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Isolation and Characterization of Some Potential Antibacterial Metabolites from Marine Microorganisms Collected in Egypt

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THIS study focuses on the isolation and characterization of bioactive compounds from marine microbial species, specifically targeting their potential as antibacterial agents. A marine sample was collected from Ain Sokhna, leading to the identification of six bacterial isolates (J8-J13) evaluated for their antagonistic activity against seven bacterial pathogens. Among these, Bacillus strain J13 exhibited the highest antibacterial activity, with a zone of inhibition measuring (30 ± 0.1 mm) against Bacillus cereus, and notable inhibition against Pseudomonas aeruginosa (28 ± 0.1 mm), Bacillus subtilis (28 ± 0.2 mm), Salmonella typhimurium (27 ± 0.2 mm), Escherichia coli (25 ± 0.2 mm), and Klebsiella pneumoniae (23 ± 0.2 mm). The chemical analysis of J13's metabolites was conducted using matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) and gas chromatography-mass spectrometry (GC-MS), revealing several bioactive compounds, including bis(2-ethylhexyl) phthalate. The Minimum Inhibitory Concentration (MIC) values ranged from 15.62 to 31.25 µg/ml, indicating the efficacy of these compounds at low concentrations. Advanced biotechnological techniques such as genomic sequencing and metabolomic profiling could further elucidate the mechanisms underlying the antibacterial properties of these compounds, maximizing their potential for therapeutic applications. By integrating these approaches, this research not only contributes valuable insights into combating antibiotic resistance but also paves the way for developing innovative treatments that could significantly impact human health and well-being.

KeyWords: *Marine microbes, Antibiotic resistance, Bioactive compounds, Inhibition effect. MALDI-TOF, GC-MS.*

Introduction

Marine cultures are regarded as significant sources of microbial variety with the potential to discover novel bioactive compounds. The rising frequency of antibiotic-resistant organisms necessitates the identification of novel antibacterial drugs in hitherto undiscovered biological niches. Marine microorganisms have produced a wide range of bioactive compounds with various activities, including cytotoxic, anticancer, photoprotective, antiproliferative, antitumor, antifouling, and antibiotic properties (Blunt et al., 2006; Mayer et al., 2011; Villa & Gerwick, 2010). In addition to being excellent natural product producers with a diverse range of structures and medicinal applications, marine microorganisms are particularly promising due to their unique adaptations in extreme environments.

To better understand the potential of marine microorganisms in combating antibiotic resistance, it is essential to recognize the unique adaptations these organisms have developed in extreme environments. Marine ecosystems, characterized by varying salinity, hydrostatic

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pressure, and temperature, have led to the evolution of specialized metabolic pathways in marine bacteria and fungi. These adaptations often result in the production of unique secondary metabolites that possess bioactive properties not typically found in terrestrial organisms. This distinct biochemical repertoire positions marine microorganisms as a promising source for novel antibacterial agents, making them a focal point for drug discovery initiatives aimed at addressing the growing threat of multidrug-resistant pathogens.

Marine microorganisms, particularly bacteria and fungi, are also a valuable source of information in the ongoing search for antibacterial chemicals to combat aquatic illnesses (Choudhary et al., 2017; Guo et al., 2008). These biologically secondary metabolites found in marine sources have been proven to have antiviral, antifungal, and antibacterial effects. As a result, they are widely used as antibiotics and may be useful in the treatment of various bacterial infections and infectious disorders, including AIDS. Ocean ecosystems differ substantially in terms of salinity, hydrostatic pressure, and temperature. To live in these specialized environments, marine microbes have evolved various adaptation methods, including the production of specific proteins. As a result, marine microorganisms appear to have a high potential for producing bioactive compounds that are not found in terrestrial environments. Extreme marine settings are unique biological niches in which extremophile bacteria develop specific metabolic pathways and characteristics. Despite accounting for 70% of the earth's surface, the bioactive compounds discovered in marine bacteria that occupy this environment are poorly understood. By using traditional screening and isolation methods to characterize a multitude of fascinating novel structures, marine bacteria have demonstrated their remarkable capacity to generate chemically distinct bioactive compounds (Gulder & Moore, 2009).

