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Isolation and Characterization of Bacteria with Antifungal Activity from Gulf of Suez

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THE MARINE microbiome consists of diverse microorganisms in ocean environments that are essential for nutrient cycling, ecosystem health, and global biogeochemical. This study focused on isolating and identifying a new bacterial strain collected from the Red Sea. The strain has the ability to produce antifungal compound as a bioactive secondary metabolite. One of six bacterial isolates from Gulf of Suez showed antagonistic activities against different fungi pathogens. The potent Bacillus strain (H19) was identified by MALDI-TOF as *Bacillus Cereus*. The pathogen fungi (*Aspergillus flavus, Penicillium glaprun, Aspergillus niger, mucor circinelloid and Candida albicans*) were all antagonistically determined by H19. The strain (H19) demonstrated the largest zone of inhibition against *Candida albicans* (29±0.1mm). Gas chromatography-mass spectrometry (GC-MS) analysis of the 2:1 methanol:chloroform extracts of the bacterial culture supernatant from strain H19 revealed the presence of two predominant bioactive compounds: Bis(2-ethylhexyl) phthalate and Octadecanoic acid, 2,3-dihydroxypropyl ester. These compounds were identified as possessing significant antimicrobial activity.

Keywords: Antifungal, Bacillus cereus, Bioactive compounds, Marine microbiome, MALDI-TOF

Introduction

The phylogenetic variety of the oceans is significantly higher than that of the terrestrial environment, despite making up 70% of the Earth's surface (Choudhary et al., 2017; Liu et al., 2019). The marine microbiome including bacteria, archaea, viruses, and microscopic eukaryotes, play critical roles in global biogeochemical cycles, and contributing to the health and stability of marine ecosystems, influencing processes such as primary production, nutrient recycling, and the degradation of organic matter (DeLong, 2009; Giovannoni & Stingl, 2005). Like many other microorganisms that play a significant role in interacting with the environment, these microbes perform add specific functions or activities of the microbes in the environment contributing to add the environmental impact or intended benefit. (Abd El-Rahim et al., 2016; El-Rahim, 2006; Moawad et al., 2019). The vast genetic and biological diversity of marine organisms makes the oceans a special and plentiful source of bioactive chemicals for the pharmaceutical industries (Martins et al., 2014). There are many different types of bioactive compounds found in marine microorganisms, such as antibacterial, antiviral, antifungal, antiquorum-sensing, and anticancer agents (Carroll et al., 2023; Valliappan et al., 2014). Marine bacteria›s ability to produce these secondary metabolites provides new opportunities for the synthesis of novel natural chemicals. Furthermore, marine bacteria are emerging as a promising source for the discovery

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of novel bioactive compounds, potentially leading to the development of new therapeutics. Their unique adaptations to diverse and extreme marine environments make them an intriguing reservoir of chemical diversity, which could be harnessed for pharmaceutical applications. For example, comprehensive analysis and rigorous screening of marine microorganisms have the potential to uncover novel antimicrobial compounds that could effectively combat drug-resistant infections for the foreseeable future, possibly extending over the next century. (Payne et al., 2007). Fungal pathogens, including *Aspergillus* and *Candida* species, can cause infections that are dangerous and can result in disease and even death. (Brown et al., 2012; El-Hossary et al., 2017; Kullberg & Arendrup, 2015). Aspergillus fumigatus has emerged as a significant pathogen in cancer patients. However, A. fumigates raises the danger of acquiring severe invasive infections in immunocompromised persons, with fatality rates ranging from 40 to 90% (Dagenais & Keller, 2009). Moreover, with a 40% fatality rate, invasive *candidiasis* is the most common fungal infection among hospitalized patients (Kullberg & Arendrup, 2015). *Bacillus* species is a common microbial flora found in many marine ecosystems (Xiao et al., 2022). *Bacillus* is one of the most significant groups of Firmcutes; *Bacillus* sp. is capable of growing rapidly in liquid culture and can withstand high temperatures. The genus *Bacillus* has been found to be widely distributed in aquatic environments, where it can form spores that can withstand a variety of unfavorable environmental conditions. *Bacillus* species have been frequently isolated from diverse environments, including agricultural waste and biogas reactor(Mohammed et al., 2020; Ziganshina et al., 2018). *Bacillus* can grow quickly and withstand a wide range of harsh environmental factors, including high pressure, salinity, pH, and nutrient deficiencies (Xiao et al., 2022). The aim of this study was to look into the possible antifungal activity of *Bacillus Cereus* that was isolated from the Gulf of Suez. In summary, *Bacillus cereus*, isolated and identified from the Gulf of Suez, demonstrated significant antifungal potential, particularly against *Candida albicans*. The bacterium's ability to inhibit fungal growth is likely due to its production of two key bioactive compounds, Bis(2-ethylhexyl)phthalate and Octadecanoic acid, 2,3-dihydroxypropyl ester. These findings suggest that *Bacillus cereus* may serve as a valuable source of antimicrobial agents.

