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Biochemical Studies on Biologically Active Secondary Metabolites from Marine *Streptomyces* Sp. and their Effect on Bacterial Contaminants in Drinking Water From the Nile River in Egypt

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In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract
This study tests the effectiveness of biological treatment for drinking water (BioDWT) as a way of removing bacterial contaminants from drinking water by inhibiting biofilm-forming bacteria using different Streptomycetes isolates from marine water and their capacity to produce biologically active secondary compounds. Samples are collected and 23 bacterial isolates from different stages of the drinking water treatment plant from River Nile of the new Mansoura water plant in Mit-Khamis, Dakahlia governorate, Egypt. Then purification of the bacterial isolates on R2A agar media. The capacity of the bacterial isolates to produce biofilms was studied. Following the sending of the nucleotide sequence to the GenBank sequence database, it was discovered that the bacterial isolate belonged to the genus Enterobacter (Enterobacter cloacae strain HMA). Marine samples were isolated and purified 12 streptomycetes from the Red Sea beach, Hurghada city, Red Sea governorate, Egypt, in different locations (marine water in the depth of ±3-5 m and sediment-water). We examined the antimicrobial activity of the purified actinomycetes acetate extracts and examined their potential to generate biologically active secondary compounds, or natural products (NPs), against biofilm-forming bacteria. Several isolates exhibited antimicrobial activity against 23 bacterial isolates but one isolate (H7) from the 12 streptomycetes isolates showed potent antimicrobial and was the most effective (82.8 %). While most crude isolates have a variable activity against 23 bacterial isolates. The antimicrobial isolate was of the genus Streptomyces (*Streptomyces* sp. strain HMA). The HMA strain was cultured on rice medium and then extracted with an ethyl acetate solution. Using flash column chromatography, the resulting extract was essentially separated into seven parts. Seven fractions obtained were biologically screened by evaluating their antimicrobial activity against *Enterobacter cloacae* strain HMA. The result of Antibacterial activity of Fractions Fr.1 (54.9 %) against Enterobacter cloacae strain HMA. Based on the antimicrobial results, the most active fragment (Fr.1) was structurally determined using GC-Ms.
Keywords: Actinomycetes, Streptomyces sp, antimicrobial activity, BioDWT, NPs.

1. Introduction

One of the greatest important supplies on the Planet is drinking water. Biochemical treatment technology is now able to meet the growing need to produce safe drinking water and high quality. Adopting this biological water treatment method raises many problems about its applicability, capability, dependability, and safety **[1]**.

However, there are several dangers to the sources of drinking water that should not be ignored. One is a reduction in the quantity and water quality available for human consumption due to human activity in industrial zones. Accordingly, a lot of micropollutants (i.e., drugs and pesticides) were discovered in raw and treated drinking water **[2]**.

The drinking water must be also cleaned of other important pollutants in addition to micropollutants, such as nitrogen compounds, heavy metals, and organic/inorganic substances.

With this technique, microorganisms are employed to yield biologically stable water and reduce contaminants in drinking water or accelerate biochemical oxidation **[3]**.

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Treatment of drinking water refers to any method used to improve the quality of water so that it can be used for a certain purpose.

By removing contaminants and unwanted substances or reducing the amount of them, the treatment of water aims to produce water suitable for the intended use.

Water treatment is necessary for the health of humans and can benefit from water and irrigation, both of which are necessary for their health.

The main reason for water contamination is untreated wastewater released by industries and companies. Wastewater from various companies is dumped in rivers and other bodies of water, containing varying levels of contaminants **[4]**.

Treated drinking water production, to generate water that is completely safe for consumption by humans without either the long- or short-term hazards of any negative effects on health includes removing pollutants from raw water and inactivating harmful bacteria.

Pathogenic bacteria, viruses, protozoa, and others can all be found in stools. Microbial pathogens must be eliminated or destroyed, which typically involves using chemically active substances such as dissolved matter to eliminate microorganisms such as algae, fungi, bacteria, viruses, and minerals such as manganese (Mn) and iron (Fe). The research team led by Professor Linda Lawton is working to improve bacteria identification **[5]**.

Due to a lack of access to effective water purification systems, many less-developed countries continue to suffer tremendously from these contaminants.

The actions performed to confirm water quality are the Purification of the water as well as the distribution of the water after treatment.

It is therefore standard procedure for keeping the remaining chemical disinfectants in the purified water to avoid and eliminate contamination by bacteria in the pipes during the distribution process and to maintain clean pipes **[6]**.

In conventional drinking water treatment, Physical and chemical techniques are typically used to pathogens eliminate, reduce turbidity, and control odor and taste problems.

Also, the amount of microbial pollutants in purified water for drinking can be reduced by these methods; however, removal is frequently insufficient. Activated carbon filtration, Reverse osmosis filtration, Nano-filtration, and Advanced oxidation are other techniques that are being studied. However, there are still concerns about the affordability and continuity of these procedures.

Recent research has discovered some microbes that are capable of biodegrading several regularly occurring and otherwise resistant micropollutants. Exploiting these new organisms' metabolic capacities could pave the way for the adoption of more effective and long-term procedures that haven’t received much thought up until now.

Natural products are organic compounds that don't directly affect an organism's normal processes of growth, development, or reproduction **[7]**.

Everything in our lives, including our ability to eat, dress, build structures, and more, depends on them. They also provide crucial defense against several diseases. The discovery of new active compounds has long time depended on natural resources.

Today, there are ten thousand antibiotics generated from microbial sources, recognized by the Fleming discovery results then continuous studies from recent **[8, 9, 10]**.

Marine environments have proven to be an excellent source of unique and novel natural products since they harbor organisms that occur nowhere else on Earth. Key features of the chemical diversity reported from marine organisms overlap with those of approved drugs, making marine NPs an important source for drug discovery **[11, 12]**. Marine drugs are becoming more prevalent; to date, 14 marine NPs or their derivatives are registered drugs, and another 23 are currently in clinical trials **[13, 14].**

Natural products, or **se**condary metabolites, are considered the most significant source of potential therapeutic leads **[15]**.

In addition, new antibiotics are desperately needed to fight life-threatening illnesses and the rise of bacteria resistant to antibiotics **[16, 17]**.

Nature remains the primary source of new antibiotics, even with notable advancements in the chemical synthesis and engineering biosynthesis of antimicrobial compounds **[18, 19].**

The success story continued in the "heroic" golden age of the 1940s and 1950s, which saw the discovery of nearly all important classes of anti-bacterial antibiotics (Cephalosporins, Tetracyclines, Aminoglycosides, and Macrolides). It appeared to researchers that the main issues with chemotherapy had been resolved at this time.

About 70 to 80 percent of all isolated biochemicals during this time were antibiotics, the majority of which were derived from Streptomyces species. These antibiotics were mostly effective against bacteria and fungi.

During this time, researchers were just beginning to identify antiviral, anticancer, and non-antibiotic enzyme inhibitory metabolites.

