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Evaluation of Efficacy of Bioactive Compounds Produced by *Streptomyces* sp. in Comparison with Commercial Antibiotics against Urinary Tract Infection Bacterial Pathogens

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BECAUSE there are few effective treatments for some bacterial pathogens, primarily those that cause infections acquired in hospitals and community, antibiotic resistance had reached a critical point, necessitating the creation of novel bioactive substances with a wide range of effects. *Streptomyces* species are important as a source of secondary metabolites with a variety of biological functions, including antibiotics. The current investigation aims at studying the antibacterial potency of the two *Streptomyces sp.* (*Streptomyces umbrosus* M1 and *Streptomyces catenulae* M6) against infectious diseases causing bacterial pathogens in comparison with commercial antibiotics. Thirty five isolates from urinary tract infections (UTIs) samples were isolated, out of them, 13 isolates (37.1%) exhibited 100% of antibiotic resistance. The inhibitory effects of extracts from two *Streptomyces* strains (M1 and M6) showed highly antibacterial activity with varying degrees. Isolates with the codes (12) and (29) demonstrated a high susceptibility towards two extracts, particularly M6. The Minimum Inhibitory Concentrations (MICs) of the extract M6 against Gram positive bacteria displayed MICs ranged from (12.5- 37.5 µg/ml), isolate no 12 had the lowest MIC (12.5 µg/ml). However, Gram negative bacteria exhibited the highest MIC values, they recorded MICs in the range of (25-100 µg/ml), isolate no. 29 had the lowest MIC (25 µg/ml). Using 16srRNA, the highly sensitive isolates to the tested extract which had the lowest MIC were identified as *Ochrobactrum grignonense* and *Enterococcus faecalis.*

The inhibitory effects of extract M6 were further confirmed by scanning electron microscopic examination, which showed considerable morphological alterations and cell membrane rupture in the tested strains. As a result, the current research proved that *Streptomyces* sp. is a promising candidate for the treatment of the UTIs pathogens.

Keywords: Antibacterial activity, Bioactive compounds, *Streptomyces* sp., Urinary tract infection.

Introduction

Urinary tract infections (UTIs) are the major cause of infection and occur when bacterial pathogens attack the urinary tract (Klumpp et al., 2006; Smelov et al., 2016). UTI accounts for approximately 40% of all infections acquired in the hospitals and 50% of bacteremia that can prolong the hospitalization and increase the morbidity and mortality rates (Saint et al., 2008). Worldwide studies indicate that the microorganisms that cause UTIs are becoming more resistant to conventional treatments.

Infectious diseases caused by multidrug-resistant (MDR) bacterial pathogens are recognized as one of the major problems in treating the disease despite advances in the health care. Many synthetic chemical substances are currently employed as drugs to prevent disorders caused by free radicals and diseases caused by pathogenic bacteria, but many of these synthetic treatments are nonspecific and fail to alleviate the diseases or disorders completely (Orrett & Davis, 2006).

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Researchers still continuously explore for discovering a novel, sustainable, most potent and broad-spectrum naturally bioactive compounds and metabolites from diverse sources. Among the well characterized pharmaceutically relevant microorganisms actinomycetes are considered one of the major microbial producers of novel and therapeutically useful natural metabolites. Along with actinomycetes, *Streptomyces* is recognized as one of the major producers of antibiotics and an underutilised species for drug production; they produce more than two-thirds of the clinically effective antibiotics from natural origin. *Streptomyces* are Gram positive, filamentous forms of soil bacteria (Ser et al., 2015).

The *Streptomycetes* are considered one of the most important prokaryotes in pharmaceutical industries and biotechnology fields, they are also considered natural producers of antibiotics with biological activities that may eventually find current therapeutic applications as anti-infective, anti- tumor, immunosuppressive agents enzymes and food supplements (Arokiyaraj et al., 2015; Arasu et al., 2017). Because they are highly effective producers of novel secondary bioactive metabolites, novel *Streptomyces* sp. must be isolated urgently in this era of continuously increasing antibiotic resistance.

The objective of the present study is to compare two bioactive compounds extracted from two distinct strains of *Streptomyces* to the selected commercial antibiotics in order to assess their antibacterial efficacy against bacterial pathogens that cause UTIs.

Materials and Methods

Fermentation and extraction of secondary metabolites

Spores of two strains, *Streptomyces umbrosus* M1 and *Streptomyces catenulae* M6, which were kindly provided from Radiation Microbiology Department, (NCRRT), were used to inoculate 1000 ml Erlenmeyer flasks with 200 ml of enriched glucose asparagine broth. After incubation at 30°C for 24 h. in an orbital incubator shaker at 200 rpm, this pre-culture was used to inoculate (5% v/v) 15 L culture medium with the same composition as the pre-culture. The culture broth was filtered after 7 days of incubation to separate mycelium and supernatant. The supernatant was then extracted with ethyl acetate and concentrated using a rotary evaporator. To obtain the ethyl acetate extract (EA extract) the supernatant was extracted twice with equal volume of ethyl acetate and the combined organic layers were evaporated (Benita & Krishnan, 2014).

Isolation and purification of the bacterial pathogens

A total of 35 isolates from UTI were studied. The isolates were non-repetitive (one per patient) and were obtained consecutively from clinical specimens in urology wards. Bacterial pathogens were isolated using standard media, including Nutrient agar, Blood agar and MacConkey‘s agar and the specimens were inoculated using standard techniques, plates were incubated at 37ºC for overnight before the plates were inspected for growth. The bacterial isolates were preserved at 4°C on slant agar and -80°C in 20% glycerol stock.

Microscopic examination of bacterial isolates

The obtained purified colonies were checked by microscopic examination using Gram’s stain.

Antibiotic susceptibility assay

Susceptibility of the purified isolates to ten different antibiotics was carried out using the disc diffusion method according to the criteria of the National Committee of Clinical Laboratory Standards (CLSI, 2010) on Muller Hinton agar (MHA). Commercially available antibiotic discs (OXOID) containing, Cefazidime (CAZ30 mcg); Azithromycin (AZM 15 mcg); Ampicillin/ sulbactam (SAM 20 mcg); Ciprofloxacin (CIP 5 µg); Clavulanic acid /Sulbactam (AMC); Cefoperazone (CFP 75); Ceftriaxone (CRD 30 mcg); Impemem (IPM 10 µg); Naladixic acid (NA); Tobramycin (TOB 10 µg) were used and inhibition zone diameters were measured after incubation at 37°C for 24 h. Results were expressed as sensitive (S), intermediate (I), and resistant (R).