Materials and Methods

Samples collection

Marine samples of various biological water were collected from the Egyptian coastal area namely Gulf of Suez on the Red Sea in February 2022 as shown in (Table 1). Samples were collected aseptically in sterile 500 ml bottles for microbiological analysis. The bottles were then transported to the laboratory and stored at 4 °C until further analysis.

Isolation, purification and cultivation of bacteria

A bacterial stock solution was prepared by using standard microbiological and serial dilution procedures (1 ml of seawater sample was suspended in 9 ml of sterile distilled water and serially diluted), the cultures of sample from Gulf of Suez was serially diluted from 10^{-1} to 10^{5} (Abdelnasser et al., 2017). Bacterial isolates were grown on medium with the following composition: Marine agar medium (pH 7.6) containing (gm l^{-1}): peptone (5); yeast extract (5); sucrose (10); agar (15); seawater (500ml). The pour plate method was used to isolate the bacteria (Abdelnasser et al., 2017). Plates were inoculated with 100µl of each dilution and incubated for 24 h at 30°C (Abdelnasser et al., 2017). The grown bacterial isolates were separated and purified using the streak method on a petri dish, in which bacterial allowed to grow for 24 hr at 30˚C. All colonies were selected and re-streaked several times on Marine agar plates to obtain pure colonies that were stored at -80°C as glycerol stocks in Marine Broth supplemented with 50% (v/v) glycerol.

TABLE 1. shows the geographical zones of marine sample collection from Suez Golf.

Geographical zones	Site	Location	Depth
GULF OF SUEZ	**Gulf of Suez (H)**	**29°01'02.5"N 33°02'31.5"E**	**40-70 cm**

Morphological Characterization

Bacterial species classification and differentiation have been greatly aided by morphological characterization. Several morphological evaluations were conducted, including gram reaction colonial characteristics on growth media (colony shape, surface, color, opacity, and consistency) (Abdelnasser et al., 2017).

Screening of antifungal activity

The antifungal assay was conducted using sterilized media, including Potato Dextrose Agar (PDA), Marine agar, Nutrient agar, and Nutrient broth. Pathogenic fungi (*Candida albicans* ATCC 10221, *Aspergillus niger* ATCC 16888, *Aspergillus flavus* AUMC 11685) were activated on PDA at pH 7.6. PDA was made using 200g of potato infusion, 20g of dextrose, 20g of agar, and one liter of distilled water. PDA was prepared with 200g Potato infusion, 20g Dextrose, 20g agar, and distilled water per liter. Marine bacteria, isolated from seawater, were cultured on Marine agar and Nutrient broth at 30°C for 24 hours. The fungi were incubated on PDA at 25°C for 5 days. Spores were transferred to sterile distilled water, vortexed, and spread on plates using a sterile swab. The well diffusion method was applied, adding 30 µL of isolated bacteria to each plate in triplicate. The plates were incubated at 30°C for 5 days to assess antifungal activity, determined by inhibition zones. One bacterial isolate (H19) was selected from 6 isolated bacteria from Gulf of Suez based on their antifungal potential.

