypt, as a promising marine remained to be explored for new bioactive compounds.

2. Experimental

2.1. Instrumentation

A Jeol Ex-500 spectrometer was used for NMR experiments. Mass spectrometry analyses were recorded on Finnigan MAT-SSQ 7000 spectrometers. Column chromatography was performed using silica gel F254 (Merck) in glass blades. Sephadex LH-20 column chromatography was used for purification, TLC was done with silica gel 60 GF254 plates (Merck, Darmstadt, Germany), then the plates were visualized by UV light using vanillin in H_2SO_4.

2.2. *Chemicals and reagents*

All solvents, standards and reagents were of highly analytical grade. Methanol, ethyl acetate, methylene chloride and other reagents were obtained from El-Nasr Pharmaceutical Chemicals Company (Cairo, Egypt). Lysogeny broth media and other chemicals were purchased from HiMedia Laboratories Pvt. Ltd (Mumbai, India).

2.3. Marine collection

Marine sponge *Crella cyathophora* was collected from Marine seawater Hurghada, red sea, Egypt during 2019. After sampling, the collected sponge was photographed, coded, and kept in sterile tubes in the refrigerator at 4ºC for further analysis.

2.4. Isolation of marine actinomycetes

Isolation of *streptomyces* from Marine sponge was carried out through surface sterilization of the sponge surface using sterile distilled water followed by cutting the sponge tissues into a small piece and plated on starch nitrate agar medium for isolation of *streptomyces* [11], containing (g/l) starch, 20.0; KNO_3, 2.0; K_2HPO_4, 1.0; $MgSO_4.7H_2O$, 0.5; NaCl, 0.5; $FeSO_4.7H_2O$, 0.01; $CaCO_3$, 3.0; agar, 20.0; and distilled water 1000 ml., and the pH was 7.2. Antifungal agent (Nystatin) was added to prevent fungi growth. The inoculated SNA plates were left for 14 days at 28ºC until the appearance of any bacterial growth. Plates showing countable single colonies were selected, purified by a streak plate technique [12].

2.5. Fermentation and extraction of the culture

To prepare the spore suspension of the isolated actinomycetes strains. The spores were inoculated in 50 ml International Streptomyces Project-2 Medium (ISP-2) broth medium in 250 ml conical flasks and incubated for 3 days on a shaker with 150 rpm rotation. Then 5 ml of the spore suspension were put in 12×1 L Erlenmeyer flasks, each containing 100-gram rice prepared in 50 % natural seawater. The inoculated flask was left for 15 days at 30ºC. The rice cultures were extracted with ethyl acetate and concentrated in a rotary vacuum [13].

2.6. Screening of crude extract

The antimicrobial activity of extract was tested using the 96 polystyrene plate method [14]. 10 µl of the crude extract was placed in the wells, then 180 µl of nutrient broth media and 10 µl of the pathogenic bacterial suspension were added. The plates were left at 37ºC and observed after 24 hrs. Ciprofloxacin and Nystatin were used as controls.

2.7. Genetic identification of the actinomycetes strain

Based on the antibacterial activity of the raw extracts, a genetic identification of one isolate coded ASD203 was done by sequencing of the 16S rRNA gene of the

chosen strains. DNA extraction was carried out with DNeasy Blood & Tissue Kit according to the recommendation of the manufacturer. Two universal primers (27F 5′-AGAGTTTGATCCTGGCTCAG-3'; 1492R 5′-GGTTACCTTGTTACGACTT-3′) were selected for amplification of PCR reactions. Volume of PCR amplification reaction was : 50 µL (5 µL of 10 × Dream Taq Green PCR buffer, 2 µL of each 10 µmol dm^{-3} primers, 5 µL of 2 mmol dm^{-3} dNTP, 0.3 µL Taq DNA polymerase, and 0.5 µL of template DNA). The conditions: 94 °C for 45 s, 55 °C for 60 s, and 72 °C for 60 s were used for the PCR reaction. Macrogen Company, South Korea was chosen for performing the sequencing of the purified PCR products. By investigation and comparing similar and the homology of the obtained 16S rRNA sequences with the comparable sequences accessible in the NCBI database by the use of the BLAST online search tools (http://www.ncbi.nlm.nih.gov/BLAST). MEGA-X software was used for the construction of the phylogenetic trees [15].

