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Bioactive secondary metabolite from marine *Streptomyces parvulus* strain MDA with potential antibacterial activity

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Abstract

The finding as well as the subsequent medical history of antimicrobials has become one of the most significant game modifications in medical history. As a result, a natural substance of 2-Methyl butyl propyl phthalate was extracted from a marine-derived *Streptomyces parvulus* strain MDA remoted originating along the Red Sea coast; Hurghada. The elimination of *Streptomyces parvulus* strain MDA via 2-Methyl butyl propyl phthalate became an exciting topic in our study; locating was tested using a minimal bactericidal concentration (MBC) of 300 µg/ml and an 80 µg/ml minimum inhibitory concentration (MIC). The kinetic time killer graph illustrated that the microorganisms damaged cells in 5 hours. The fractionation and purification of 2-Methyl butyl propyl phthalate were completed by the use of silica gel column G254, thin layer chromatography (TLC), liquid chromatography (LC), and nuclear magnetic resonance (NMR), which helped in the elucidation of the structure of 2-Methyl butyl propyl phthalate with the molecular components of $C_{16}H_{22}O_4$.

Keywords: *Streptomyces parvulus*, Bioactive metabolite, Antibacterial**,** 2-Methyl butyl propyl phthalate

1. Introduction

Actinomycetes are a kind of gram-positive filamentous bacteria that have a complex life cycle. They are members of the phylum Actinobacteria, which is one of the 18 main phyla recognized in the study of bacteria and one of the vast taxonomic groupings. The actinomycetes share characteristics with the bacterial group in terms of their internal structure and cell wall, which connects bacteria and fungus and has characteristics with both [1].

Actinobacteria exhibit several characteristics from a morphological perspective. In fact, we can identify microorganisms with a coccoid or rod-coccoid-shaped species from the suborder of *Micrococcineae*, or with a fragmenting hyphal form from the Corynebacterineae suborder (including *Nocardia* spp.), or with a persistent, highly differentiated branched mycelium from the *Streptomyces* spp.

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Actinomycetes exhibit decomposing behaviors and break down organic materials including chitin, complex polysaccharides, hemicellulose, etc. They generate 1000 bioactive secondary metabolites, or 45% of all bioactive microbial metabolites known to science [2], [3]. Powerful antibiotics are among these bioactive substances. In 1928, Fleming and colleagues developed penicillin, the first natural antibiotic. Upon their return from vacation, the scientists discovered one of their Petri dishes that contained a *Staphylococcus* colony had been infected with a mold that was subsequently determined to be *Penicillium notatum*. Fleming was observant enough to think the incident was extraordinary while being startled that no germs were able to develop around the mold. Many of the best-known antibiotics, including amphotericin, neomycin, novobiocin, chloramphenicol, and tetracycline, are produced by members of the actinomycetes. *Mycobacterium*, *Corynebacterium*, *Streptomyces*, and *Actinomyces* are a few of their members. Actinomycete cells have a significantly smaller diameter (1-2 m) than branching fungi (between 5 m and 10 m) [1]. Bioactive metabolites are byproducts of primary and secondary metabolism in various organisms (plants, animals, fungi and bacteria). They frequently show signs of biological activity [4]**.**

Secondary metabolites come in a variety of active compounds and, in many cases, have a low molecular weight mass [5], [6]**.** Bioactive compounds, as opposed to primary metabolites, are used to identify specific groups of organisms and have no role in the cell's life cycle. Optimizing nutrient needs and culture conditions may have an impact on the production of secondary metabolites from the species *Streptomyces*. These conditions are vital to the creation of secondary metabolites [7]. Since then, research in this area has been conducted by academic institutions and pharmaceutical businesses, leading to the discovery of various novel bioactive compounds. The discovery of the best-known and significant classes of antibiotics marked the beginning of the antibiotic era, which had its "golden period" during the 1950s and 1970s [1]. Different types of fungi and bacteria may naturally create these bioactive chemicals, but Actinobacteria, and in particular *Actinomycetes*, are the most alluring group of microorganisms capable of doing so. Due to their capacity to generate several kinds of antibiotics concerning the chemical structure and modes of action, this order is significant. Antibiotic resistance in a variety of pathogenic bacteria necessitates the development of contemporary antibiotics to overcome these bacteria. Secondary metabolites produced by bacteria keep going to pique the interest of researchers because of their complex active compounds and extremely specialized antimicrobial properties. Soil microbes of the genus Streptomyces have a high concentration of bioactive natural products and are frequently used as antimicrobial agents. Streptomyces species produce approximately 75% of the beneficial antibiotics [8].

