The Open Access Journal of Science and Technology
Vol. 3 (2015), Article ID 101140, 14 pages
doi:10.11131/2015/101140

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Research Article

Biological and Histological Studies of Purified Product from *Streptomyces janthinus* M7 Metabolites

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Received 23 September 2014; Accepted 17 October 2014

Academic Editors: Grażyna Biała and Paul Higgins

Abstract. Fifteen clinical samples were taken out from patients suffering cancer, these patients being under the treatment with radio- and/or chemotherapy. The samples were used for the isolation of bacterial cells surrounding tumor; the samples were collected from Center of Cancer Therapy, Ain Shams University, Cairo, Egypt. The clinical bacterial isolates were purified and identified according to Bergey's manual of determinative bacteriology ninth edition (1994). The bacterial isolates were found to be *Klebsiella oxytoca* m1; *Enterobacter cancerogenus* m2; *P. aeruginosa* m3; *Citrobacter diversus* m4; *Enterobacter agglomerans* m5; *Klebsiella oxytoca* m6; *Enterobacter dissolvens* m7; *Serratia fonticola* m8; *Escherichia coli* m9; *Citrobacter freundii* m10; *Staphylococcus aureus* m11; *Escherichia coli* m12; *P. aeruginosa* m13; *Staphylococcus aureus* m14; and *Bacillus cereus* m15. In the present study both primary and secondary screening methods were used to screen the antibacterial activity of *St. janthinus* M7 against fifteen clinical bacterial isolates. The *St. janthinus* M7 showed an increase in antibacterial activity against all the tested human bacterial pathogens. In this study Gamma irradiation at dose levels (0.5 and 1.5 kGy) was used for the enhancement of the antibacterial activity of *Streptomyces* strain against the clinical isolates. Several commercial antibiotic discs (Doxorubicin, Augmentin, Norfloxacin, Ofloxacin, Oxacillin, and Cefazolin) were used for comparing their antimicrobial activity with purified product. The results declared a significant increase in the antibacterial activity in most cases. The physiochemical properties of the purified product were carried out for determination of R_f, empirical formula, M.W, and chemical structure of product and then analyzed by thin layer chromatography, elemental analysis, UV, Mass, and NMR. The result exhibited brown color, one spot, R_f (0.76), M.W (473), while it recorded 270 nm in UV region and the calculated empirical formula was ($C_{22}H_{19}NS_2O_7$). In *in vitro* antitumor activity of the purified product against human tumor cell lines (Hepg2) the IC_{50} was measured to be 3.0 μg/ml and in *in vitro* antitumor activity of purified product measured against Ehrlich ascites carcinoma (EAC) the IC_{50} was measured to be 33.0 μg/ml. In *in vivo* study of the cytotoxic effect of *St. janthinus* M7 purified product MYN7 was investigated. The result of histopathological alteration in female mice infected with Ehrlich tumor showed a significant effect on abnormal cells.

Keywords: Ehrlich; Streptomyces; Gamma irradiation

1. Introduction

Natural products are produced by a wide range of different organisms. Microorganisms, plants, marine species, and animals employ such compounds for several purposes such as building blocks, coenzymes and cofactors, host-defense against microbial infection and predators, protection of ecological niches, communication between and within

species, pigments, cellular signaling, gene expression, and homeostasis maintenance natural products, such as the antimalarial and several anticancer agents, the lipid-lowering statins, and immune-suppressors used to prevent the rejection of tissue grafts [1].

Actinomycetes are Gram positive bacteria frequently filamentous with DNA rich in GC ratio from 55–75% [2].

The streptomyces are the dominant among actinomycetes. They are responsible for the production of about half of the discovered bioactive secondary metabolites [3] and antibiotics [4].

Cancer is a class of diseases characterized by out-of-control cell growth. There are over 100 different types of cancer, and each is classified by the type of cell that is initially affected. Cancer harms the body when damaged cells divide uncontrollably to form lumps or masses of tissue called tumors (except in the case of leukemia where cancer prohibits normal blood function by abnormal cell division in the blood stream). Tumors can grow and interfere with the digestive, nervous, and circulatory systems and they can release hormones that alter body function. Tumors that stay in one spot and demonstrate limited growth are generally considered to be benign [5].

Cancer is a chronic, metabolic disease that is obvious. It isn't an infectious disease, which is caused by bacteria or viruses. It is a disease that is metabolic in origin [6].

Cancer patients are particularly susceptible to nosocomial infections because of their compromised immune system, and because of the nature of treatment practices they experience. Recently, a shift of the microbial spectrum of cancer patients from Gram negative to Gram positive has been demonstrated [7].

Bacterial infections traditionally have not been considered a major causes of cancer. Bacteria have been linked to cancer by two mechanisms: induction of chronic inflammation and production of carcinogenic bacterial metabolites. Bacterial infection of urinary tract has been reported to increase the risk of bacterial cancer. Significant etiological factors have not been identified, but chronic inflammation caused by infectious agent appears to be playing a role in this disease [8].

Antibiotics are the typical secondary metabolites produced by microorganisms. Secondary metabolites are main products of microorganisms; antibiotics are very often used in medicine for suppression of pathogenic bacteria, fungi and viral diseases. Their use marked a revolution in medicine, saved millions of lives and helped [9].

The benzoxazole derivative nataxazole was isolated from *Streptomyces* sp. (strain Tü 6176). Nataxazole is related in structure to the potent antitumor compounds UK-1 and AJI9561 and showed similar strong growth inhibitory activity against various human tumor cell lines [10].

Cancer patients are more susceptible to infections associated with health care because of their compromised immune system, use of invasive technologies, and their being subjected to surgical operations and chemotherapy. New tools, aggressive practices, and technologies for the treatment of cancer patients can facilitate the onset of infections by microorganisms that were once considered as nonpathogenic or saprophytic [11].

This study aimed to evaluate the antibacterial and the antitumor activity of *St. janthinus* M7 purified product as pharmaceutical product and its activity against newly isolated and characterized clinical bacterial isolates from patients suffering cancer and under the treatment with radio and/or chemotherapy.

2. Material and Methods

2.1. Isolation of clinical bacterial. Fifteen clinical samples were collected from different patients suffering cancer and treated with chemo- and/or radiotherapy. The samples were used for isolation of bacteria living in or surrounding tumor. All samples were collected from Ain Shams University, Center of Cancer Therapy, Cairo, Egypt.

2.2. Bacterial purification. The samples were inoculated on nutrient agar medium (Yeast extract 2.0, Lab lemco powder 1.0, Peptone 5.0, NaCl 5.0, Agar 15.0, D.W. up to 1000 ml. and the pH adjusted to 7.0–7.4) and incubated at 37˚C for 24 hr. Single separated colony of distinct shape and color was picked up and restricted again for several consecutive times on the surface of agar plate to ensure its purity; finally fifteen clinical bacterial isolates were selected according to difference in shape and morphology.

2.3. Scanning electron microscope of morphological character of clinical bacterial isolates. Bacterial cells morphology were scanned using Scanning Electron Microscopy (SEM) JEOL, 5400 (Japan), in the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt.

2.4. Characterization of bacterial isolates. Characterization by biochemical parameters was as follows: Gram stain, Catalase test, Coagulase test, and Oxidase test. And according to the methods recommended in Bergey's manual of determinative bacteriology ninth edition 1994, the bacterial isolates were characterized and assessed by API20 system.

2.5. Microorganism: Used for screening its ability for production of antibacterial and antitumor agents. In previous study [12] the *Streptomyces janthinus* M7 was isolated from Toshka Area, Egypt, and identified according to Bergey's manual of determinative bacteriology ninth edition, 1994.

2.6. Irradiation unit. The spore suspensions of *St. janthinus* M7 were irradiated to enhancement of the production of

antitumor and antibacterial agent in Co^{60} facility at Gamma irradiation unit (4000 A) in the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. (Russian unit and dose rat at the time of the experiment was 1.845 k Gy/hr.)

