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Evaluation of the Antimicrobial-antitumor Activity of Newly Isolated Melanin Pigment Streptomyces Strain from Egyptian Localities and Effect of Gamma Irradiation on Antagonistic Process

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BOTH BACTERIA and fungus are important pathogens that have a significant impact on human health. It has always been crucial to investigate new medicinal compounds and other bioactive secondary metabolites from Streptomyces species for use in pharmaceutical and industrial applications. Streptomyces, a biologically potential melanin pigment, and its antagonistic activity against microbes were the focus of the current research. To isolate actinomycete species that can produce the pigment melanin, eight soil samples from different regions of the Kafr El Sheikh Governorate were collected. *Streptomyces* isolates were characterized based on morphological, biochemical and cultural features and identified as *Streptomyces aureofasciculus* and given the name *Streptomyces aureofasciculus* M20. In the present study, a secondary screening method was used to screen the antimicrobial activity of actinomycete metabolites against bacterial and fungal strains. Additionally, gamma irradiation at dose levels of 2.0 and 4.0 kGy were used as well as small doses as stress factor to investigate the effect of *Streptomyces* extract against bacterial and fungal strains. The SEM was used to exhibits distraction effect of both irradiated and non-irradiated extract. The results showed a significant antibacterial and antifungal effect through morphological alteration on bacterial cells shape and size and also the morphological alteration on substrate and aerial hypha of *Aspergillus niger* and significant effect on its sporangium. The chemical structure of the extract was analyzed using GC-MS which revealed the fractionation of extract as (Octadecenoic acid, Hexadecanoic acid Hexadecanoic acid methyle ester, Octadecenoic acid(2-phenyl-1,3 Diooxolan-4-Y-)methyle ester Cis,9- Octadecenoic acid methyle ester,1- Naphthalenoacetic acid-methyle ester,10- Octadecenoic acid methyle ester and - Octadecanoic acid methyle ester) respectively. It also shows the molecular formula ($C18H34O2$, $C16H32O2$, $C17H34O2$, $C28H44O4$, $C19H36O2$, $C13H12O2$, $C19H36O2$ and $C19H38O2$) respectively. Moreover, the thin-layer chromatography and UV spectroscopy were used. The extract exhibited a brown color, one spot, RF (0.8), wavelength 255 nm. The antitumor activity of the extract was studied against HCT-116 and MCF-7 cell line, the colon carcinoma cell line exhibits IC_{50} = 15.1±0.9 µg/ml and the breast carcinoma cells exhibits IC_{50}= 24.7 ±1.2 µg/ml. Thus the current study exhibited antimicrobial activity of the extract against both Gram negative Gram positive bacterial strains and fungal strain. It also showed a significant effect after irradiation and a small dose level and also a significant antitumor effect against both the colon and breast carcinoma cells in vitro.

Keywords: Breast carcinoma cell line, Colon carcinoma cell line, Gamma irradiation, SEM, *Streptomyces*.

Introduction

It has long been recognized that microorganisms are crucial for the development of drugs from natural products. The huge availability of structurally different natural products may have its origins in the number of species. When compared to the chemical production method, it will be more cost-

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effective to extract these chemicals from microbial sources (Ab Mutalib et al., 2020).

The microorganisms have undergone screening for the ability to produce anticancer substances or leads. According to theory, the anticancer effect of these microbial natural chemicals could control immune response, restrain cell growth, and trigger apoptosis (Rayan et al., 2017).

Gram positive bacteria called *streptomyces* are usually filamentous and sporulating, and their DNA has a high G C ratio of 55-75%. More than 70% of commercially available antibiotics are produced by bacteria from the genus *Streptomyces* and other therapeutically valuable metabolites, such asanti-tumor medicines and immunosuppressive medications (Raja et al., 2010).

The melanin pigments, which are of a high-molecular-weight metabolites, brown to black color and are made from phenolic or indolic chemicals, are frequently found in complexes with proteins or carbohydrates (Ser et al., 2016). Tyrosinase and polyketide synthase, two essential melanogenesis-related enzymes, play a major role in their production. Both of these processes can lead to the formation of melanin.

The microbial resistance is recognized as one of the biggest hazards to public health and a source of worry on a global scale. With increasing morbidity and mortality due to multidrug-resistant bacteria, it is imperative that new, more effective medications be developed. In contrast, cancer is a complex human disease that is on the rise and calls for novel medications with minimal or no adverse effects. The majority of medications currently utilized in healthcare systems is either synthetic versions of or derived from Streptomyces. Streptomyces research on natural products has been truly amazing in recent years (Kui Hong et al., 2019).

A variety of endogenous and external stimuli is involved in the complex process of human cancer, which involves changes to cells and molecules' structure. In other words, these triggers lead to the unchecked proliferation of malignant cells, which then invade other organs and no longer respond to normal cell signalling. The early detection, with effective treatment with radio and or chemotherapy and prevention are some of the variables that have helped reduce cancer-related mortality (Tan et al., 2019).

Among these, finding potent anticancer medications is a top priority for cancer therapy. We have been successfully battling cancer with natural materials derived from a variety of sources, including plants, ecosystems, and microbes. Approximately 60% of today's anticancer/ antitumor medications came from these natural sources (Nobili et al., 2009).

The microbial natural products can not only suppress or inhibit cancer progression, but also can reverse its progression and can be considered an alternative solution to overcome side effect of chemotherapeutic, such as distinguishing between normal and cancer cells and removing only cancer cells (Rayan et al., 2017).

The present study aims at evaluating the antimicrobial and the antitumor activity of the newly isolated *Streptomyces* strain and extraction the purified product as a natural product and studying the effect of gamma irradiation as stress factor on antagonistic properties.

Materials and Methods

Isolation of actinomycetes producing melanin pigment

Eight soil samples were aseptically collected from agricultural regions from the soil at Kafr El sheikh governorate, for isolation of bacterial species capable of producing melanin pigment. The collected soil samples were dried at room temperature overnight, then 10 gm of soil was suspended in basic lauryl-sulfate buffer solution. An amount of 0.1 ml of suspension was spread on the surface of the tyrosine- yeast extract broth, glycerol- tyrosine agar and peptone yeast extract-iron agar. The media producing black-brown colored melanin pigment after incubation at 28^0C for 7 days were selected. The pure isolates were observed for their cultural characteristics after cultivation on ISP 2 agar (yeast extract 4.0 g, malt extract 10.0 g, Dextrose 4.0 g, and agar 20.0 g, pH 7.3) at 28°C for 7 days. The selected isolates were preserved in 20% (v/v) glycerol solution at −20°C until further use (Butler et al., 2010; Tawfik et al., 2015).

Morphological characterization of Streptomyces isolates

Colony and spore morphology

The morphology, color and pigment production of the *streptomyces* isolates were considered by

growing them on different media as exhibited in Table 1. The plates were inoculated with 0.2 ml of selected colonies suspended on 1 ml physiological saline and incubated at 28°C for 7 days, the growth of isolates was observed at regular intervals. The nature of the specific colony, color and pigmentation were recorded.

Biochemical characteristics

The biochemical features of the selected isolate were investigated to characterize the genus and species level of the organism.

*The bacterial and fungal strain***s**

Gram negative *Enterobacter cancerogenus, Klebsiella oxytoca* (Tawfik et al., 2015), *Pseudomonas aeruginosa* MMCC13*,* and *Escherichia coli MMCC24*. Gram positive *S. aureus MMCC21* and *Bacillus cereus* MMCC11. The fungal strains were *Aspergillus niger* and *Aspergillus terreus* were isolated and identified from a previous study at Bacteria and Viruses lab. Radiation Microbiology Department, the National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority.

Gamma irradiation

Irradiation of the extract was preformed using a ^{60}Co-source Indian gamma cell (GE 4000A) located at the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Egypt. The dose rate was 1 kGy/h at the time of experiment. The *Streptomyces* extract was irradiated at dose levels of 2.0 and 4.0 kGy.

Extraction and purification of Streptomyces metabolites

This method recommended by Tawfik et al. (2015). A spore suspension of a *Streptomyces* strain was added to glucose-asparagine broth (20.0 g D-glucose, 5.0 g L-asparagine, 1.0 g MgSO4.7H2O, 0.7 g KH2PO4, 2.0 g yeast extract, 1 L distilled water, pH 6.8).) at 28 °C for 8 days, the broth was filtered through Whatman No1 filter paper and centrifuged at 5000 rpm for 10 min. The supernatant was adjusted to pH 7.0 and extracted using ethyl acetate at a concentration of 1:1 (v/v). The organic phase was collected and rotary evaporated under reduced pressure.

Characterization of purified product

This method was recommended by (Tawfik et al., 2015). Melting points were determined on a Stuard melting point apparatus. Purified product solubility dissolved in ethyl alcohol, deionized water dimethyl sulfoxide (DEMSO), and acetone.

Spectroscopic analysis via Spectroscopic apparatus

For determination of function groups and chemical structure of the product (GC-MS, UV and IR)**,** Infrared (IR) spectra were recorded using technique on JASCO FT/IR/- 6300 apparatus, was carried out at the National Center for Radiation Research and Technology, (NCRRT), Cairo, Egypt.

TABLE 1. Cultural characteristics of the *Streptomyces* selected isolate

Medium	Growth	Color of aerial mycelium	Color of substrate mycelium	Soluble pigment	Type of growth
Starch nitrate agar	moderate	whitish-brown	brown	negative	cottony
Inorganic salt starch agar	moderate	Gray	Gray	negative	Powdery
Starch casein agar	weak	whitish	Gray	negative	cottony
Nutrient agar	weak	white	Gray	negative	cottony
Glucose nitrate agar	no growth				
Malt yeast extract agar	no growth				
Glucose asparagine agar	moderate	Grayish	dark-yellow	negative	cottony
Oat meal agar	weak	Gray	white	negative	cottony
Glycerol asparagine agar	moderate	Creamy	creamy	negative	leathery
Peptone yeast extract iron agar	weak	white	Yellow	Positive	cottony
Glycerol tyrosine agar	moderate	white	white	Positive	cottony
Czapek,s agar	weak	white	white	negative	cottony

Ultra-violet (UV) spectra were recorded using Streptomyces strain purified product that dissolved in acetone on UV JASCO/V-560 apparatus, it was carried out at the National Center for Radiation Research and Technology, (NCRRT), Cairo, Egypt.