Streptomyces species' capacity to generate secondary metabolites has received considerable research interest from Scientists **[20, 21]**. Streptomyces strain clusters are often responsible for this capability because they are known for encoding

enzymes for a variety of secondary metabolic processes) **[22]**.

However, several alimentary variables and growth circumstances also have a significant effect on the generation of secondary metabolic products in microorganisms **[23]**.

The current study concentrated on isolating streptomycetes from marine and sediment-water obtained from various locations throughout Egypt and screening Streptomyces spp. for their capacity to produce antimicrobial compounds.

Many media, such as starch nitrate agar and starch casein agar have been proposed for the streptomycetes isolation **[24, 25]**.

When several carbon and nitrogen sources were examined shown that starch nitrate agar medium is the most effective at isolating streptomycetes. Streptomycetes are extracted from marine and sediment-water samples using a variety of media, including Glucose Asparagine, Starch Casein, Starch Peptone, ZoBell, and Grein-Meyer's.

Secondary metabolite synthesis may be enhanced and also patterns affected by changing the environmental conditions that influence strain growth, like changing the medium composition.

Several studies conducted by researchers showed that actinomycetes have exhibited obvious antimicrobial activities against almost all tested pathogenic strains **[26]**. Many actinomycetes were isolated and examined for their ability to generate antimicrobial compounds.

2. Experimental

2.1. Collecting Water samples from the stages of the drinking water treatment plant

Samples were taken for bacteriological examination from the stages of water treatment as follows: (Raw water, Sedimentation/Clarification, Filtration, and Disinfection) at Dakahlia Drinking Water Company, new Mansoura water plant in Mit-Khamis, Dakahlia governorate, Egypt **[27]**. Fourteen samples are taken from each stage of drinking water treatment; samples are taken in 250-500 mL sterilized glass containers. It is stored in the ice box at a temperature of 2-8 °C for a holding time of 6-8 hours.

2.2. Isolation and purification of bacterial isolates

Isolation is done by Serial dilution method by Dilution Water (buffered water). The sample is taken in a test tube and five other test tubes each containing 9 ml of sterile diluted water (buffered water). Add 1 ml of sample to 9 ml of buffered water. Next, using a serial dilution method until the ideal dilution is achieved, the final dilution of bacteria/cells is 10-5 (1 in 100,000) **[28]**. Isolation is done by Membrane filter technique By Using a membrane filtration device with membrane filter paper. Put membrane filter paper onto sterile Petri dishes (6 cm) containing R2A agar media by sterile forceps and beside the flame. Incubation of all samples at 35˚C ± 0.5 for 24 hours.

2.3. Collecting marine samples

Marine samples (marine water in a depth of ±3-5 m and sediment-water) were obtained from different locations along the Red Sea beach in Hurghada City. Samples were stored at 4°C waiting for further analysis after being aseptically transported to the laboratory in a dry, sterile, and insulated container.

2.4. Isolation and purification of streptomycetes

0 ml of sediment-water and marine water samples have been added into 90 ml of sterile saline (0.85% w/v aqueous NaCl solution).

To isolate streptomycetes, suspensions were plated using the serial dilution technique [28]. To remove the moisture film from the agar surface, Petri dishes were prepared the day before plating and incubated at 37 °C overnight [29].

A sterilized glass rod was used to disseminate 0.1 ml of the appropriate diluted inoculum on each Petri dish. Utilizing starch nitrate agar, streptomycetes were successfully isolated **[24]**. For marine samples (50%) seawater was used in the isolation medium.

2.5. Preparation of streptomycetes extracts

The streptomycetes isolate (H1-H12) was fermented on rice-solid media at a small volume for 7 days at 30 °C using 150 ml of 50% natural seawater and 100 grams of commercial rice.

Following incubation, each strain's culture medium was separately extracted using ethyl acetate, decanted, and filtered.

After being concentrated in vacuo, the organic extracts were applied for biological antibacterial activities.

2.6. Antimicrobial activity screening by disk diffusion method

To assess the antibacterial activity of the various extracts (H1–H12). Experiments took place on 96-well flat polystyrene plates were used for the tests. Following the addition of 10 µl of test extracts (final concentration 500µl/ml) to 80 µl of lysogeny broth (LB broth) and 10 µl of a suspension of an isolated bacterial culture (log phase), the plates were incubated for a whole night at 37 °C. Following incubation, the tested substance's effective antibacterial effects were seen as clearance in the wells, but substances with no such effects caused the growth media in the wells to appear opaque.

Untreated pathogens serve as the control. The absorbance was measured at OD600 after around 20

hours using a SPECTROstar Nano-Microplate Reader from BMG LABTECH's GmbH in Allmendgrun, Germany **[30, 31]**.

2.7. Identification of potent Streptomycetes isolate

Based on its physiological, morphological, and biochemical properties, the chosen Streptomyces species (H7), which has a greater antibacterial activity, was identified [32, 33, 34].

At the National Research Center in Egypt, transmission electron microscopy was conducted using (HR-TEM-2100, JEOL, and Japan).

2.8. 16S rRNA confirmation for the potent isolates

In a Polymerase Chain Reaction, the forward primer 5 AGAGTTTGATCMTGGCTCAG 3 and the reverse primer 3 TACGGTACGTACGTTGTTACGACTT 5 were utilized [35, 36, 37].

The sequencing was done using a large dye Terminator Cycle Sequencing Kit from US-based Applied BioSystems.

An automated DNA sequencing device from Applied Biosystems, model 3730XL (Applied BioSystems, USA), was used to analyze the sequencing findings.

The GenBank database received new information. Using the BLAST tool (https://www.ncbi.nlm.nih.gov/), the DNA sequence was compared to the GenBank database. Under the accession number OQ784267.1, The sequence of the 16S rRNA gene of the streptomyces isolate is available in the DDBJ/EMBL/GenBank nucleotide sequence databases. While, for bacterial isolate the accession codes were OQ179907.1.

2.9. Scale up Fermentation and Extraction of Bioactive Compounds

The selected Streptomyces strain (H7), which has a stronger antimicrobial activity, was cultivated on ISP-2 agar media plates for 3 days at 28 °C In a large-scale fermentation experiment to produce secondary metabolites. 100 grams of rice soaked in 100 mL in 1 liter Erlenmeyer was autoclaved and Inoculated with a portion of the Streptomyces strain's well-grown agar subculture.

Before being collected, the flasks were kept for 15 days at 28°C. With the use of ethyl acetate, secondary metabolites were extracted from the rice medium, and the crude extract was then obtained through filtration and rotary evaporation **[38]**.

2.10. Purification and Clarification of the Structure of the biologically active Compound

Flash column chromatography was initially used for purification. A normal-phase silica-filled 7 cm column received 3.8 grams of the resultant crude extract. The ratio of silica gel (Adsorbent) to the crude extract (Solute) is (20:1).

Thin-layer chromatography (TLC) was used to examine 115 fractions of 5 ml each and determine which fractions contained the chemicals of interest.