2.7. Scanning electron microscope depending on morphological character of clinical bacterial isolates. The basic principles of isolation, fixation, dehydration, drying, mounting, and photographing have many variations; scanning electron microscopy can be used for viewing microorganisms under study; however, the concentration of cells is critical. The result in Figures 1(a)–(o) showed morphology shape of clinical bacterial isolates.

2.8. Effect of gamma irradiation on the antibacterial activity of the Streptomyces janthinus M7 . The spore suspensions of the *Streptomyces janthinus* M7 were irradiated at dose levels (0.5 and 1.5 kGy) to study the effect of γ-irradiation on the production of antibiotic-like substances.

2.9. Extraction and purification of Streptomyces janthinus M7 metabolites. The spore suspensions of the *Streptomyces janthinus* M7 were inoculated on glucose-asparagine broth medium (D-glucose 20.0 g. L-asparagine 5.0 g, $MgSO_4.7H_2O$ 1.0 g, KH_2PO_4 0.7 g, yeast extract 2.0 g and distilled water up to 1 L, pH adjust at 6.8) and incubated at 28 °C for 8 days; the broth was then filtrated through filter paper Whatman no. 1 and followed by centrifugation at 5000 rpm for 10 min. The clear filtrate containing the active metabolite was adjusted at pH 7.0 and then extraction process was carried out using ethyl acetate at the level of 1:1 (v/v). The organic phase was collected and evaporated under reduced pressure using rotary evaporator.

2.10. Thin layer chromatography. Used for determination purity of product via optimization of mobile phase which is ethyl acetate: iso-propanol: acetonitrile (1:4:5) (v/v) for unknown antibiotics was done by using 5 × 20 cm, 1 mm thick silica gel plates, where it was prepared and activated at 110 °C for half an hour.

2.11. Screening of antibacterial activity of Streptomyces janthinus M7 metabolites against clinical bacterial isolates. The antibacterial activity was determined according to the methods recommended by [13].

2.12. Screening of antitumor activity of Streptomyces janthinus M7 purified product against liver cell (Hepg2). Human tumor cell lines, liver cells (Hepg2) were obtained frozen in liquid nitrogen (−180 °C), the American type from culture collection. The tumor cell lines were maintained in the National Cancer Institute, Cairo, Egypt, by serial subculturing according to methods by [14].

2.13. Screening for antitumor activity of Streptomyces janthinus M7 purified product against EAC by using trypan blue exclusion method. The method was carried out according to methods by [15]. Trypan blue is a vital stain (diazo dye) used to select color dead tissues or cells with blue color. It detects cell membrane damage due to the loss of membrane polarity; that is, viable cells exclude the days whereas dead cells take it up and give distinctive blue color under a light microscope. Measurement of survival is achieved by direct counting of viable and dead cells.

2.14. Drug preparation for in vivo cytotoxic effect. Purified product MYN7 and commercial drug Doxorubicin were dissolved in DEMSO and administrated intramuscular (IM) in female mice with concentration (2.3 mg/Kg) to mice over a period of two weeks day by day.

2.15. Screening for in vivo cytotoxic effect of the purified product MYN7. The method was carried out according to that of [16]. In this study *in vivo* cytotoxic effect of *St. janthinus* M7 purified product was examined via six groups of female Swiss albino mice which were kept on animal house under supervision of Biology Department, National Center for Radiation Research and Technology (NCRRT), Egypt, with good aeration and ventilation conditions (these animals were housed seven animals per cage and allowed to become acclimatized to laboratory condition for one week before the experiment). A line of Ehrlich ascites carcinoma (EAC) cells was supplied from NCRRT, Biology Department, and maintained by slowly Intraperitoneal (I.P) transplantation of about 2.5×10^6 cells/mouse. The tumor was left to grow for 7 days before starting treatment.

2.16. Determination of physical properties of the purified product from Streptomyces janthinus M7.

- Melting point: was determined on a Stuart melting point apparatus.
- Solubility: purified product was dissolved in dimethyl sulphoxide (DEMSO) without heating for *in vitro* and *in vivo* experiments.
- Elemental analysis: elemental percentage of C, H, O, N, and S % elements was carried out in National Research Center (NRC), Cairo, Egypt, on Vario El Elementar system, Germany.

2.17. Spectroscopic analysis. The analysis is for determination of molecular weight, wavelength, function groups, and chemical structure of product.

Infrared (IR) spectra were recorded using potassium bromide discs technique on JASCO FT/IR/-6300 Spectroscopic apparatus.

Ultraviolet (UV) spectra were recorded by using *Streptomyces janthinus* M7 purified product that dissolved in

dimethyl sulphoxide (DEMSO) without heating on UV JASCO/V-560 Spectroscopic apparatus.

Mass spectrum (MS) was run on HP Model MS-5988 and on Varian MAT 311-A70 e.v.

Proton nuclear magnetic resonance (H^1-NMR) spectrum was performed on Mercury −300BB "NMR30" apparatus using dimethyl sulphoxide (DEMSO) as solvent.

3. Results

The results in Tables 1, 2, and 3 showed morphological, biochemical, and physiological characters of fifteen clinical bacterial isolates, including pigment production; the isolates were characterized by the following classical tests according to Bergey's Manual of determinative bacteriology ninth edition, 1994; the result showed that the isolates nos. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 13 were Gram negative, whilst isolates nos. 11, 14, and 15 were Gram positive.

3.1. Characterization of clinical bacterial isolates. It is clear from the previously mentioned results and from Figures 1(a)–(o) and according to the tested methods recommended in Bergey's manual of determinative bacteriology ninth edition 1994 that it's likely to be *Klebsiella oxytoca*, called *Klebsiella oxytoca* m1, *Enterobacter cancerogenus*, called *Enterobacter cancerogenus* m2, *P. aeruginosa*, called *P. aeruginosa* m3, *Citrobacter diversus*, called *Citrobacter diversus* m4, *Enterobacter agglomerans*, called *Enterobacter agglomerans* m5, *Klebsiella oxytoca*, called *Klebsiella oxytoca* m6, *Enterobacter dissolvens*, called *Enterobacter dissolvens* m7, *Serratia fonticola*, called *Serratia fonticola* m8, *Escherichia coli*, called *Escherichia coli* m9, *Citrobacter freundii*, called *Citrobacter freundii* m10, *Staphylococcus aureus*, called *Staphylococcus aureus* m11, *Escherichia coli*, called *Escherichia coli* m12, *P. aeruginosa*, called *P. aeruginosa* m13, *Staphylococcus aureus*, called *Staphylococcus aureus* m14, and *Bacillus cereus*, called *Bacillus cereus* m15, respectively.

3.2. Physicochemical characteristics of the purified product (illustrated in Table 4). The elemental percentage of the purified product revealed the following: C = 23.52%; H = 4.37%; N = 2.64%; and S = 12.12%. This analysis suggested the empirical formula to be $C_{22}H_{19}NS_2O_7$.

3.3. Spectroscopic characteristics. The spectroscopic analysis of the purified product had been determined. The infrared (IR) spectrum showed characteristic band corresponding to peaks in (Figure 3), the ultraviolet (UV) spectrum recorded a maximum absorption peak at 270 nm (Figure 2), NMR spectrum indicated the presence of amide, hydroxyl group, and a benzene ring (Figure 4), and the mass spectrum suggested the molecular weight was 474 (Figure 5).

3.4. Antibacterial activities of both unirradiated and irradiated Streptomyces janthinus M7 filtrated broth. Data recorded in Table 6 indicated that the *Streptomyces janthinus* M7 filtrated broth was active against both Gram negative and Gram positive examined bacteria, while it exhibited a significant increase in the antibacterial activity at dose levels 0.5 and 1.5 kGy of γ-irradiation with some cases.

3.5. Antibacterial activities of the St. janthinus M7 purified product and some commercial antibiotic discs. Data recorded in Table 7 illustrated the antibacterial activity of both purified product and commercial antibiotic discs against both Gram negative and Gram positive bacteria.