Gas chromatography–mass spectrometry (GC-MS) analysis

The chemical composition of the extract was identified by using trace GC1310-ISQ MS (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The initial temperature of the column oven held at 50^0 C and was increased by 5°C / min up to 230°C hold for 2 min. increased to the final temperature 290°C by 30°C /min and hold for 2 min. The line temperatures of the injector and MS transfer were kept at 250, 260°C respectively. The Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The delay of the solvent was 3 min and the samples of 1 µl were injected using auto-sampler AS1300 connected with GC in the split mode. The EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source for temperature was set at 200 °C. The components were identified by comparison of their mass spectra and retention times according WILEY 09 and NIST 11 mass spectral database, apparatus was carried out at the National Center for Mycological Research and its applications, Cairo, Egypt.

Thin Layer Chromatography

The purity of the extracted product was determined by optimizing the mobile phase of TLC, which was ethyl acetate. Isopropanol:Acetonitrile (1:4:5) (v/v) was performed on unknown products using 5 x 20 cm, 1 mm thick silica gel plates, where they were prepared and activated at 110 °C for 30 min .

Scanning Electron Microscope depending on morphological character of clinical bacterial and fungal strains

Scanning Electron Microscopy, EVO 15 ZIESS, UK was used. The basic principles of isolation, fixation, dehydration, drying, mounting, and photographing have many variations, scanning electron microscopy can be used for viewing microorganisms which under the present study; however, the concentration of cells is critical.

Antimicrobial activity of partially purified compound

The antagonistic activity was determined by measuring the diameter of the inhibition zone in mm. 0.2 ml of both un-irradiated and irradiated *Streptomyces* extract with conc. 50 mg/2ml of DEMSO, this method was recommended by Blair et al. (2015).

In vitro screening the antitumor activity of *Streptomyces* extract against the colon HCT-116 and breast MCF-7 carcinoma cell line was carried out. The method was carried out according to Mosmann (1983).

The cell line obtained frozen in liquid nitrogen (-180^0C) from the American type culture collection, Which were maintained at the National Cancer Institute, Cairo, Egypt.

Results and Discussion

Isolation of actinomycetes was carried out by serial diluting of the soil sample and inoculating on the tyrosine- yeast extract broth, glycerol- tyrosine agar and peptone yeast extract-iron agar, one isolate producing melanin pigment after incubation at 28^0C for 7 days was selected.

Identification of the selected isolate depends on the following characteristics

Morphological properties: Exhibited spiral sporophore and smooth spore (Fig.1a and Fig1b).

Physiological properties:

Production of melanin pigment: The *Streptomyces* isolate was positive for melanin pigment production on tyrosine- yeast extract broth, glycerol- Tyrosine agar and peptone yeast extract-iron agar media.

The *Streptomyces* isolate exhibits a positive reaction toward Gelatin liquefaction, coagulation of milk and reduction of Nitrate, whilst exhibited negative results towered H_2s production.

Exoenzymes production: The Streptomyces isolate was able to produce amylase, caseinase and cellulosae enzymes.

Carbon source utilization: The *Streptomyces* isolate exhibited utilization of D-glucose, D-mannose, raffinose, D-xylose, fructose, sucrose, L-rhaminose, -mannose, L- inositol, L-arabinose and lactose, whilst D-mannitol not utilized.

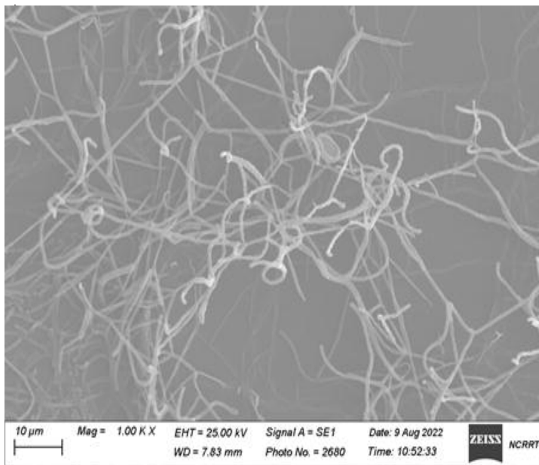


Fig. 1a. Scanning Electron Micrograph, showing spiral sporophore of *Streptomyces* isolate

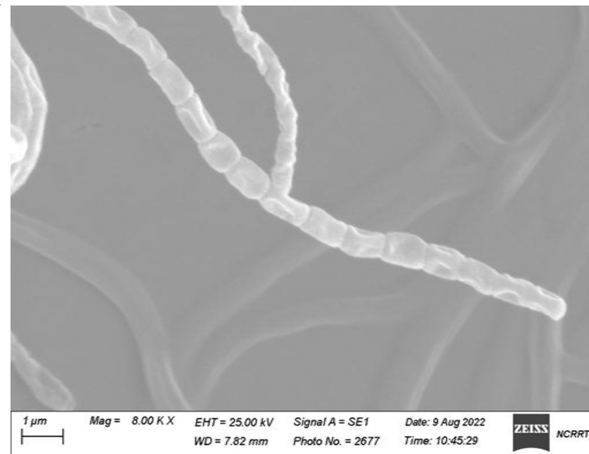


Fig. 1b. Scanning Electron Micrograph, showing smooth spore surface of *Streptomyces* isolate

The results illustrated in Table 1, show that the growth behavior of *Streptomyces* strain was not observed on glucose nitrate agar medium and malt yeast extract agar medium, while weak growth on media other than glucose asparagine agar medium, glycerol asparagine agar medium, and inorganic salt starch agar medium Agar showed a moderate growth. The isolate had visible white aerial hyphae on all media used except inorganic salts starch agar, oatmeal agar, and glucose-asparagine agar. The growth pattern of the isolates was flocculent on all media except that growth on glucose nitrate agar was leathery. The substrate mycelium of the isolate was brown on starch nitrate agar, yellow on peptone yeast extract iron agar, starch casein agar and glucose asparagine agar and nutrient agar, and powdery on mineral salt starch agar indicated. A diffusible brown pigment could be detected in all media except glycerol tyrosine agar. The results shown in Table 2 revealed that the *Streptomyces* isolate was able to tolerate NaCl concentrations up to 6%.

TABLE 2. The NaCL tolerance for *Streptomyces* selected isolate

NaCl concentration (%)	Growth
2	+++
4	++
6	+
8	-
10	-
12	-

+++ Heavy growth, + Weak growth, ++ Moderate growth, - No growth

Characterization of Streptomyces isolate

According to the abovementioned results of the morphological cultural, physiological analyses and following the diagnostic simple working keys for the classification and identification of Normal Taxa such as Szabo key and following the description of the *streptomyces* species included in the International *Streptomyces* Project (ISP) (1972), this isolate was characterized to be likely *Streptomyces aureofasciculus* and given the name *Streptomyces aureofasciculus* M20 (Bergey's manual, 1994).

Spectroscopic characteristics

The melanin's appealing potentiality to react with various metals in a process requiring the links between metals and the pigment's carboxyl, hydroxyl, and amine functional groups facilitates its contribution in heavy metal bioremediation (Gomha et al., 2015). Melanin has a variety of uses in the pharmacological, medicinal, and beauty industries related to human health, including radioprotection, anticancer, antioxidant, and antimicrobial (Mattoon et al., 2021).

The physiochemical properties of the extracted product using UV recorded a maximum absorption peak at =255.nm in the ultraviolet region (Table 3). The spectroscopic analysis of the *Streptomyces aureofasciculus* M20 extract was carried out, GC-MS which exhibited antimicrobial production capacity in the genus *Streptomyces* might be related to the presence of multiple secondary metabolite 8 chemical compounds with significant area percentages namely, (Octadecenoic acid, Hexadecanoic

acid Hexadecanoic acid methyle ester, Octadecenoic acid(2-phenyl-1,3_Diooxolan-4-Y-)methyle ester Cis,9- Octadecenoic acid methyle ester,1- Naphthalenoacetic acid-methyle ester,10- Octadecenoic acid methyle ester and -Octadecanoic acid methyle ester) respectively (Fig. 2 and Table 4).

TABLE 3. Physiochemical properties of *Streptomyces aureofasciculus* M20 purified product

Characteristic	Results
Color	Brown
Purity	One spot (RF=0.8)
Melting point (^{0}C)	248^{0}C
Ultra-violet	255 nm

TABLE 4. Groups absorbing in IR region of *Streptomyces aureofasciculus* M20 purified product

Range (cm^{-1})	Assignment
3263	OH or N-H (2°-amide-amines)
2961	C-H str.-alkane
1639	C=O amide
1563	N-H (2¡-amide) II band
1448	-O-H
1398	-O-H
1146	-N=N
1069	CH_3
945	CH_3
848	CH_3

Priyanka & Debajit (2021) reported that the derived compound was identified using the GC-MS chromatogram peak area, molecular weight and molecular formula, where the peak area is directly proportional to magnitude of the bioactive extract existing in the partially purified composites. GCMS analysis exhibits the presence of 17 compounds, most of which were earlier documented for its antibacterial, antifungal, antitumor, anticancer and antioxidant activities

Another study on the anti-inflammatory by 8-oxo-9-octadecenoic acid by Min-Cheol et al. (2018) reported that the anti-inflammatory activity of 8-oxo-9-octadecenoic acid (OOA) which isolated from *Undaria peterseniana* by examining its ability to inhibit the lipopolysaccharide (LPS)-induced production of inflammatory mediators in RAW 264.7 macrophage cells. Moreover, they found that OOA significantly suppressed the LPS-induced production of nitric oxide (NO) and inflammatory cytokines. OOA down regulated the LPS-induced expression of inducible nitric oxide synthase and cyclooxygenase-2 proteins.

In addition, the extract was recognized from the TLC which exhibits the same at 3 line for conformation only one band was recorded with R.f= 0.85 (Fig. 3j).