Viruses, bacteria, and fungi are the source of many terrible diseases and pose a major threat to public health despite amazing advancements in medical sciences for treating these germs. The lack of access to medications has a widespread effect in underdeveloped nations and contributes to the rise in widespread drug resistance. Microbes, on the other hand, have a positive side because certain of them, such as fungi, are utilized to clean the environment (Abd El-Rahim 2006; Abd El-Rahim et al., 2016; (Moawad et al., 2019)Whereas the need for creating novel potent antimicrobial chemicals stems from how microbial diseases have adapted to antibiotics. Antibiotic-resistant microorganisms have been targeted by researchers using novel medications, primarily antibiotics. Multidrug resistance has spread more quickly because of widespread antibiotic usage. According to a 2010 study, whereas 30% of strains were resistant to ciprofloxacin (which has been used since 1987), 79% of strains of *Escherichia coli* were resistant to ampicillin (which has been used since 1961). In America, it was discovered that 43% of Staphylococcus aureus infections between 1999 and 2000 were resistant to methicillin. The mortality rate from infectious diseases has increased again due to bacterial resistance to drugs (Singer et al., 2003). Infectious diseases continue to be the leading cause of morbidity and death rates in developing nations such as Indonesia. Approximately 40–50% of medications currently available were created using natural chemical compounds such as marine microbes. Numerous research studies conducted in the previous few decades have concentrated on the bioactive components of marine goods such as marine microorganisms (Webster & Taylor, 2012). It has been suggested that the marine environment is a rich and diverse source of strong bioactive compounds (Singer et al., 2003; Webster & Taylor, 2012). It is equivalent to a medication (Haefner, 2003). The extraction of novel and potential bioactive compounds from marine microorganisms is increasing quickly these days (Chellaram & Prem Anand, 2010).

Given the pressing need for new antimicrobial agents, this study aims to isolate and characterize novel bacterial strains from marine environments that exhibit significant antagonistic activity against common bacterial pathogens. Specifically, we will investigate the bioactive compounds produced by these strains, assessing their efficacy and safety profiles. By focusing on both the isolation of these microorganisms and the characterization of their bioactive metabolites, this research seeks to contribute valuable insights into developing new therapeutic options for treating antibiotic-resistant infections.

Materials and Methods

Sample collection from marine water

Seawater samples were collected from Ain -Sokhna (J) El Zohor beach (29°47'31.6"N 32°26'35.7"E) in August 2021 at a depth of

approximately 1.0 meter in sterile bottles, transferred to the laboratory, and refrigerated at 5°C until the following day.

Isolation and purification marine micro-organisms

Using serial dilution agar plate technique, microorganisms were separated from water. Each water sample was diluted up to six to ten times with sterile water ranging from one to nine milliliters. For bacterial isolation, an aliquot of 0.1 ml from each dilution was inoculated in marine agar (Mulango, 2020).

Screening of marine bacterial isolates against standard pathogens

Using the conventional agar disc diffusion method, the microbial isolates were evaluated for their ability to produce antibacterial agents against seven standard pathogen strains. *Salmonella typhimurium (ATCC 6539)*, *Bacillus subtitles (ATCC 6633)*, *Bacillus cereus (ATCC 11778)*, *Escherichia coli (ATCC 8739)*, *staphylococcus aureus (ATCC 6538)*, and *Pseudomonas aeruginosa (ATCC90274) and Klebsiella pneumoniae (ATCC 13883)* were among the investigated bacterial isolates whom the American Type Culture Collection (ATCC) pays. The test strains were cultured for 24 hours shaker incubator at 37°C in 100 ml of nutrient broth medium (Mulango, 2020)**.**

Bioactive compounds extraction from culture filtrates

One of the most effective isolates demonstrated a strong broad-spectrum action standard pathogen bacterium The bioactive compounds from the culture filtrates were extracted using a variety of solvents. The best extraction solvent was determined to be a 2:1 mixture of chloroform and methanol, as well as ethyl acetate and ethanol. Three parts were taken from each crude culture filtrate, and each solvent system was given an equal volume was included into every section. Each combination was then split into three sections: the first was made acidic to pH 2.0 by adding acetic acid or HCl; the second was left at pH 8.0; and the third was made alkaline to pH 10.0 by adding NaOH. After 15 minutes of agitation of the solvent-supernatant mixture, the organic phase was separated from the soup using a separating funnel. To get rid of any remaining fermentation broth, the solvent was centrifuged for 15 minutes at 5000 rpm. The antibacterial activity of each extract was tested using the appropriate solvents and an agar well disk technique control.