The GC/MS analysis was performed using Thermo Scientific's Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1 mm film thickness).

For GC/MS detection, helium gas was employed as the carrier gas in an electron ionization system with an ionization energy of 70 eV. The helium gas flow was kept constant at 1 mL/min.

Both the transfer line and MS injector temperature were fixed at a temperature of 280 °C.

The oven's temperature was programmed to increase from a starting temperature of 45 °C to 150 °C at a rate of 7 °C per minute (hold for 2 minutes). Then that to 270 °C at a rate of 5 °C per minute (hold for 2 minutes), and finally to 310 °C at a rate of 3.5 °C per minute (hold for 10 minutes) to reach the ultimate temperature.

Quantification of every component found was assessed using the percentage of the relative peak area.

Preliminary identification of the compounds was made based on a comparison of retention times and mass spectrum of the compounds as well as those in NIST and WILLY library information from the GC/MS instrument.

2.11. Physicochemical properties of ADME predicted in silico

SwissADME web resources were used to forecast the derived compound's ADME-related physical and chemical properties **[39]**.

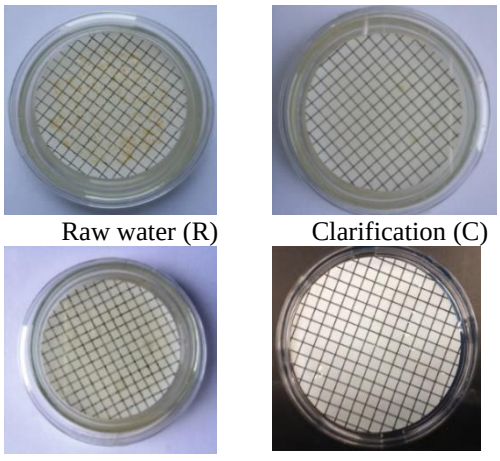
2.12. Prediction of toxicity in silico

In silico substance toxicity prediction was performed using the web server ProTox ii, According to recent research **[40, 41]**.

3. Results

3.1. Sampling, isolation, and Purification of the bacterial isolates

Twenty-three bacterial isolates were recovered from different stages of water treatment Raw water (14 isolates), Sedimentation & Clarification (8 isolates), Filtration (1 isolate), and Disinfection (0 isolate). All samples isolated from different stages of water treatment (Twenty-three bacterial isolates) were grown on R2A agar media by membrane filtration technique and Purification of all samples also grown on R2A agar media (Figure 1).



Raw water (R) Clarification (C)

Filtration (F) Disinfection (D)

Figure (1): Isolation stages of bacteria by Membrane filter technique with membrane filter paper on R2A agar media**.**

3.2. Isolation, fermentation, and extraction of streptomycetes

Marine samples have been collected from the Red Sea beach, Hurghada city in Egypt. Twelve streptomycetes were isolated from Hurghada. The obtained actinomycetes strains were grown on a rice media for small-scale fermentation to acquire the bioactive components. Using ethyl acetate, cultures were extracted. To employ the ethyl acetate phase for further study, it was evaporated until it was entirely dry.

3.3. Screening antimicrobial activity of different crude extracts

The isolated actinomycetes acetate extracts' antimicrobial activity was assessed. Several isolates exhibited antimicrobial activity against 23 bacterial isolates but one isolate (H7) from the 12 streptomycetes isolates showed potent antimicrobial and was the most effective. While most crude isolates have a variable activity against 23 bacterial isolates **(Table 1).**

3.4. Taxonomical study of the most potent isolates

Streptomycetes isolate (H7) has been identified with morphological, physiological, biochemical, and chemo-taxonomical characteristics. The strain was identified primarily by visualizing its spore morphology using TEM, and the obtained result showed that the strain has rectiflexibility spore chains with a smooth spore surface, according to spore chain morphology **(Figure 2)**. Tyrosine agar does not produce melanoid pigments in most cases. The spore mass is whitish and produces yellow diffusible pigments **(Table 2).** This strain's affiliation with the genus Streptomyces spp. and a neighboring joining-based phylogenetic tree supported the findings. The nucleotide sequence has been submitted to the sequence database at GenBank. *Streptomyces* sp. strain HMA is the name we gave it. The strain has been assigned the accession number OQ784267.1 in the GenBank database **(Figure 3)**. While the bacterial isolate belonged to the Enterobacter genus, it was recognized as *Enterobacter cloacae* strain HMA (Accession no. OQ179907.1) when entering its nucleotide sequence into the sequence database of the GenBank **(Figure 4)**.

Table (1): Antimicrobial activities of natural products derived from Streptomycetes isolates %

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12
R1	92.49	81.27	86.08	82.19	91.71	93.77	**87.05**	30.92	1.53	90.20	78.14	41.80
R2	78.11	81.05	79.97	88.38	85.81	91.96	**90.22**	32.64	47.04	87.63	78.69	61.04
R3	55.67	73.71	78.81	52.23	62.04	79.47	**86.93**	57.30	44.28	14.25	71.34	65.73
R4	42.78	54.27	0.00	48.91	29.04	62.27	**85.29**	48.48	44.81	74.20	54.06	49.14
R5	81.90	71.96	78.77	56.48	63.13	82.50	**58.42**	42.09	35.43	65.18	29.14	46.28
R6	72.67	65.39	67.94	60.16	35.27	84.32	**82.03**	30.39	8.53	76.40	71.77	50.44
R7	87.60	86.15	73.92	84.84	79.68	89.50	**86.68**	52.05	31.39	89.06	14.14	58.05
R8	0.00	48.79	29.24	81.32	0.00	82.43	**85.37**	34.80	36.21	77.74	41.10	45.73
R9	70.67	69.04	34.86	68.36	78.74	74.00	**75.33**	38.13	30.36	22.48	38.60	17.74
R10	89.54	65.83	53.51	86.99	87.98	88.57	**92.33**	55.66	42.71	64.39	61.67	23.61
R11	89.68	78.64	87.11	87.83	66.52	76.39	**94.54**	69.07	46.14	91.94	60.36	56.56
R12	86.28	76.07	71.80	59.09	21.68	69.30	**67.03**	52.86	6.86	88.77	14.30	49.10
R13	44.30	75.89	34.70	80.38	68.34	80.53	**92.49**	68.43	24.71	85.03	64.74	69.52
R14	27.99	58.48	70.69	77.95	50.69	70.10	**84.70**	63.16	53.78	89.28	29.50	64.52
C1	27.68	81.06	28.78	33.69	24.77	82.57	**53.33**	4.11	19.01	6.16	6.89	54.54
C2	70.89	64.68	39.16	68.90	69.73	76.21	**62.32**	58.53	47.53	67.23	43.48	62.93
C3	68.37	14.77	55.07	86.11	74.50	85.33	**90.70**	67.83	49.12	93.26	45.43	68.78
C4	91.16	6.28	14.68	79.77	84.38	78.63	**92.49**	52.65	45.67	89.97	46.89	34.02
C5	19.63	65.07	43.72	70.83	19.35	72.41	**91.83**	61.85	64.34	79.43	78.64	65.35
C6	86.14	0.00	0.00	8.62	82.47	80.31	**91.28**	51.72	5.97	85.52	6.00	32.65
C7	90.05	0.00	48.42	81.66	88.94	90.08	**94.24**	66.63	15.00	81.43	83.95	63.51
C8	84.76	0.00	0.00	79.11	46.94	75.26	**90.41**	59.23	20.19	89.61	62.45	53.61
F1	65.73	79.55	73.13	59.61	59.69	79.74	**69.39**	36.06	21.20	3.79	5.92	8.83
Average%	**66.27**	**56.43**	**50.02**	**68.84**	**59.63**	**80.25**	**82.80**	**49.33**	**32.25**	**70.13**	**47.27**	**49.72**