Antibacterial activity assay

The inhibitory activity of the two bioactive extracts from *Streptomyces* strains against the purified isolates was evaluated using agar well diffusion method (Krishna et al., 2015). Initially, 100 µl of autoclaved distilled water was taken in autoclaved eppendorf and a loop full of test inoculums was immersed in it and vortexed to match its turbidity with McFarland 0.5. Then, 10 µl of this inoculum were taken and poured on

solidified agar surface and spread with sterilized spreader by rotating in clockwise direction. Agar wells of 5 mm were made with sterilized stainless steel cork borer. The wells were loaded with 10 µl of two tested extracts (M1 and M6). After 24 h. of incubation at 37 ºC, the diameter of inhibition zone (DIZ) was measured in millimeters, (DMSO was used as a negative control). Tests were carried out in triplicate.

Minimum inhibitory concentration (MIC) determination

The MIC of the studied extract required for the inhibition of the purified bacterial isolates was determined using the broth dilution method, according to Panacek et al. (2006). A control test containing inoculated broth supplemented with only DMSO was also performed. The MIC was determined as the lowest concentration of the extract that demonstrated no visible growth after 24 h. incubation time.

Molecular identification of the chosen isolates using 16S rRNA PCR-sequencing

The rapid amplification of nucleic acid targets from relatively lower starting material makes PCR one of the most sensitive techniques available for detection of bacterial targets. PCR-based identification of bacterial DNA through amplification and sequencing of the 16S rRNA gene has become a standard molecular method, both in the laboratory as well as in clinical settings. The 16S rRNA gene is highly specific to each bacterial species and this makes it an ideal target for identification. The standard method involves PCR amplification of the 16S rRNA gene, followed by sequencing and comparison to known databases for identification (James, 2010).

Scanning electron microscopic examination

Scanning electron microscopic (SEM) observations were carried out to determine the inhibitory effects of extract M6 (at MIC) on morphology of the two identified bacterial pathogens. Control samples were prepared without the extract, after incubation at 37°C for 18 h., cells were harvested by centrifugation for 10 min. at 4,500 rpm/min and washed twice using 0.9% phosphate buffer pH 7.2, and then were resuspended in water containing 2.5% glutaraldehyde and kept at −4°C for 10 h to fix the cells. After centrifugation, the cells were further dehydrated in water–alcohol solutions at various alcohol concentrations (30, 50, 70, 80, 90, and 100%) for 10 min. Finally, the samples were fixed and then sputter coated with gold under vacuum, and were examined in the JEOL- JSM -5400 scanning electron microscope (Japan) at the NCRRT, Cairo, Egypt.

Characterization of Streptomyces M6 extract

Spectral analysis

Elucidation of the chemical structure of the purified extract includes the use of the following physical methods:

Ultraviolet (UV) spectra

This was done using a purified *Streptomyces* M6 extract dissolved in dimethylsulfoxide (DMSO) without heating on a UV-JASCO/V-560 spectroscopy system at the NCRRT, Cairo, Egypt.

Infrared absorption spectrum (FTIR)

A purified extract of *Streptomyces* M6 was evaluated by FTIR using the potassium bromide disc technique on a Shimadzu IR-110 spectrometer at the NCRRT, Cairo, Egypt.

Determination of the elemental analysis

Elemental analysis for C, H, O, N, and S were performed at the Vario El Elementar System, Germany at the National Research Center (NRC), Cairo, Egypt.

Thin layer chromatography

Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. Optimization of mobile phase ethyl acetate, isopropanol, and acetonitrile (1:4:5) unknown bacterial extracts (v/v) were performed using 20 x 20 cm, 1 mm thick silica gel plates prepared and activated at 110°C for 30 min. Chromatograms were generated by loading 10 µl of each fraction and running for 30 min. After the experiment, the spots are visualized. this can be often done simply by projecting ultraviolet light onto the sheet.

Results and Discussion

Incurable diseases are becoming increasingly commonly, with the rise in non-sensitivity of microbes to antibiotics; the lack of new alternatives poses a threat to world health (Jones et al., 2008). This serious rise in antibiotic resistance of microbes necessitates research on unconventional methodologies for discovering new drugs (Kumar et al., 2017).

Urinary tract infections are common infections in both outpatients and hospitalized patients. This may be brought on by an increase in immune-compromised patients, prolonged hospital stays, more sophisticated equipment, and insufficient personal and environmental hygiene (Showlag et al., 2019). JebaMercy et al. (2019) reported that, the main microbial pathogens that contribute in UTIs are bacteria.

Over the past 80 years, *Streptomyces* have entered the medical field through the production of antifungal, antiparasitic, anticancer, and bioactive compounds, as well as the production of natural metabolites used as antibacterial agents. Recent isolates of *Streptomyces* from traditional medicine reveal that these microbes may have been crucial to human health for a longer period of time than previously thought (Quinn et al., 2020).

Antibiotic susceptibility test

This experiment was carried out to provide an antibiogram of the isolated bacteria against several antibiotic classes. For each isolate, the mean inhibition zones for every antibiotic employed were recorded. Antibiotic resistance profile of the collected isolates against 10 different standard antibiotics has been shown in Table 1. Out of them, 16 (45.7%) isolates were resistant to CAZ and AMC; 18 (51.4%) were resistant to SAM, 19 (54.3%) were resistant to AZM and IPM, 20 (57.1%) were resistant to CIP and CFP; 25 (71.4%) were resistant to CRD and TOB, 29 (82.8%) were resistant to NA, while 13 isolates were resistant to all tested antibiotics (100%). The present findings revealed a remarkable rise in the incidence of antimicrobial resistance in uropathogenic bacteria, as evidenced by the appearance of multidrug-resistant (MDR) isolates among all uropathogens. All uropathogenic species have noticed a significant rise in the emergence of multidrug-resistant (MDR) isolates in recent years, which has been attributed to the extensive use of antibiotics. Numerous bacteria, including Gram-positive and Gram-negative bacteria, are responsible for UTIs.

Distribution of the purified bacterial isolates using Gram's stain

The results of Gram-stain of the 13 isolates which appeared 100 % resistant towards all the tested antibiotics are shown in Table 2 and Fig. 1. The results illustrated that 5 isolates (38.5%) exhibited Gram-positive cocci and 8 isolates (61.5 %) were Gram-negative bacilli and short rods. One of the primary microbial techniques used to identify the bacteria is Gram staining. According to the present results, 61.5% of the tested isolates were Gram-negative bacteria, which is consistent with earlier investigations (Kothari & Sagar, 2008). Furthermore, the data in this experiment demonstrated that the prevalence of Gram-positive isolates (38.5%) were less than those of Gram-negative one; this is similar to other studies **(**Amin et al., 2009). Mollick et al. (2016) reported that Gram-negative bacteria were mainly responsible for urinary tract infections.