3.6. In vitro experiments on St. janthinus M7 purified product using liver cell line Hepg2 and trypan blue methods. The calculated IC_{50} of the EAC cells previously treated with different concentration of MYN7 was found to be 33 μg/ml (Figure 6) and The IC_{50} of the purified product against liver cell line Hepg2 calculated to be 4.6 μg/ml (Figure 8); in this study doxorubicin (DOX) was used for comparison and its IC_{50} against Hepg2 was 3.0 μg/ml (Figure 7).

3.7. Histopathological findings. In this study *in vivo* cytotoxic effect of *St. janthinus* M7 purified product MYN7 showed through six groups of female's mice a histopathological alteration of Ehrlich tumor after injection of cancer cell in mice femur and after 7 days ago as detailed below.

Group 1 of mice kept as control, this group contains seven female mice with average body weight of 24±3 g; the result of histological analysis showed there was no histopathological alteration observed and the normal histological structure of the skeletal muscle bundles was recorded in Figure 9(A) and 9(B).

Group 2 of mice implanted with Ehrlich tumor cells, this group contains seven female mice with average body weight of 23 ± 3 g. The femur diameter measured before injection was found to be 0.6 × 0.5, 0.6 × 0.6, 0.8 × 0.7, 0.9 × 0.9, 1.0 × 1.1, 0.8 × 0.9, and 0.8 × 0.7 cm and after 7 days of tumor injection it was 1.2 × 0.9, 1.6 × 1.5, 1.65 × 1.6, 1.7 × 1.4, 1.3 × 1.5, 1.6 × 1.4, and 1.4 × 1.6 cm, respectively. The histological analysis showed intact Ehrlich tumor cells (90%) were implanted in the hyperemic muscle bundles (Figure 9(C) and 9(D)).

Group 3 of mice implanted with Ehrlich tumor cells and treated with 2.3 mg/Kg of MYN7, this group contains seven female mice with average body weight of 23.3 ± 3 g. The femur diameter measured after 1 day of tumor injection was found to be 1.0×0.9, 1.4×1.4, 1.0×1.1, 0.9×1.2, 1.0×1.3, 1.2×1.5, and 0.9×1.2 cm and after 7 days of tumor injection it was 1.3 × 1.4, 1.6 × 1.5, 1.65 × 1.6, 1.7 × 1.4, 1.4 × 1.6, 1.6 × 1.3, and 1.5 × 1.4 cm, respectively. The histological analysis showed 80% of the intact implanted Ehrlich tumor

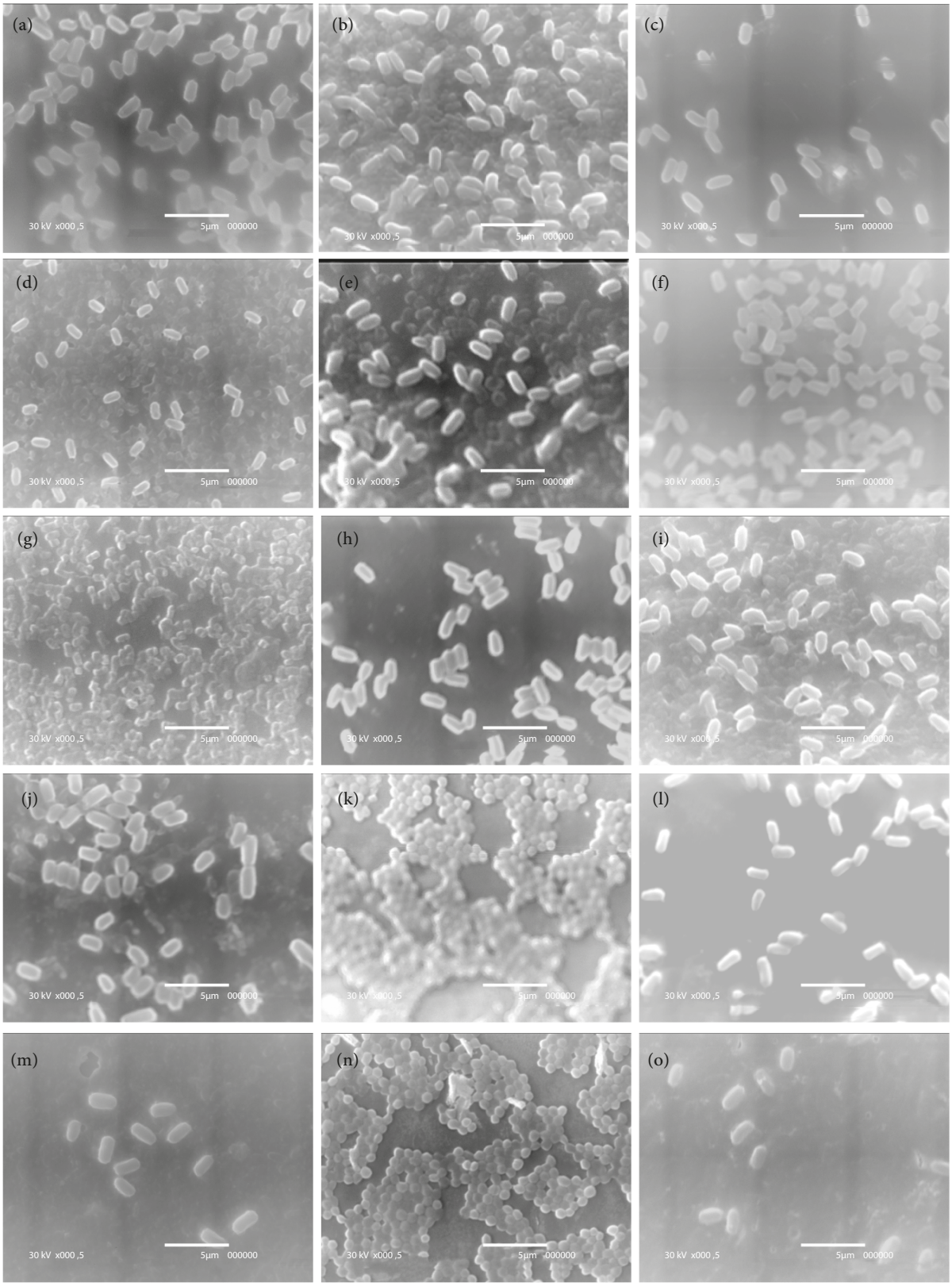

Figure 1: (a) SEM of Clinical Isolates no. 1, (b) SEM of Clinical Isolates no. 2, (c) SEM of Clinical Isolates no. 3, (d) SEM of Clinical Isolates no. 4, (e) SEM of Clinical Isolates no. 5, (f) SEM of Clinical Isolates no. 6, (g) SEM of Clinical Isolates no. 7, (h) SEM of Clinical Isolates no. 8, (i) SEM of Clinical Isolates no. 9, (j) SEM of Clinical Isolates no. 10, (k) SEM of Clinical Isolates no. 11, (l) SEM of Clinical Isolates no. 12, (m) SEM of Clinical Isolates no. 13, (n) SEM of Clinical Isolates no. 14, and (o) SEM of Clinical Isolates no. 15.

Table 1: Morphological and biochemical characteristic of clinical isolates.