Table (2): Chemical and physiological taxonomic characteristics of the isolate H7

Isolate No.		**H7**
Production of Melanin pigment	Peptone iron agar	Positive (+)
	Tyrosine agar	Positive (+)
Activities of Enzyme	Proteolysis	Negative (-)
	Lipolysis	Negative (-)
	Lecithinase	Positive (+)
Utilizing various sources of carbon	No sugar (-)	Positive (+)
	D-Glucose (+)	Positive (+)
	D-Fructose	Positive (+)
	Sucrose	Negative (-)
	Rhamnose	Negative (-)
	D-Mannitol	Negative (-)
	D-Xylose	Positive (+)
	Raffinose	Negative (-)
	I-inositol	Negative (-)
	Galactose	Negative (-)
	L-Arabinose	Positive (+)
Nitrate reduction		Positive (+)
H2S production		Positive (+)
Starch hydrolysis		Positive (+)
Cellulose decomposition		Positive (+)
Gelatin liquification		Positive (+)

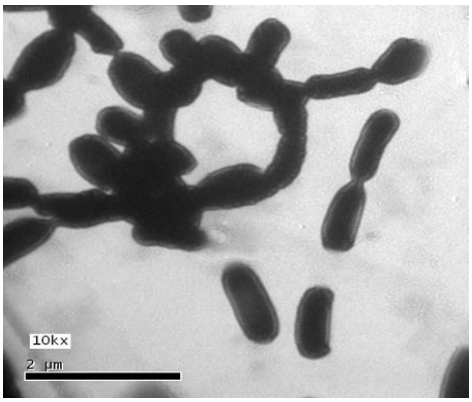


Figure (2): Transmission electron microscope of H7

3.5. Large-scale fermentation, fractionation, and antimicrobial activity of bioactive compounds from Streptomyces sp. strain HMA

On a rice medium, the Streptomyces sp strain HMA was grown and extracted by ethyl acetate, then the ethyl acetate evaporated, yielding 3.8 g of extract. Seven fractions were principally separated from the resultant extract using flash column chromatography. The biological screening of the 7 fractions obtained involved testing them against the Enterobacter cloacae strain HMA for antimicrobial activity (Table 3). Using GC-Ms, the most active fraction (Fr.1) was structurally identified based on the antimicrobial results.

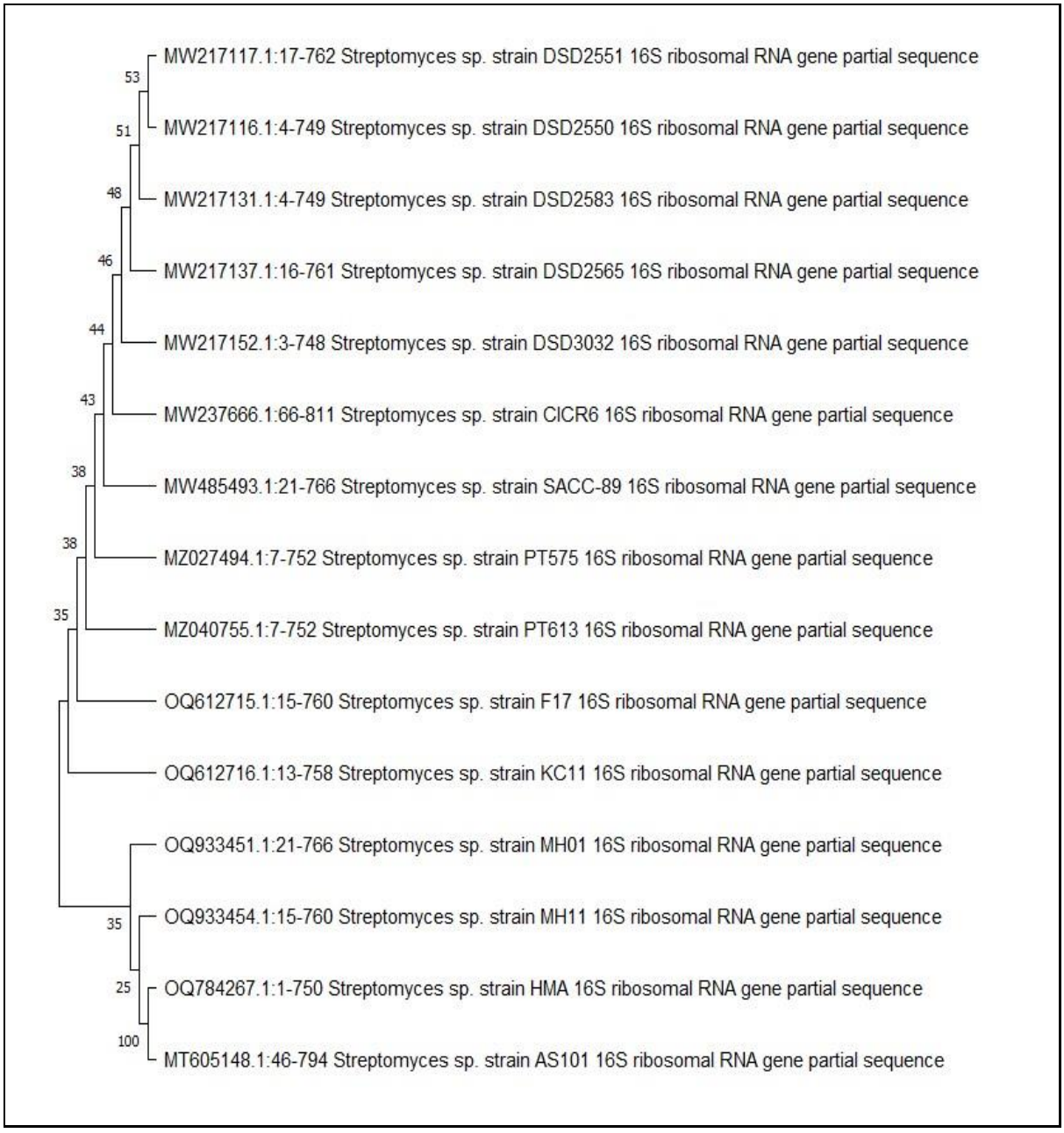


Figure (3): phylogenetic tree *of Streptomyces* sp. strain HMA, (Accession number OQ784267.1)