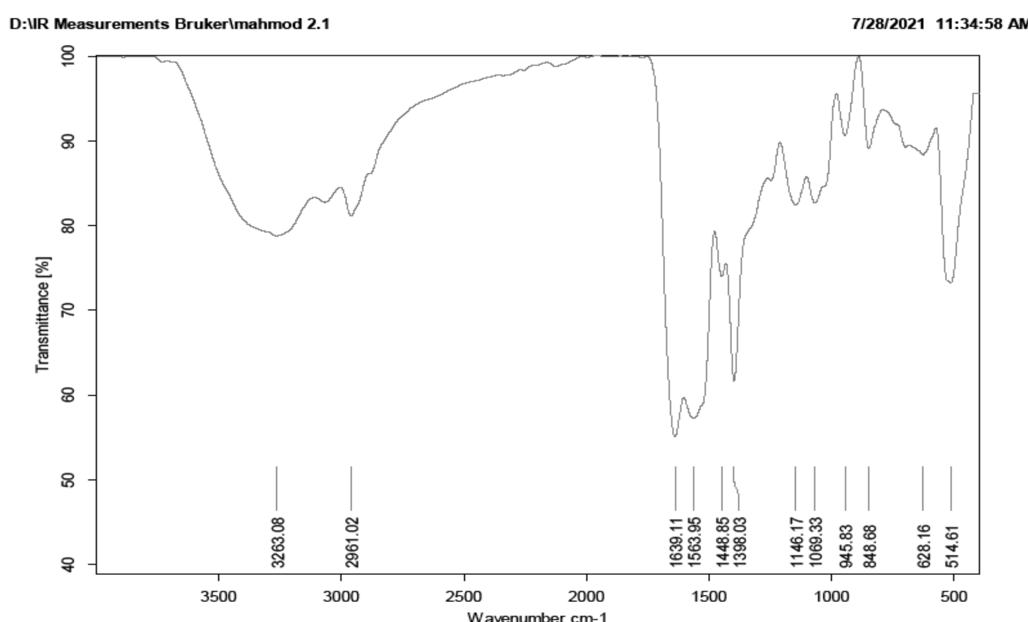


Fig. 2. Infra-Red spectrum of *Streptomyces* aureofasciculus M20 purified product

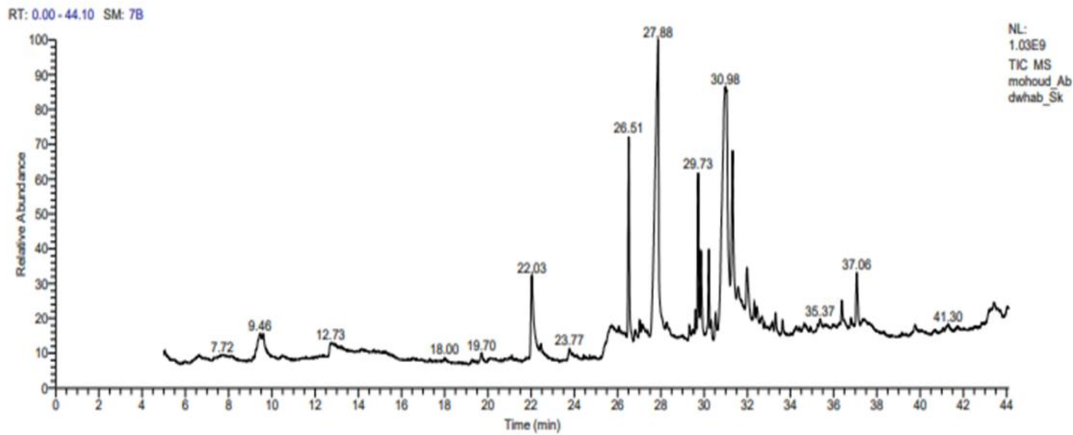
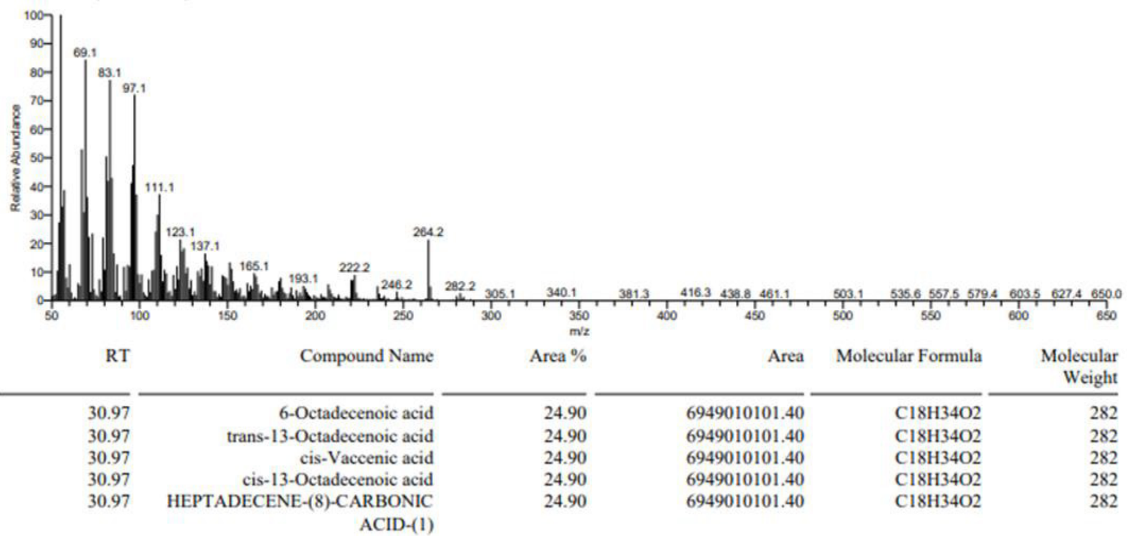
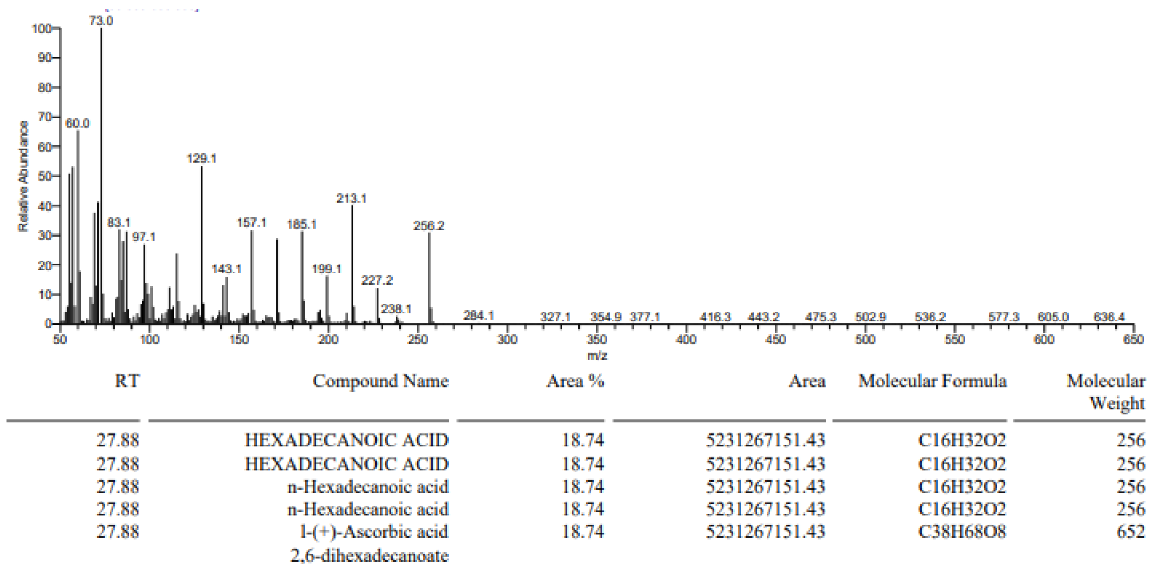


Fig. 3. GC MS Report of *Streptomyces aureofasciculus* M20 purified product



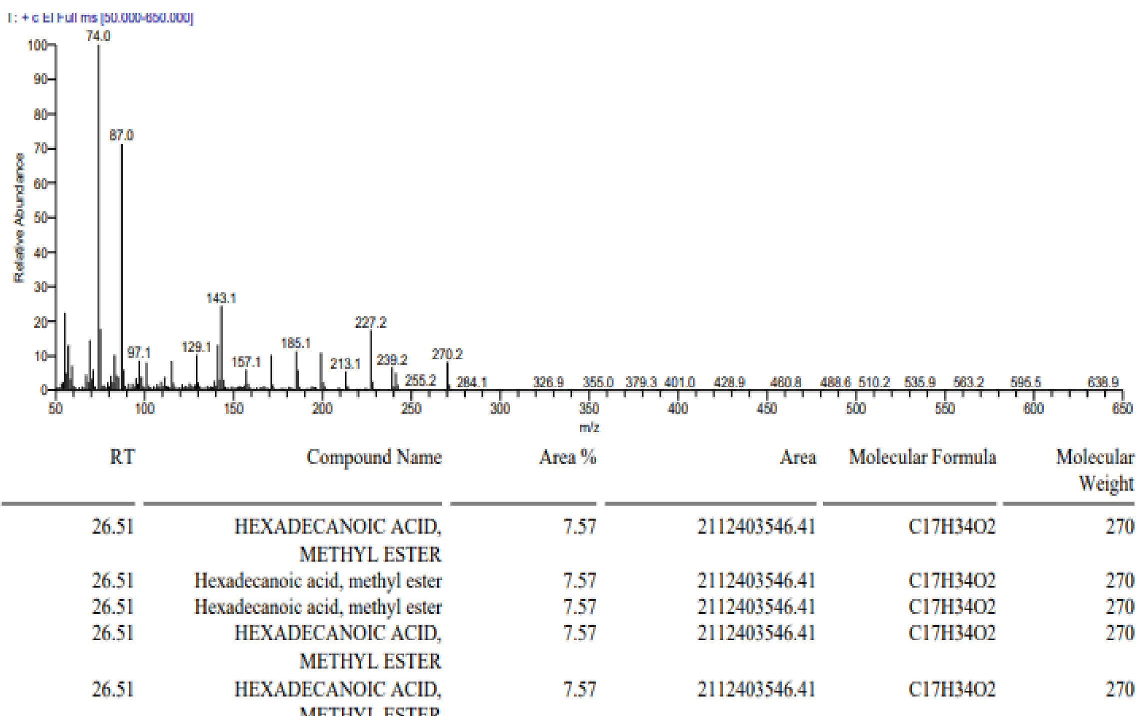
RT	Compound Name	Area %	Area	Molecular Formula	Molecular Weight
30.97	6-Octadecenoic acid	24.90	6949010101.40	C18H34O2	282
30.97	trans-13-Octadecenoic acid	24.90	6949010101.40	C18H34O2	282
30.97	cis-Vaccenic acid	24.90	6949010101.40	C18H34O2	282
30.97	cis-13-Octadecenoic acid	24.90	6949010101.40	C18H34O2	282
30.97	HEPTADECENE-(8)-CARBONIC ACID-(1)	24.90	6949010101.40	C18H34O2	282