The medical literature employs diverse concepts of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan drug-resistant (PDR) bacteria to categorize several types of antibiotic-resistant microbes that cause issues to healthcare services. The European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) collaborated to establish standardized terminology for characterizing a variety of bacterial species, including *Staphylococcus aureus*, *Enterococcus* spp., *Pseudomonas aeruginosa*, and *Acinetobacter* spp., have evolved resistance profiles [9], [10].

2. Materials and Methods

2.1. Collection of marine samples

Along the Red Sea shore including, Hurghada, marine samples were collected (marine water at depths of ±3-5 m and sediment water). The samples were sent aseptically to the lab in a dry, enclosed box, and they were maintained there at 4°C pending additional laboratory evaluation.

2.2. Isolation and purification of Streptomycetes

Suspensions were plated for Streptomycetes isolation using the serial dilution method described by Hayakawa and Nonomura [11]. The day before plating, agar plates were planned and incubated overnight at 37 °C to remove moisture films on the surface of the agar [12]. Each plate received 0.1 ml of the appropriate dilution and was scattered with a sterile glass rod. Streptomycetes were isolated using starch nitrate agar [13]. The isolation medium for the marine water sample (50%) was seawater.

2.3. Preparation of streptomycetes extracts

Streptomycetes isolates (H1-H16) were fermented using a rice-solid medium for 7 days at 30°C (100 g commercial rice and 150 ml of 50% natural seawater). After cells were incubated, ethyl acetate was used to extract the culture media from each strain, which was then decantation and filtered. The organic extracts were concentrated in a vacuum.

2.4. Antimicrobial activity of the streptomycetes extracts

To investigate the potential antibacterial effect of different extracts (H1-H16). Experiments were conducted in 96-well flat polystyrene plates. 10µl of sample extracts (final concentration of 500 g/ml) were introduced to 80 µl of lysogeny broth (LB broth), followed by 10 µl of isolated bacterial culture suspension (log phase) and incubated overnight at 37 °C. Bacterial isolates of *pseudomonas aeruginosa* (B1-B12) were collected from the Microbiology and Immunology Department at Benha University Hospital which was recognized using VITEK2 system version: 0701 **(table 1)**. This effective antimicrobial of the isolated samples showed up as clear in the wells, after incubation, whereas compounds that did not affect the bacteria caused the growth media in the wells to become opaque. As a control, the pathogen was not treated. In a Spectrostar Nano Microplate Reader (BMG LABTECH GmbH, Allmendgrun, Germany), the absorbance was measured after around 20 hours at OD600 in the form of a mean standard deviation (± SD) [14]**.**

2.5. Identification of the most potent Streptomycetes isolate

Based on its morphological, physiological, and biochemical characteristics, the chosen Streptomyces strain (H2), which has a greater antibacterial activity, was identified [15]–[17]. At the National Research Center in Egypt, transmission electron microscopy (HR-TEM-2100, JEOL, Japan) was carried out. Spore surface ornamentation was investigated using transmission electron microscope (Philips 10 Technai with wave length (λ) of 0.0251 and an accelerating voltage of around 180 keV) at magnifications of 10.000 to 80.000X, and the spore surface ornamentation was classed as (smooth, warty, spiny, or hairy).

2.6. Confirmation by 16srRNA

Genomic DNA extraction, PCR-mediated amplification of the 16S ribosomal DNA, purification of PCR products, and sequencing of the PCR products were performed. The forward primer, 5' AGAGTTTGATCMTGGCTCAG 3', and the reverse primer, 3' TACGGTACGTTGTTACGACTT 5', were used in a Polymerase Chain Reaction [18]. In 50 µL of polymerase buffer, 50 µL of each primer, 10 µg of chromosomal DNA, 200 M dNTPs, and 2.5 units of Taq polymerase were mixed. The PCR was run for 30 cycles at 94 ºC for 1 minute. A big dye Terminator Cycle Sequencing Kit was used for the sequencing. (Applied BioSystems, USA). Sequencing results were resolved on an automated DNA sequencing system from Applied Biosystems, model 3730XL (Applied BioSystems, USA). Data was added to the GenBank database. Using the BLAST tool, the DNA sequence was compared to the GenBank database (https://www.ncbi.nlm.nih.gov/). The DDBJ/EMBL/GenBank nucleotide sequence databases contain the 16S rRNA gene sequences of the bacteria under the accession codes OQ179632.1.