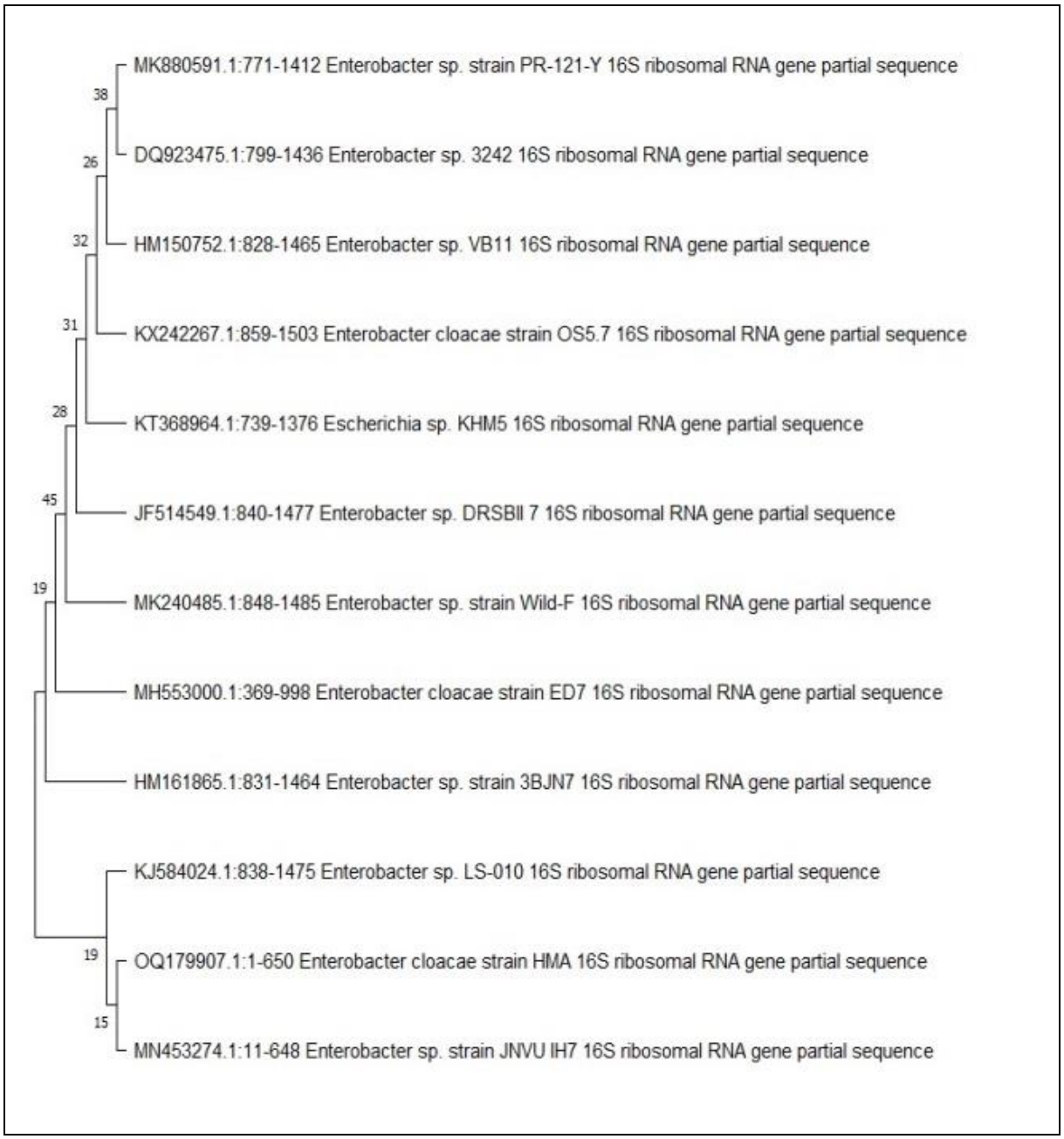


Figure (4): Phylogenetic tree of the *Enterobacter cloacae* strain HMA, (Accession number OQ179907.1)

Table (3): antibacterial activity of Fractions F1 (%) against *Enterobacter cloacae* strain HMA

Inhibition (%)						
F1	F2	F3	F4	F5	F6	F7
54.9	**10.5**	**19.5**	**25.8**	**22.4**	**19.7**	**32.6**

3.6. Metabolomic profiles of the Streptomyces sp. strain HMA

Gas chromatography-mass spectrometry (GC-MS) analysis played a pivotal role in the comprehensive examination of the chemical constituents found within the Streptomyces sp. strain HMA cultures during the fermentation process. This analytical technique enabled a detailed exploration of the chemical landscape within the axenic culture of *Streptomyces* sp. strain HMA, resulting in the identification of various compounds. The collective total peak areas, as visualized in (Figure 5), underscore the significance of these constituents, accounting for a substantial 54.9 % of the overall chemical composition (Table 4), meticulously cataloged the chemical structures of these discerned compounds, providing a valuable reference for their characterization and further investigation. Among the identified compounds, several stand out as the major constituents of interest. Chief among them is (Methylsulfonyl)dichloromethylsulfinylchloride, constituting 2.89% of the total chemical makeup.

Additionally, 2,7,12,17-tetrabromcyclotetrathiophen, a rather rare compound, was observed at a concentration of 0.5%. In particular, 7-Bromo-1-methyl-1,2,3,4-tetrahydrobenzo [e] [1,4] diazepine-5-one was a prominent substance, accounting for a significant 7.27% of the total chemical profile. The utilization of GC-MS allowed for a thorough and precise examination of the chemical diversity present within the Streptomyces sp. strain HMA culture, leading to the identification and quantification of these noteworthy compounds, with particular attention given to the interesting 7-Bromo-1-methyl-1,2,3,4-tetrahydrobenzo [e] [1,4] diazepine-5-one.