Fig. 3a. GC –MS Report at RT 30.97 of *Streptomyces aureofasciculus* M20 purified product



RT	Compound Name	Area %	Area	Molecular Formula	Molecular Weight
27.88	HEXADECANOIC ACID	18.74	5231267151.43	C16H32O2	256
27.88	HEXADECANOIC ACID	18.74	5231267151.43	C16H32O2	256
27.88	n-Hexadecanoic acid	18.74	5231267151.43	C16H32O2	256
27.88	n-Hexadecanoic acid	18.74	5231267151.43	C16H32O2	256
27.88	l-(+)-Ascorbic acid 2,6-dihexadecanoate	18.74	5231267151.43	C38H68O8	652

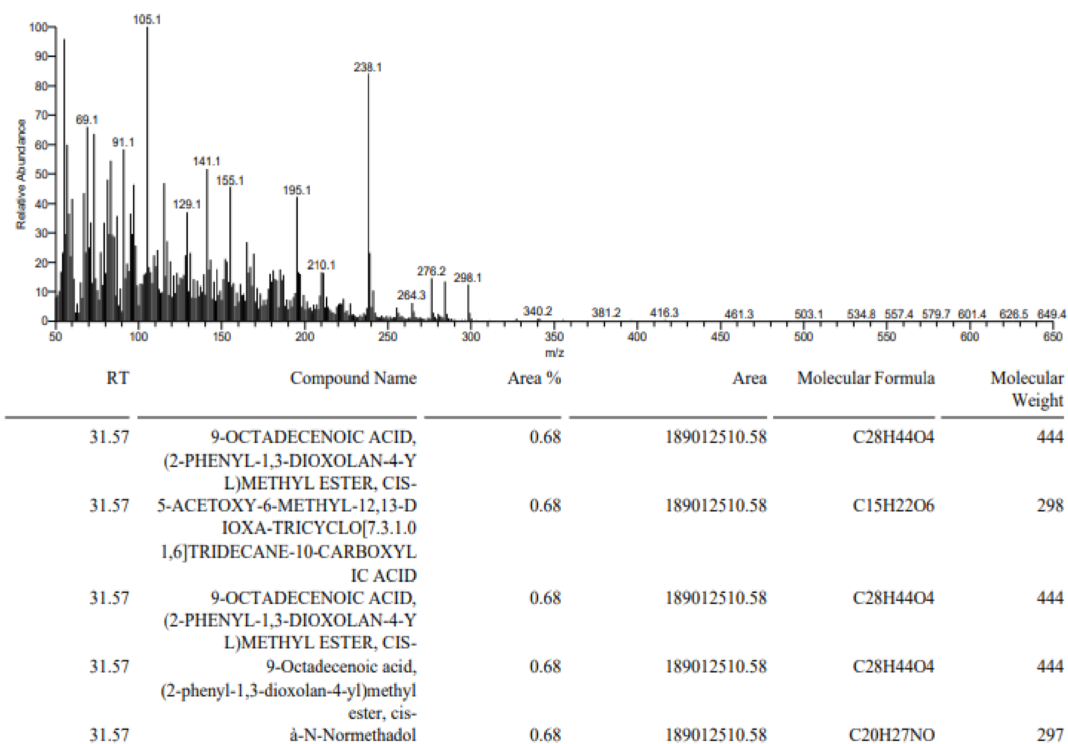
Fig. 3b. GC –MS Report at RT 27.88 of *Streptomyces aureofasciculus* M20 purified product



RT	Compound Name	Area %	Area	Molecular Formula	Molecular Weight
26.51	HEXADECANOIC ACID, METHYL ESTER	7.57	2112403546.41	C17H34O2	270
26.51	Hexadecanoic acid, methyl ester	7.57	2112403546.41	C17H34O2	270
26.51	Hexadecanoic acid, methyl ester	7.57	2112403546.41	C17H34O2	270
26.51	HEXADECANOIC ACID, METHYL ESTER	7.57	2112403546.41	C17H34O2	270
26.51	HEXADECANOIC ACID, METHYL ESTER	7.57	2112403546.41	C17H34O2	270

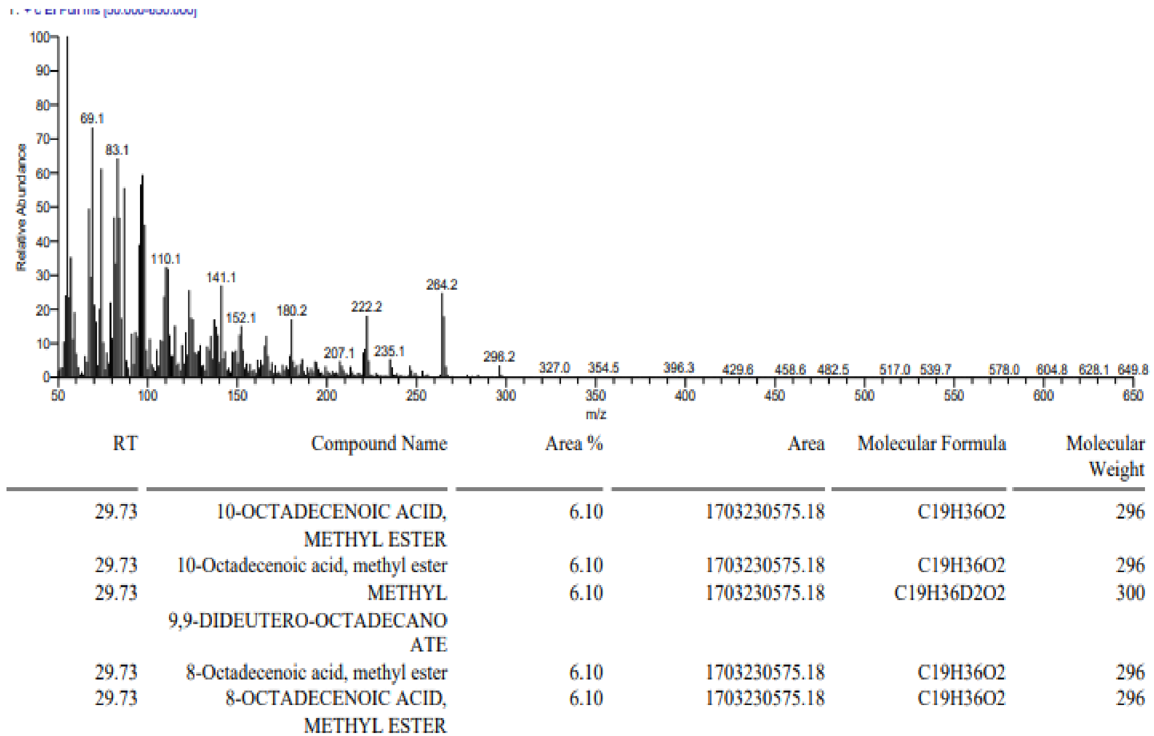
Fig. 3c. GC –MS Report at RT 26.51 of *Streptomyces aureofasciculus* M20 purified product

product



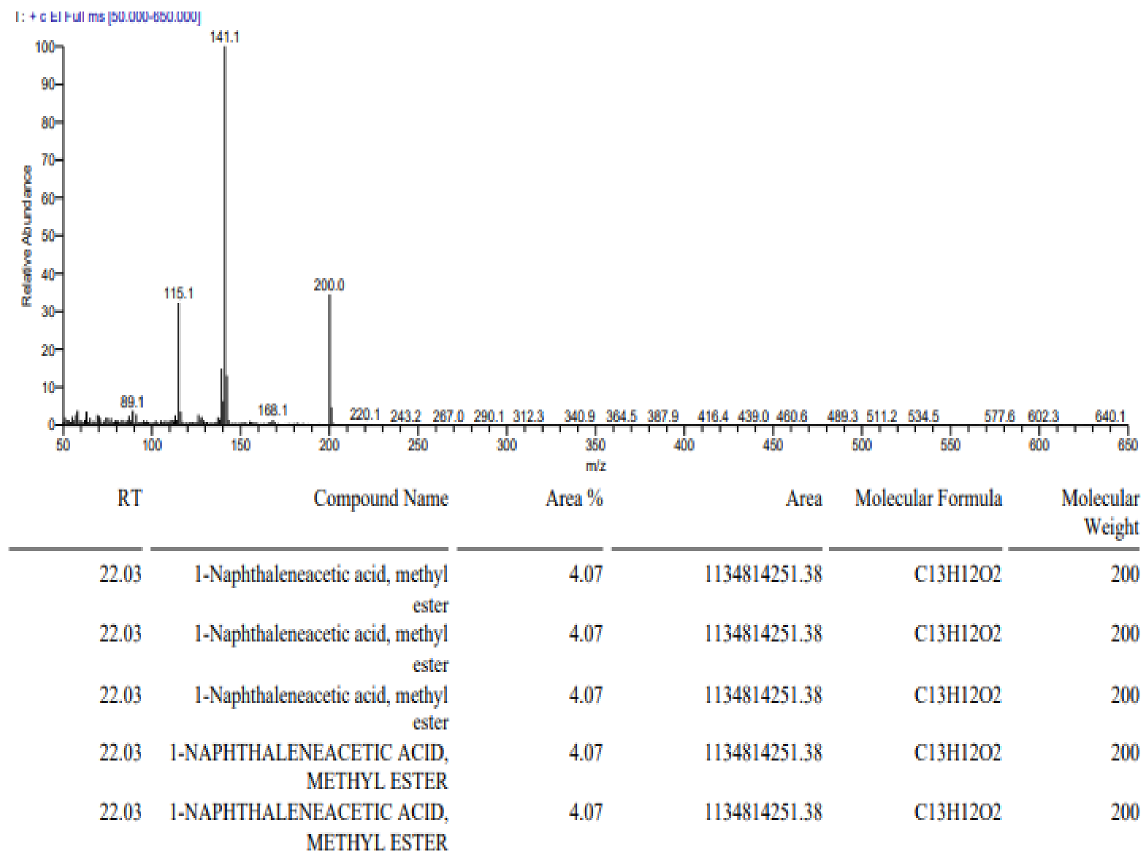
RT	Compound Name	Area %	Area	Molecular Formula	Molecular Weight
31.57	9-OCTADECENOIC ACID, (2-PHENYL-1,3-DIOXOLAN-4-YL)METHYL ESTER, CIS-	0.68	189012510.58	C28H44O4	444
31.57	5-ACETOXY-6-METHYL-12,13-DIOXA-TRICYCLO[7.3.1.0 1,6]TRIDECANE-10-CARBOXYLIC ACID	0.68	189012510.58	C15H22O6	298
31.57	9-OCTADECENOIC ACID, (2-PHENYL-1,3-DIOXOLAN-4-YL)METHYL ESTER, CIS-	0.68	189012510.58	C28H44O4	444
31.57	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	0.68	189012510.58	C28H44O4	444
31.57	à-N-Normethadol	0.68	189012510.58	C20H27NO	297