Isolation and Purification of the Antifungal Agent

H19 isolate was grown onto nutrient broth culture for 3 days at 30°C and centrifuged at 5000 rpm for 10 minutes, then the supernatant was sterilized by passing it through a Millipore membrane filter (0.45µm pore size). 250 ml from H19 isolate supernatant was mixed with the several extracts at the same volume of 2:1 methanol: chloroform extract, the same volume of the ethyl acetate extract and ethanolic extract. The mixture was shaken for one hour at room temperature, then the mixture was transferred to a separator funnel and allowed to stand until the aqueous phase was separated from the solvent phase. 2:1 methanol: chloroform was then evaporated at room temperature and the remaining precipitate was dissolved on dimethyl sulfoxide (DMSO) for antifungal activity measurement. Antifungal activity of 2:1 methanol: chloroform extract was detected qualitatively by agar well diffusion assay using 100µl of the extract, while DMSO alone served as a negative control (Helal et al., 2022).

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

The fungal strains were cultured on Sabouraud Dextrose agar slants at 25°C. Inoculums were prepared by collecting sporulated fungi from the agar slant using a loop and suspending them in 10 mL of sterile water. These fungal suspensions were filtered through sterile gauze to remove hyphae. The resulting conidia suspensions were vigorously vortexed and adjusted with sterile distilled water to a concentration of (10^5) CFU/mL using a hemocytometer cell counting chamber, confirmed by a serial dilution plate count. These fungal suspensions were then diluted 1:5 with RPMI to obtain 2× final suspensions, resulting in a final conidial concentration of (10^4) CFU/mL when mixed with antifungal solution. Minimum inhibitory concentrations (MICs) were determined using round-bottomed 96-well plates. The mold conidial suspensions were prepared in RPMI 1640 and adjusted to a final concentration of (0.4 –5) × (10^4) CFU/mL as previously described. The inoculated plates were incubated for 48 hours at 35°C, and MICs were read at 24 or 48 hours as the drug concentration that resulted in a 100% reduction in growth compared to a drug-free control well (Cuenca-Estrella et al., 2010). Minimum fungicidal concentration (MFC) was evaluated by plating 0.01 mL of the sample from each concentration with no visible fungal growth onto Sabouraud glucose agar plates (SGA), followed by incubation at 26°C for 72 hours. The concentration at which no growth was observed after MIC readings was determined. Microplates were agitated using a vortex and an automatic plate shaker, and 100 µL aliquots were removed from each growth-negative well (micro dilution) and spread on SGA Petri dishes. These plates were incubated at 25°C for 72 hours, and the resulting fungal colonies were counted(Izzo et al., 2020).

GC-MS analysis

The chemical composition of the samples was analyzed using a Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature program started at 35°C, followed by an increase of 3°C/min to 200°C, held for 3 minutes, and then further increased to a final temperature of 280°C

at 3°C/min, held for 10 minutes. The injector and MS transfer line temperatures were maintained at 250°C and 260°C, respectively. Helium served as the carrier gas at a constant flow rate of 1 mL/min. A solvent delay of 3 minutes was applied, and 1 µL of diluted samples was automatically injected using an Auto sampler AS1300 coupled with the GC in split mode. Electron ionization (EI) mass spectra were collected at 70 eV ionization voltage over a mass range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200°C. Components were identified by comparing their retention times and mass spectra with those in the WILEY 09 and NIST 11 mass spectral databases (Huwaimel et al., 2023).

Using MALDI TOF MS for Identification

Whole bacterial cell protein profiles are compared to reference spectra using the well-known MALDI-TOF MS technique. Presumptive Bacillus isolates were verified using proteomic spectra produced by MALDI-TOF MS (VITEK®MS, database version 3, BioMerieux, France). As an internal identification control and calibration measure, *E. Coli* ATCC 8739 was injected on the calibration spots (Sabeq et al., 2022). The manufacturer's guidelines were adhered to when interpreting the results. In order to identify the (H19) isolate, the peaks from the spectrum were compared to the standard spectrum for a certain species, genus, or family of microbe.