Anti-bacterial from extract isolates j13 by agar well diffusion

The antibacterial activity of one marine bacterial extract was evaluated against seven clinical infections. *Salmonella typhimurium* ATCC 6539, *Bacillus subtitles* ATCC 6633, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538*, Pseudomonas aeruginosa* ATCC 90274 and *Klebsiella pneumoniae* ATCC 13883 were among the investigated bacterial isolates whom the American Type Culture Collection (ATCC) pays.

Using sterile cotton swabs, Overnight-grown clinical pathogen cultures in nutrient broth were used to swab the marine agar plate surface uniformly. The clinical pathogen cells that were actively growing on Marine Agar plates were seeded with marine isolates from the agar slants. The plates were incubated at 37 °C for the entire day. Following the incubation period, the zones of inhibition on the plates were examined and the results documented.

Using the agar-well diffusion method, the antibacterial activity of marine isolates was evaluated. Using sterile cotton swabs, the surface of the Marine Agar plates was uniformly swabbed with the overnight cultures of the clinical pathogens in nutrient broth. Using a sterile cork borer, four 6 mm wells were created on the seeded plates. Each well was aseptically incubated for 24 hours at 37°C after adding approximately 100µl of overnight developed cultures using nutrient Broth. The inhibitory zone was then noted and recorded. For additional identification, the marine strains exhibiting encouraging activity against standard pathogens were chosen (Magaldi et al., 2004).

Determination of Minimum Inhibitory Concentration (MIC)

Further incubation of the cultures (96-well plates) that were used in the experiments was performed for 12 h (total 24 h) and the turbidity was measured as before. The lowest concentration of fraction needed to inhibit the visible growth of a test microorganism after 24 h was considered as the MIC.(Majali et al., 2019).

Determination of Minimum Bactericidal Concentration (MBC)

Minimal bactericidal concentration (MBC) was determined by transferring and spreading the treated culture broth of the wells containing the concentrations of equal to and higher than the MIC

on agar plates. The lowest concentration of the fraction required to destroy test microorganisms (no growth on the agar plate) after incubation at 37 °C for 24 h (bacteria) or at room temperature.

Gas chromatography mass spectrometry (GC-MS) analysis

The material was evaluated using the GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a capillary column TG-5MS (30 m x 0.25 mm x 0.25 µm film thickness). A 1µl sample was introduced by an all-glass injector in split mode, with Helium as the carrier gas and a constant flow rate of 1 ml/min. The components were identified by comparing their retention durations and mass spectra to those from the WILEY 09 and NIST 11 mass spectra databases.

Identification isolated by MALDI TOF MS

The isolated microorganism was identified at the outsourced 57357 Children's Cancer Hospital employing MALDI-TOF MS (VITEK®MS, database version 3, BioMerieux, France) for confirmation. As a calibration and internal identification control, *E. Coli* ATCC 8739 was injected into the calibration spots. The manufacturer's guidelines were followed for interpreting the results. To identify the isolation, the peaks from the spectrum were compared to the normal spectrum for a certain species, genus, or family of microorganism.

Results and Discussion

The exploration of marine microorganisms as sources of novel antibacterial agents is increasingly relevant in the context of rising antibiotic resistance. This study successfully isolated six bacterial strains from marine environments, with a particular focus on the Bacillus strain J13, which exhibited significant antagonistic activity against several pathogenic bacteria. The findings underscore the potential of marine ecosystems, often overlooked in pharmaceutical research, as reservoirs for bioactive compounds that could lead to new therapeutic options.

Sample collection from marine water

The collection of marine water samples from Ain Sokhna provided a unique opportunity to explore the diverse microbial populations present in this marine environment. This specific location, harbor microorganisms with unique adaptations that contribute to their ability to produce bioactive compounds. One sample of marine water was taken for the current investigation. Following the serial dilution method, various bacteria were isolated from about 6 distinct colonies and were chosen. Each colony received a strain code until the species was identified.

Isolation and purification marine micro-organisms

The isolation of six distinct bacterial colonies using the serial dilution agar plate technique demonstrated an effective method for obtaining pure cultures from complex marine samples. Each colony was assigned a strain code for identification, facilitating subsequent analyses. The preservation of these isolates at -20°C in nutrient broth supplemented with 20% glycerol is a robust approach, ensuring the viability of cultures for future studies. This method not only allows for the long-term storage of isolates but also maintains their metabolic activity, which is essential for further screening and characterization.