Fig. 3d. GC –MS Report at RT 31.57 of *Streptomyces aureofasciculus* M20 purified product



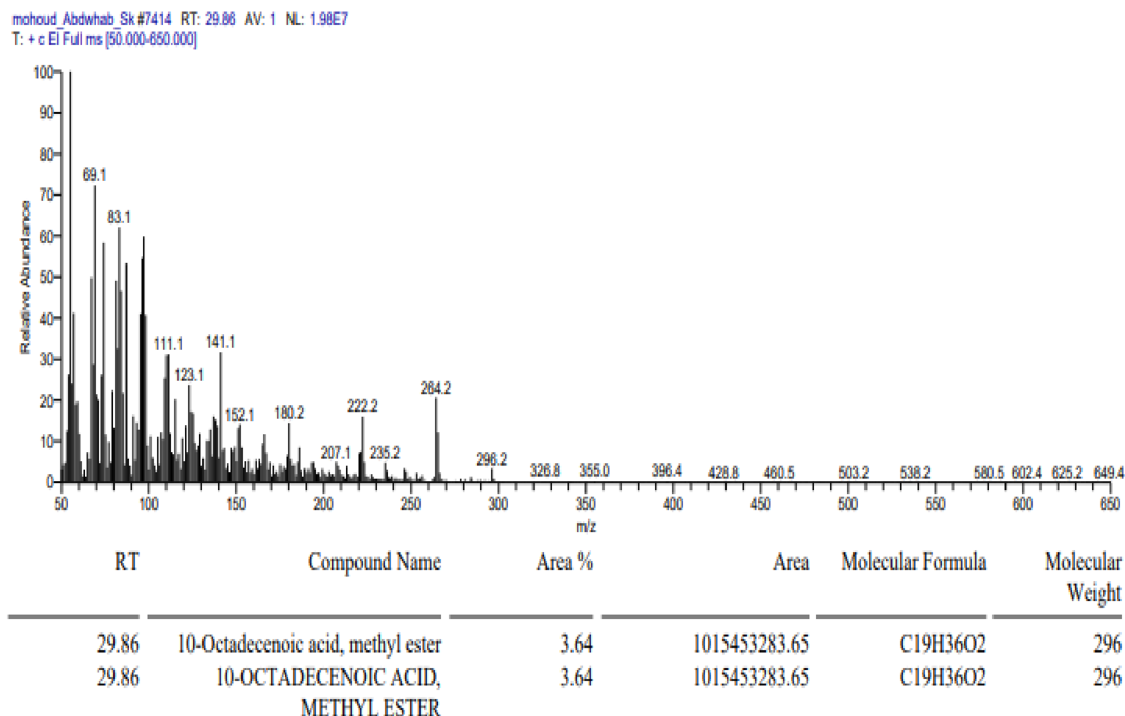
RT	Compound Name	Area %	Area	Molecular Formula	Molecular Weight
29.73	10-OCTADECENOIC ACID, METHYL ESTER	6.10	1703230575.18	C19H36O2	296
29.73	10-Octadecenoic acid, methyl ester	6.10	1703230575.18	C19H36O2	296
29.73	METHYL 9,9-DIDEUTERO-OCTADECANOATE	6.10	1703230575.18	C19H36D2O2	300
29.73	8-Octadecenoic acid, methyl ester	6.10	1703230575.18	C19H36O2	296
29.73	8-OCTADECENOIC ACID, METHYL ESTER	6.10	1703230575.18	C19H36O2	296

Fig. 3e. GC –MS Report at RT 29. 73 of *Streptomyces aureofasciculus* M20 purified product



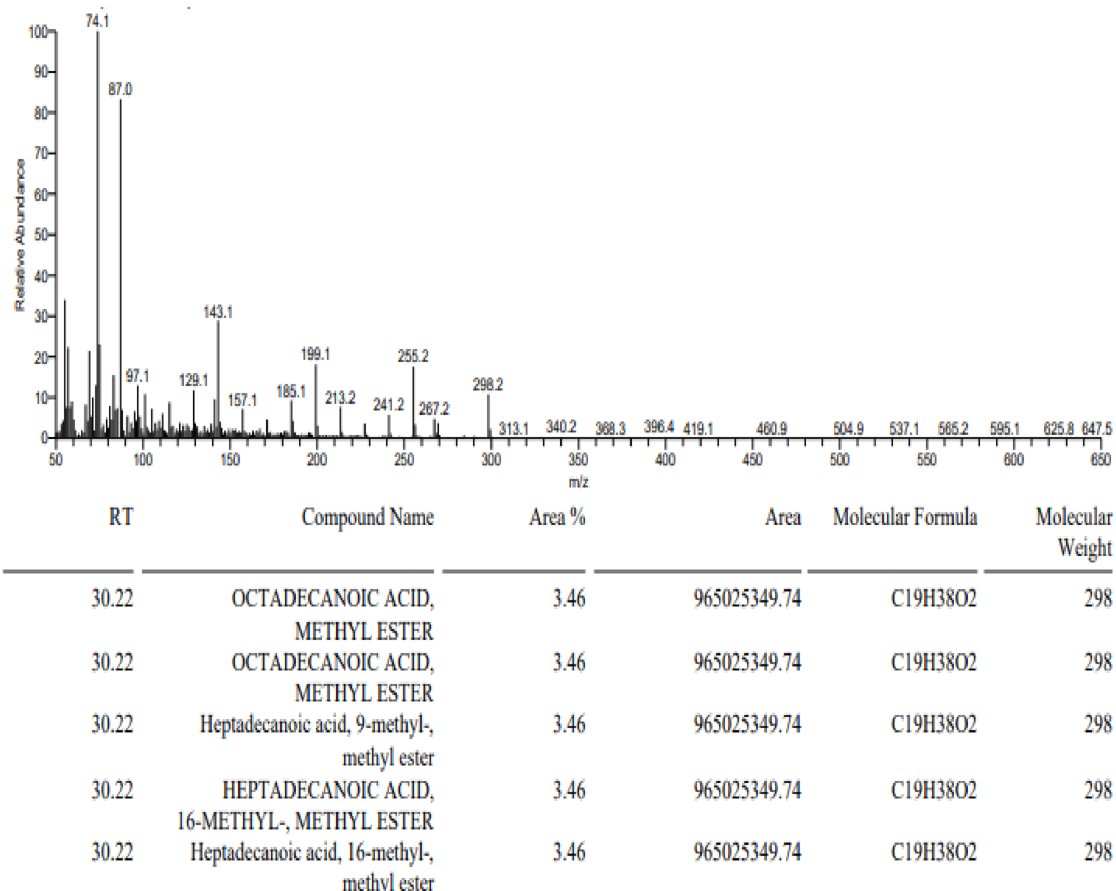
RT	Compound Name	Area %	Area	Molecular Formula	Molecular Weight
22.03	1-Naphthaleneacetic acid, methyl ester	4.07	1134814251.38	C13H12O2	200
22.03	1-Naphthaleneacetic acid, methyl ester	4.07	1134814251.38	C13H12O2	200
22.03	1-Naphthaleneacetic acid, methyl ester	4.07	1134814251.38	C13H12O2	200
22.03	1-NAPHTHALENEACETIC ACID, METHYL ESTER	4.07	1134814251.38	C13H12O2	200
22.03	1-NAPHTHALENEACETIC ACID, METHYL ESTER	4.07	1134814251.38	C13H12O2	200

Fig. 3f. GC –MS Report at RT 22.03 of *Streptomyces aureofasciculus* M20 purified product



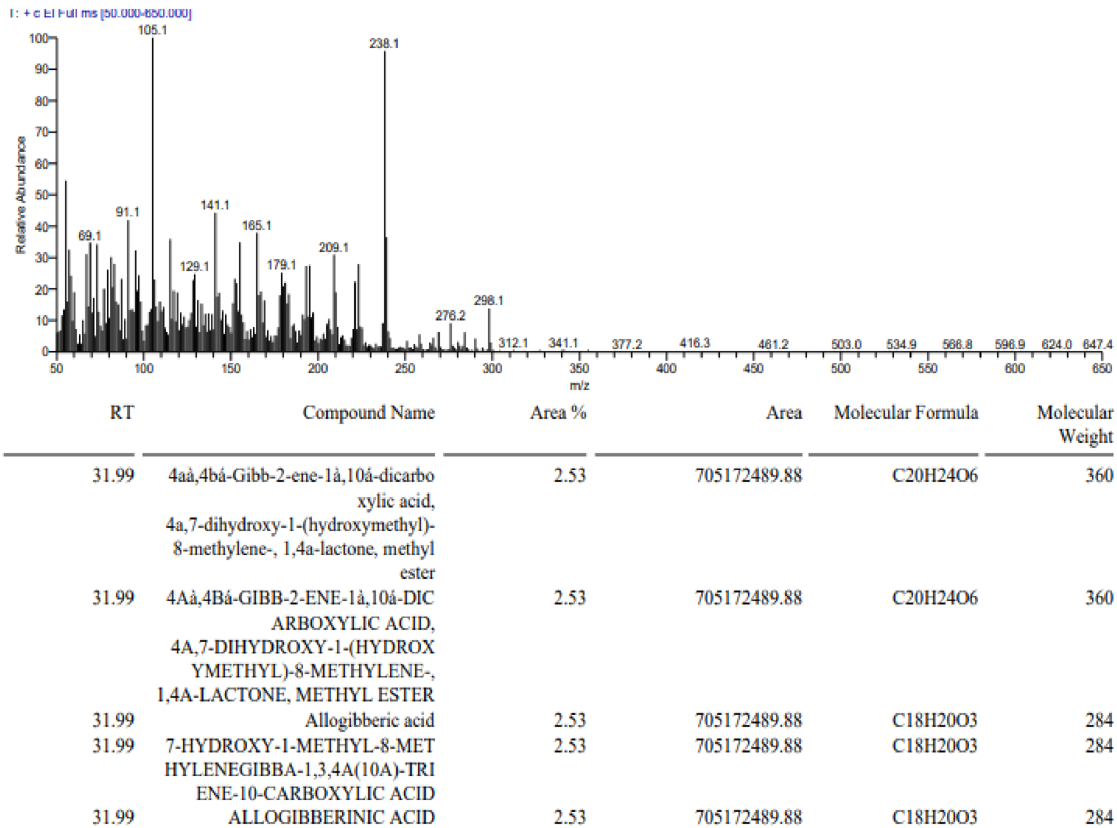
RT	Compound Name	Area %	Area	Molecular Formula	Molecular Weight
29.86	10-Octadecenoic acid, methyl ester	3.64	1015453283.65	C19H36O2	296
29.86	10-OCTADECENOIC ACID, METHYL ESTER	3.64	1015453283.65	C19H36O2	296