2.8. Cultivation of selected streptomyces and preparation of crude extract

Based on the antimicrobial screening, the most potent actinomycetes isolate *Streptomyces sparsus* ASD203 was cultivated on sterilized rice media (20 flasks 1L each contain 100g rice) rice and incubated for 15 days. After incubation, the rice culture was extracted with ethyl acetate till exhaustion and evaporated under reduced pressure to yield (10 g) of crude extract. Part of this extract was subjected to column chromatography to efford isolated compounds and part was stored for biological studies.

2.9. Isolation of the compounds

The crude extract was submitted to silica gel column chromatography and eluted with *n*-hexane: ethyl acetate step-gradient, starting with100% *n*-hexane with increasing polarity, in the ratio (100:0, 8:2, 6:4, 4:6, 2:8, 0:100). Fractions were monitored by TLC, UV light and 1% vanillin/5% H_2SO_4/ EtOH reagent. Similar fractions were combined based on TLC profiles, to give four major fractions (F1- F4), which were subjected to Sephadex LH-20 column using ethyl acetate/hexane as solvent system and TLC silica gel to give 5 compounds. The four fractions were eluted by different concentrations of ethyl acetate/ n-hexane. F1 eluted by 20% ethyl acetate in hexane was purified on Sephadex LH-20 column using ethyl acetate/hexane (1:9) giving compound 1, while F2 eluted by 40% ethyl acetate in hexane was purified by TLC on silica gel "G" plate with ethyl acetate/methylene chloride (2:8) as a developing system, giving compounds C2 and C 3. On the other hand, F3 eluted by 60% ethyl acetate in hexane was subjected to Sephadex LH-20 column with ethyl acetate/hexane (3:7) to afford compound 4, while F4 eluted by 80% ethyl acetate in hexane was purified on Sephadex LH-20 column with ethyl acetate /hexane (4:6) giving compound 5. The compounds were identified with their physical and spectroscopic data and comparing data with reported literature.

2.10. Determination of the antimicrobial activity

Crude extract and pure compounds were evaluated toward pathogenic microbes comprises two Gram-positive bacteria (*Bacillus subtilis* ATCC6633 and *Staphylococcus aureus* NRRLB-767), four Gram-negative bacteria isolates (*Escherichia coli* ATCC25955, *Pseudomonas aeruginosa* ATCC10145, *Klebsiella pneumonia* ATCCBAA-1705, and *Proteus vulgaris* ATTC7829), obtained from Microbiology and Immunology Department, Faculty of Medicine, Al-Azhar University, Egypt, and used as test organisms [3]. Tests were performed in 96 well flat polystyrene plates. First, 10 mL of each compound (100 µg/ml) was transferred to 80 µL of lysogeny broth then we add10 µL of bacterial suspension, and all inoculated plates were incubated overnight at 37ºC. The positive effect of the tested compounds was detected as clearance in the wells, and in compounds that showed no activity on the bacteria, the growth medium in the wells seemed opaque. The absorbance was observed after 20 h at OD600 in a Spectrostar Nano Microplate Reader (BMG LABTECH GmbH, Allmendgrun, Germany).

2.11. Minimum inhibitory concentration (MIC)

MIC of the compounds that displayed a good antibacterial activity was measured using the serial dilution microplate method [16].

2.12. Assssment of antioxidant activity

The free radical scavenging activity (RSA) was assessed by the decolouration of ethanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and evaluated spectrophotometrically at 517 nm according to [17]. Briefly, 100 µL sample extract diluted in methanol was mixed with 1 ml of DPPH solution (0.5 mM) and after 30 min, the absorbance was at OD517 nm using Spectrostar Nano Microplate Reader (BMG LABTECH GmbH, Allmendgrun, Germany).