Results and Discussion

A unique source of biological diversity, the Red Sea contributes organisms and genetic lines to aquatic biodiversity systems around the world(Soliman et al., 2022). The majority of marine species discovered in the last five years have been found along Egypt's coasts, accounting for 58% of all species found there compared to other Red Sea locations (Soliman et al., 2022). The Egyptian environment's high level of biodiversity is probably the cause of this predominance (Soliman et al., 2022).

Isolation, purification and cultivation of bacteria

The Gulf of Suez on the Red Sea is one of the coastal ecosystems in Egypt where marine water sample was collected for this study. After being incubated for 48 hours at 30°C, all isolated strains demonstrated robust growth on marine agar medium. Gram staining was used to confirm the selection of roughly six different pure colonies based on morphological variations.

Morphological Characterization

The most promising marine bacterial isolate from Gulf of Suez was phenotypically characterized using microscopic examination, gram stain, color, shape, surface, opacity, and consistency, as indicated in Table 2. This table show the phenotypic characterization of the isolates from the Gulf of Suez from the Red Sea. Bacterial isolates were identified based on the colony, gram stain and microscopy shape. Aerobic organisms can produce endospores, which allow them to endure extreme climatic conditions (Piewngam et al., 2018; Sharp et al., 2021; Stein, 2005). It has been widely reported that extracellular secondary metabolites from isolates can inhibit pathogenic microbes. After isolation and purification, the isolates were assessed for their production of antifungal activities. According to Das et al. (2019), there were differences in the morpho-physio-biochemical properties, involving colony characteristics, which suggested that the bacteria had different structures and functions(Das et al., 2019). All marine isolates in our study had their physiological, morphological, and microscopic features determined, and studies of their antifungal activity were carried out.

TABLE 2. shows the Phenotypic characterization of strains isolated from GULF OF SUEZ

Geographical zones	Code Strain	Morphology	Gram stain	Bacterial Colony Morphology				
				Color	Shape	Surface	Opacity	Consistency
GULF OF SUEZ	H3	Long rod	-	creamy	Irregular	Smooth	Translucent	Mucous
	H5	Short rod	+	Yellow	Irregular	glistening	Opaque	Viscid
	H8	Coccus	-	Light yellow	Irregular	Smooth	Opaque	Friable
	H15	Long rod	+	Gray	Irregular	Smooth	Opaque	Viscid
	H19	Short rod	+	Creamy	Irregular	Wrinkled	Opaque	Mucous
	H20	Short rod	-	Yellow	Rhizoid	Smooth	Translucent	Viscid

Screening for antifungal activity

The ability to produce antifungal agents against pathogenic fungi was assessed in the purified selected bacterial isolates. One of the main factors in the selection of these pathogens was the wide variety of antimicrobial activity(Al Amoudi, 2016). Marine bacteria commonly demonstrate broad-spectrum activity(El-Gamal et al., 2015). In our study, one out of six screened isolates demonstrated high potential with large inhibition zones against the tested pathogens (*Candida albicans* (ATCC 10221), *Aspergillus niger* (ATCC 16888), *Aspergillus flavus* (AUMC 11685) as shown in Table 3. With regard to the tested pathogenic indicators, isolates H19 demonstrated notable antifungal activity. According to Helal et al., seven of eighty bacterial isolates collected from the Red Sea demonstrated antimicrobial activity against five reference strains. Bacillus amyloliquefaciens was the most potent isolate, demonstrating notable activity against yeasts, Gram-positive bacteria, and Gram-negative bacteria (Helal et al., 2018). Table 3 presents the screening results of the most highly purified bacterial strains, selected for their ability to produce antifungal agents effective against the tested pathogenic indicators.