Fig. 3g. GC –MS Report at RT 29.78 of *Streptomyces aureofasciculus* M20 purified product



RT	Compound Name	Area %	Area	Molecular Formula	Molecular Weight
30.22	OCTADECANOIC ACID, METHYL ESTER	3.46	965025349.74	C19H38O2	298
30.22	OCTADECANOIC ACID, METHYL ESTER	3.46	965025349.74	C19H38O2	298
30.22	Heptadecanoic acid, 9-methyl-, methyl ester	3.46	965025349.74	C19H38O2	298
30.22	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	3.46	965025349.74	C19H38O2	298
30.22	Heptadecanoic acid, 16-methyl-, methyl ester	3.46	965025349.74	C19H38O2	298

Fig. 3h. GC –MS Report at RT 29.78 of *Streptomyces aureofasciculus* M20 purified product



RT	Compound Name	Area %	Area	Molecular Formula	Molecular Weight
31.99	4aà,4bá-Gibb-2-ene-1à,10á-dicarboxylic acid, 4a,7-dihydroxy-1-(hydroxymethyl)-8-methylene-, 1,4a-lactone, methyl ester	2.53	705172489.88	C20H24O6	360
31.99	4Aà,4Bá-GIBB-2-ENE-1à,10á-DICARBOXYLIC ACID, 4A,7-DIHYDROXY-1-(HYDROXYMETHYL)-8-METHYLENE-, 1,4A-LACTONE, METHYL ESTER	2.53	705172489.88	C20H24O6	360
31.99	Allogibberic acid	2.53	705172489.88	C18H20O3	284
31.99	7-HYDROXY-1-METHYL-8-METHYLENEGIBBA-1,3,4A(10A)-TRIENE-10-CARBOXYLIC ACID	2.53	705172489.88	C18H20O3	284
31.99	ALLOGIBBERINIC ACID	2.53	705172489.88	C18H20O3	284

Fig. 3i. GC –MS Report at RT 31.99 of *Streptomyces aureofasciculus* M20 purified product

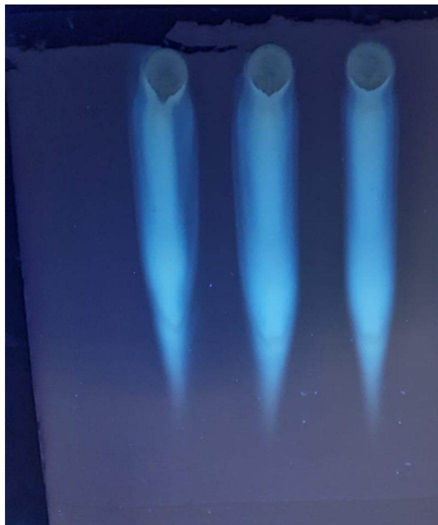


Fig. 3j. Thin layer chromatography of *Streptomyces aureofasciculus* M20 purified product

The results confirmed by Afifi et al. (2012) showed that the antibacterial extract obtained from *Streptomyces crystallinus,* AZ151 suggested the calculated empirical formula to be $C_{15}H_{30}N_2O_{10}$, the UV recorded a maximum absorption peak at 225 nm and the Mass spectrum indicated that the molecular weight was 432.36.

Antimicrobial activities of Streptomyces aureofasciculus M20 purified product

The results of SEM illustrated in Fig. 4 shows normal shape of Gram negative *Klebsiella oxytoca,* while Fig. 4a shows the morphological changes in cell shape and size after treatment with purified extract. On the other hand Fig. 4b and 4c at doses 2.0 and 4.0 kGy respectively exhibits no change was detected in cell morphology. Additionally Fig. 5 exhibits normal shape of Gram negative *E.coli*, while Figs. 5a and 5b shows variation on cell shape and size after the treatment with purified extract and irradiated one at doses levels 2 and 4 kGy. moreover, SEM, Fig. 6 shows normal shape of Gram positive *S.aureus* whilst, Fig. 6a, 6b and 6c shows variation on cell shape and size of the cells after treated with purified extract and irradiated extract at dose levels of 2 and 4 kGy. However, Fig. 7 exhibits normal sporangium and aerial mycelium of *Aspergillus niger*, whilst Fig. 7a shows destruction on sporangium and release of the spores after treatment with purified extract, on other hand a complete destruction of sporangium and aerial mycelium after treatment with the irradiated extract at doses 2 and 4 kGy which is shown in Figs. 7b and 7c, respectively.

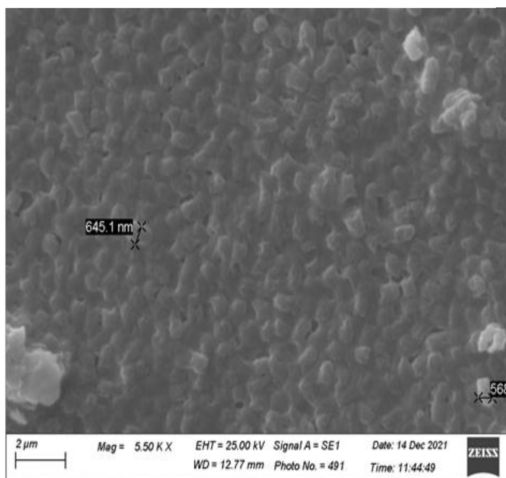


Fig. 4. SEM photo of *Klebsiella oxytoca*

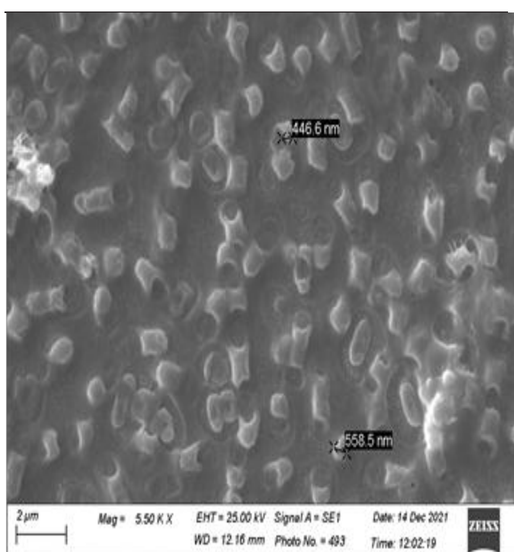


Fig. 4a. SEM photo of *Klebsiella oxytoca* treated with non-irradiated purified extract

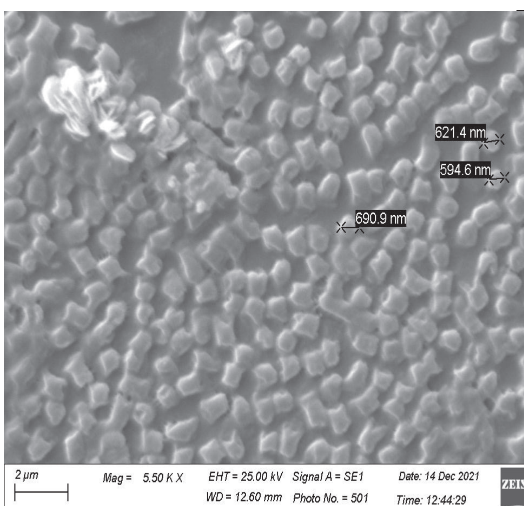


Fig. 4b. SEM photo of *Klebsiella oxytoca* treated with irradiated of purified extract at dose 2 kGy

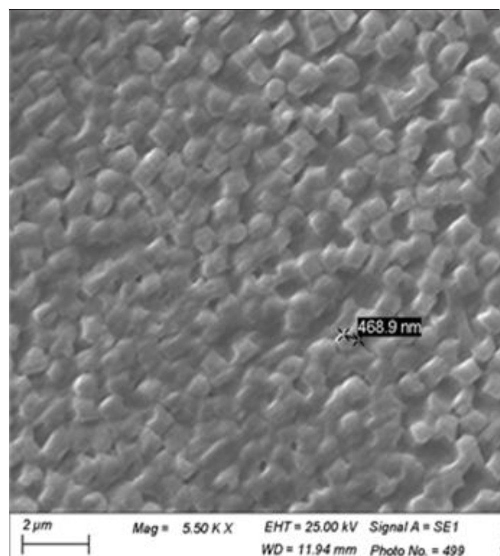


Fig. 4c. SEM photo of *Klebsiella oxytoca* treated with irradiated of purified extract at dose 4 kGy

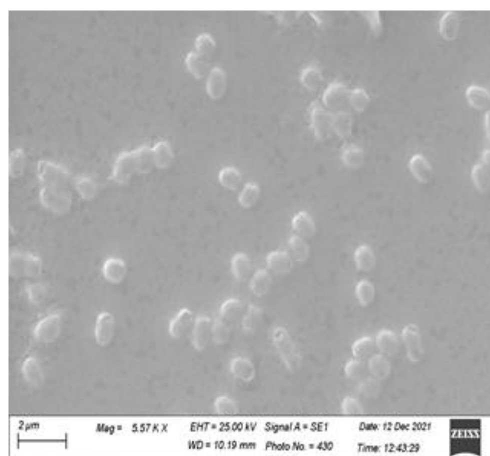


Fig 5. SEM photo of *E.coli* MMCC24

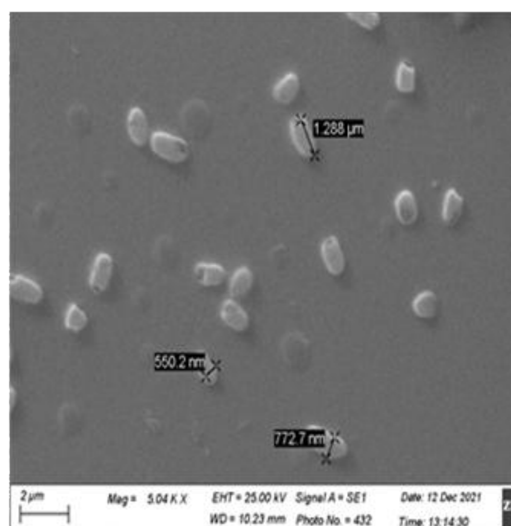


Fig. 5a. SEM photo of *E.coli* MMCC24 treated with non-irradiated purified extract