The scavenging activity was calculated as follows:

$$\text{Scavenging ability (\%)} = (A_{517\ \text{of control}} - A_{517\ \text{of sample}} / A_{517\ \text{of control}}) \times 100.$$

2.13. In vitro antibiofilm activity

The microtiter plate assay (MTP) method was used to measure the biofilm inhibitory activity of the

isolated compound in 96 well-flat bottom polystyrene titer plates and four clinical microbes (*E. coli*, *P. aeruginosa*, *S. aureus*, and *B. subtilis*) according to [18,19]. Each well of the 96 well-plate was filled with 180 µL of lysogeny broth (LB), with the following composition (g L^{-1}): tryptone, 10.0; yeast extract, 5.0; NaCl, 10.0. At pH 7.2, 10 µL of overnight growing test bacteria, 10 µL of the isolated pure compound at a concentration of 100 µg mL^{-1} sample, along with the negative control (*i.e.*, filtrate without sample) and then the plate was incubated for 24 h at 37 °C. The content of each well was removed, and each well was washed with 200 µL of phosphate buffer saline pH 7.2 to eliminate the floating bacteria. Crystal violet (0.1%, w/v) was added to each well for 1 h, for staining, then 200 µL of distilled water was used, for washing, and then the plate was kept for drying in the laminar flow. For measuring the optical density (OD) at 570 nm, 95% ethanol was added to the dried plate, and by using (SPECTROstar nano absorbance plate reader – BMG LABTECH).

3. Results and Discussion

3.1. Isolation of actinomycete isolates from different marine samples.

Actinomycetes vary between sponge sources in their recoverability; example, four actinomycetes from *Amphimedon* spp. collected from Ras Mohamed, Egypt [20], 16 actinomycetes from *Amphimedon* spp. collected from Hurghada ,Egypt [21]. In the current study, 7 actinomycetes cultures were isolated from marine sponge *Crella cyathophora* (**Fig. 1)** collected from Hurgada marine sea, The *Streptomycetes* isolated according to the morphological characteristics of the colony which are usually round, convex, shaped colonies.

Fig.1: Crella cyathophora image [22]

3.2. Pre-screening

To select the most potent isolate, the 7 isolates were screened for their antimicrobial activity. To obtain the microbial extracts of the isolated strains, small scall fermentation has been carried out by incubation of isolated bacterial strain in 50 ml of International System Project (ISP2) medium. After incubation, the microbial extracts were obtained by extraction with ethyl acetate. To evaluate the antimicrobial activity of the bacterial crude extracts, 96 polystyrene plates were used. The most active extract based on the antibacterial result was isolate coded as ASD203. (Table 1).

Table 1: Antimicrobial activity of the isolated actinomycetes

	Inhibition ratio (%)			
Isolates codes	***B. subtills***	***E. coli***	***S. aureus***	***P. aeruginosa***
ASD200	22%	20 %	30%	20%
ASD201	13%	12 %	20%	15%
ASD202	--	-	-	-
ASD203	70%	85%	62%	75%
ASD204	50%	50%	42%	45%
ASD205	35%	45 %	20%	53%
ASD206	10	9 %	-	-

3.3. Morphological identification

The morphological characteristics of strain *ASD203* have been shown that the strain *ASD203* produced pale grey mycelium. The spores are short, rod-shaped, straight to flexuous with sizes ranged from (0.5x 1.5 mm) and the spore surface was smooth (Fig. 2).

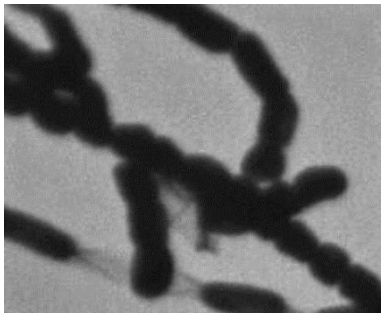


Fig. 2: Scanning electron micrograph showing spores and spore chains of strain ASD203

3.4. Genetic identification of the most active strain

Based on the preliminary evaluation of the isolated actinomycetes, the most active strain was selected and 16S rRNA gene sequence was obtained, identified, and aligned against other sequences identified available in the GeneBank database using the BLAST tool to identify the similarity score and to calculate the statistical significance of the matches