TABLE 1. **Antibiotic resistance profile of the purified clinical isolates**

Antibiotics	No. of resistant isolates from 35 isolates	% of resistant isolates from 35 isolates
CAZ	16	45.7
AZM	19	54.3
SAM	18	51.4
CIP	20	57.1
AMC	16	45.7
CFP	20	57.1
CRD	25	71.4
IPM	19	54.3
NA	29	82.8
TOB	25	71.4

Cefazidime (CAZ30mcg); Azithromycin (AZM 15 mcg); Ampicillin/sulbactam (SAM 20 mcg); Ciprofloxacin(CIP 5 µg); Clavulanic acid /Sulbactam (AMC); Cefoperazone (CFP 75); Ceftriaxone (CRD 30mcg); Impemem (IPM 10 µg); Naladixic acid (NA); Tobramycin (TOB10 µg)

TABLE 2 Some morphological characteristics of the purified isolates

Isolate code	Characteristics		
	Gram stain	Shape	Motility
1	-ve	Bacilli	Motile
3	-ve	Short rods	Non-motile
4	-ve	Bacilli	Motile
8	-ve	Short rods	Non-motile
12*	-ve	Short rods	Non-motile
15	+ve	Cocci	Non-motile
20	+ve	Cocci	Non-motile
23	+ve	Coccobacilli	Non-motile
28	-ve	Bacilli	Motile
29*	+ve	Cocci	Non-motile
30	-ve	Bacilli	Non-motile
31	-ve	Bacilli	Motile
33	+ve	Cocci	Non-motile

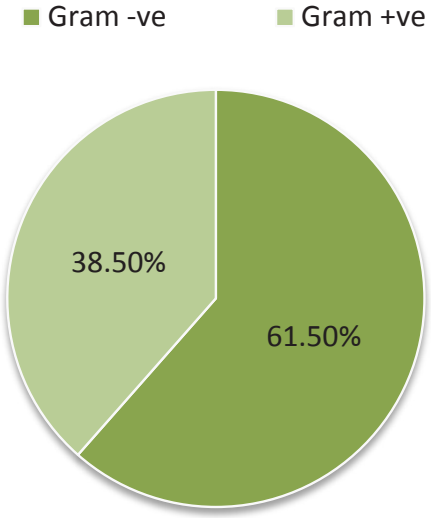


Fig. 1. Percentages of the purified isolates according to Gram's stain

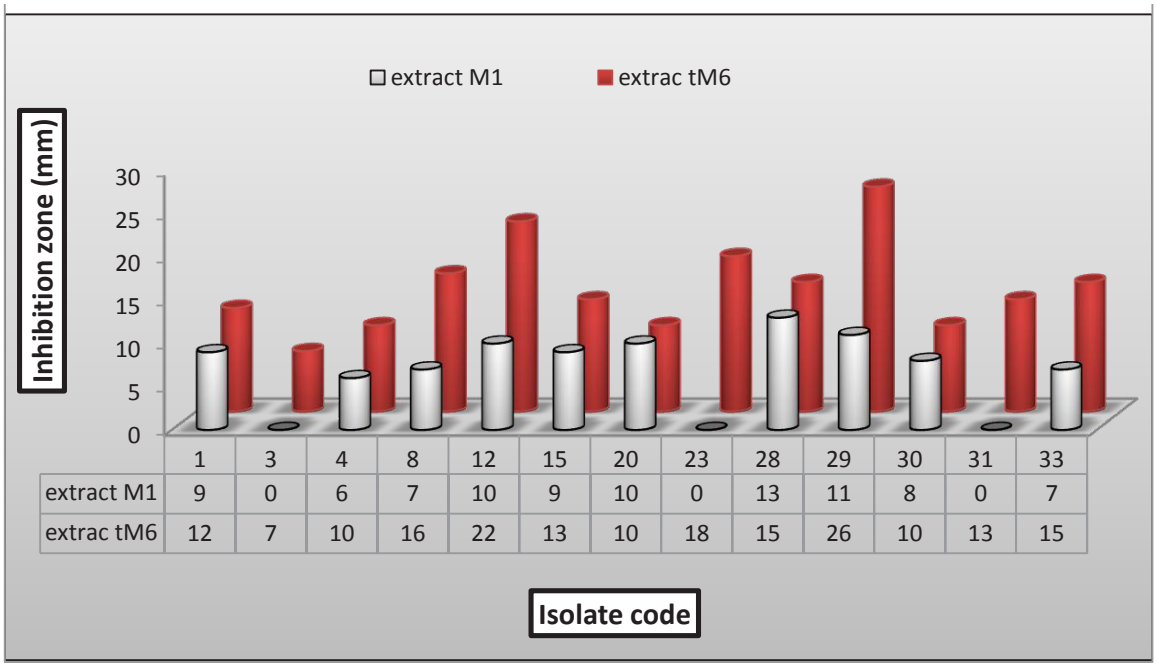
Antibacterial activity of the extracellular metabolites

In order to avoid microbial infections, several antibiocidal drugs were used, however over time, the microbe acquired a resistance (Abdel Maksoud et al., 2021). To produce newly unexplored antimicrobial compounds, particularly those used to prevent the spread of infections, new techniques and materials were applied (Jin & He, 2011).

Actinomycetes are potential sources of secondary metabolites with a wide variety of biological and antibacterial properties which are used to treat both humans and animals. The genus *Streptomyces* is well known for producing a variety of bioactive metabolites, including volatile organic compounds, enzymes, insecticides, herbicides, and antibiotics (Mysoon et al., 2019). However, the production of new therapeutics is delayed in recent days and the isolation of new *Streptomyces* species has drawn attention.

According to Vonothini et al. (2008) and Syed et al. (2009), enzymes such as proteases, lipases, cellulases, amylases, pectinases, and xylanases are the most important products of *Streptomyces* after antibiotics.

Initially, the agar well diffusion method was used to evaluate the antimicrobial properties of the two bioactive metabolites against the thirteen bacterial isolates. The results in Fig. 2 showed that the two extracts (M1 and M6) demonstrated high antibacterial activity with a variety of effects. For ten isolates, extract (M1) displayed antibacterial activity in the inhibition zone ranged from (6-11 mm), but with the remaining three isolates (3, 23, and 31), the extract does not exhibit any antibacterial activity. However, extract (M6) had strong efficacy against thirteen isolates, with an inhibition zone ranged from (7- 26 mm). Isolates with code numbers (12) and (29) demonstrated great sensitivity to two extracts, particularly M6. Our findings revealed that the tested extracts had a satisfactory ability to inhibit various pathogenic isolates including Gram positive and Gram negative one.



	1	3	4	8	12	15	20	23	28	29	30	31	33
extract M1	9	0	6	7	10	9	10	0	13	11	8	0	7
extrac tM6	12	7	10	16	22	13	10	18	15	26	10	13	15

Fig. 2. Antibacterial activity of *Streptomyces* M1 and M6 extract against thirteen bacterial isolates

The MIC values of the extract M6 were estimated by standard methods. The ethyl acetate extract from the fermentation broth obtained from *S. catenulae* M6 demonstrated various degrees of antibacterial activity, as seen in Fig. 3. Among the studied isolates, the Gram positive bacteria displayed MIC ranged from (12.5- 37.5 µg/ml), isolate no 12 had the lowest MIC (12.5 µg/ml), whereas Gram negative bacteria had the highest MIC values. They recorded the MIC in range (25-100 µg/ml), isolate no 29 had the lowest MIC (25 µg/ml).