2.7. Scale up Fermentation and Extraction of Bioactive substances

The selected *Streptomyces* strain (H2), which has a stronger antimicrobial activity, was cultured on ISP2 agar plates at 28 °C for 3 days in a scale-up fermentation experiment to produce secondary metabolites. Grams of rice were autoclaved and inoculated with a portion of the Streptomyces strain's well-grown agar subculture in 1 liter Erlenmeyer. Before being collected, the flasks were kept at 28°C for 15 days. To obtain the crude extract, secondary metabolites were extracted from rice medium using ethyl acetate, followed by filtration and evaporation using a rotary evaporator.

2.8. purification and Structure elucidation of the bioactive compound

The resultant extract was subjected to silica gel G254 extract column chromatography, and the drops at the bottom of the column were collected and fractions investigated via thin layer chromatography (TLC). The collected fractions' antibacterial efficacy was assessed. The greatest active components against bacteria were given top priority, and the highest-yielding antibacterial fraction was extracted further on a Sephadex LH-20 column. Using preparative TLC and the bioactivity of the Sephadex LH-20 column fractions, the subfraction with the highest antibacterial properties underwent extra purification. LC-Mass spectrometry (MS), which analyzes the mass-to-charge ratio (*m/z*) of charged particles, was used for the structural elucidation. (ions). Finally, the pure compounds' rapid structural elucidation was accomplished using Nuclear Magnetic Resonance (NMR). The Center for Drug Discovery Research and Development at the Faculty of Pharmacy at Ain Shams University conducted this investigation.

2.9. Calculating the MIC and MBC (Minimum Inhibitory and Bactericidal Concentrations)

MIC, or minimum inhibitory concentration, is measured by preparing 5 ml of nutrient broth medium that can be sterilized in test tubes to confirm the MIC of a bacterial secondary metabolite. Each

tube was then filled with 0.1 ml of bacterial culture and varying amounts of pure isolate that had been cultivated for 24 hours in a rotary shaker at 150 rpm and 37 °C. Microbial development was calculated at 620 nm and the results were displayed as a percentage of the inhibitory zone. whereas the only component of the control sample is bacterial culture. By sub-culturing from plates having concentrations equal to or more significant than the MIC on fresh broth culture, the minimum bactericidal concentration was found (MBC). The tubes were placed in an incubator at 37 °C for 18–24 hours. The various tubes were subsequently distributed on fresh nutrient agar plates, then incubated at 37 °C for a further 18 to 24 hours to identify noticeable agar colonies. The minimal concentration of the extract was determined, and no bacterial growth was seen [19]**.**

2.10. Kinetic time killer experiment

The extracted pure compound's time-kill kinetics were studied using the methods of [20], [21]. After 90 minutes of cultivation in nutrient broth at 37 °C, all bacteria were in the logarithmic growth phase. Microbes are measured in colony-forming units per milliliter (CFU/ml). Aliquots were taken at 0, 0.5, 1, 2, 3, 4, 6, and 8 hours, and 100 µL of inoculums were distributed on MacConkey agar plates. The plates were immersed for twenty-four hours at 37 °C to test the derived pure compound's inhibitory activity, and colony-forming units (CFUs) were examined to count living cells.