Isolation and Purification of the Antifungal Agent

To identify the compound(s) responsible for antifungal activity, the extracted was by different solvents (Chloroform: Methanol (2:1) extract, the Ethyl acetate extract and ethanolic extract). The largest inhibition zone observed for strain H19 was Chloroform: Methanol (2:1) solvent and other solvent gave negative result shown in fig1.. The extract was qualitatively assessed using an agar well diffusion assay with 100µl of the extract. The extract inhibited fungal growth, indicating a positive result. The antagonistic performance of Burkholderia species is well known and relies on the production of a diverse range of antifungal compounds. Moreover, the antagonistic activity of H19 isolate against pathogenic fungi is likely due to their production of secondary metabolites and volatile compounds, which play a crucial role in inhibiting fungal growth. Isolate H19 exhibited significant antagonistic activity against various pathogenic fungi, including *Candida albicans* (ATCC 10221), (*Aspergillus niger* (ATCC 16888), (*Aspergillus flavus* (AUMC 11685), (Penicillium glabrum (Op694171), and (Mucor circinelloides (AUMMC 11656). The maximum zone of inhibition for H19 was against (*Candida albicans*) (29±0.1 mm), followed by (*Penicillium glabrum*) (29±0.2 mm), (*Mucor circinelloides*) (27±0.1 mm), (*Aspergillus niger*) (19±0.2 mm), and (*Aspergillus flavus*) (12±0.1 mm).

Evaluation of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC).

The minimal inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial agent required to inhibit visible growth of a microorganism in a broth dilution susceptibility test. The minimum fungicidal concentration (MFC) is indicating the lowest concentration of an antifungal agent that kills the fungus. A stock solution containing 10 mg of scaffold sample was dissolved and suspended in 10 ml distilled water, resulting in a concentration of 1000 µg/ml. MIC values were determined using concentrations prepared through serial twofold dilutions, following a base 2 logarithmic scale (e.g., 125, 250, 500, 1000 µg/ml). Results are expressed in µg/ml. Table 5 presents the MIC and MFC value for H19 extracts against pathogenic fungi, including *Candida albicans* (ATCC 10221), *Aspergillus niger* (ATCC 16888), *Aspergillus flavus* (AUMC 11685), *Penicillium glabrum* (Op694171), *and Mucor circinelloides* (AUMMC 11656).

TABLE 3. Measurement of the Inhibition Zone Diameter Demonstrating the Antagonistic Activity of Strain H19 Against Human Pathogens.

Isolates	*Candida albicans* (ATCC 10221) (mm)	*Aspergillus niger* ATCC 16888 (mm)	*Aspergillus flavus* AUMC 11685 (mm)
H3	28±0.1	23±0.1	18±0.1
H5	30±0.1	15±0.1	27±0.1
H8	20±0.1	No inhibition zone	15±0.1
H15	32±0.1	No inhibition zone	20±0.1
H19	35±0.1	40±0.1	45±0.1
H20	25±0.1	No inhibition zone	No inhibition zone