Earlier research demonstrated that the *actinomycetes* isolated from termite gut samples were selectively active against some Gram-positive and Gram-negative pathogenic bacteria as well as pathogenic yeast (Arango et al., 2016).

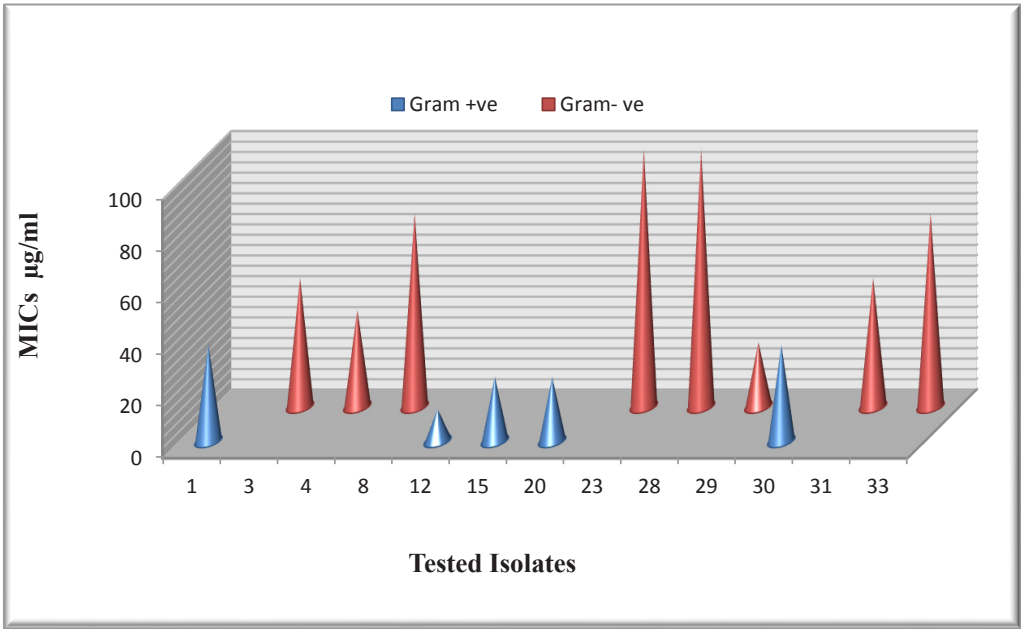


Fig. 3. Minimum Inhibitory Concentrations for *S. catenulae* M6 extract against the selected pathogens

Pandey et al. (2004) and Sapkota et al. (2020) stated that ethyl acetate extracts of actinomycetes isolated from Nepalese soils at different altitudes can produce secondary metabolites that exhibit antibacterial activity against different ATCC strains.

The hypothesized antibacterial mechanism is represented diagrammatically in Fig. 4. It is evident that the studied extract (bioactive metabolites) start acting by adhering to the bacterial cell's outer surface, rupturing the membrane, and altering transport behavior. After that, each bioactive molecule is distributed independently inside each bacterial cell, along with the dispersion of all intracellular constituents like DNA, plasmids, and other essential organelles.

Molecular identification of the chosen bacterial isolates using 16$_S$ rRNA

Neighbor-joining phylogeny trees of the output of BLAST showed that the submitted gene corresponding to rRNA sequence is identical by 99.4% to *Ochrobactrum grignonense* 16S ribosomal RNA gene, partial sequence (Table 3, Fig. 5) and by 99.3% to *Enterococcus faecalis* strain 16S ribosomal RNA gene, partial sequence (Table 4, Fig. 6).

Scanning electron microscopic analysis (SEM)

Morphological alterations may take place on the cell surface of microbial pathogens when treated with antimicrobial agents. Hence, a SEM examination was carried out to further visualize the impact of treatment of extract M6 at MIC on the morphology of the two tested strains. *Ochrobactrum grignonense* cells were found to have a smooth surface when untreated (Fig. 7a), whereas treated cells were more rugged and their surfaces became irregular. It was also observed that treated cells displayed lysis of the outer surface followed by cell deformations (Fig. 7b).

Additionally, SEM images showed that untreated cells of *Enterococcus faecalis* exhibited distinctive morphologies (spherical shaped), with typical smooth-walled cocci clustered in an organized manner (Fig. 8a). As opposed to the untreated cells, the studied strain displayed morphological changes such as the elongation of cells with rigid cell walls and cell destruction after being incubated with extract M6 at MIC for 18 h. (Fig. 8b). In general, it is obvious from the images that treated cells demonstrated observable structural alterations and membrane integrity compared to untreated cells.

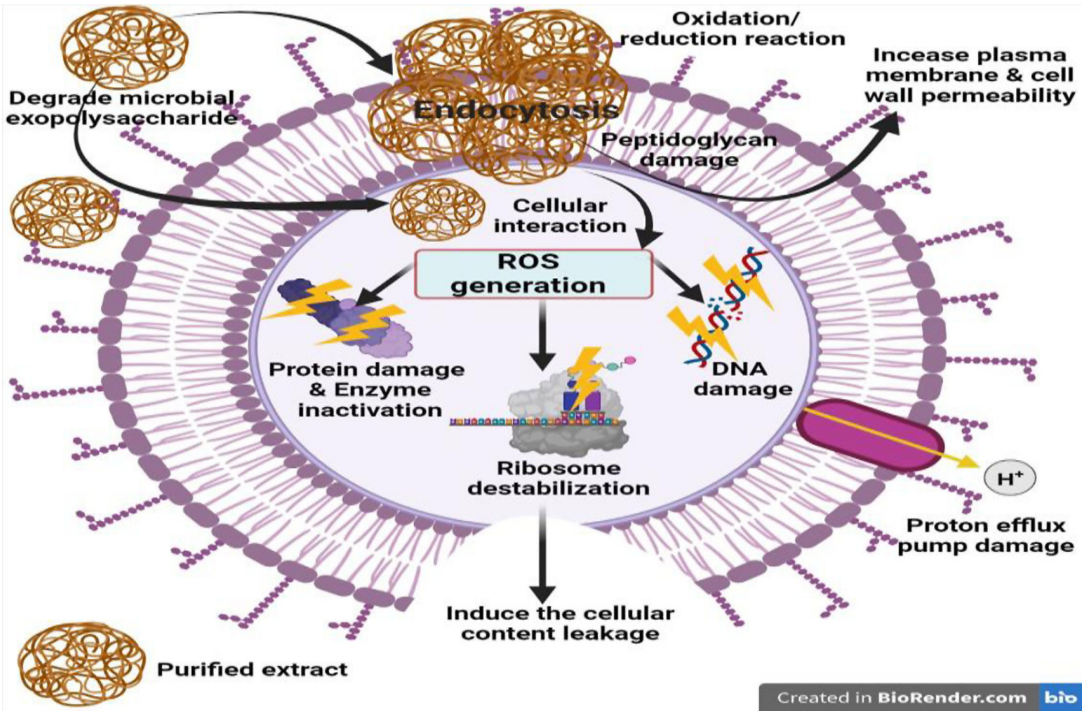


Fig. 4. A hypothesized model illustrating possible mechanisms of bioactive metabolites antibacterial action