3. Results and Discussion

3.1. Isolation of streptomycetes isolates and antimicrobial activity

One of the main reasons for discovering new marine bioactive substances is to prevent the spread of bacterial resistance which might be resistant to currently utilized treatment [22]. Genes found in marine microorganisms offer a promising avenue for prescription treatments and target discovery. Secondary metabolites generated by marine microbes have resulted in chemicals and medicines [23]**.** So, sixteen isolates (H1-H16) were isolated again from the Red Sea in Hurghada. The crude ethyl acetate samples from these isolates showed biological antibacterial action with twelve different bacteria (B1-B12). The isolated actinomycetes acetate extracts were tested for their antimicrobial activity. Several isolates exhibited antimicrobial activity against different bacterial isolate but one isolate (**H2**) from the 16 actinomycetes isolates showed potent antimicrobial and were the most effective**.** While, most crude isolates have variable activity against bacterial isolates **(table 2).** There is a global problem with antibiotic resistance, but unfortunately, some countries lack proper surveillance and are unaware of the issue public health agencies have released reports on action plans to combat antibiotic resistance and proposed incentives for pharmaceutical investments in antibiotic research as a result of the decline in antibiotic development and increasing reports of resistance. Drug resistance rates vary significantly between different geographic locations and within the same industry's states, as well as across different centuries [24]**.** Therefore, novel therapeutic compounds from secondary metabolites with high effectiveness and low toxicity must be discovered at this moment. When it comes to discovering and developing new secondary metabolites, synthetic biology has a crucial role. To test a therapy for pseudomonas aeruginosa the researchers in this study employed a variety of secondary metabolites derived from microbes. Thus, antibiotic-resistant bacteria are at an alarmingly high level worldwide, especially in underdeveloped countries [25], [26]**.** The antimicrobial activity of ethyl acetate extract from *streptomyces* sp. strain isolated from Raf Raf forest (Tunisia) showed significant antimicrobial activity against *micrococcus luteus* and *staphylococcus aureus* and listeria monocytogenes and *pseudomonas aeruginosa* [27].

Antibiotic prescription is less strictly regulated in Egypt, and as a result, multidrug-resistant bacteria are appearing at an alarming rate. When you buy antibiotics from a drug store or pharmacy without a prescription, you're putting yourself at risk of contracting an infection. Antibiotics may be purchased without a prescription from drug stores and pharmacies [28], [29]**.**

As a result, ten bacterial isolates from the Microbiology and Immunology Department at Benha University Hospital were collected and tested for cultural, physiological, and biochemical characteristics. It was also utilized to verify the traditional biochemical identification of the ten bacterial isolates, some of the naturally occurring antimicrobial substances found in plants, herbs, and spices, as well as in animal products, have been shown in laboratory studies [30]**.** Lertcanawanichakul and Sahabuddeen study the effect of various cultural conditions on the production of bioactive compounds from *Streptomyces* sp. KB1, and determine the optimal cultural conditions for maximum production. findings that bioactive compounds from *Streptomyces* sp. KB1 are important and efficient compounds that may be used in the discovery of new anti-MRSA drugs [2].

3.2. Taxonomical study of the most potent streptomycetes isolate

A variety of methods were used to pinpoint the origins of these rapidly spreading streptomyces and pseudomonas aeruginosa isolates. A wide range of genotypic and phenotypic approaches are presently utilized for the taxonomic study of microbial species, but the most useful method is the close inspection of the 16S and 18S ribosomal RNA (rRNA) oligonucleotides of the 16S and 18S rRNAs [31]. Morphological, physiological, biochemical, and chemo-taxonomical characteristics of streptomycetes isolate H2. The strain was identified primarily by visualizing it"s spore morphology using TEM and the obtained result showed that the strain has arectiflexibility spore chains with a smooth spore surface, according to spore chain morphology **Figure (1 &2).**

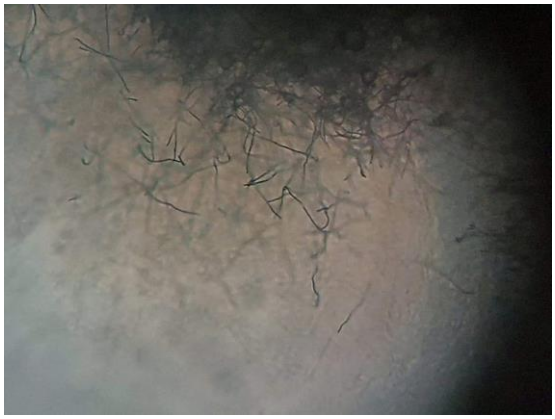

Figure 1. Light microscope of *Streptomyces parvulus* strain MDA showed arectiflexibility spore chains

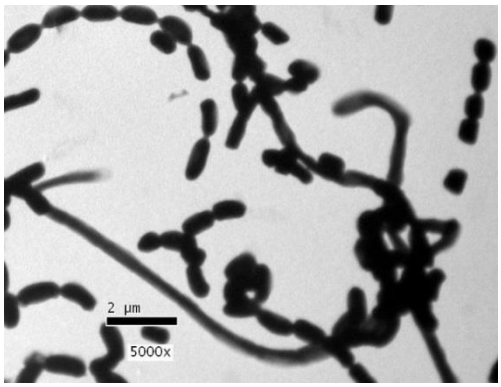

Figure 2. Electron microscope of *Streptomyces parvulus* strain MDA showed smooth spore surface