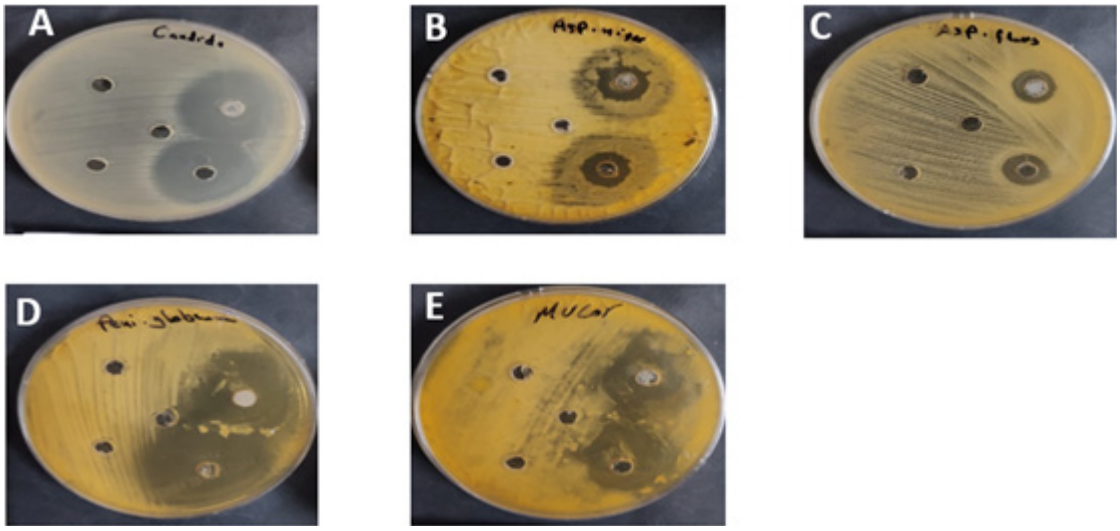

Fig.1. Antifungal activity of H19 against (A) Candida albicans, (B) Aspergillus niger, (C) Aspergillus flavus, (D) Penicillium glabrum and (E) Mucor circinelloid by Chloroform: Methanol (2:1) extract.

TABLE 4. MIC and MFC quantifications values for H19 extract against the indicated pathogens.

Pathogenic microorganism	MIC for Sample (H19) Ch:M(2:1) Ext. (µg /ml)	MFC for Sample (H19) Ch:M(2:1) Ext. (µg /ml)
Candida albicans **(ATCC 10221)**	15.62	31.25
Aspergillus niger (**ATCC 16888)**	62.5	62.5
Aspergillus flavus **(AUMC11685)**	500	1000
Penicillium glabrum (**Op694171)**	15.62	31.25
Mucor circinelloid **(AUMMC 11656)**	15.62	15.62

H19 isolate was tested as an antifungal activity against filamentous mold and yeasts, including *Candida albicans* (ATCC 10221), *(Aspergillus niger* (ATCC 16888), *(Aspergillus flavus* (AUMC 11685), *(Penicillium glabrum* (Op694171), *and (Mucor circinelloides* (AUMMC 11656).The maximum zone of inhibition for H19 was against (*Candida albicans*) (29±0.1 mm) inhibition zone, MIC of 15.62µg/mL, and MFC of 31.25µg/mL, followed by (*Penicillium glabrum*) (28±0.1 mm) inhibition zone, MIC of 15.62µg/mL, and MFC of 31.25µg/mL, (*Mucor circinelloides*) (27±0.1 mm) inhibition zone, MIC of 15.62µg/mL, and MFC of 15.62µg/mL, (*Aspergillus niger*) (19±0.2 mm) inhibition zone, MIC of 62.5µg/mL, and MFC of 62.5µg/mL, and (*Aspergillus flavus*) (12±0.1 mm) inhibition zone, MIC of 500µg/mL, and MFC of 1000µg/mL. as shown in Table 4,5. (Alharbi et al., 2024) demonstrated that the exopolysaccharide EPSR2 exhibits strong antifungal activity against (*Candida albicans*), with a 28 mm inhibition zone, MIC of 7.8 µg/mL, and MFC of 15.62 µg/mL. However, *Mucor circinelloides* and (*Trichoderma harzianum*) were resistant to EPSR2, while (*Aspergillus niger*) and (*Penicillium glabrum)* showed moderate susceptibility. Further research should target its potential in treating candidiasis and yeast infections due to its high efficacy against (*C. albicans*)(Alharbi et al., 2024).

Identification of potentially bioactive compounds of H19 by GC-MS

Gas chromatography-mass spectrometry (GC-MS) is a fundamental method for metabolite profiling that has substantially advanced our understanding of the metabolome. Since its introduction as an essential tool for metabolite analysis, GC-MS has been consistently employed in functional genomic studies of plants and microorganisms, including bacteria and fungi, to identify both known and novel metabolic traits (Kopka, 2006). In this study, GC-MS analysis was performed on extracts to identify