TABLE 3. Similarity analysis of 16S rRNA gene sequence of *Ochrobactrum grignonense*

Description	Max score	Total score	Query cover	E value	Per.Ident.	Accession
Ochrobactrum grignonense strain NBRC102586 16s ribosomal RNA gene partial sequence	900	900	100%	0.0	99.40 %	NR 114149.1
Ochrobactrum pituiosum strain CCUG 30717 16s ribosomal RNA gene partial sequence	900	900	100%	0.0	99.40 %	NR 115043.1
Ochrobactrum pseudogrigonnense strain OgA9a strain 16s ribosomal RNA gene partial sequence	900	900	100%	0.0	99.40 %	NR 042589.1
Ochrobactrum grignonense strain QgA9a 16s ribosomal RNA gene partial sequence	894	894	100%	0.0	99.40 %	NR 028901.1
Ochrobactrum rhizosphaerae strain PR17 16s ribosomal RNA gene partial sequence	894	894	100%	0.0	99.20 %	NR 022600.1
Ochrobactrum grignonense strain QgA9a 16s ribosomal RNA gene partial sequence	894	894	100%	0.0	99.20 %	NR 115044.1
Ochrobactrum thiophenovoranas strain DMS7216 16s ribosomal RNA gene partial sequence	894	894	100%	0.0	99.20 %	NR 042599.1
Ochrobactrum galinifaecis strain ISO 196 16s ribosomal RNA gene partial sequence	878	878	100%	0.0	99.59 %	NR 025576.1
Ochrobactrum anthropi strain ATCC 49188 16s ribosomal RNA gene partial sequence	863	863	100%	0.0	98.00 %	NR 074243.1
Ochrobactrum anthropi strain NBRC 15819 16s ribosomal RNA gene partial sequence	863	863	100%	0.0	98.0%	NR 133811.1
Ochrobactrum tiotio strain Sc1124 16s ribosomal RNA gene partial sequence	863	863	100%	0.0	98.0%	NR 114980.1
Ochrobactrum anthropi strain LMG 3331 16s ribosomal RNA gene partial sequence	863	863	100%	0.0	98.0%	NR 114979.1
Ochrobactrum cysti strain ESCI 16s ribosomal RNA gene partial sequence	863	863	100%	0.0	98.0%	NR 043184.1
Ochrobactrum tnici strain SC ii 24 16s ribosomal RNA gene partial sequence	863	863	100%	0.0	98.0%	NR 028902.1
Ochrobactrum lupine strain LUP 21 16s ribosomal RNA gene partial sequence	863	863	100%	0.0	98.0%	NR 042911.1
Ochrobactrum pecoris strain 08RB2839 16s ribosomal RNA gene partial sequence	861	861	100%	0.0	97.99%	NR 117053.1
Ochrobactrum tritici strain NBRC 102585 16s ribosomal RNA gene partial sequence	859	859	100%	0.0	97.80%	NR 114148.1
Ochrobactrum haematophilum strain CCUG 38531 16s ribosomal RNA gene partial sequence	857	857	100%	0.0	97.80%	NR 042588.1
Ochrobactrum aestuani strain LG 29090 16s ribosomal RNA gene partial sequence	830	830	100%	0.0	97.80%	NR 156984.1
Ochrobactrum ovis strain 81315 16s ribosomal RNA gene partial sequence	828	828	100%	0.0	97.80%	NR 135736.1
Ochrobactrum composti strain Nis3 16s ribosomal RNA gene partial sequence	813	813	100%	0.0	96.79%	NR 113183.1

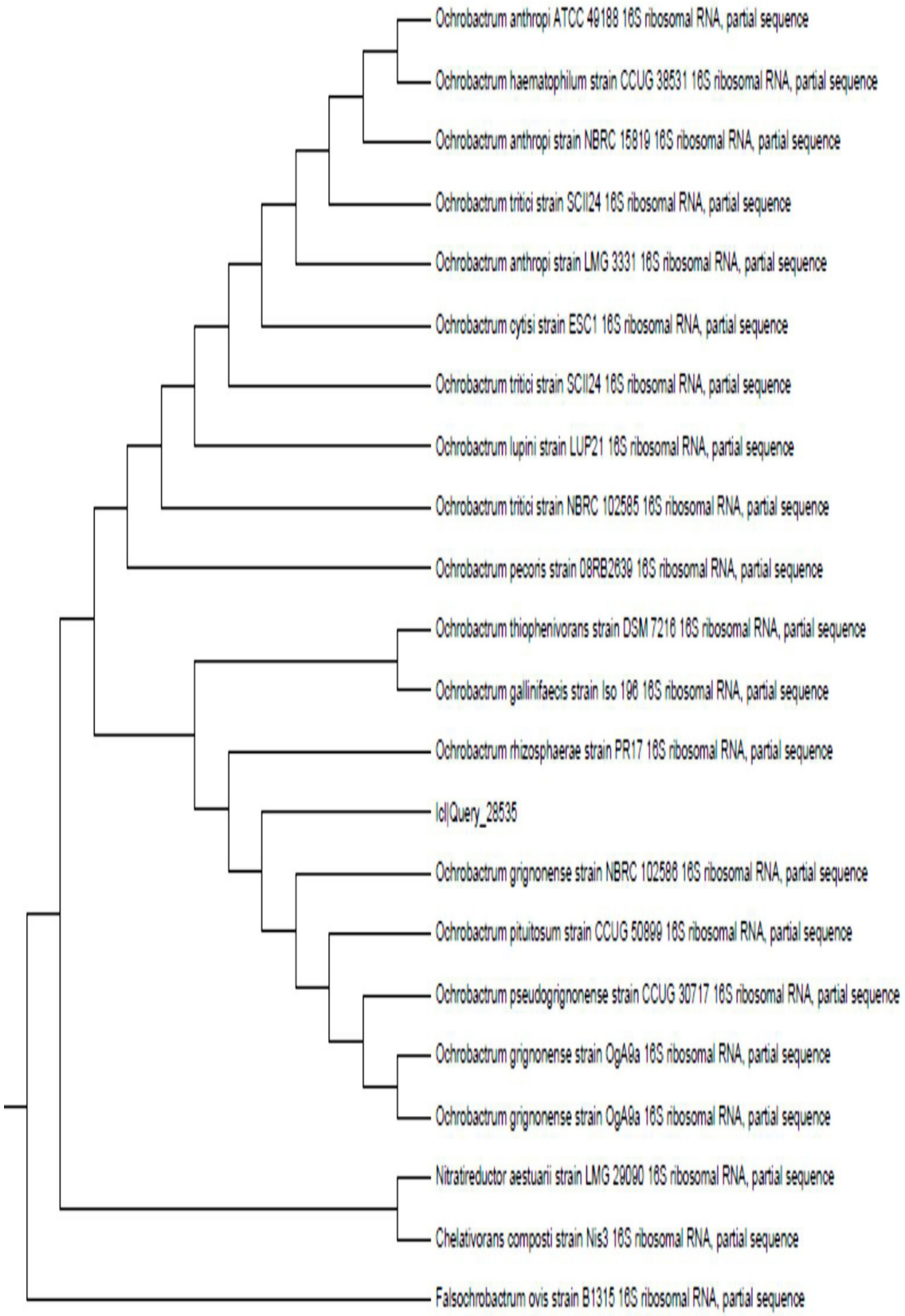


Fig. 5. The phylogeny tree of *Ochrobactrum grignonense* PCR molecular identification