Test parameters	**Clinical bacterial isolates no.**														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Gram stain	−ve	−ve	−ve	−ve	−ve	−ve	−ve	−ve	−ve	−ve	+ve	−ve	−ve	+ve	+ve
Shape	Bacilli & rods	Bacilli	Rods	Rods	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	cocci	Bacilli	Rods	Cocci	Rods
Motility	Nonmotile	Nonmotile	Motile	Motile	Nonmotile	Nonmotile	Motile	Motile	Nonmotile	Motile	Nonmotile	Nonmotile	Motile	Nonmotile	Motile
Catalase test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	−ve	+ve	+ve	+ve
Oxidase test	−ve	−ve	+ve	−ve	−ve	−ve	−ve	−ve	−ve	−ve	−ve	−ve	+ve	−ve	−ve
Coagulase test	+ve	+ve	+ve	+ve	+ve	−ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	−ve
NO_3 Nitrate reduction	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Indol production	−ve	−ve	−ve	+ve	+ve	+ve	+ve	−ve	−ve	+ve	+ve	+ve	−ve	+ve	+ve
Glucose fermentation	+ve	−ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	−ve	+ve	+ve	+ve	+ve
Galactose fermentation	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	−ve	+ve	+ve	+ve	+ve	+ve
Rhamnose fermentation	−ve	+ve	−ve	−ve	+ve	−ve	−ve	+ve	−ve	−ve	+ve	+ve	−ve	−ve	−ve
Raffinose fermentation	−ve	−ve	−ve	−ve	−ve	+ve	+ve	−ve	+ve	−ve	+ve	+ve	+ve	+ve	+ve
Arginine dihydrolase	−ve	−ve	+ve	+ve	−ve	+ve	+ve	+ve	−ve	+ve	−ve	+ve	+ve	−ve	+ve
Urease	−ve	+ve	+ve	+ve	−ve	+ve	+ve	+ve	−ve	+ve	−ve	+ve	+ve	+ve	+ve
Esculin hydrolysis	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Gelatin hydrolysis	+ve	+ve	+ve	−ve	+ve	+ve	−ve	−ve	−ve	+ve	−ve	+ve	+ve	+ve	−ve
Beta-glucosidase	−ve	−ve	−ve	+ve	+ve	−ve	+ve	−ve	+ve	+ve	−ve	+ve	−ve	+ve	+ve
D-glucose assimilation	+ve	+ve	+ve	+ve	+ve	+ve	+ve	++ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
L-arabinose assimilation	+ve	+ve	−ve	−ve	+ve	+ve	+ve	−ve	+ve	−ve	+ve	+ve	−ve	+ve	+ve
Mannose assimilation	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Manitol assimilation	+ve	+ve	+ve	+ve	+ve	−ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
N-acetyl-glucosamine assimilation	+ve	+ve	+ve	+ve	+ve	−ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
D-maltose assimilation	+ve	+ve	−ve	+ve	+ve	+ve	+ve	−ve	+ve	+ve	+ve	+ve	−ve	+ve	+ve
Potassium gluconate assimilation	+ve	+ve	+ve	+ve	+ve	−ve	+ve	−ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Capric acid assimilation	+ve	−ve	+ve	+ve	+ve	+ve	+ve	++ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Adipic assimilation	+ve	−ve	+ve	+ve	+ve	−ve	+ve	−ve	+ve	+ve	+ve	+ve	+ve	+ve	−ve
Malic assimilation	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	−ve	+ve	+ve	+ve	+ve	+ve	+ve
Trisodium citrate assimilation	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Phenylacetic acid assimilation	−ve	−ve	−ve	+ve	+ve	−ve	+ve	+ve	+ve	+ve	+ve	+ve	−ve	+ve	+ve
H_2S production	−ve	−ve	−ve	−ve	−ve	−ve	−ve	−ve	−ve	−ve	−ve	−ve	−ve	+ve	−ve

+ve = Positive −ve = Negative.

Table 2: Morphological and physiological characteristic of clinical isolates.

Clinical isolates no.	Starch hydrolysis	Blood hemolysis	6.5 NaCl %	MacConkey Agar	Manitol salt agar	Baird-Parker Medium	Trypticase soy agar
1	+ve	Gamma -hemolysis	+ve	+ve	−ve	−ve	+ve y
2	+ve	Gamma -hemolysis	+ve	+ve	−ve	−ve	+ve y
3	−ve	Beta-hemolysis	+ve	+ve	−ve	−ve	+ve G
4	−ve	Beta-hemolysis	+ve	+ve	−ve	−ve	+ve y
5	−ve	Gamma -hemolysis	+ve	+ve	−ve	−ve	+ve y
6	−ve	Gamma -hemolysis	+ve	+ve	−ve	−ve	+ve y
7	−ve	Gamma -hemolysis	+ve	+ve	−ve	−ve	+ve y
8	−ve	Gamma -hemolysis	+ve	+ve	−ve	+ve/yas	+ve y
9	−ve	Gamma -hemolysis	+ve	+ve	−ve	−ve	+ve y
10	+ve	Beta-hemolysis	+ve	−ve	−ve	−ve	+ve C
11	−ve	Beta-hemolysis	+ve	+ve	−ve	+ve/yas	+ve y
12	−ve	Beta-hemolysis	+ve	+ve	−ve	−ve	+ve G
13	+ve	Beta-hemolysis	+ve	+ve	+ve	+ve/yas	+ve y
14	−ve	Beta-hemolysis	+ve	−ve	−ve	+ve/no	+ve C
15	−ve	Beta-hemolysis	+ve	+ve	+ve	−ve	+ve y

- MacConkey Agar (Positive = lactose fermentation)
- Manitol salt agar (positive = yellow colour mannitol fermentation)
- Baird-Parker Medium (positive yas = Blackening with clear zone & positive/no = Blackening with nuclear zone)
- Trypticase soy agar (+ve y = growth yellow & +ve G = growth green & +ve g = growth gray and +ve C = growth creamy).

Table 3: Physiological characteristic of clinical isolates on triple sugar iron agar medium.

Isolated organisms no.	Result	Interpretation
1	Yellow/Yellow with bubbles	Glucose and lactose and/or sucrose fermentation, gas produced
2	Red/Yellow with bubbles	Glucose fermentation only, gas produced
3	Yellow/Yellow with bubbles	Glucose and lactose and/or sucrose fermentation, gas produced
4	Yellow/Yellow with bubbles	Glucose and lactose and/or sucrose fermentation, gas produced
5	Red/Red	No fermentation, peptone catabolized
6	Yellow/Yellow with bubbles	Glucose and lactose and/or sucrose fermentation, gas produced
7	Yellow/Yellow with bubbles	Glucose and lactose and/or sucrose fermentation, gas produced
8	Yellow/Yellow with bubbles	Glucose and lactose and/or sucrose fermentation, gas produced
9	Yellow/Yellow with bubbles	Glucose and lactose and/or sucrose fermentation, gas produced
10	Red/Yellow	Glucose fermentation only, peptone catabolized
11	Yellow/Yellow with bubbles	Glucose and lactose and/or sucrose fermentation, gas produced
12	Yellow/Yellow with bubbles	Glucose and lactose and/or sucrose fermentation, gas produced
13	Yellow/Yellow with bubbles	Glucose and lactose and/or sucrose fermentation, gas produced
14	Red/Yellow with black precipitate	Glucose fermentation only, H_2S produced
15	Yellow/Yellow with bubbles	Glucose and lactose and/or sucrose fermentation, gas produced

cells observed adjacent to the congested, muscle bundles necrosis and granulation tissue formation (Figure 10(E) and 10(F)).

While group 4 of mice was implanted with Ehrlich tumor cells and injected with 20 mg/Kg of antibiotic doxorubicin, this group contains seven female mice with average body weight of 24.3 ± 3 g. The femur diameter measured after 1 day of tumor injection was found to be 0.6 × 0.5, 0.5 × 0.4, 0.4 × 0.5, 0.5 × 0.5, 0.5 × 0.6, 0.7 × 0.8, and 0.5 × 0.7 cm and after 7 days of tumor injection it was 1.2 × 1.0, 0.7 × 0.8, 1.0 × 0.8, 1.0 × 0.9, 1.3 × 1.0, 1.0 × 0.8, and 1.3 × 1.0 cm, respectively. The result of histological analysis showed necrosis in 75% of the implanted Ehrlich tumor cells (Figure 10(G) and 10(H)).