Table (4): Streptomyces sp. strain HMA

No	RT	Area %	Molecular Weight	Molecular Formula	Identified compounds
1	5. 42	0.05	640	C16H4Br4S4	2,7,12,17-Tetrabromo-(all-às)Cyclotetrathiophen (2,7,12,17-TetrabromCycloocta[1,2-b:4,3-b':5,6-b":8,7-b"']Tetrathiophen
2	5.51	0.04	100	C7H16	Pentane, 2,4-dimethyl-(CAS)
3	5.87	0.03	188	C8H16N2O3	N-dl-Leucylglycine
4	6.49	7.27	254	C10H11BrN2O	7-Bromo-1-Methyl-1,2,3,4-TetraHydroBenzo[e][1,4]Diazepin-5-one
5	6.73	6.25	78	C2H6OS	Methane, Sulfinylbis-(CAS)
6	6.96	0.01	58	C3H6O	Acetone-O
7	7.12	0.71	78	C6H6	Benzene (CAS)
8	7.22	0.11	190	C9H6N2O3	4-Formyl-3-(phenyl)Sydnone
9	7.31	0.84	169	C3H8NO5P	N-(Phosphonomethyl)Glycine
10	7.43	1.84	76	CH4N2S	di(15)N-Thiourea
11	7.52	2.25	182	C4H10N2O4S	(N-(-2-Acetamido))-2-aminoethanesulfonic acid
12	7.66	8.32	136	C4H3D6Br	SEC-BromoButane-1,1,1,2,3,3-D6
13	10.59	0.31	223	C10H9NO3S	N-[(2'-Thiapropyl)oxy]Phthalimide
14	7.88	0.46	78	C2H6OS	Methane, Sulfinylbis-(CAS)
15	8.03	2.89	691	C2H3Cl3O3S2	(Methylsulfonyl) DichloroMethylSulfinylchloride
16	8.30	17.04	169	C3H8NO5P	N-(Phosphonomethyl)Glycine
17	8.73	0.05	198	C14H14O	9-Oxa-Cis(1,8),Cis(2,7),Trans(7,8)-Tricyclo[6.3.0, 0(2,7)]Undeca-3,5,10-Triene
18	9.22	0.10	118	C5H10O3	EthylMethoxyAcetate
19	9.02	0.70	313	C14H23NO5Si	N-Acetyl-4-Trimethylsilyl-2,3-Dicarbomethoxy-1,2,3,4 Tetrahydropyridine
20	9.35	0.13	144	C6H8O2S	3-Thiabicyclo[3.2.0]Hept-6-ene3,3-Dioxide
21	9.49	0.40	123	C3H6ClNO2	á-Chloro-D-alanine
		T% 49.8			

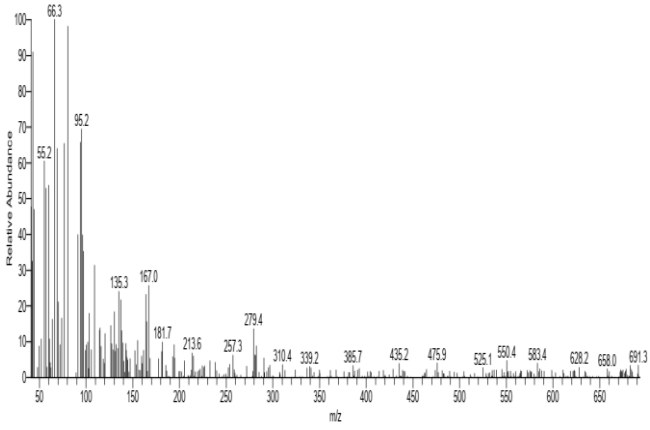


Figure (5): GC-chromatogram of the *Streptomyces* sp. strain HMA crude extract

3.7. The physicochemical characteristics of ADME

The most important substance, 7-Bromo-1-methyl-1,2,3,4-tetrahydrobenzo [e] [1,4] diazepin-5-one, underwent an in-depth assessment of its physicochemical characteristics related to ADME. This evaluation was carried out employing the SwissADME online server, as detailed by Daina et al. 2017 [40]. The scrutiny centered on characterizing the purified compound by established drug-likeness criteria.

In adherence to these criteria, the compound successfully navigated Lipinski's rules, with only a single deviation concerning Ghose's rule (specifically, with atoms < 20). Notably, C2 exhibited compliance with the Egan, Veber, and Muegge rules, boasting an absence of any violations. These findings point to a promising oral medication potential, with an estimated oral bioavailability of 0.55%, as delineated in (Table 5).

To speed up the assessment of drug similarity, and the biological effectiveness of both compounds, a radar plot was created that included six important physicochemical factors: solubility, polarity, size, flexibility, lipophilicity, and saturation. For every parameter, the ideal desired value range is shown by the pink area of the plot. Interestingly, all parameters save for instauration was within the compound's optimum range, as illustrated in (Figure 6).

Another critical physicochemical factor is Lipophilicity, signifying the compound's permeability across cell membranes, a pivotal aspect in drug absorption. Here, the compound displayed a favorable log Po/w value of 1.56, indicative of robust permeability and absorption across cell membranes. Moreover, solubility emerged as a pivotal parameter profoundly influencing compound absorption during the formulation process. Utilizing the ESOL topological model, the compound was found to possess a moderate degree of solubility.

For the stringent evaluation of medicinal chemistry and Lead likeness, the compound successfully adhered to the Rule of Three (RO3). As for the assessment of synthetic accessibility score (SA score), which is calculated using component likeness and complication actions, the compounds showed a medium degree of synthetic availability, with a value of 2.03, highlighting their potential for use in drug development projects. According to Daina et al. 2017 [39], the vector machine algorithm (SVM) model was used to determine the compound's pharmacokinetic parameters, which are shown in Table 5. It is worth noting that Compound C1 exhibited a distinct pattern of inhibitory activity, specifically targeting the CYP1A2 isoenzyme.

In Figure 7, we introduce the BOILED-Egg model, which compares WLOGP (the partition

coefficient's logarithm) with TPSA (the topological polar surface area), a concept that was inspired by Daina et al.'s work from 2017. This model provides valuable insights into drug permeation, especially through the brain or intestinal membranes. Remarkably, our compounds displayed a remarkable level of absorption within the human gastrointestinal tract (GI), signifying their potential as efficacious oral agents. Furthermore, these compounds proved to be non-P-gp substrates, as indicated by the presence of red dots, and exhibited permeation across the blood-brain barrier (BBB), with TPSA values falling below 75 Å2. This suggests the possibility of these compounds influencing the central nervous system (CNS), an important aspect highlighted by Daina and Zoete in 2016 [42]. According to the methodology provided by Potts and Guy, 1992 [43], the skin permeability coefficient evaluation (Kp) for these compounds was performed.

Notably, the compound displayed a log (Kp) value of -6.93 cm/s. It's worth mentioning that a more negative log Kp indicates lower skin permeability for the compounds, underlining a distinctive characteristic of their skin interaction properties.

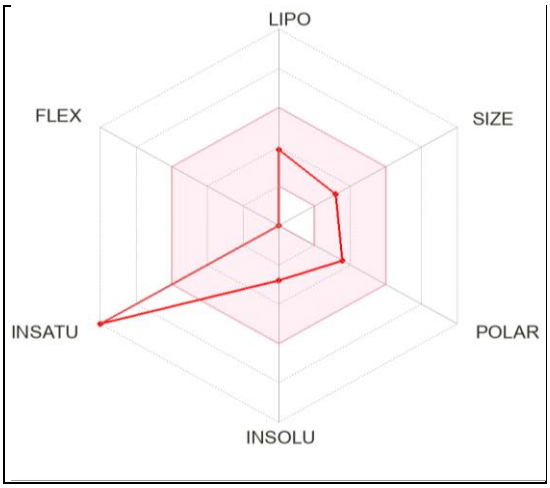


Figure (6): Bioavailability The obtained compound's radar plot.