TABLE 4. Similarity analysis of 16S rRNA gene sequence of *Enterococcus faecalis*

Description	Max score	Total score	Query cover	E value	Per.Ident.	Accession
Enterococcus faecalis strain NBRC100480 16s ribosomal RNA gene partial sequence	1138	1138	100%	0.0	99.36 %	NR 113901.1
Enterococcus faecalis strain ATCC 1943316s ribosomal RNA gene partial sequence	1138	1138	100%	0.0	99.36 %	NR 115765.1
Enterococcus faecalis strain LMG 7937 16s ribosomal RNA gene partial sequence	1138	1138	100%	0.0	99.36 %	NR 114782.1
Enterococcus wangshanyuanii strain MNO05 16s ribosomal RNA gene partial sequence	1116	1116	100%	0.0	98.73 %	NR 159231.1
Enterococcus rivonum strain S299 16s ribosomal RNA gene partial sequence	1116	1116	100%	0.0	98.73 %	NR 117040.1
Enterococcus faecalis strain GCM5803 16s ribosomal RNA gene partial sequence	1112	1112	100%	0.0	98.73 %	NR 040789.1
Enterococcus moraviensis strain NBRC 100710 16s ribosomal RNA gene partial sequence	1107	1107	100%	0.0	98.41 %	NR 113937.1
Enterococcus haemoperoxidus strain NBRC 100709 16s ribosomal RNA gene partial sequence	1105	1105	100%	0.0	98.41 %	NR 113936.1
Enterococcus plantanum strain NBRC 100709 16s ribosomal RNA gene partial sequence	1105	1105	100%	0.0	98.41 %	NR 118050.1
Enterococcus termitis strain LMG8895 100709 16s ribosomal RNA gene partial sequence	1105	1105	100%	0.0	98.41 %	NR 042406.1
Enterococcus haemoperoxidus strain 440 16s ribosomal RNA gene partial sequence	1099	1099	100%	0.0	98.25 %	NR 028795.1
Enterococcus crotali strain ETRF1 16s ribosomal RNA gene partial sequence	1094	1094	100%	0.0	98.09%	NR 156981.1
Enterococcus silesaacus strain R-23712 16s ribosomal RNA gene partial sequence	1094	1094	100%	0.0	98.09%	NR 022405.1
Enterococcus caccae strain 2215-02 16s ribosomal RNA gene partial sequence	1094	1094	100%	0.0	98.09%	NR 043285.1
Enterococcus rotai strain CCM 4630 16s ribosomal RNA gene partial sequence	1094	1094	100%	0.0	98.09%	NR 108137.1
Enterococcus ureilyicus strain CCM 4926 16s ribosomal RNA gene partial sequence	1094	1094	100%	0.0	98.09 %	NR 125485.1
Enterococcus moraviensis strain 330 16s ribosomal RNA gene partial sequence	1090	1090	100%	0.0	97.93 %	NR 028794.1
Enterococcus massiliensis strain AM1 16s ribosomal RNA gene partial sequence	1072	1072	100%	0.0	97.45%	NR 144723.1
Enterococcus saigonesis strain VE80 16s ribosomal RNA gene partial sequence	1072	1072	100%	0.0	97.45%	NR 152049.1
Enterococcus saccharolyticus subsp.*taiwanensis* strain 812 16s ribosomal RNA gene partial sequence	1072	1072	100%	0.0	97.45%	NR 132299.1
Enterococcus asini strain NBRC 10068116s ribosomal RNA gene partial sequence	1072	1072	100%	0.0	97.45%	NR 113929.1