Tyrosine agar does not produce melanoid pigments in most cases. The spore mass is whitish in color and produces yellow diffusible pigments (**Table 3).** This strain's affiliation with the genus *Streptomyces spp*. A neighborjoining-based phylogenetic tree supported the findings. The nucleotide sequence has been submitted to the GenBank sequence database. *Streptomyces parvulus* strain MDA is the name we gave it. The strain has been assigned the accession number OQ179632.1 in the GenBank database. *Streptomyces parvulus* strain MDA 16S rRNA gene nucleotide sequence. The isolated strain Streptomyces parvulus strain MDA has a high similarity score to other known sequences in GenBank. **Figure (3)**. *Streptomyces parvulus* strain MDA phylogenetic tree (Accession no. OQ179632.1)**.**

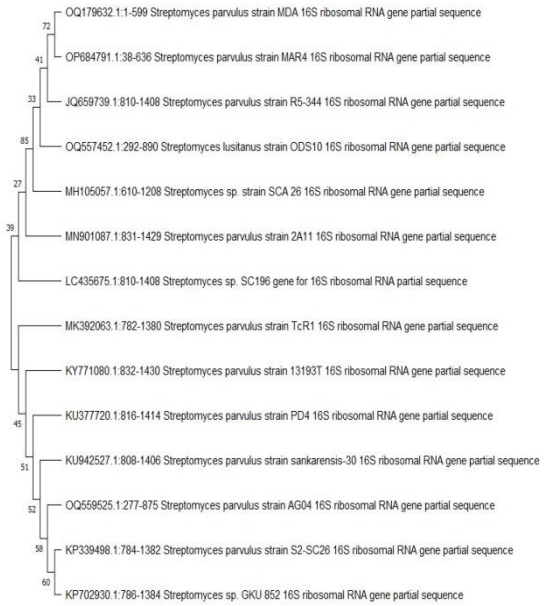

Figure 3. Phylogenetic tree of the *Streptomyces parvulus* strain MDA

(Accession no. OQ179632.1)

3.3. purification and structure elucidation

Streptomyces parvulus MDA was grown on a rice medium. Following extraction and scaling and the obtained extract was (3.8 g). the extract was rinsed using a variety of chromatography techniques to give nine fractions and with an antimicrobial effect against twelve Bacterial isolates **(Table 4)**. The most potent fraction (F6) was then rinsed which use column chromatography techniques to produce a pure substance that has strong antibacterial activity with twelve bacterial isolates. Among them, the subfraction (SF2) was the most potent as it showed broad-spectrum activity toward all tested microbes **(Table 5)**. The most potent sephadex subfraction (SF2) was further purified to remove any impurities and the obtained compound was structurally identified using NMR and LC-Ms.

Table 1. vitik 2 system results of *pseudomonas aeruginosa*

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	+
23	ProA	+	26	LIP	+	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	+	62	ELLM	-	64	ILATa	-			