Screening of marine bacterial isolates against standard pathogens

Six isolates were screened for antibacterial activity against seven bacterial pathogens. Marine isolates from agar-slants were spotted onto Marine Agar using the disc diffusion method for every marine isolate, inhibitory efficacy against a minimum of one standard pathogen was found the J13 isolate showed the greatest zone of inhibition against all tested organisms among the seven clinical pathogens evaluated against isolated strains. The disc diffusion method was used for screening, and then for confirming the antibacterial activity. For four marine isolates, inhibitory efficacy against at least one standard pathogen was found Table 1**.**

An antagonistic activity test was performed on each of the 6 isolates against seven bacterial pathogens *Bacillus cereus*, *Bacillus subtitles, staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia***,** *Pseudonymous aeruginosa* and *Salmonella typhimurium one* possible isolate of Bacillus, strain code J13 were chosen based on their capacity to suppress the common pathogen. J13, one possible strain, exhibited the highest level of action against normal bacteria J13 demonstrated the highest zone of inhibition against *Bacillus cereus* (50mm) followed by *staphylococcus aureus* (25mm) and followed by *Escherichia coli* (25mm) and followed by *Pseudonymous aeruginosa* (24mm) and followed by salmonella (12mm) and followed by Bacillus subtitles (10mm) and followed by *Klebsiella pneumoniae* (13mm) show in the Table 1**.**

TABLE 1. Antibacterial activity of the marine isolates by disc diffusion Method

Bacterial strains		J38	J7	J36	J13	J2	J37
Bacillus cereus	G+	13	17	13	50	14	8
Bacillus subtitles	G+	18	12	10	10	7	-
Pseudonymous aeruginosa	G+	15	12	10	24	10	13
Escherichia coli	G-	17	9	12	25	20	10
Salmonella typhimurium	G-	35	16	13	12	13	9
staphylococcus aureus	G-	20	10	-	25	12	8
Klebsiella pneumoniae	G-	10	-	-	13	10	20

These organisms' aggressive behavior may be caused by the extracellular metabolites they produce. The Pacific Institute of Bio-organic Chemistry (Vladivostok, Russia) taxonomically evaluated a group of marine Bacillus strains capable of producing physiologically active compounds (Ivanova et al., 1998; Jensen et al., 1996). Many researchers have actively experimented with Bacillus species because of its anti-fungal capabilities (Cubeta et al. 1985). According to (Loeffler et al., 1986), the antagonistic action mechanism results from antibiotic production. According to (Berrue et al., 2009), surfactin molecules isolated from *B. pumilis* inhibited *S. aureus, P. vulgaris,* and *E. faecalis*. According to Darabpour et al. (2010).

Bioactive compounds extraction from powerful culture filtrates

Several organic solvents, including chloroform : methanol 2:1, ethanol, and ethyl acetate, were used to extract a culture. Out of all of them, only the ethyl acetate extract shown noteworthy antibacterial activity against all test pathogenic bacteria gram-positive bacteria or gram-negative bacteria (Uzair et al., 2006).

Anti-bacterial from extract isolates j13 by Ethyl Acetate

Diameter of the Zone of inhibition Table.2 Antagonistic activity was detected of J13 against bacterial pathogens; therefore, J13 demonstrated the highest zone of inhibition against *Bacillus cereus (*30±0.1) followed by *Bacillus Subtitles (*28±0.2), *Pseudonymous. Aeruginosa* (28±0.1). According to the findings, marine isolation can produce secondary metabolites that are biologically active against human infections. According to previous research, the marine bacterial isolate can produce secondary metabolites that are effective against *bacillus cereus*, *bacillus substiles*, *pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium,* and *Klebsiella pneumoniae* isolate J13 in MALDI TOF MS *bacillus subtills* (Wu et al., 2013).

Fig.1 The antibacterial activity of j13 against seven distinct bacterial pathogens using Ethyl acetate was investigated using the Agar Well Diffusion Assay Method. Bacillus isolate j13 had the maximum inhibition zone (30±0.1) against Bacillus cereus. The Bacillus genus produces antimicrobial chemicals against several diseases (Wu et al., 2018; Saber Kelany et al., 2019).