Group 5 of mice administrated with 2.3 mg/Kg of purified product MYN7, this group contains seven female mice with

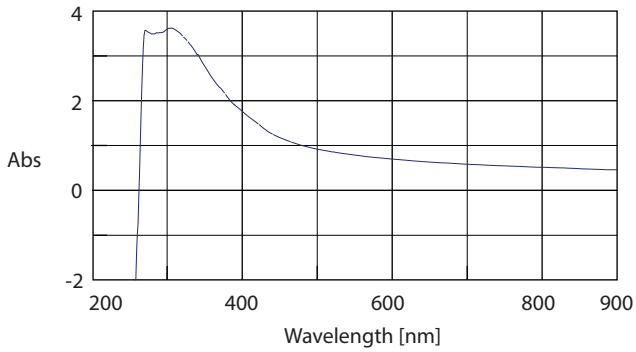

Figure 2: Ultra-violet aberrance of purified product MYN7.

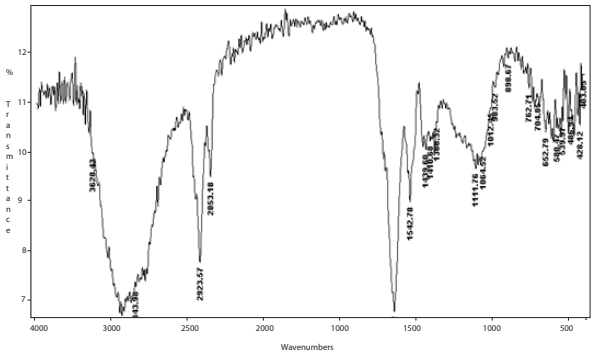

Figure 3: Infra-red spectrum of purified product MYN7.

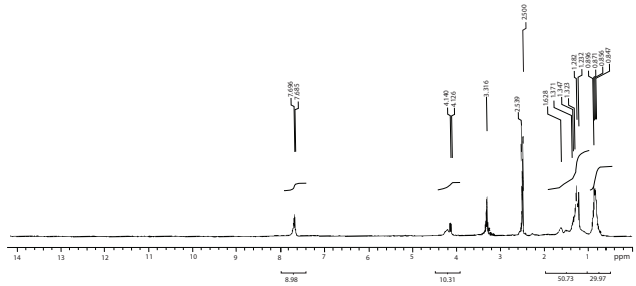

Figure 4: NMR spectrum of *St. janthinus* M7 purified product MYN7.

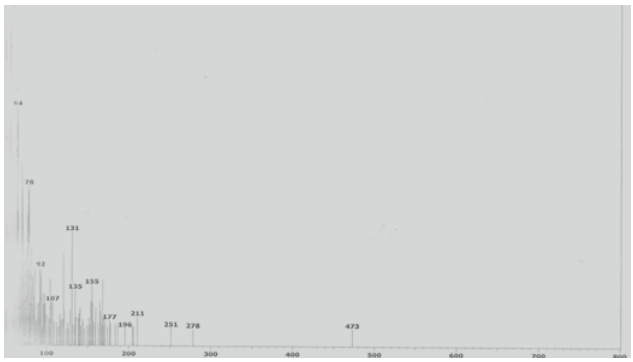

Figure 5: Mass spectrum of *St. janthinus* M7 purified product MYN7.

Table 4: Physiochemical properties of purified product MYN7.

Characteristic	**Extract**
Color	Brown
Purity	One spot
R_f	0.76
Melting point (˚C)	226 – 230˚C
Molecular weight	473 Dalton
Ultra-violet	270 nm
Crystallinity	Crystal
Suspected empirical formula	$C_{22}H_{19}NS_2O_7$
Microanalysis %	
N	2.64
C	23.52
S	12.12
H	4.37

Table 5: Groups absorbing in IR region of purified product MYN7.

Range (cm^{-1})	**Assignment**
3628	O-H (free)
3348	N-H (2˚-amines) stretching
2923	CH_3, CH_2 & CH2 or 3 bands
2853	CH_3, CH_2 & CH 2 or 3 bands
1636	N-H (2¡-amide) II band bending
1439	-N=N
1410	-O-H
1388	CH_3

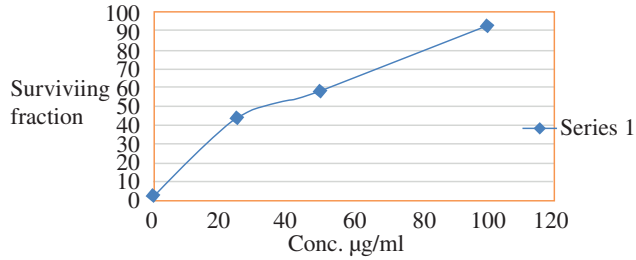

Figure 6: % of mortality of EAC cells as affected by different concentration of MYN7.

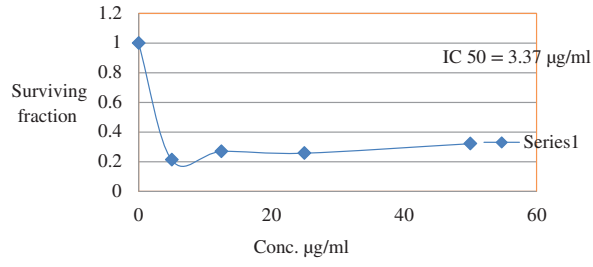

Figure 7: % of mortality of drug cytotoxicity-Hepg2-DOX.

Table 6: Antibacterial activity of unirradiated and irradiated *Streptomyces janthinus* M7 against clinical bacterial isolates.

Inhibition zone mm of 0.2 ml broth of unirradiated and irradiated *Streptomyces janthinus* M7 at dose levels (0.0, 0.5, 1.5 kGy)			
Clinical isolates	**Dose level**		
	0.0	0.5 kGy	1.5 kGy
Klebsiella oxytoca m1	20	20	16
Enterobacter cancerogenus m2	18	20	20
P. aeruginosa m3	16	16	−ve
Citrobacter diversus m4	18	18	12
Enterobacter agglomerans m5	20	16	16
Klebsiella oxytoca m6	12	15	12
Enterobacter dissolvens m7	−ve	−ve	−ve
Serratia fonticola m8	14	12	−ve
Escherichia coli m9	18	16	12
Citrobacter freundii m10	18	18	12
Staphylococcus aureus m11	16	12	−ve
Escherichia coli m12	−ve	14	−ve
P. aeruginosa m13	11	14	11
Staphylococcus aureus m14	−ve	−ve	−ve
Bacillus cereus m15	−ve	−ve	−ve

Table 7: Antibacterial activity of purified product and antibiotic discs.

Inhibition zone of mm of							
Clinical isolates	0.2 ml of conc. 30 mg/1.5 ml DEMSO	**Commercial antibiotic discs**					
	MYN7	DOX	NOR	AUG	OX	OFX	CZ
Klebsiella oxytoca m1	21	−ve	12	16	−ve	10	−ve
Enterobacter cancerogenus m2	14	−ve	11	25	−ve	12	−ve
P. aeruginosa m3	18	12	−ve	18	−ve	13	−ve
Citrobacter diversus m4	22	−ve	16	−ve	−ve	11	−ve
Enterobacter agglomerans m5	21	−ve	−ve	16	−ve	−ve	−ve
Klebsiella oxytoca m6	16	19	21	16	−ve	12	−ve
Enterobacter dissolvens m7	11	−ve	16	−ve	−ve	24	−ve
Serratia fonticola m8	−ve	10	11	18	−ve	13	−ve
Escherichia coli m9	−ve	−ve	11	20	−ve	11	−ve
Citrobacter freundii m10	22	19	19	18	12	14	−ve
Staphylococcus aureus m11	13	−ve	−ve	17	−ve	10	−ve
Escherichia coli m12	−ve	10	26	−ve	−ve	18	−ve
P. aeruginosa m13	13	19	23	18	−ve	16	−ve
Staphylococcus aureus m14	14	19	19	16	−ve	13	17
Bacillus cereus m15	−ve	−ve	20	11	−ve	16	−ve

MYN7 = Purified product; NOR = Norfloxacin; Ox = Oxacillin; AUG = Augmentin; OFX = Ofloxacin; CZ = Cefazolin; DOX = Doxorubicin

average body weight of 25.3 ± 3 g; the result exhibited focal wide area of the skeletal muscle which showed inflammatory cells infiltration (Figure 11(I) and 11(J)).