Table 2. Antibacterial effects of different *Streptomycetes* crude extracts

	Antimicrobial activity (%)											
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**	**B11**	**B12**
H1	**0.0**	**15.66 ±1.40**	**0.0**	**21.43 ±1.32**	**16.82 ±1.77**	**26.77 ±1.23**	**32.01 ±1.70**	**17.40 ±1.28**	**10.94 ±1.42**	**25.57 ±1.52**	**31.06 ±1.30**	**21.30 ±1.18**
H2	**62.70 ±1.20**	**79.31 ±1.30**	**63.44 ±1.32**	**67.50 ±2.10**	**60.02 ±1.94**	**73.61 ±1.32**	**68.11 ±1.22**	**69.70 ±1.17**	**64.51 ±0.94**	**77.40 ±2.01**	**62.09 ±1.43**	**84.99 ±1.55**
H3	**0.0**	**21.55 ±1.63**	**0.0**	**26.90 ±1.90**	**0.0**	**19.50 ±1.34**	**17.49 ±1.77**	**0.0**	**32.08 ±1.19**	**0.0**	**0.0**	**0.0**
H4	**11.66 ±2.11**	**43.09 ±2.05**	**38.65 ±1.60**	**32.99 ±1.43**	**41.89 ±1.94**	**18.50 ±1.63**	**37.11 ±1.73**	**25.84 ±1.60**	**35.20 ±1.82**	**22.98 ±1.79**	**28.55 ±1.38**	**31.04 ±1.41**
H5	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
H6	**47.32 ±1.55**	**53.08 ±1.18**	**48.35 ±1.31**	**39.42 ±1.88**	**44.11 ±1.83**	**52.28 ±1.53**	**64.39 ±1.62**	**47.63 ±1.39**	**42.77 ±2.31**	**59.20 ±1.83**	**44.10 ±1.39**	**52.40 ±1.42**
H7	**0.0**	**21.69 ±1.93**	**24.18 ±1.73**	**26.72 ±1.69**	**0.0**	**29.32 ±1.44**	**0.0**	**19.90 ±1.26**	**20.82 ±1.63**	**0.0**	**18.63 ±1.29**	**13.86 ±1.33**
H8	**38.71 ±1.72**	**35.49 ±2.16**	**41.50 ±1.92**	**44.18 ±1.50**	**37.49 ±1.75**	**44.16 ±1.62**	**41.64 ±1.86**	**50.42 ±1.70**	**46.54 ±2.11**	**36.19 ±1.40**	**42.06 ±1.35**	**49.38 ±1.75**
H9	**53.39 ±1.29**	**47.55 ±0.93**	**43.27 ±1.08**	**58.43 ±1.48**	**60.20 ±1.69**	**46.39 ±1.80**	**42.70 ±2.1**	**51.18 ±1.08**	**47.20 ±1.87**	**44.63 ±1.70**	**57.49 ±1.50**	**41.86 ±1.60**
H10	**0.0**	**33.27 ±1.27**	**26.66 ±1.49**	**31.82 ±1.38**	**0.0**	**29.85 ±1.74**	**41.58 ±1.54**	**0.0**	**36.91 ±1.20**	**38.72 ±1.40**	**0.0**	**0.0**
H11	**0.0**	**28.19 ±1.62**	**0.0**	**38.39 ±0.84**	**31.41 ±1.38**	**26.09 ±1.62**	**34.92 ±1.59**	**0.0**	**38.52 ±1.02**	**28.50 ±1.53**	**39.52 ±1.53**	**33.17 ±1.97**
H12	**48.85 ±1.40**	**42.42 ±1.73**	**56.41 ±1.32**	**52.54 ±1.50**	**47.69 ±1.90**	**53.14 ±1.22**	**44.07 ±1.90**	**41.23 ±1.53**	**50.77 ±1.77**	**58.42 ±1.38**	**45.99 ±1.41**	**39.53 ±2.10**
H13	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
H14	**0.0**	**14.77 ±1.94**	**0.0**	**16.93 ±1.73**	**0.0**	**0.0**	**0.0**	**21.50 ±1.91**	**0.0**	**0.0**	**22.98 ±1.30**	**31.53 ±0.95**
H15	**0.0**	**19.80 ±1.15**	**0.0**	**23.54 ±1.09**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**26.55 ±1.52**	**20.05 ±1.52**	**24.90 ±1.11**
H16	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Table 3. Physiological and chemo-taxanomical properties of the isolate H2

Isolate no.		**H2**
Melanin pigment production	Pepton iron agar	-
	Tyrosine agar	-
Enzyme activities	Proteolysis	+
	Lipolysis	-
	Lecithinase	-
Utilization of different carbon source	No suger (-)	+
	D-Glucose (+)	+
	D-Fructose	+
	Sucrose	-
	Rhamnose	-
	D-Mannitol	-
	D-Xylose	-
	Raffinose	-
	I-inositol	-
	Galactose	-
	L-Arabinose	-
Nitrate reduction		+
H2S production		-
Starch hydrolysis		+
Cellulose decomposition		-
Gelatin liquification		-