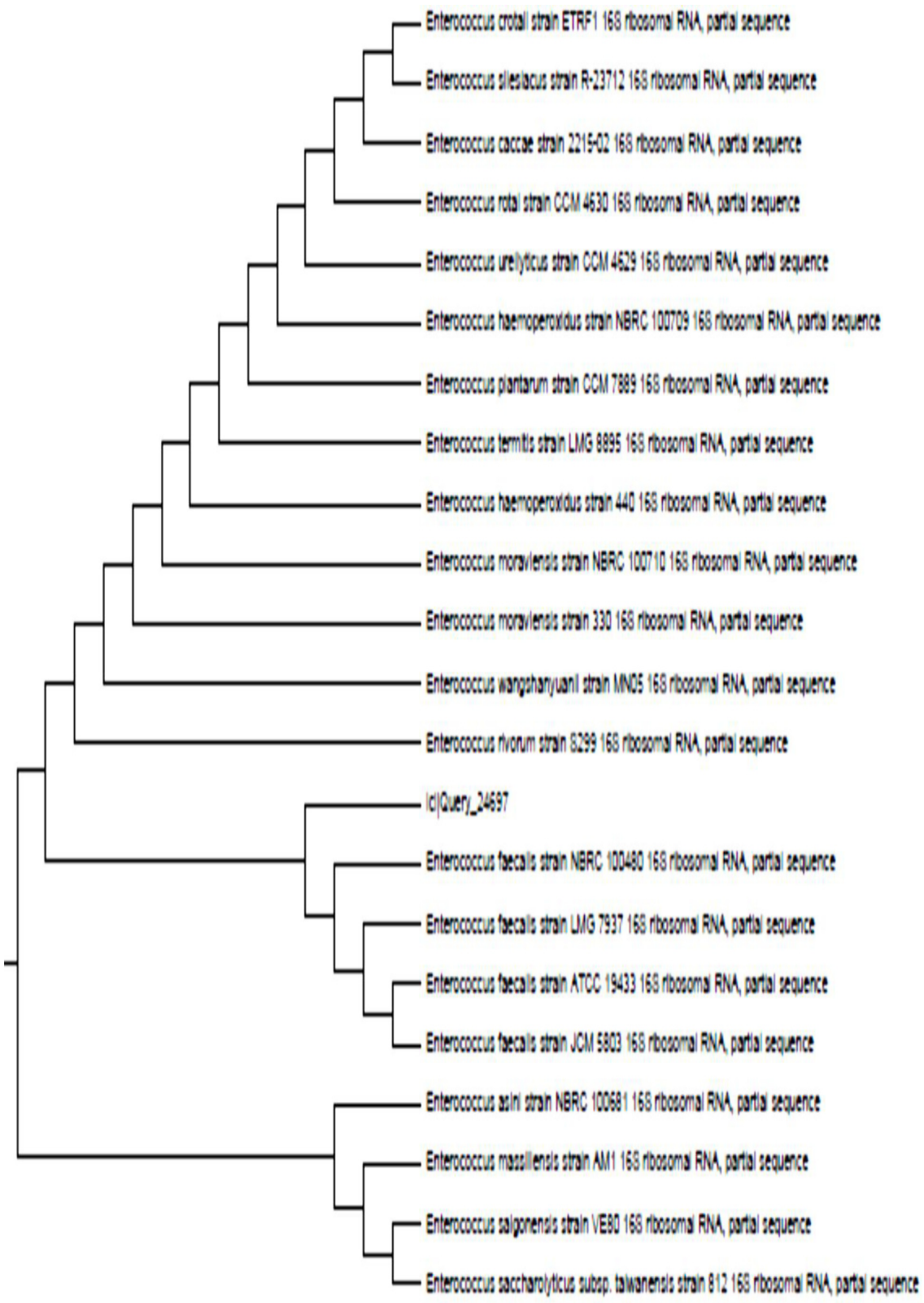


Fig. 6 The phylogeny tree of *Enterococcus faecalis* PCR molecular identification

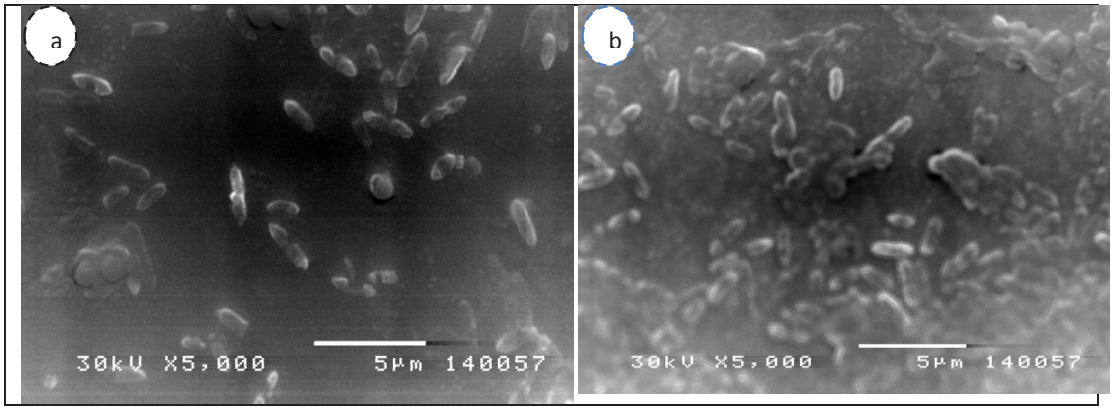


Fig. 7. Scanning electron micrographs of *Ochrobactrum grignonense* (a) untreated cells (b) cells treated with extract M6 at MIC

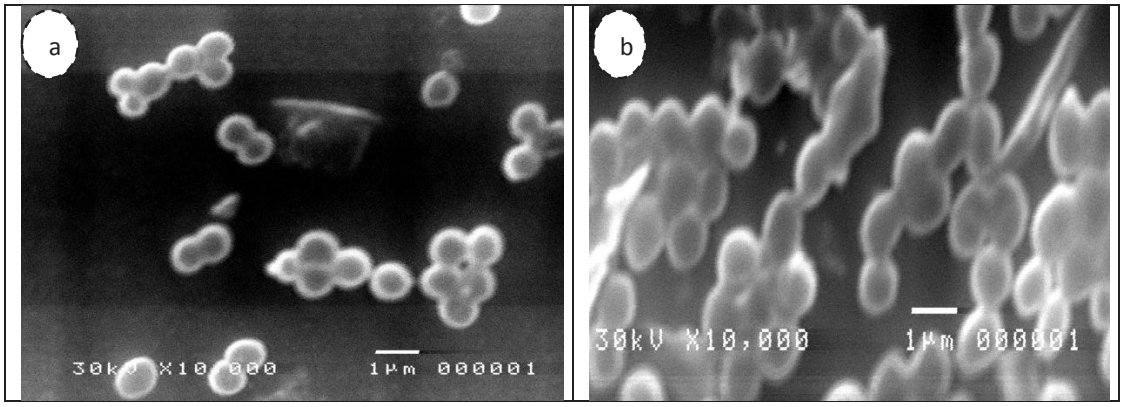


Fig. 8. Scanning electron micrographs of *Enterococcus faecalis* (a) un treated cells (b) and b) cells treated with extract M6 at MIC

Characterization of Streptomyces M6 Extract

Spectroscopic analysis

FTIR spectroscopy: Because FTIR provides a strong absorption pattern at a certain frequency for a specific functional group, it was utilized to identify the functional groups present in pure compounds or mixtures of compounds. In the present study, the FTIR spectrum of the purified extract M6 is shown in Fig. 9 and Table 5. The mid-infrared, approximately 4000-400 cm^{-1}, was used to study the fundamental vibrations and associated rotational-vibrational spectrum. Amid, Amine, Hydroxyl, and Methyl groups can be found in the spectrum. The IR spectrum illustrated the functional groups O-H (free) at wave number (WN) 3996 and O-H (very broad). Moreover, it exhibits a wave number at 2999, which indicated the presence of C-H alphatic and aromatic groups, as well as a peak at 1749 cm^{-1} indicating the presence of carbonyl (C=O), saturated aldehydes, or an amide group, and NH2 scissoring (1°-amines) at WN 1608.The Whilst the fictional group CH2 bending, C-C-C bending, and =C-H & =CH2 may appear at wave numbers 1477, 1430, 1106, 1019, and 895, respectively. In another study, *Streptomyces sp*. metabolite isolated from a Bangladeshi soil sample represented as , 2-hydroxy-3-(hydroxymethyl)-4Hpyran-4-one, and the structure of the metabolite were chemically modified and spectroscopically confirmed using IR techniques (Bytul et al., 2002).

Singh et al. (2018) reported that the bands recorded based on wavelength indicated the presence of carbonyl groups, ketones, aldehydes, esters and carboxylic acids in the extracted compounds.

Table 6 illustrates the physicochemical properties of *S. catenulae* M6 extract, which exhibits a brown color and a melting point in the range of 270°C. Additionally, the data revealed that the elemental percentage of the purified product was C = 12.9%, H = 4.1%, N = 1.55%, and S = 9.3%, implying that the empirical formula was $C_{10}H_{37}NS_3O_{46}$.