Group 6 of mice administrated with 20 mg/Kg of Doxorubicin, this group contains seven female mice with average body weight of 24.5 ± 3 g and injected with 0.1 ml of Doxorubicin; the histological finding in focal area of muscle bundles showed few inflammatory cells infiltration (Figure 11(K) and 11(L)).

4. Discussion

Bacteria from the genus *Streptomyces* are very important for the production of natural bioactive compounds such as

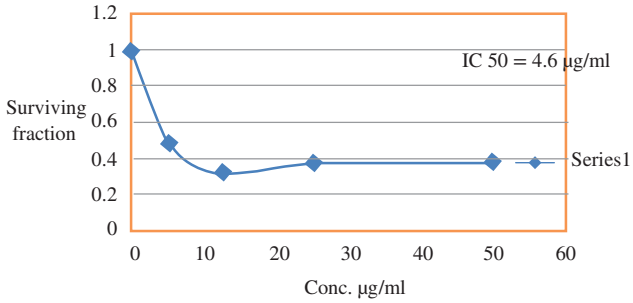

Figure 8: % of mortality of drug cytotoxicity MYN7 on liver cell line Hepg2.

antibiotic, antitumor, or immunosuppressant drugs. Around two-thirds of all known natural antibiotics are produced by these bacteria. Bleomycin is a chemotherapeutic agent commonly used to treat curable diseases such as germinative tumors and Hodgkin's lymphoma [17].

In this study fifteen clinical bacterial isolates from different body organs and localities were taken out from patients suffering skin, umbilical, breast, urinary bladder, and prostatic tumor who are treated with chemo- and/or radiotherapy and had history of repeated bacterial infection. Fifteen clinical bacterial isolates were characterized according to Bergey's manual of determinative bacteriology ninth edition, 1994, to called (*Klebsiella oxytoca* m1, *Enterobacter cancerogenus* m2, *P. aeruginosa* m3, *Citrobacter diversus* m4, *Enterobacter agglomerans* m5, *Klebsiella oxytoca* m6 *Enterobacter dissolvens* m7, *Serratia fonticola* m8, *Escherichia coli* m9, *Citrobacter freundii* m10, *Staphylococcus aureus* m11, *Escherichia coli* m12, *P. aeruginosa* m13, *Staphylococcus aureus* m14, and *Bacillus cereus* m15).

The result was found to be in agreement with that obtained by [7] who demonstrated that Gram negative bacteria are still the predominant pathogens causing bacteremia in febrile neutropenia patients and *E. coli* was the most frequently isolated Gram negative bacteria and *Staph. epidermidis* was the most commonly isolated Gram positive bacteria.

Also the result was found to be in agreement with that obtained by [18] who revealed that Gram positive bacteria isolated from different clinical specimens of cancer patients were mainly of *Streptococcal* or *Staphylococcal* sp. The main isolated Gram positive bacteria from sputum were α-hemolytic *Streptococci* (46.8%) and CNS (37.4%). Gram positive bacteria from blood were CNS (66.9%; 93 CNS isolates out of 139 total Gram positive blood isolates), followed by *Staph. aureus* (26.6%; 37 CNS isolates out of 139 total Gram-positive blood isolates). The main isolated Gram positive bacteria from all specimens were CNS (46.5%; 350 out of 752 total Gram positive isolates), followed by α-hemolytic *Streptococci* (29.4%) and *Staph. aureus* (18.6%).

Our result was in agreement with that obtained by [19] who reported that Gram negative bacteria are still the predominant pathogens causing bacteremia in febrile neutropenia patients; this result was similar to the one that had been reported in other studies. *E. coli* was the most frequently isolated Gram negative bacteria and *Staphylococcus epidermidis* was the most commonly isolated Gram positive bacteria.

Our results declared that *St. janthinus* M7 showed antibacterial activity against *Klebsiella oxytoca* m1, *Enterobacter cancerogenus* m2, *P. aeruginosa* m3, *Citrobacter diversus* m4, *Enterobacter agglomerans* m5, *Klebsiella oxytoca* m6, *Enterobacter dissolvens* m7, *Serratia fonticola* m8, *E. coli* m9, *Citrobacter freundii* m10, *Staphylococcus aureus* m11, *E. coli* m12, and *P. aeruginosa* m13. The inhibition zone was measured to be 20, 18, 16, 18, 20, 12, 14, 18, 18, 16, and 11 mm, respectively.

In the present study γ-irradiation was used at dose levels 0.5 and 1.5 kGy for the enhancement of the antibacterial activity of *St. janthinus* M7 filtrated broth. The antibacterial activity of *St. janthinus* M7 exhibited negative result before and after irradiation at dose levels 0.5 and 1.5 kGy against *Staphylococcus aureus* m14 and *Bacillus cereus* m15. On the other hand, they showed an increase in the antibacterial activity after exposure to γ-irradiation at dose level 0.5 kGy against *Enterobacter cancerogenus* m2 and *P. aeruginosa* m13. Whilst after the exposure to γ-irradiation at dose level 1.5 kGy declared negative results against *P. aeruginosa* m3, *Serratia fonticola* m8, and *Staphylococcus aureus* m11.

The result was found to be in agreement with that obtained by [12] who studied the effect of $\boldsymbol{\gamma}$-irradiation on the antimicrobial activity of *Streptomyces* stains. The result indicated some sort of mutation could be induced due to the exposure of *Streptomyces* strains to γ-irradiation at dose levels 0.5, 2, and 4 kGy. Some of *Streptomyces* strains exhibited negative result, while after the exposure to $\boldsymbol{\gamma}$-irradiation at dose levels ranging from 1 to 4 kGy they declared antimicrobial activity as in the case of *St. umbrosus* M1 and *St. rocemochromogenus* M9 against *Sarcina* sp. On the other hand some lost their sensitivity completely as in the case of *S. umbrosus* M1 and *S. griseoruber* M5 against *Bacillus cereus*.

In this study, *Streptomyces* metabolites were extracted by using ethyle acetate and then the extracellular metabolites was collected to evaluate their physiochemical properties and also checked for antimicrobial and antitumor activity.

The extracted product was dried via oven for 1 hr. at 80 °C. The purity of the extracted products was investigated by using TLC. The product MYN7 exhibited one spot, the calculated R_f was 0.76, and melting point was 226–230 °C.

In this study, the antimicrobial activity of *St. janthinus* M7 purified product MYN7 against clinical bacterial isolates was investigated in comparing with several commercial antibiotic discs, namely, Doxorubicin, Augmentin, Norfloxacin, Ofloxacin, Oxacillin, and Cefazolin.

The purified product exhibited antibacterial activity against all bacterial isolates except in case of *Serratia*