Determination of Minimum Inhibitory Concentration (MIC) By Ethyl Acetate(µg/ml)

In isolate J13, the MIC values from 15.62 to 31.25 µg/ml. *Salmonella typhimurium, Escherichia coli* and *Pseudonymous Aeruginosa* had the lowest MIC of 15.62*, while Bacillus Subtitles*, *Bacillus cereus*, *Staphylococcus aureus* and *Klebsiella pneumoniae* had the highest MIC 31.25 µg/ml in Table 3

Determination of Minimum Bactericidal Concentration (MBC) by Ethyl Acetate (µg/ml):

MBC values varied between 31.5 to 125 µg/ml the greatest value was recorded against *Staphylococcus aureus* and *Klebsiella pneumoniae* 125 µg/ml*,* and the lowest value recorded against *Escherichia coli* and *Salmonella.typhimurium* 31.25 µg/ml in Table 4

TABLE 2. Zone of inhibition J13 (mm).

Pathogenic bacteria	J13	Control
Bacillus Subtitles (ATCC6633)	28±0.2	29±0.1
*Bacillus cere*us (ATCC 11778)	30±0.1	30±0.2
Staphylococcus aureus (ATTCC6538)	20±0.1	24±0.1
Escherichia coli (ATTCC87390)	25±0.2	19±0.1
Salmonella typhimurium (ATTCC6539)	27±0.2	22±0.1
*Pseudonymous. Aeruginos*a (ATTCC90274)	28±0.1	26±0.1
Klebsiella pneumoniae (ATCC 13883)	23±0.2	21±0.1