The pink region indicates the ideal range for each characteristic. [Saturation: fraction of carbons in the sp3 hybridization not less than 0.25, Solubility: log S not greater than 6, Flexibility: no more than 9 rotatable bonds, Polarity: TPSA between 20 and 130 Å2, Size: MW between 150 and 500 g/mol, and Lipophilicity: XLOGP3 between 0.7 and +5.0].

Log S represents the logarithm of molar solubility in water, while Log Po/w signifies the partition coefficient between n-octanol and water. The Lipinski (RO5) criteria encompass the following ranges: Lipophilicity (log Po/w) should be ≤ 5, molecular weight (MW) should be ≤ 500, hydrogen bond donors should be ≤ 5, and hydrogen bond acceptors should be ≤ 10. The Ghose filter criteria involve a log Po/w range between -0.4 and +5.6, a molecular refractivity (MR) range between 40 and 130, a MW range between 180 and 480, and the number of atoms ranging from 20 to 70. The Veber rule criteria specify that the number of rotatable bonds (RB) should be ≤ 10, and the topological polar surface area (TPSA) should be ≤ 140 Å². The RO3 requirements dictate that XLOGP3 should be ≤ 3.5, MW should be ≤ 350, hydrogen bond donors should be ≤ 3, hydrogen bond acceptors should be ≤ 3, and RB should be ≤ 3. Synthetic accessibility (SA) scores range from 1 (very easy) to 10 (very difficult). GI (HIA) assesses human gastrointestinal absorption, BBB evaluates blood-brain barrier penetration, P-gp measures permeability glycoprotein, and log Kp pertains to the skin permeability coefficient.

Table (5): ADME-related physical-chemical characteristics of the compound that were obtained

Predictive model attributes		Isolated compound
Chemical and Physical Properties	M.Wt	253.05
	Csp3 Fraction	0.00
	Rotatable Bonds	0
	Hydrogen bond acceptor sites	3
	Hydrogen bond donor sites	1
	Refraction properties per mole	56.33
	TPSA	62.82 Å²
Ability to dissolve	log *S* (ESOL)	− 2.46
	Solubility	9.69e-03 mg / ml ; 3.83e-05 mol /l
	type	Moderately Soluble
Lipophilicity	log $P_{o/w}$ (XLOGP3)	1.28
	log $P_{o/w}$ (WLOGP)	1.05
	log $P_{o/w}$ (MLOGP)	1.60
Lead likeness	Rule of three (RO3)	Yes
	Synthetic Accessibility	2.56
Pharmaceutical suitability	Lipinski (RO5)	Yes
	Ghose	One detected violation: # atoms < 20
	Veber	Yes
	Bioavailability Assessment	0.55
Pharmacokinetic Parameters	Gastrointestinal (HIA) uptake	High
	Blood-Brain Barrier Penetrator	Yes
	Permeability Glycoprotein Substrate	ND
	Substance that impedes CYP1A2 activity	Yes
	Substance that impedes CYP2C19 activity	ND
	Substance that impedes CYP2C9 activity	ND
	Substance that impedes CYP2D6 activity	ND
	Substance that impedes CYP3A4 activity	ND
	log K_p (Skin Permeation: cm /s)	-6.93 cm / s

The yellow area, denoting the yolk, indicates a high likelihood of BBB permeability, whereas the white segment, symbolizing GI, suggests a strong potential for HIA (GI) absorption. The outer gray zone encompasses compounds with limited or negligible ability to penetrate the brain. Furthermore, in the online color illustration, the dots are distinguished by a blue shade to represent anticipated P-gp substrates (PGP +), and a red hue signifies anticipated non-substrates for P-glycoprotein (PGP).

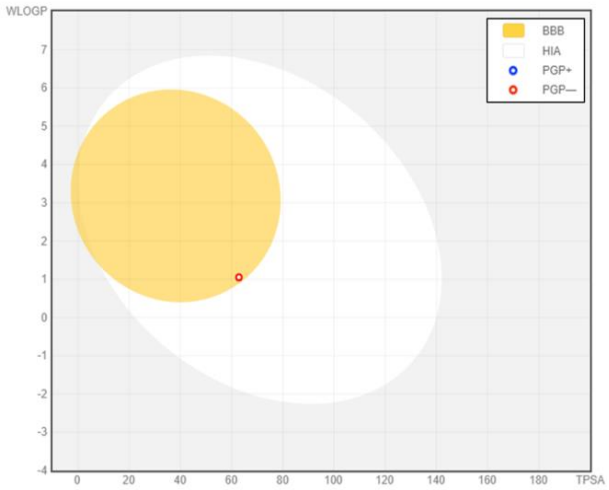


Figure (7): BOILED-EGG plot for C1 and C2, respectively:

3.8. Predicting Toxicity

To assess the potential toxicity of the compounds we obtained, we employed the ProTox II web server **[40]**. The outcome of this toxicity evaluation is meticulously documented in Table 6, revealing intriguing insights into the safety profile of these compounds.

Specifically, the analysis indicates that the obtained compound exhibited a notably low level of toxicity. This assessment encompasses various aspects of toxicity, offering a comprehensive understanding of the compound's safety profile, which is paramount for further considerations in medicinal and pharmaceutical applications.

4. Discussion

Safe water is essential for people, animals, and plants. Water is necessary for humans to break down large compounds into smaller ones and transfer them to different parts of the body as part of the metabolic process.

Toxic substances must be removed from water before they can be used for human consumption. Similar to how people become exposed to risks when they drink polluted water, animals and plants also become exposed to risks if they consume it.

The agricultural sector, illegal waste disposal, industrial effluent discharge, and sewage are a few of the major causes of water contamination [44]. Although the majority of industrial companies possess plants for sewage treatment, if the wastewater is released daily, the amount of contaminants will grow with time.

Only conventional treatment techniques, such as screening, flocculation, coagulation, Sedimentation/Clarification, filtration, and chlorination or fluoridation, are employed in most DWTPs worldwide. These treatment methods cannot effectively remove all contaminants, including nitrogen compounds, organic carbon, heavy metals, and microorganisms in high concentrations.

Table (6): Prediction of the obtained compound's toxicity using in silico analysis

Target		Toxicity Predictions
liver toxicity		NT
Cancer induction		NT
Effects on the immune system		NT
The ability to cause genetic mutation		ND
Cellular Signalling Pathways Involving Nuclear Receptors in the Tox21 Framework	AhR	NT
	AR	NT
	AR-LBD	NT
	Aromatase	NT
	ER	NT
	ER-LBD	NT
	PPAR-Gamma	NT
Stress response pathways within the Tox21 context	Nuclear-related factor (Erythroid-derived 2)-like 2(Nrf2)/Antioxidant Responsive Element (ARE)	NT
	HSE	NT
	MMP	NT
	Phosphoprotein (Tumour Suppressor) P53	NT
	ATAD5	NT

NA: No toxicity, ND: Not detected, AhR: (Aryl hydrocarbon Receptor), AR: (Androgen Receptor), AR-LBD: (Androgen Receptor Ligand Binding Domain), ER: (Estrogen Receptor), ER-LBD: (Estrogen Receptor Ligand Binding Domain), PPAR-Gamma: (Peroxisome Proliferator-Activated Receptor Gamma), HSE: (Heat Shock factor response Element), MMP: (Mitochondrial Membrane Potential), ATAD5: (ATPase family AAA domain-containing protein 5).