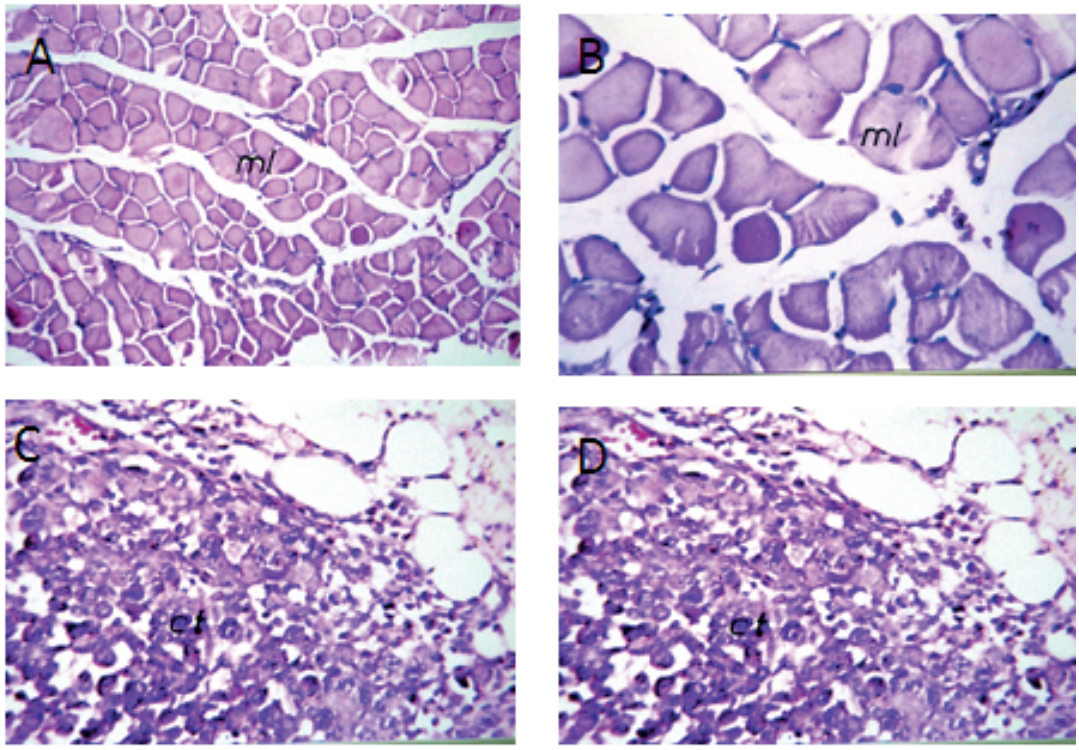

Figure 9: Figure (A) Skeletal muscle of thigh in mice go (1) Showing normal histological stucture of the muscle bundles (HPE × 40), figure (B) The magnification of figure (A) (HPE × 80), figure (C) gp (2) Showing Ehrlich tumor cells (te) implanted in hyperemic (V) muscle bundle (ml) (HPE × 40), figure (D) The magnification of figure (C) as (HPE × 80).

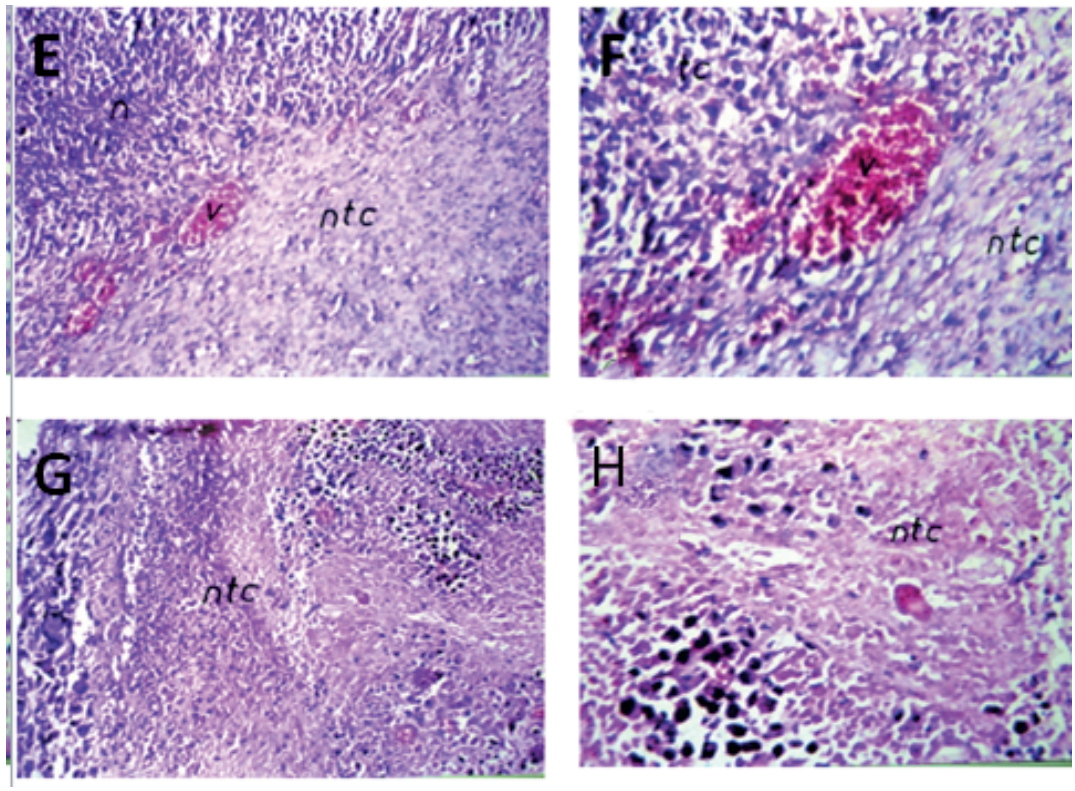

Figure 10: Figure (E) gp (3) Showing 80% intact tumor cells (n) in adjacent ingested (v) and necrosis (ntc) 20 and granulation tissue formaticn (g) (HPE × 40), figure (F) Showing the magnification of figure (E) (HPE × 80), figure (G) gp (4) Showing necrosis in 75% of Ehrlich tumor cells (ntc) (HPE × 40), figure (H) Showing the magnification of figure (G) (HPE × 80).

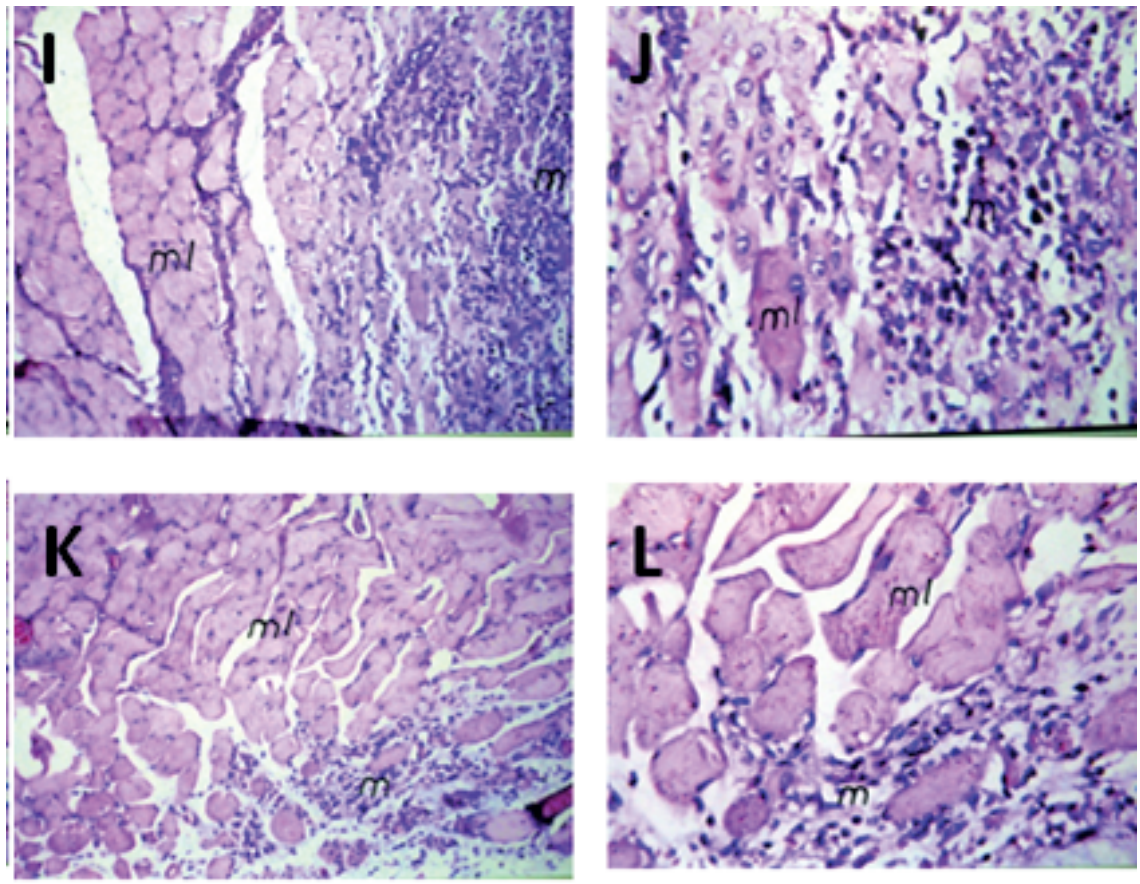

Figure 11: Figure (I) gp (5) Showing focal inflammatory cells infiltration (m) in the muscle bundle (ml) (HPE × 40), figure (J) Showing the magnification of figure (I) (HPE × 80), figure (K) gp (6) Showing focal few inflammatory cells infiltration in between the bundles (HPE × 40), figure (L) showing the magnification of figure (K) (HPE × 80).

fonticola m8, *Escherichia coli* m9, *E. coli* m12, and *Bacillus cereus* m15. On the other hand the antibiotic Norfloxacin, Ofloxacin, and Doxorubicin exhibited negative antimicrobial activity against *Serratia fonticola* m8 and *E. coli* m12, while Cefazolin showed negative result against the tested isolates except in the case of *Staphylococcus aureus* m14.