(http://www.blast.ncbi.nlm.nih.gov/Blast). The results revealed that the 16S rRNA gene sequence was extremely comparable with 99.86% homology of the isolate ASD203 with *Streptomyces sp*. The phylogenetic analysis and tree were constructed using the neighbor-joining method (Fig.3) by the MEGA 7 program according to [15]. Based on the analysis of the DNA sequence and the morphological characteristics of the strain ASD203 was identified as *Streptomyces sparsus ASD203* and deposited in GenBank with accession no. MT605147

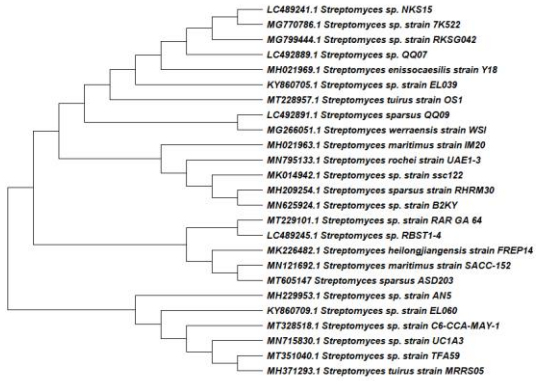


Fig. 3: Phylogenetic tree of the *Streptomyces sparsus ASD203* strain

3.5. Large scale fermentation and Isolation of the bioactive metabolites

To prepare the seeded culture media, pure single colonies of *Streptomyces sparsus ASD203* were grown in 50 mL (ISP2) medium and left for 4 days at 30 °C. The seeded culture media obtained was used to inoculate 12×1 L Erlenmeyer flasks, each containing 100 mL (rice) prepared in 50 % natural seawater. The flasks were kept at 30 °C for 15 days and extracted with ethyl acetate. The extracts were concentrated and dried in vacuo giving 10 g of a yellowish-brown crude extract.

3.6. Identification of the compounds

Actinomycetes, and especially Streptomyces species, are commonly known as a prolific secondary metabolite source [23], because of their large dispersion and exceptional ability to make a large spectrum of bioactive compounds [24]. In this study five compounds from the ethyl acetate extract *were* identified for the first time from the marine-associated actinomycete *Streptomyces sparsus* as: Stearic acid, 9 heptadecenoic acid, palmitoleic acid, cedarmycin A, and phytol acetate (Fig. 4). The compounds were identified by the mass spectrum, ^{1}H and ^{13}CNMR [$CDCl_3$: 500 MHz, 125 MHz] respectively: and compared with the literature. The following compounds were identified with their physical and spectroscopic data:

Stearic acid (C1): White powder. EI-MS *m/z* 284, molecular formula $C_{18}H_{36}O_2$. ^{1}H NMR: δ 0.88 (3H, t, 6.5Hz, H-18), 7.3 (s, COOH, H-1), 2.34 (2H, t, 7.5Hz, H-2), 1.63 (2H, m, H-3), 1.26 (28H, m, H 4: H 17). ^{13}C NMR: δ 178.5, 33.5, 24.6 [C(1,2,3,)] respectively, 29.1-29.7 [C4: C15], 31.7, 22.6 ,14.1 [C(16,17,18)] respectively. The spectral data were compared with those previously reported [25].

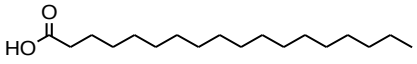
9-heptadecenoic acid (C2): White powder, [M]+ *m/z* 268, molecular formula $C_{17}H_{32}O_2$. ^{1}H NMR: δ 7.3 (s, COOH, H-1) , 0.88 (3H, t, 7.0Hz, H-17), 2.32 (2H, t, 7.1Hz, H-2), 1.61 (2H, m, H-3), 5.32–5.36 (2H, m, H- 9,10), 2.07 (4H, m, H- 8,11), 1.26–1.24 (m, 18H). ^{13}C NMR: δ177.5, 33.8, 31.4,14.3 [C(1,2,3,17], 129.5,129.7(C9, 10), 29.6(C8,11), 29.2-29.8 [C(4,5,6,7,12,13,14,15,16)] respectively. The spectral data was compared with those previously reported [26].