Table 4. Antimicrobial activity for fractions

	Antimicrobial activity (%)											
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**	**B11**	**B12**
F1	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
F2	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
F3	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
F4	**49.56 ±1.33**	**52.08 ±1.54**	**47.48 ±2.20**	**58.18 ±1.49**	**35.72 ±1.72**	**61.11 ±1.75**	**64.36 ±1.60**	**57.08 ±2.15**	**48.33 ±1.74**	**64.90 ±1.69**	**43.03 ±1.20**	**59.22 ±0.97**
F5	**24.77 ±1.26**	**28.37 ±0.98**	**19.83 ±1.55**	**33.42 ±2.12**	**18.38 ±1.70**	**41.90 ±1.43**	**27.52 ±1.51**	**29.63 ±1.44**	**35.16 ±1.35**	**27.70 ±1.54**	**21.44 ±1.32**	**36.72 ±2.05**
F6	**67.29 ±1.76**	**82.53 ±2.14**	**71.15 ±1.71**	**75.40 ±2.11**	**69.15 ±2.26**	**80.57 ±1.85**	**73.91 ±1.50**	**74.71 ±1.62**	**69.83 ±1.94**	**82.49 ±1.88**	**66.82 ±1.41**	**87.80 ±1.19**
F7	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
F8	**22.22 ±1.19**	**15.51 ±1.13**	**27.50 ±1.92**	**23.77 ±1.77**	**19.54 ±1.50**	**20.05 ±1.61**	**25.57 ±1.97**	**18.29 ±1.10**	**25.29 ±1.08**	**20.77 ±1.90**	**21.49 ±1.37**	**28.60 ±2.16**
F9	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Table 5. Antimicrobial activity for subfractions

	Antimicrobial activity (%)											
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**	**B11**	**B12**
SF1	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
SF2	**73.54 ±1.34**	**85.44 ±2.10**	**76.28 ±1.73**	**83.19 ±1.83**	**74.05 ±1.09**	**87.48 ±1.32**	**77.17 ±1.14**	**81.17 ±1.49**	**73.23 ±1.33**	**85.50 ±1.76**	**68.73 ±1.80**	**88.50 ±1.78**
SF3	**45 ±1.67**	**42 ±1.55**	**61.68 ±1.88**	**55.72 ±1.49**	**49.41 ±0.89**	**52.20 ±1.74**	**58.42 ±1.66**	**46.46 ±1.90**	**52.67 ±1.54**	**59.90 ±1.04**	**47.82 ±1.05**	**53.90 ±1.63**
SF4	**34.18 ±1.89**	**38.28 ±1.54**	**42.39 ±1.80**	**28.21 ±2.21**	**40.27 ±1.83**	**34.41 ±1.39**	**44.61 ±1.31**	**37.33 ±1.89**	**31.54 ±1.50**	**41.16 ±1.31**	**30.19 ±1.19**	**25.32 ±1.73**
SF5	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
SF6	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

The compound (SF2) 1H-NMR spectrum revealed 9 signals at 7.71 (m, 2H); 7.52 (m, 2H); 4.30 (t, 2H, J = 6.46 Hz); 4.07 (d, 2H, J = 7.27 Hz); 2.04 (m, 1H); 1.71 (t, 3H, J = 7.27 Hz); 1.44 (m, 2H); 0.98 (m, 6H); and 0.86. (m, 3H) The compound's MS analysis yielded a molecular ion *m/z* of 278. Bioactive compound 1 was defined as 2-Methyl butyl propyl phthalate according to the spectral data presented above. C16H22O4 is the molecular formula in **Figures (4&5&6)**. Di-n-butyl phthalate ester is a common phthalate ester (1, 2-Benzenedicarboxylic acid dibutyl ester). It cannot be dissolved in water. At 760 mm Hg, it has a boiling point of 644 °F, and a melting point of -31°. The substance is flammable, even though it may need some effort to start. There are several applications for it, including in paints, polymers, and chemical reactions. To esterify n-butyl phthalic acid with phthalic acid, sulfuric acid or p-toluene sulfonic acid is used as a motivator [32].