The results were found to be in agreement with that obtained by [17] who reported that Norfloxacin was used for treatment of certain types of infections, including infections of the urinary tract and prostate (a male reproductive gland).

Similarly [20] illustrated that Cefazolin is mainly used to treat bacterial infections of the skin (cellulitis). It can also be used to treat moderately severe bacterial infections involving the lung, bone, joint, stomach, blood, heart valve, and urinary tract. It is clinically effective against infections caused by staphylococci and streptococci which are commonly found on human skin. Cefazolin had been showed to be very effective in treating Methicillin-susceptible Staphylococcus aureus (MSSA) but it does not work in cases of Methicillin-resistant S. aureus (MRSA). It was reported by [21] that Doxorubicin (DOX) antitumor agent was used in the treatment of bladder, liver, and colon and prostatic cancer is an anthracycline-type polyketide, typically produced by *St. peucetius* ATCC 27952.

In this study the physical and chemical properties of the purified product were investigated. The TLC exhibited brown color and one spot which indicated pure product and R_f = 0.76 and absorption spectra in UV region recorded characteristic maximum absorption peak at 270 nm.

Infra-red (IR) spectrum of purified product MYN7 at IR region showed characteristic band corresponding peak at 3628, 3439, 2923 and 2853 = cm^{-1} which indicated the presence of hydroxyl group and absorption at 1542 and 1439 cm^{-1} indicated the presence of a benzene ring which supported NMR spectrum results and the mass spectrum recorded molecular weight of 473 Dalton.

In our result the elemental percentage of the purified product revealed that C = 23.52; H = 4.37; N = 2.64; and S = 12.12%. This analysis suggested calculated empirical formula of $C_{22}H_{19}NS_2O_7$.

The elemental analytical data of the antibacterial agent produced by *Streptomyces crystallinus*, AZ151 by [22], have been revealed as follows: C = 46.43; H = 7.46; N = 6.81; O = 39.43; and S = 0.0%; this analysis indicates a suggested

empirical formula of $C_{15}H_{30}N_2O_{10}$. And the spectroscopic analysis of purified antimicrobial agent was determined. The infrared (IR) spectrum showed characteristic band corresponding to 21 peaks, the ultraviolet (UV) spectrum recorded a maximum absorption peak at 225 nm, and the mass spectrum indicates that the molecular weight was 432.36.

In this study, the calculated IC_{50} of the EAC cells previously treated with different concentration of MYN7 was found to be 33 μg/ml and the IC_{50} of the MYN7 against Hepg2 was found to be 4.6 μg/ml); in this study Doxorubicin (DOX) was used for comparison and its IC_{50} against Hepg2 was 3.0 μg/ml.

The results was in agreement with that obtained by [23] who illustrated that compounds of Chromomycin B exhibited the $IC_{50} = 0.007 \pm 0.0004$ μg/ml, Chromomycin A_2 exhibited the $IC_{50} = 0.0005 \pm 0.00003$ μg/ml, and Chromomycin A_3 $IC_{50} = 0.1 \pm 0.006$ μg/ml were tested for cytotoxic activity against, Hepg2, based on IC_{50} values from compounds 1–3; the result showed strong cytotoxicity against Hepg2 and Doxorubicin as comparing drugs exhibited the $IC_{50} = 0.084 \pm 0.004$ μg/ml.

In this study histological analysis of six mice groups is detailed below. The result of histological analysis in group 1 showed no histopathological alteration observed and normal histological structure of the skeletal muscle bundles. While group 2 of mice implanted with Ehrlich tumor cell, result showed intact Ehrlich tumor cells (90%) implanted in the hyperemic muscle bundles.

Also result of group 3 of mice implanted with Ehrlich tumor cells and treated with MYN7 showed 80% of the intact implanted Ehrlich tumor cells observed adjacent to the congested, muscle bundles necrosis and granulation tissue formation.

Additional result of group 4 of mice implanted with Ehrlich tumor cells and treated by antibiotic doxorubicin showed necrosis in 75% of the implanted Ehrlich tumor cells, while the result of group 5 of mice administrated with MYN7, in focal wide area of the skeletal muscle, showed inflammatory cells infiltration.

Final result of group 6 of mice administrated with doxorubicin in focal area of muscle bundles showed little inflammatory cells infiltration.

Our results were found to be in agreement with that obtained by [24, 25] which illustrated the cytotoxicity toward nonmalignant cells in *in vivo* models; (R)-goniothalamin was reported to have tumoricidal and tumoristatic activities in Sprague-Dawley rats with 7, 12-dimethylbenzanthracene-(DMBA-) induced mammary tumors and in Ehrlich solid tumor in mice.

It was reported that, by [16] after encouraging effect of ethanol extract *Gracilaria edulis* (EEGE) in inhibiting cancer progression *in vivo*, the result evaluated the undesired side effects of the i.p. administration of daily doses of 100, 200, and 300 mg/kg of (EEGE) for 35 days in healthy adult Swiss albino mice. Drug toxicity was assessed by clinical signs of gross toxicity, behavioral changes, and mortality, including hematological, biochemical, and histopathological parameters. No animal death was observed in any of the groups during the experimental period of 35 days. No abnormal clinical signs or behavioral changes were observed in any of the groups, and changes in body weights of the EEGE-treated groups were not significantly different between any groups including the control group after 35 days of treatment period.

It was reported by [26, 27] that bacterial products can also combat cancer through inhibition of the HDAC enzyme. Romidepsin (FK228), a naturally occurring depsipeptide isolated from *Chromobacterium violaceum*, is a potent HDAC inhibitor with anticancer activity against leukemia, colon cancer, and neuroblastoma cell lines, human tumor xenografts and murine tumors, and is therefore expected to be a novel and promising anticancer drug, currently under clinical evaluation in the USA.

Similarly, early study demonstrated that doxorubicin showed relatively dense tumor cells pressing on the muscle tissue. The inhibitory rate of AP19-2a against P388 mouse leukemia cells, BEL-7402 human liver cancer cells, and A-549 human lung cancer cells was up to 81.6%, 92.3%, and 100%, respectively, at a concentration of 10–8 mol/L, which were consistent with the profound antitumor effects of Chromomycin A3 [28].

5. Conclusion

The present study exhibits or discovers newly bacterial metabolites isolated from *Streptomyces* in a previous study from Toshka area. Study showed newly physical and chemical properties of the purified product and showed antibacterial activity against newly isolated clinical bacterial isolates from different patients suffering tumor and treaded with radio- and/or chemotherapy, and also the newly purified product exhibits cytotoxic activity in both *in vitro* and *in vivo* level of study.

6. Recommendation

1. Further studies should be carried out for the identification of the clinical bacterial isolates phylogenetically using 16S RNA.

2. Further studies will be carried out for identification of the purified product and determination of specific active groups.

3. It's necessary to search for newly natural metabolites from biological origin having antimicrobial and antitumor effect, nontoxic and nonexpensive.

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