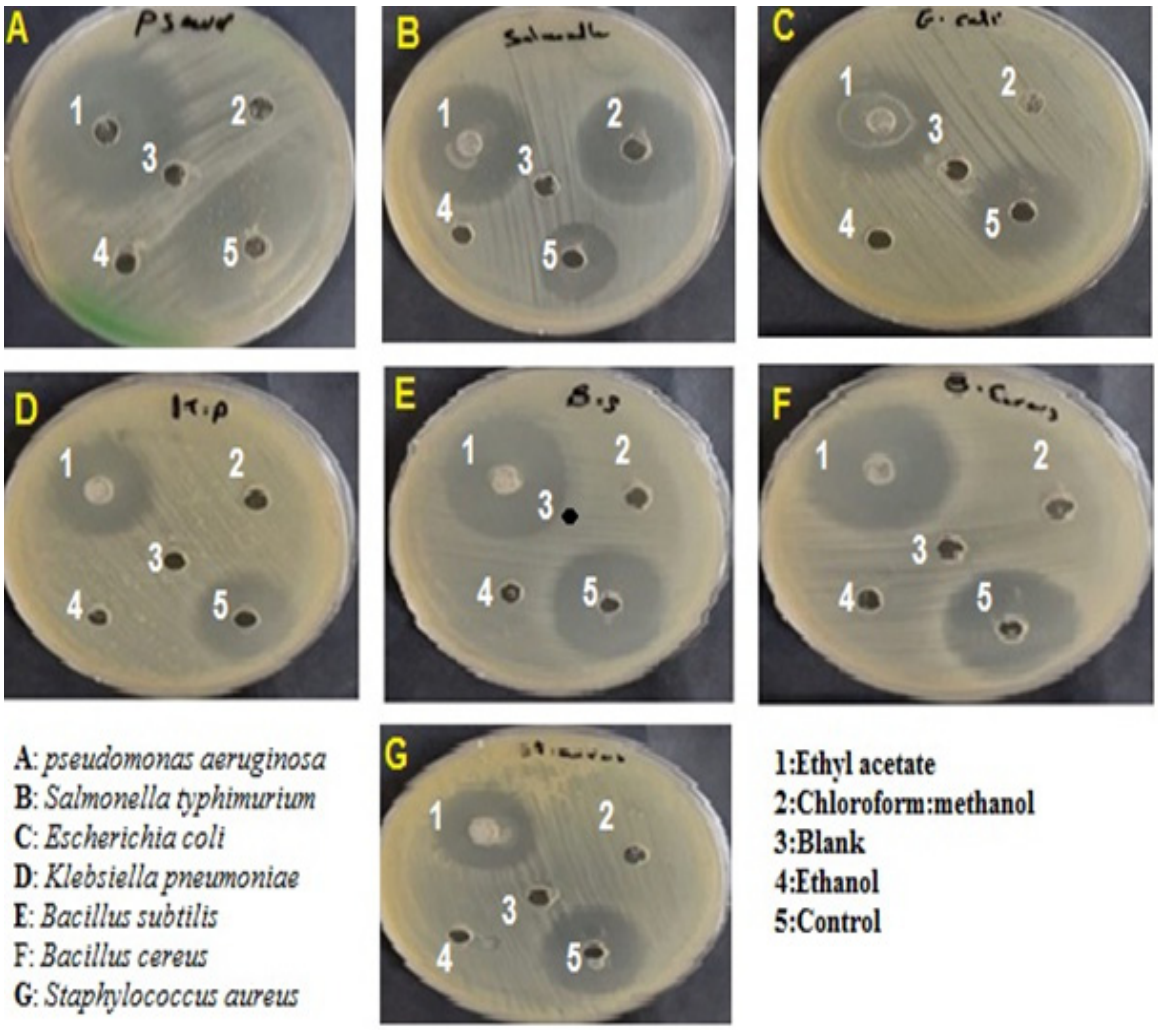

Fig.1. J13 against different bacterial pathogens .

TABLE 3: MIC values J13 (µg/ml)

Pathogenic bacteria	J13
Bacillus Subtitles (ATCC6633)	31.25
Bacillus cereus (ATCC 11778)	31.25
*Staphylococcus aure*us (ATTCC6538)	31.25
*Escherichia col*i (ATTCC87390)	15.62
*Salmonella.typhimuriu*m (ATTCC6539)	15.62
*Pseudonymous. Aeruginos*a (ATTCC90274)	15.62
*Klebsiella pneumonia*e (ATCC 13883)	31.25

TABLE 4: MBC values J13 (µg/ml)

Pathogenic bacteria	J13
Bacillus Subtitles (ATCC6633)	62.5
Bacillus cereus (ATCC 11778)	62.5
*Staphylococcus aureu*s (ATTCC6538)	125
*Escherichia col*i (ATTCC87390)	31.25
*Salmonella typhimuriu*m (ATTCC6539)	31.25
Pseudonymous. Aeruginosa (ATTCC90274)	62.5
Klebsiella pneumonia (ATCC 13883)	125

Gas chromatography mass spectrometry (GC-MS) analysis GC MS profiling:

GC-MS-based metabolite profiling of biological samples is one of the advanced metabolite profiling techniques that has significantly enriched our understanding of metabolic profile. Since its introduction as a prominent method for metabolite profiling, GC-MS has been widely used in functional genomic studies of bacteria, fungi, and plants to search for obvious or previously unknown metabolic traits (Kopka, 2006), (Wani et al., 2010). The findings suggested that bacteria could be a source of bioactive chemicals and should be investigated to discover natural antibiotics. The ethyl acetate extract and the primary component were identified by GC-MS analysis. The study aimed to assess their efficacy as antibacterial agents Fig.2 shows the bioactive compounds found in isolated j13. There are numerous biological actions, including antibacterial. antioxidant, anticancer, and anti-inflammatory properties, are possessed by the discovered compounds.

Table 5 The majority of the compounds discovered in the ethyl acetate extract of the bacterium Bis (2-Ethylhexyl) Phthalate were identified by GC-MS analysis, as reported by Lotfy et al. (2018). The highest proportion known to display antibacterial and cytotoxic activity is 79.98.

identification of marine bacteria by Maldi Tof MS:

MALDI-TOF-MS analysis revealed a notable peak cluster between m/z 3280 and 3520 in the butanoic extracts of *Bacillus spizizenii* culture supernatants. They remain standing for H+, Na+, and K+. Adducts of sactipeptide subtilisin, lanthipeptide subtilin, and its succinylated derivative (Chan et al., 1993; Heinzmann et al., 2006). The lipoheptapeptide surfactin's many isoforms were found using MALDI-MS testing because of their different m/z values.

Surprisingly, surfactin was generated in all investigations on *Bacillus subtilis* and *Bacillus Spizizenii* strains. Surfactin was found in the culture supernatants of all Bacillus strains studied, so the phenotype "surfactin production" cannot be used as a biomarker for subspecies classification or differentiation (e.g., between *Bacillus subtilis* and *Bacillus spizizenii*) (Kalinovskaya et al., 2002; Peypoux et al., 1999; Torres et al., 2016). Furthermore, bacteria belonging to the *Bacillus* genus often seem to be surfactin makers. When surfactin was found in Bacillus strains that regularly generate surfactin (Peypoux et al. 1999; Kalinovskaya et al. 2002). According to Torres et al. (2016), there is little use of the phenotypic "surfactin production" as a biomarker for subspecies classification or differentiation (between, for example, *Bacillus subtilis* and *Bacillus spizizenii*).