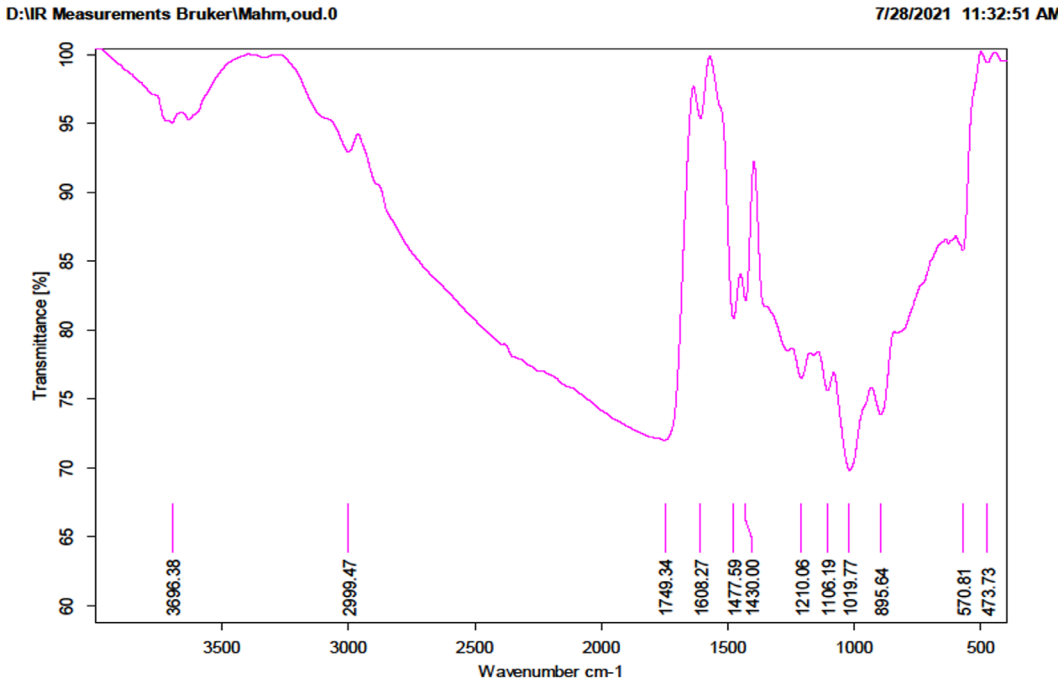


Fig. 9. Infra-Red spectrum of *S. catenulae* M6 extract

TABLE 5. Groups absorbing in IR region of *S. catenulae* M6 extract

Range (cm^{-1})	Assignment
3996	O-H (free)
2999	O-H (very broad)
1749	C=O (saturated aldehyde)
1608	NH_2 scissoring (1°-amines)
1477	α-CH_2 bending
1430	α-CH_2 bending
1106	C-C-C bending
1019	C-C-C bending
895	=C-H & =CH_2

TABLE 6. Physical properties of *S. catenulae* M6 extract

Characteristic	Extract
Color	Brown
Melting point (^{0}C)	270^{0}C
Purity by TLC	Pure extract (one band)
Ultra-violet	250
Molecular formula	$C_{10}H_{37}NS_3O_{46}$
Microanalysis %	
N	1.55
C	12.9
S	9.3
H	4.1

Afifi et al. (2012) reported an elemental analysis of the antibacterial agent AZ151 produced by *Streptomyces crystallinus* C = 46.43; H=7.46; N = 6.81; O = 39.43; this analysis indicates that the proposed empirical formula is $C_{15}H_{30}N_2O_{10}$.

Additionally, in the current study, the purity of *S. catenulae* M6 extract was tested using thin layer chromatography which exhibits one band (which may be a pure extract) (Fig. 10).

Furthermore, the ultraviolet (UV) spectrum (Fig. 11) recorded a maximum absorption peak at 250 cmm. According to Singh et al. (2012), the UV absorption measured ranged between 241 and 251 nm, with the maximum absorption was measured at 251 nm for the partially purified ethyl acetate extract of *Streptomyces* sp. SW72IV.

Conclusion

Globally, multidrug resistant bacteria provide a severe threat, particularly in cases of UTIs. *Streptomyces* species have played a critical role as a source of various antimicrobial bioactive components against many pathogenic bacteria. According to the information on the antibacterial activities of some *Streptomyces* spp. provided by the present study, the bioactive metabolites extracted from *S. catenulae* appears to be a very promising candidate for eradicating the pathogenic bacteria.

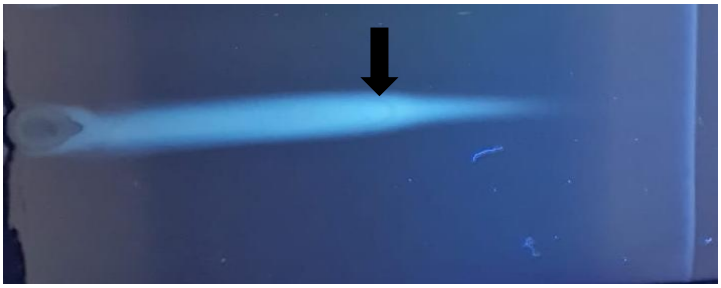


Fig. 10. Thin layer chromatography of *S. catenulae* M6 extract showing one band (pure extract)

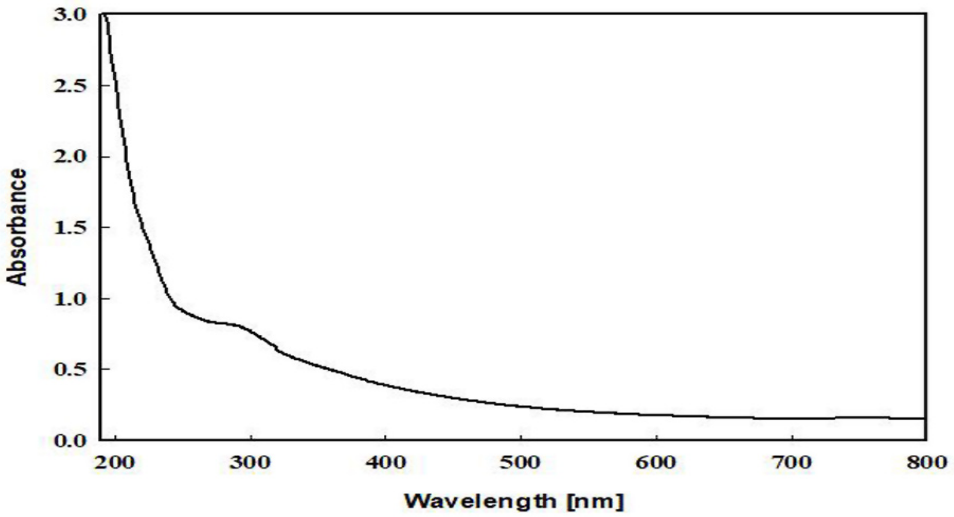


Fig. 11. Ultra-violet aberrance of *S. catenulae* M6 extract

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