Palmitoleic acid (C3): Colorless oil. [M]+ *m/z* 254, molecular formula $C_{16}H_{30}O_2$. ^{1}H NMR: δ 0.89 (3H, J = 6.8 Hz, t, H-16), 5.41–5.32 (2H, m, H- 9,10), 2.35 (2H, J = 7.5 Hz, t, H- 2), 1.9-2.01 (4H m, H-8,11), 1.61 (2H, m, H-3), 1.25–1.37 (m, 16H, 4,5,6,7,12,13,14,15). ^{13}C NMR: δ177.4, 33.7, 24.6,14.1 [C (1,2,3,16] respectively, 129.5, 130 (C-9, 10), 27.1 (C-8, 11), 22.3-31.7 [C(4,5,6,7,12,13,14,15)] respectively. The spectral data was compared with those previously reported [27].

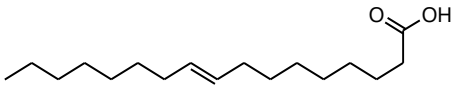
Cedarmycin A (C4): Colourless oil. [M]+ *m/z* 241, molecular formula $C_{13}H_{20}O_4$. ^{1}H NMR: δ 3.39(m, H-3),4.19-4.48 (dd, J= 9.4,1.5 Hz ,H-4), 6.39 (d, 2.7Hz, H-5),4.18-4.25 (dd, J= 11.2, 7.3Hz, H-6), 2.31(t, J=7.6Hz, H-8),1.62 ,1.19,1.55 (m, H-9,10,11), 0.88(d, J=6.6Hz, H- 12,13).^{13}C NMR: δ169.8, 134.6,38.1 [C(1,2,3,)], 68.1, 124.1, 64.7,173.5 [C(4,5,6,7] respectively, 34.3, 22.8 , 38.3, 27.7, 22.5, 22.5 [C(8,9,10,11,12,13)] respectively. The spectral data was compared with those previously reported [28].

Phytol acetate (C5): Colourless liquid. [M]+ *m/z* 338, molecular formula $C_{22}H_{42}O_2$. ^{1}H NMR: δ 0.88–0.91 (12H, m, CH_3), 2.21 (s, 3H, CH_3), 1.76 (s, 3H, CH_3), 1.96 (2H, t, *J* =7.3Hz , CH_2), 1.62-1.65 (m, 1H), 1.21- 1.35(m, H), 4.51 (d, J=7.2 Hz, 2H, *CH_2* OAc), 5.34 (1H, m, *CH*CH_2OH). ^{13}C NMR: δ CH3 [16.4, 19.7,19.7, 21.0,22.7, 22.7], CH_2[24.4, 24.8, 24.9], 28.1(CH), CH_2 [33.2, 33.2, 37.8, 37.7,37.7, 39.3, 39.9, 60.5], two olefinic carbons: 118.1(CH), 142.8(C) and 171.2 (C=O). Compound was

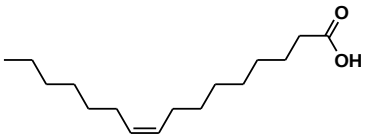
confirmed to be phytol acetate by comparing its spectral data with the literature [29].



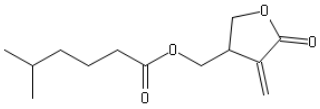
Stearic acid



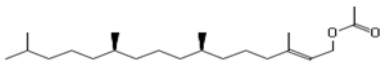
9-heptadecenoic acid



Palmitoleic acid



Cedarmycin A



Phytol acetate

Fig. 4: Isolated compounds from the ethyl acetate extract of *Streptomyces sparsus* ASD203

3.7. Antimicrobial activity

The Antimicrobial Resistance (AMR) of pathogenic microbes usually occurs when the microbes become resistant to antibiotics which make them no longer respond to medicines. The antimicrobial activity of the crude extract and compounds 1-5 were measured toward two Gram-positive and four Gram-negative bacteria isolates. The antimicrobial results revealed that compound (4) is the most potent one against all test microorganisms (*S. aureus*, *B. Subtilis*, *P. aeruginosa*, *E. coli*, *P. vulgaris*, *K. pneumonia)*, while compound (1) displayed good inhibition activity against (*S. aureus*, *P. aeruginosa* and *B. Subtilis)* Meanwhile, compound (3 and 5) showed only activity against *B. Subtilis* and *S. aureus*, while no activity against the rest microbes (Fig. 5).