The biological treatment was first introduced in the early 20th century [45], but it was not utilized for the purification of drinking water. Although biological drinking water treatment (BioDWT) has been used since the 1800s [3], its application is still very limited on an international level. There have only been a few studies conducted in developing nations, compared to some developed nations like the United States which have recently used BioDWT for clean and safe drinking water treatment.

Natural products are organic substances that do not directly contribute to an organism's typical processes of growth, development, or reproduction **[7]**. They are crucial to everything in our lives, including food,

clothing, structures, and more. In addition, they offer important defense against several ailments. Natural resources have played a significant role in the creation of novel active compounds for a long time.

During this period, antibiotics largely effective against bacteria and fungi were found in 70 to 80 percent of all identified compounds, most of which were derived from Streptomyces species. Research on the discovery of antiviral, anticancer, and non-antibiotic enzyme-inhibitory metabolites had just started during this period [8].

Researchers have been especially interested in Streptomyces species because of their capacity to produce secondary metabolites [46]. This capacity is mainly due to the presence of Streptomyces strain clusters, which encode enzymes for numerous secondary metabolic processes [47]. However, several nutritional factors and growth conditions are also very potent regulators of secondary metabolite production in microorganisms [23].

Our study aims to determine the ability of marine microbial isolates to yield biologically active secondary compounds that can be used for the biological treatment of drinking water by inhibiting biofilm-forming bacteria from the drinking water treatment plant of the Nile River in Egypt. Several studies conducted by researchers showed that actinomycetes have exhibited obvious antimicrobial activities against almost all tested pathogenic strains. [26] has isolated many actinomycetes isolates and screened their ability to produce antimicrobial compounds. One of these strains is marine Streptomyces misakiensis, which showed high antimicrobial activities. It had been reported that this strain produced many bioactive compounds such as mikamycin; a mammalian cell growth-stimulating substance [48]. BQ-153 and B150-SIGMA) [49, 50], and some other compounds which have an anticancer activity such as BE-18257A and BE-18257B [51]. In his work also, the marine Streptomyces aminophilus exhibited activities against almost all test organisms and high activity against Aspergillus niger. Our study, employs a starch-nitrate agar medium to isolate actinomycetes, following the methodology illustrated by [52, 53, 54].

Also, all of the isolates were tested for their potential to create bioactive compounds. According to the International Streptomycete Project (ISP) (1964) and the keys of Nonomura (1974), Szabo et al. (1975), or Bergey (1989) [55, 34, 56], based on morphological, biochemical characteristics, and chemical taxonomical methods, the most effective isolate was discovered to belong to Streptomyces sp.

The streptomycetes isolate required a small-scale fermentation on rice solid media.

After incubation, ethyl acetate was used to extract each strain's culture media, decanted, and put through filtering.

The antibacterial activity of the different extracts was evaluated using the disk diffusion method in 96-well flat polystyrene plates **[30]**.

The isolated actinomycetes acetate extracts were tested for their antimicrobial activity. Several isolates exhibited antimicrobial activity against 23 bacterial isolates, but one isolate streptomyces sp strain HMA (H7) from the 12 streptomycetes isolates showed potent antimicrobial activity and was the most effective. The majority of crude isolates have variable activity against 23 bacterial isolates.

The most potent isolate, Streptomyces isolate (H7), which showed a high level of antimicrobial activity in an initial evaluation, was sequenced and its 16S rDNA was analyzed. This process starts with DNA isolation [35], and amplifying the 16S rRNA gene using the polymerase chain reaction [36]. The fragments of DNA are sequenced directly. A DNA sequencer is used to carry out the sequencing procedures to confirm the base arrangement along the sample's length [37]. Using blast search algorithm and alignment of these sequences through matching with reported 16S rRNA gene sequences in GenBank, the obtained data by GenBank, the assignment of the (H7) isolate was proposed as a new species related to genus Streptomyces sp. and deposited in GenBank as Streptomyces sp. HMA under accession no. (OQ784267.1). The accession number for the bacterial isolate was OQ179907.1.

The bioactive components of Streptomyces sp strain HMA (H7) were cultivated on rice medium, large-scale fermentation, fractionated, and extracted with ethyl acetate to determine their antibacterial activity. Next, the evaporation of ethyl acetate was followed by the evaporation of the resultant extract. The obtained extract was primarily fractionated from the selected isolate using Flash column chromatography into (7) fractions. All fractions were chemically screened using TLC. The obtained 7 fractions were biologically screened against Enterobacter cloacae strain HMA to evaluate their antimicrobial activity (Table 3). Based on the antimicrobial results, the most active fraction (Fr.1) was structurally identified using GC-Ms. In their study, pathogenic bacterial biofilm is considered an important factor for bacterial virulence and is a primary virulence factor for a variety of bacteria that cause persistent illnesses. Researchers found that there is an extracellular polymeric material (EPS) that surrounds biofilms, which are highly complex structures made of polysaccharides, proteins, and DNA. Because biofilms are so intricate, bacteria tend to stick to damaged medical devices and cause persistent illnesses. Due to the biofilm formation's acidic pH and decreased oxygen content, which limit the antibiotics' ability to diffuse and penetrate, EPS is critical in the fight against antibiotics. All of these factors are in opposition to the effects of antibiotics. The presence of a biofilm containing over 80% of the bacterial biomass caused the bacteria to become

multi-drug resistant (MDR). Biofilms are formed by both gram-positive and gram-negative bacteria **[57]**. In our study, the obtained pure compounds from Streptomyces sp HMA (H7), showed antimicrobial activity against Enterobacter Cloaca HMA biofilms with an inhibition ratio of 69.3% and other types of bacteria.

5. Conclusion

Actinomycetes produce potent antibacterial compounds that help in the development of antibiotics and antimicrobial agents. Their bioactive metabolites are essential for fighting bacterial infections and treating drug resistance.

The results demonstrate the diversity of antimicrobial activity against different drinking water-borne bacterial isolates by displaying a wide range of inhibitory effects, from high to low inhibition and changing inhibition ratios. This indicates that the antimicrobial agent Streptomyces sp HMA (H7) showed effectiveness as a potent antibacterial agent by showing a strong inhibitory effect against a variety of bacterial isolates, including Enterobacter Cloaca HMA biofilms, from the filtration stage of drinking water treatment. These results indicate a promising avenue for developing antimicrobial agents or therapeutics with potential applications in various fields, including pharmaceuticals and drinking water treatment. Further research is warranted to identify the specific compounds or conditions responsible for these inhibitory effects and to explore their broader practical

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