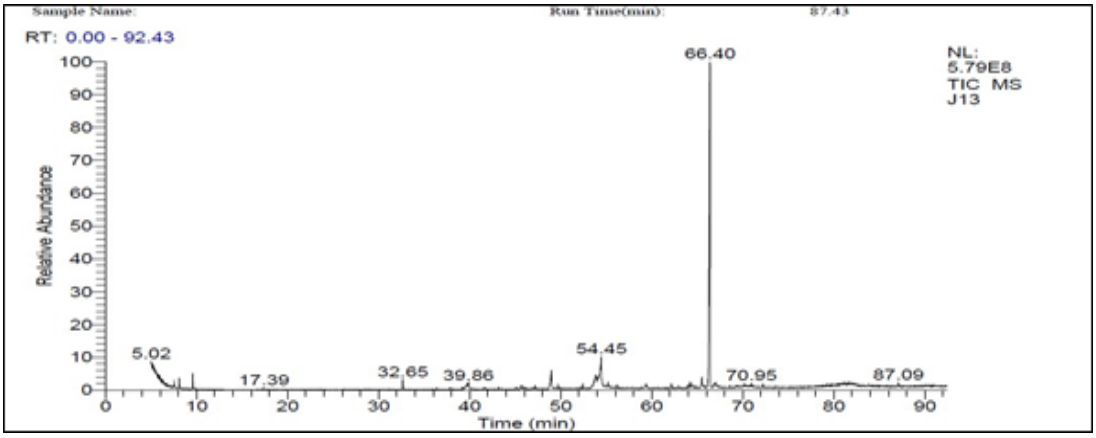

Fig.2 shows the bioactive compounds found in isolated j13, GC-MS was used to identify the bioactive substances in the bacterial ethyl acetate extracts chemically based on the molecular weight, peak regions, retention times, and molecular formulas.

TABLE 5 Gc bioactive compounds in isolates j13

RT min.	Compound name	Area	Area%	Molecular formula	molecular weight g/ mol	Proposed function
28.48	Tetradecane	2621086.15	7.81	$C_{14}H_{30}$	198	Antifungal and Antibacterial (Ozdemir et al., 2004)
36.36	Hexadecane	12080951.1	1.49	$C_{16}H_{34}$	226	Antifungal, Antibacterial, Antioxidant activity (Akpuaka et al., 2013)
43.17	1-Docosene	17099843.65	0.10	$C_{22}H_{44}$	308	Antibacterial activity Beevi et al. (2014)
43.5	Octadecane	9833607.14	0.37	$C_{18}H_{38}$	254	Anti-fungal, anti-tumor, anti-bacterial, and anti-microbial properties. (Jasim et al., 2015)
43.5	Pentacosane	9833607.14	0.46	$C_{25}H_{52}$	352	Antimicrobial activity (Salih et al., 2021)
47.18	Hexadecenoic acid, methyl ester	20765979.62	0.78	$C_{17}H_{34}O_2$	270	Exhibited strong anti-microbial, hypocholesterolemia, anti-inflammatory, anti-fungal, and antioxidant properties (Thelma & Balasubramanian, 2021)
47.98	9-Octadecenoic acid (z)-	2813291.69	0.11	$C_{18}H_{34}O_2$	282	Antibacterial (Mujeeb et al., 2014)
47.98	Oleic acid	2813291.69	0.37	$C_{18}H_{34}O_2$	282	Antibacterial and antifungal qualities (Seidel and Taylor 2004)
48.99	N-hexadecenoic acid	123493657.4	0.11	$C_{16}H_{32}O_2$	256	Anti-inflammatory, antispasmodic, anticancer and antiviral (Al-Imara & Al-Gazzawy, 2016)
54.44	Cis-vaccenic acid	207109616.8	4.66	$C_{18}H_{34}O_2$	282	Antibacterial activity and hypolipidemic (Semwal et al., 2018)
55.19	Octadecanoic acid	39430587.78	0.78	$C_{18}H_{36}O_2$	284	Antioxidant, anti-inflammatory, nemaicide, pesticide, Anti-androgenic flavor, hemalytic, 5- Alpha reductase inhibitor (Balasundari & Boominathan, 2018)
56.17	1-eicosanol	21945392.39	0.64	$C_{20}H_{42}O$	298	Antimalarial, antifungal and antioxidant activities (Albratty et al., 2021)
59.35	Ergotamine	40300655.89	0.83	$C_{33}H_{35}N_5O_5$	581	Not reported
66.39	Bis (2ethylhexyl) phthalate	2120707731.53	79.98	$C_{24}H_{38}O_4$	390	Antimicrobial and cytotoxic activities (Lotfy et al., 2018)