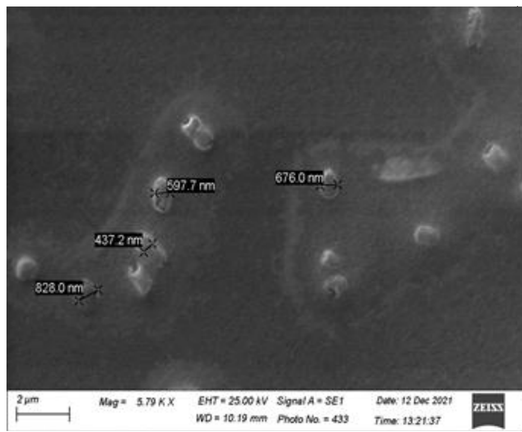


Fig. 5b. SEM photo of *E.coli* MMCC24 treated with irradiated purified extract at dose 2 kGy

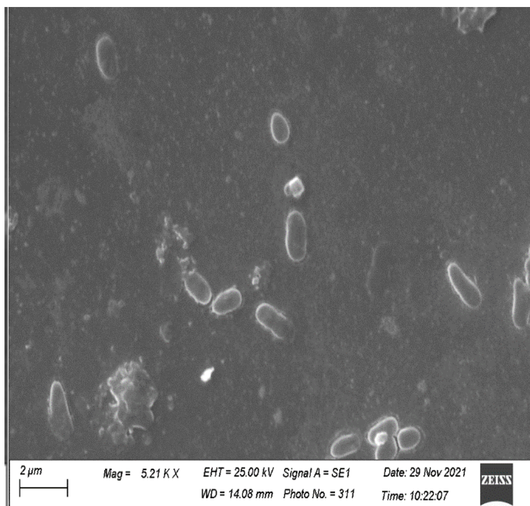


Fig. 5c. SEM photo of *E.coli* MMCC24 treated with irradiated purified extract at dose 4 kGy

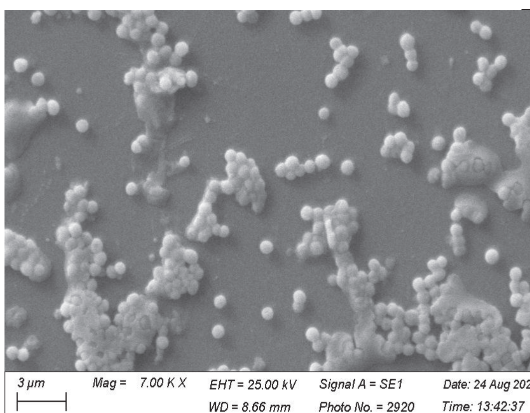


Fig. 6. SEM photo of *S .aureus* MMCC21

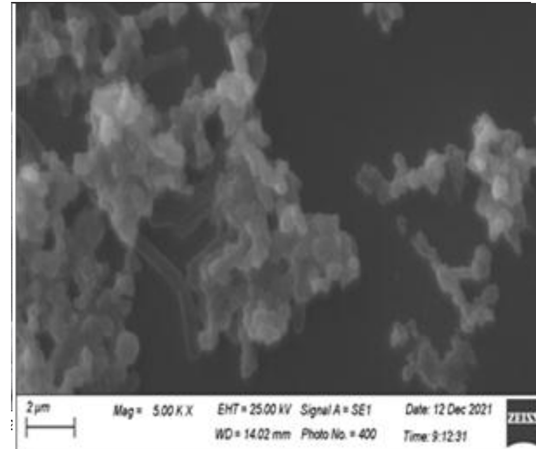


Fig. 6a. SEM photo of *S .aureus* MMCC21 treated with non-irradiated purified extract

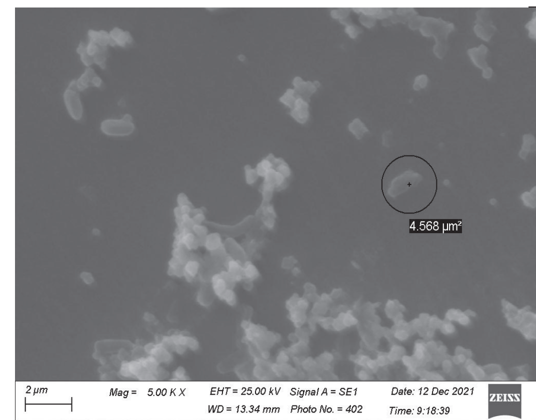


Fig. 6b. SEM photo of *S .aureus* MMCC21 treated with irradiated of purified extract at dose 2 kGy

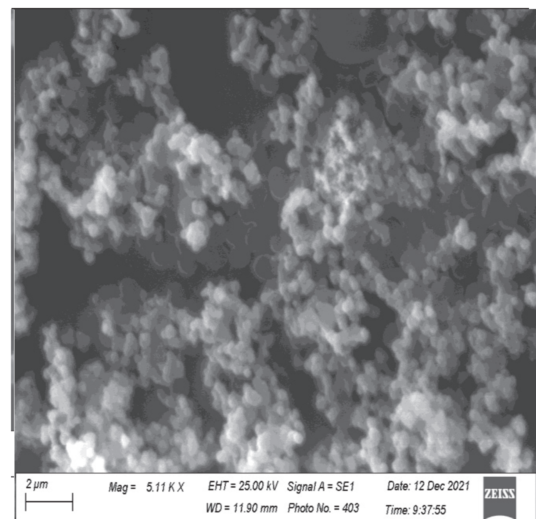


Fig. 6c. SEM photo of *S. aureus* MMCC21 with irradiated of purified extract at dose 4 kGy

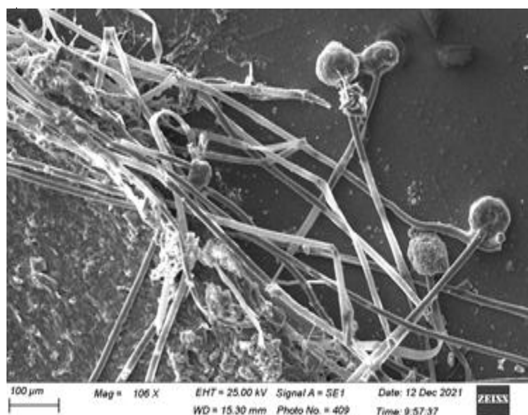


Fig. 7. SEM photo of *Aspergillus niger*

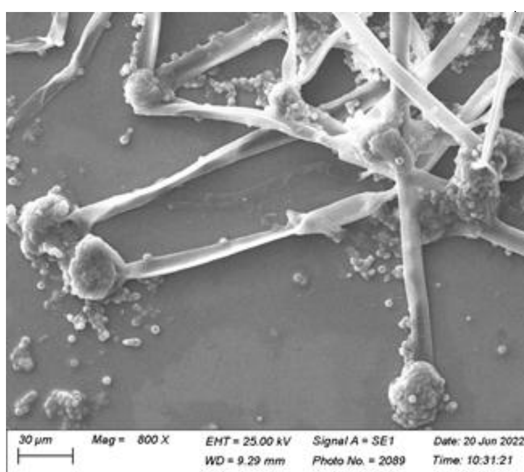


Fig. 7a. SEM photo of *Aspergillus niger* with non-irradiated purified extract

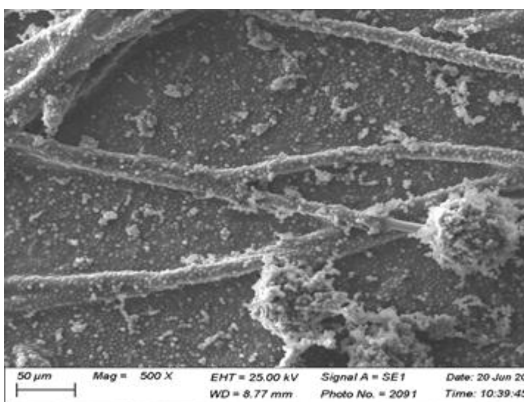


Fig. 7b. SEM photo of *Aspergillus niger* treated with 2 kGy of purified extract

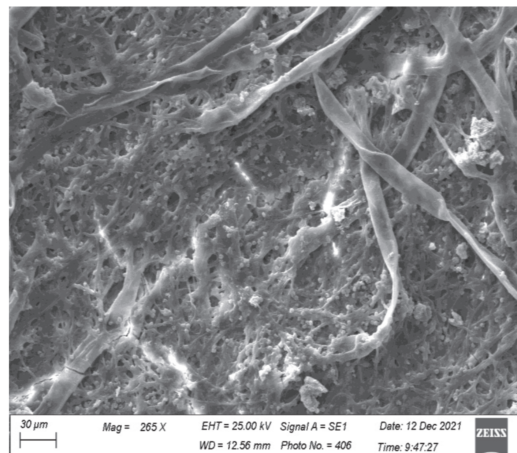


Fig. 7c. SEM photo of *Aspergillus niger* treated with 4 kGy of purified extract

Table 5 exhibits the antimicrobial activity of the purified product and irradiated one at doses levels 2.0 and 4.0 kGy, γ-irradiation was used for the study its effect on the antimicrobial activity of *Streptomyces aureofasciculus* M20 extract against Gram negative, Gram positive bacteria and fungal strains. The antibacterial activity of *Streptomyces aureofasciculus* M20 exhibited a negative result before and after irradiation at dose levels 2.0 and 4.0 kGy against Gram negative *Klebsiella oxytoca* and Gram positive *S. aureus MMCC21* which also showed antimicrobial activity against *Aspergillus niger* with purified extract and irradiated one at dose levels 2 and 4 kGy. On the other hand, they showed antibacterial activity against *E. cancerogenus* and *Pseudomonas aeruginosa* after treatment with the extract. Whilst after the exposure to γ-irradiation at doses level 2 and 4 kGy negative results were revealed. In addition, from results given in Table 5 both extract and irradiated one do not show antimicrobial activity against both *Bacillus cereus*, *Enterobacter dissolvens* and *Asperegillus niger*.