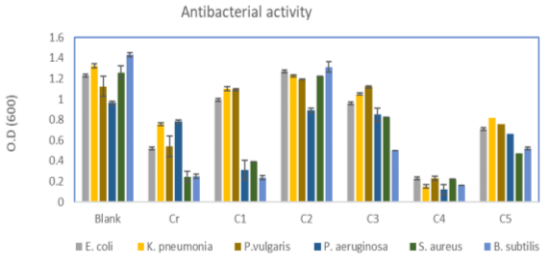


Fig. 5.: Antibacterial activity of crude and pure compounds of *Streptomyces sparsus* ASD203 against pathogenic bacterial strains

The results were compared with literature showed antimicrobial activity comparable to conventional drugs, which were in agreement with [30], saying that the genome sequence of an Streptomyces sp. showed a clinically interesting chemical compound. Also, the results revealed that cedarmycin A is the most potent one against all test microorganisms, in contrast to [31], which recorded a weak activity against gram-positive and negative bacteria and moderate activity against yeasts. Also, [32] said that cedarmycin A isolated from streptomyces sp. has antifungal activity. Furthermore, stearic acid displayed good inhibition activity against *S. aureus*, *P. aeruginosa*, and *B. Subtilis* in contrast to [33], which found that stearic acid has no inhibitive effects on FabI, a key of bacterial fatty acid synthesis, a bacterial enoyl-acyl carrier protein reductase which was a promising target for antibacterial medicines. Palmitoleic acid showed activity against *B. Subtilis* and *S. aureus* this result is in accordance with [33], which showed that palmitoleic acid inhibited bacterial enoyl-acyl carrier protein reductase (FabI). Moreover, this study revealed that phytol acetate showed antimicrobial activity against *B. Subtilis* and *S. aureus*, showed inhibitory activity against biofilm, and has antioxidant activity. This result is in accordance with [34], who reported that phytol compound showed antimicrobial, antioxidant activity. While, there is no available data about the inhibitory activity of 9-heptadecenoic acid so, this paper is considered the first report of this compound.

3.8. Minimum inhibitory concentration

The MIC of pure compounds isolated from *Streptomyces sparsus* ASD203 were determined, and the data were presented in (Table 2).

Table 2: Minimum Inhibitory Concentrations of the compounds isolated *Streptomyces sparsus* ASD203 against bacterial pathogens.

Cpd	Minimum Inhibitory Concentration (MIC, µg/mL)*					
	S. aureus	***B. subtilis***	***E. coli***	***K. pneumonia***	***P. vulgaris***	***P. aeruginosa***
1	25	50	-	-	-	50
2	-	-	-	-	-	-
3	100	100	-	-	-	-
4	25	25	50	25	25	25
5	125	125	-	-	-	-
Cip	0.62	0.31	1.25	1.25	1.25	2.5

* Results are the average of two independent replicates; positive controls: Cip (Ciprofloxacin); - (not detected)

3.9. Antioxidant activity

Oxidative stress is a phenomenon that resulted from the overproduction of reactive nitrogen species (RNS) and reactive oxygen species (ROS). Oxidative stress is described as an imbalance between the rate of production of free radicals and enzymatic antioxidants in the human body, which may be associated with a variety of health problems such as cancer, cardiovascular disease, inflammation, and Alzheimer's disease [14]. Several reports have found a strong relationship between the antioxidant activities of endophytic and associated actinomycetes extracts and the existence of certain chemical groups, such as flavonoid and quinone derivatives and Quinones [35, 12]. The antioxidant activity of compounds 1-5 based on DPPH assay (200 µg/ml) showed that C4 is the most active one with maximum DPPH scavenging activity 71.54%, after that C1 showed 59.10 %, then C3 showed 40.02%. In contrast, the remaining compounds showed less antioxidant activity (Table 3) showed the antioxidant activity of the *Streptomyces sparsus* ASD203 crude extract and pure compounds.