Conclusion

This study successfully isolated and characterized six bacterial strains from marine environments, with strain J13 demonstrating exceptional antibacterial activity against multiple pathogens, including *Bacillus cereus, Pseudomonas aeruginosa,* and *Escherichia coli*. The agar disc diffusion method revealed that J13 produced the largest zones of inhibition, particularly against Bacillus cereus (30 ± 0.1 mm). Gas chromatography-mass spectrometry (GC-MS) analysis identified several bioactive compounds, notably bis(2-ethylhexyl) phthalate, suggesting a diverse chemical profile with potential therapeutic applications. Additionally, matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) confirmed the identification of strain J13 as a Bacillus species. The Minimum Inhibitory Concentration (MIC) values ranged from 15.62 to 31.25 µg/ml, indicating the efficacy of these compounds at low concentrations. The discovery of bioactive compounds from marine microorganisms like Bacillus strain J13 holds significant promise for addressing critical challenges in healthcare, agriculture, and biotechnology. Continued exploration and characterization of these compounds will not only enhance our understanding of their potential but also pave the way for innovative solutions to pressing global issues such as antibiotic resistance and environmental sustainability.

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عزل و توصيف لبعض المواد الأيضية المحتملة المضادة للبكتيريا من الكائنات الحية الدقيقة البحرية المجمعة في مصر

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تعتمد هذه الدراسة على عزل وتوصيف بعض المركبات النشطة بيولوجيا من الأنواع الميكروبية البحرية وتستهدف على وجه التحديد قدرتها على إنتاج المضادات البكترية ؛ تم تجميع العينات البحرية من منطقة العين السخنة مما أدى إلى التعرف على ستة عزلات بكتيرية ومن بينهما الأرقام (J13 -J 8) حيث تم تقيم نشاطها المضاد للبكتريا ضد سبعة أنواع مختلفة من البكتريا الممرضة ، وأوضحت النتائج أن أعلى نشاط مضاد للبكتريا للمنطقة J13 مع السلالات البكتيرية كالتالى*Bacillus cereus* (30±0.1mm) يليه*Pseudomonas* aeruginosa (28±0.1mm) يليه *Bacillus subtilis* (28±0.2mm) يليه *Salmonella typhimurium* (27±0.2mm) يليه *Escherichia coli* (25±0.2mm) يليه *Klebsiella pneumonia* (23±0.2mm).

تم تعريف تلك العينة وراثيا باستخدام جهاز MALDI-TOF-MS ووجد أنها من ضمن أنواع البكتريا العصوية ، أيضا تم التعرف على العديد من المركبات النشطة بيولوجيا باستخدام جهاز GC-MS والتي من أهمها مركب Bis (2-ethylhexyl) phthalate. وتراوحت قيم التركيز المثبط الأدنى MIC من ١٥,٦٢ الى ٣١,٢٥ ميكروجرام/ مللي مما يشير إلى فعالية هذه المركبات عند التركيزات المنخفضة. ويمكن لتقنيات التكنولوجيا الحيوية المتقدمة مثل التسلسل الجيني ودراسات التمثيل الغذائي أن تزيد من توضيح الخصائص المضادة للبكتريا لهذه المركبات مما يزيد من إمكانية استخدامها في التطبيقات العلاجية. ومن خلال دمج هذه الأساليب البحثية المختلفة يساهم هذا البحث برؤيا قيمة فى مكافحة مقاومة المضادات الحيوية كما يمهد الطريق أيضا لتطوير علاجات مبتكرة يمكن أن توثر بشكل كبير على صحة الإنسان ورفاهيته.

الكلمات الدالة: الميكروبات البحرية، حساسية المضاد الحيوي، المركبات النشطة بيولوجيا، التأثير المثبط.