In the present study, the hypothesized antibacterial mechanism is illustrated diagrammatically in Fig. 8. It is clear that the extract mechanism of action start acting by adhering to the bacterial outer surface then rupturing the cell membrane, through changing the cell wall's and membrane's transport behavior. Following this, each extracted molecule is distributed independently into each bacterial cell, and all intracellular elements including plasmid, DNA, and other significant organelles are attached..

TABLE 5. Antimicrobial activity of un-irradiated and irradiated *Streptomyces aureofasciculus* M20 extract against clinical bacterial isolates [Inhibition zone in (mm) of 0.2 ml extract with conc. 50 mg/2 ml of DEMSO of both un-irradiated and irradiated *Streptomyces aureofasciculus* M20 at dose level (non-irradiated, 2.0 and 4.0 kGy)]

Clinical isolates	Culture extract		
	non-irradiated	2.0 kGy	4.0 kGy
Enterobacter cancerogenus	14	-ve	-ve
Pseudomonas aeruginosa MMCC13	16	-ve	-ve
Klebsiella oxytoca	20	20	18
Enterobacter dissolvens	-ve	-ve	-ve
Escherichia coli MMCC24	20	18	14
Staphylococcus aureus MMCC21	25	22	22
Bacillus cereus MMCC11	-ve	-ve	-ve
Aspergillus niger	25	30	30
Asperegillus terreus	-ve	-ve	-ve

-ve = no inhibition zone

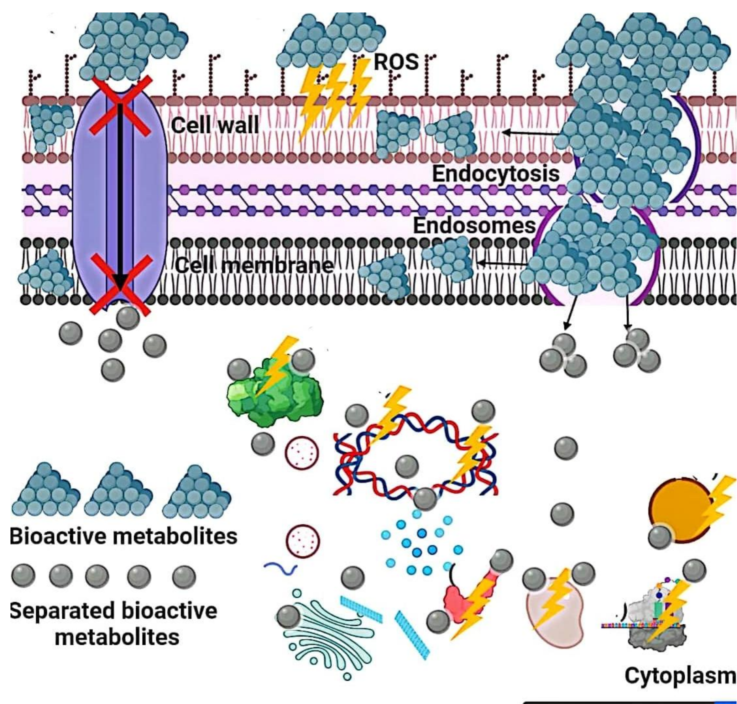


Fig. 8. A hypothesized model illustrating possible mechanisms of bioactive metabolites antibacterial action

These results were confirmed by Sripreechasak & Athipornchai (2019) who reported that the *Streptomyces* extract could not inhibit Gram-negative bacteria, *P. aeruginosa* and *E. coli*, while exhibits inhibitory activity against *M. luteus* (79.92%), *B. subtilis* (69.23%) and *S. aureus* (46.15%), also shows moderate antifungal activity against *C. albicans* (15.38%). They also reported that the *Streptomyces* extract obtained from Humic acid–vitamin agar medium (HV) were more active than from Starch Casein agar medium. Thus, *Streptomyces* isolated from Humic acid–vitamin agar medium is considered the best choice for producing bioactive metabolites against Gram-positive bacteria.

Another study reported by Butler et al. (2010) illustrated that the *Streptomyces* sp.*VITBRK2* isolated from marine sediment of Chennai and India exhibited antibacterial activity against drug resistant MRSA and VRE strains. The fractionation of EA extract by HPLC DAD for

the identification of indole type of the extracted compounds along with amicoumacin antibiotic exhibits that the indolo compounds showed a significant antibacterial activity against drug resistant Gram-positive and Gram-negative bacteria pathogens.

An attempt was made in previous study to screen the antimicrobial activity of *Streptomyces purpeofuscus* against bacteria such as *Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* (Anupama et al., 2012).

In vitro cytotoxic effect of *Streptomyces aureofasciculus* M20 purified product against the breast carcinoma MCF-7 and the colon carcinoma cell line (HCT-116) were studded (Figs. 9, 10).

Lack of new anti-cancer and anti-infective agents directed the pharmaceutical research to natural products' discovery especially from actinomycetes as one of the major sources of bioactive compounds.

The results exhibited in Tables 6, 7 and Figs. 9, 10, respectively showed the cytotoxic effect of the *Streptomyces aureofasciculus* M20 extract against colon carcinoma cell line (HCT-116) and Breast carcinoma MCF-7. The IC of the colon carcinoma cells detected under these experimental conditions was IC50= 15.1±0.9 µg/ml, and inhibitory activity against breast carcinoma cells was IC50= 24.7 ±1.2 µg/ml.

The crude extracts were tested by Osama et al. (2022) who reported that the cytotoxic effects against two cancer cell lines, HepG2 and MCF-7, and their normal cell lines, THLE2 and MCF-10A, respectively. All the tested *Streptomycetes* extract demonstrated anti-tumor activity against MCF-7, with weak inhibitory activity against MCF-10A. It worth noting that the isolates, the extract of isolates SH10, SH8 and SH12 exhibited potent anti-tumor activity against MCF-7, with IC_{50} values of 2.22, 4.12 and 7.37 µg ml^{-1}, respectively. Other extracts from *Streptomycetes* isolates SH12, SH4 and SH10 demonstrated a strong anti-tumor activity against the HepG2 cell line, with IC_{50} values of 1.31, 7.27 and 9.7 µg ml^{-1}, respectively, compared with their inhibitory activities on the liver normal cell line THLE2.

In another study reported by Wu et al. (2007) it was found that the cytotoxic activity against, Hepg2 exhibits a strong cytotoxicity based on IC50 values of *Streptomyces* bioactive compound (Chromomycin B) exhibited the IC50 = 0.007 ± 0.0004 *µ*g/ml, Chromomycin A2 exhibited the IC50 = 0.0005 ± 0.00003 *µ*g/ml, and Chromomycin A3 IC50 = 0.1 ± 0.006 *µ*g/ml . The commercial doxorubicin was used as comparing drugs which exhibit the IC50 = 0.084 ± 0.004 µg/ml.

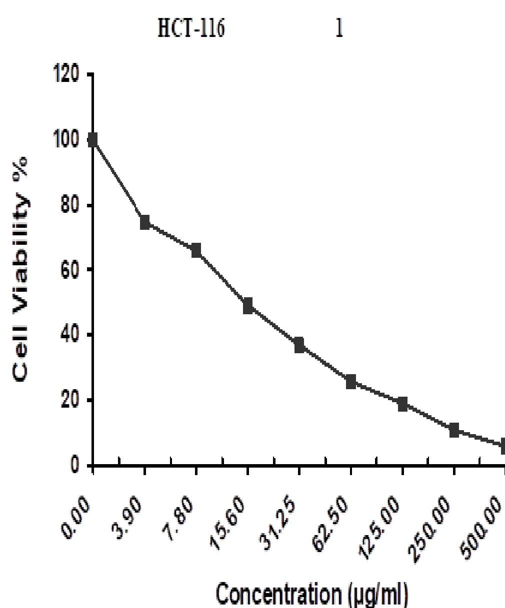


Fig. 9. Evaluation of cytotoxicity against HCT-116 cell line

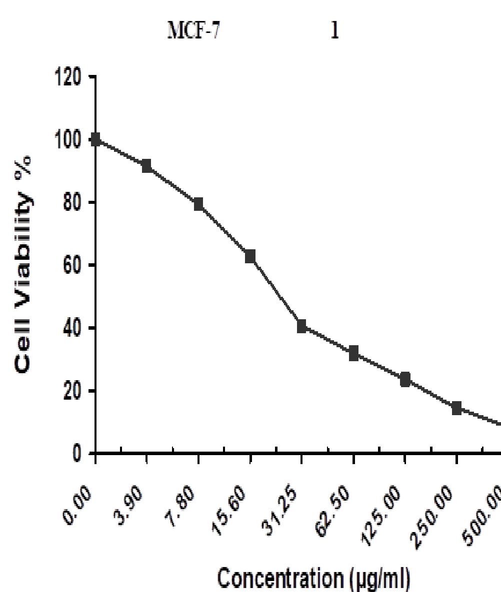


Fig. 10. Evaluation of cytotoxicity against MCF-7 cell line

TABLE 6. Evaluation of cytotoxicity against HCT-116 cell line

Sample conc. (µg/ml)	Viability %	Inhibitory %	S.D. (±)
500	5.84	94.16	0.28
250	10.72	89.28	0.36
125	18.96	81.04	0.72
62.5	25.61	74.39	0.39
31.25	36.87	63.13	0.75
15.6	48.95	51.05	1.89
7.8	65.81	34.19	2.78
3.9	74.58	25.42	0.64
0	100		

TABLE7. Evaluation of cytotoxicity against MCF-7 cell line

Sample conc. (µg/ml)	Viability %	Inhibitory %	S.D. (±)
500	8.60	91.4	0.46
250	14.56	85.44	0.39
125	23.79	76.21	1.05
62.5	31.94	68.06	0.88
31.25	40.72	59.28	2.36
15.6	62.84	37.16	2.72
7.8	79.25	20.75	0.61
3.9	91.43	8.57	0.25
0	100		

Conclusion and Recommendation

The present study demonstrates, for the first time, the potential of *Streptomycetes* isolated from unique environmental niches to produce antimicrobial compounds. Further studies will be carried out for the identification the *Streptomyces* isolate, also for the identification of the extract to further determination of the final chemical structure. It is also necessary to find new natural metabolites from biological origin having antimicrobial and antitumor activity, nontoxic and non-expensive. Moreover, the study exhibits antimicrobial positive effect of gamma irradiation and its effective role in sterilization more medical, industrial and agriculture products.

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