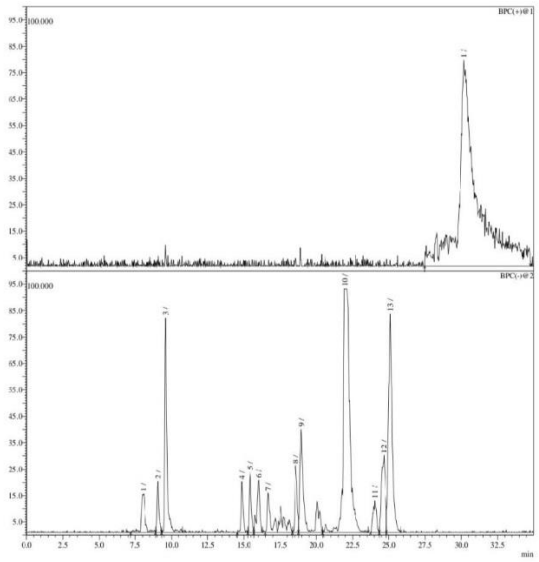

Figure 4. Mass spectrum of 2-Methyl butyl propyl phthalate

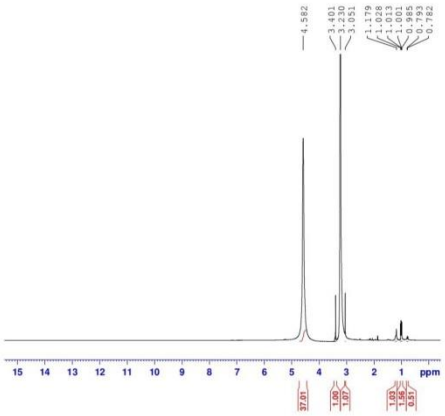

Figure 5. Proton NMR of 2-Methyl butyl propyl phthalate

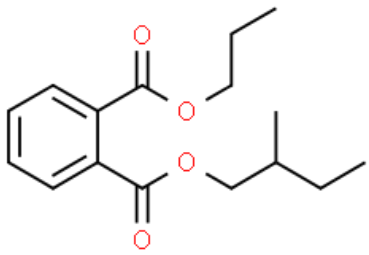

Figure 6. Chemical structure of 2-Methyl butyl propyl phthalate

3.4. Methyl butyl propyl phthalate's MIC, MBC, and time-kill kinetics

An intriguing finding in our research is the inhibition of *pseudomonas aeruginosa* (B12) by 2-Methyl butyl propyl phthalate. Because preliminary antibacterial analysis indicates inhibitory activity with effective antimicrobial parameters, the MIC and MBC against *pseudomonas aeruginosa* (B12) were

measured, and the tests were repeated three times to verify the MIC and MBC data. The MIC of the extracts was discovered to contain about 80 µg/ml, with an MBC of 300 µg/ml. The extract had a bactericidal effect on the examined isolates, according to the results. 2-Methyl butyl propyl phthalate killed *pseudomonas aeruginosa* (B12) in 5 hours **Figure (7)**.

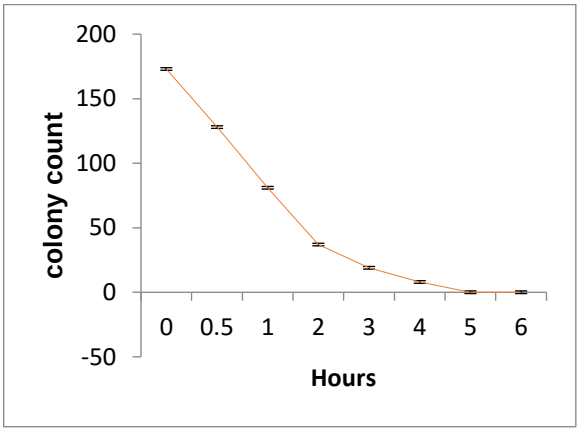

Figure 7. Time-kill kinetics of 2-Methyl butyl propyl phthalate against pseudomonas aeruginosa (B12). Data presented as mean ± SE. ANOVA one-way was used for data analysis (n=3, P<0.05).

Methyl butyl propyl phthalate exhibited antibacterial activity against a variety of gram-positive and gram-negative bacteria, for which the MIC values ranged from 4 to 256 µg/ml. Among the facultative and pathogenic gram-positive bacteria, Methyl butyl propyl phthalate was active against all the bacteria tested and the best activity of this compound was recorded against Staphylococcus aureus (8 µg/ml) followed by Streptococcus mutans and Bacillus subtilis (16 µg/ml) [33].

\\

4. Conclusion

Marine microorganisms are an extremely rich source of structurally diverse classes of secondary metabolites. *Streptomyces parvulus* MDA isolated from Hurghada with accession numbers OQ179632.1. therefore, the large-scale fermentation and working up of the crude extract, according to the antibacterial activity against different *pseudomonas aeruginosa* isolates and based on its chromatographic properties, proton and carbon spectra, it led to the isolation of 2-Methyl butyl propyl phthalate

5. Conflicts of interest: "There are no conflicts to declare"

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