Table 3. DPPH scavenging activity (%) of compounds 1-5

Compounds	DPPH scavenging activity (%)
Cr	45.00
C1	59.10
C2	17.25
C3	40.02
C4	71.54
C5	10.55

3.10. Antibiofilm activity

Biofilm plays a significant role in bacterial persistence [36]. This process protects the bacteria by increasing their attachment ability to living and non-living surfaces [19]. In our efforts to find secondary metabolites with antibiofilm activity, the isolated pure compounds have been assayed using MTP assay, the biofilm inhibition activity was studied towards four pathogenic bacteria (*P. aeruginosa*, *S. aureus*, *E. coli*, and *B. subtilis*). In general, the results showed that (Fig.6) only three compounds (C1: C4) have biofilm inhibition activity against microbes. *S. aureus*, *B. subtilis*, and *P. aeruginosa*, biofilm formation were inhibited by C1 over 50.47, 41.91, and 57.39 %, respectively. However, C2 showed no activity against tested pathogenic bacteria. While C3 displayed a very low response toward biofilm formation of *B. subtilis* and *S. aureus.* C4 lessened the biofilm formation of *S. aureus* up to 60.21%, while with *B. subtilis* the inhibition ratio was up 65.23 %, this percent decreased with *E. coli* and *P. aeruginosa* to reach 54.67 and 53.98%, respectively. In contrast, C5 has low inhibitory activity (Table 4) with all tested microbes ranged from 7.29 % with *B. subtilis*, to 11.20% with *S. aureus.*

Table 4. Antibiofilm activity of (C1:C5) compounds on different strains of microorganisms:

Tested compounds	S. aureus	B. subtilis	E. coli	P.aruginosa
Cr	50.21	43.00	30.40	35.02
C1	50.47	41.91	NA	57.39
C2	NA	NA	NA	NA
C3	12.13	20.91	NA	NA
C4	60.21	65.23	54.67	53.98
C5	11.20	7.29	9.47	9.52

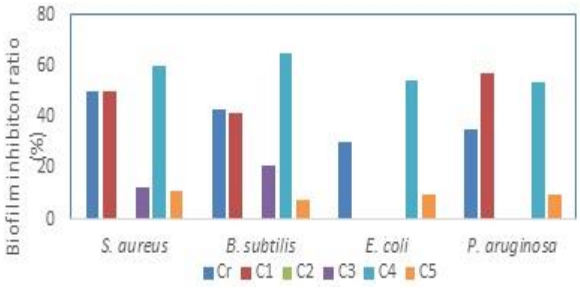


Fig.6 Biofilm inhibitory activity of crude and pure compounds of *Streptomyces sparsus* ASD203 against nosocomial pathogenic bacterial strains

4. Conclusion

In our effort to isolate bioactive metabolites with potential antibacterial, antioxidant, and antibiofilm, seven associated actinomycetes strains were isolated from the sponge *Crella cyathophora*. Based on antibacterial screening, one actinomycete isolate has been selected identified using 16s rDNA analysis as *streptomyces* analysis lead to the isolation of 5 compounds: stearic acid (1), 9-heptadecenoic acid *sp.* ASD203. Large scale fermentation, chromatographic

purification, and structural (2), palmitoleic acid (3), cedarmycin A (4), phytol acetate (5). The isolated pure compounds were evaluated toward some pathogenic bacteria and the result showed that some compounds exhibited a pronounced antimicrobial activity. Moreover, the antioxidant and antibiofilm activity of the pure compounds were also evaluated the activity of the isolated pure compounds also evaluated. These results make *Streptomyces sparsus* an attractive strain for further exploration and industrial use.

Conflicts of interest

The authors declare no conflicts of interest.

Abbreviations

rRNA Ribosomal Ribonucleic Acid; DNA: Deoxyribonucleic Acid; BLAST: Basic Local Alignment Search Tool; NCBI: National Center for Biotechnology Information; NMR: Nuclear Magnetic Resonance; ^{1}H: Proton; ^{13}C: Carbon 13; ATCC: American Type Culture Collection; Gram+ve: Gram-Positive; Gram-ve: Gram-Negative; TLC: Thin Layer Chromatography; J: Coupling Constant; Hz: Hertz; ppm: Parts Per Million; HRESIMS: High Resolution Electrospray Ionization Mass Spectrometry; O.D.: Optical Density.

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