their components. The analysis results, including compound percentages, formulae, retention times, and molecular weights, are presented in Table 5 and fig.2 for H19 strain. Various solvents, including ethyl acetate, ethanol, and Chloroform: Methanol (2:1) extract, were tested for their efficiency in extracting desired bioactive compounds. The crude extract was evaluated for antifungal activity against previously mentioned pathogenic microbial indicators from strain H19. Results indicated that Chloroform: Methanol (2:1) extract, was the most efficient solvent for extracting the desired compounds, exhibiting the highest antifungal activity from strain H19. GC-MS analysis identified several bioactive compounds in this extract. The active principle in H19 included Bis(2-ethylhexyl) phthalate and Octadecanoic Acid. Bis(2-ethylhexyl) phthalate demonstrated antimicrobial activity (Lotfy et al., 2018). Masrukhin et al., 2021. also identified bis (2-ethylhexyl) phthalate as a predominant compound in the GC-MS profile of active compounds extracted from Bacillus spp. (Bacillus siamensis) using ethyl acetate (Masrukhin et al., 2021). Studies have confirmed the antibacterial(Lotfy et al., 2018), antifungal and anticancer activities of bis (2-ethylhexyl) phthalate (Masrukhin et al., 2021; Siddharth, 2019). And Octadecanoic Acid demonstrated antifungal and antibacterial properties as seen in (Akpuaka et al., 2013) (Akpuaka et al., 2013). K. Marimuthu, N. Nagaraj, and D. Ravi, reported that the 9-Octadecenoic acid (Z)-, 2-hydroxy-1- (hydroxy methyl) ethyl ester demonstrated Antimicrobial(Marimuthu et al., 2014). According to Viswanathan and Shobi (2018), phthalate compounds, specifically Bis-(2-ethylhexyl) phthalat, have been shown to block the growth of a number of bacteria, including S. epidermidis, *S. aureus*, *S. pneumoniae, E. coli, M. luteus, K. pneumonia, S. flexneri, V. cholerae, and P. aeruginosa* (Shobi & Viswanathan, 2018). According to Kanjana et al,2019 that the Bis (2-ethyl hexyl) phthalate exhibited antioxidant, antifungal, and antimicrobial properties (Kanjana et al., 2019).

Identification Using MALDI-TOF MS.

MALDI-TOF mass spectrometry operates by comparing the protein spectrum of the analyzed specimen to a reference database of known spectra (Wilkins et al., 2006). The Spectra Bank (http://www.spectrabank.org) is a database containing mass lists specific to various species or strains. Currently, it holds data for approximately 200 specimens. These characteristic mass lists can be analyzed and compared using the SPECLUST tool (Alm et al., 2006). The bacterial isolate H19 was identified at genus level as *Bacillus sp. (Bacillus Cereus*) through the MALDI-TOF/MS analysis.

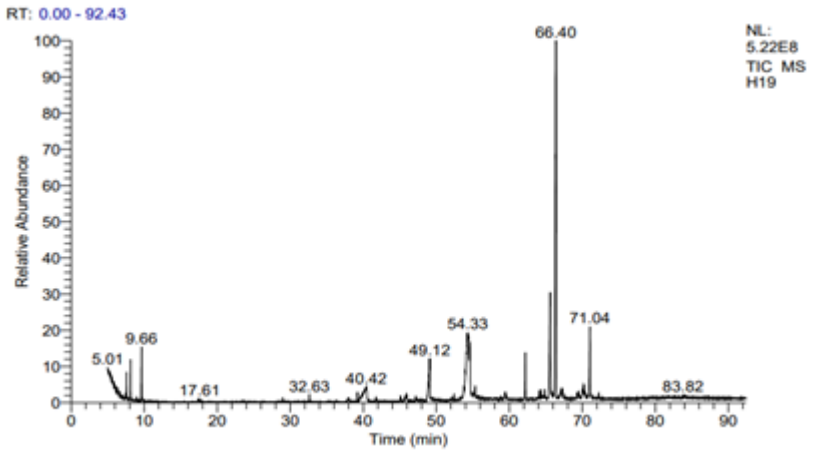

Fig.2: GC-MS profiles of H19

TABLE 5. GC-MS analysis of the Chloroform: Methanol (2:1) extract of H19

RT	Area %	Compound name	Molecular formula	Molecular weight g/ mol	Proposed function
38 .01	0.51	1,4-diazabicyclo [4.3. 0] nonan-2,5-dione, 3-methyl	C8H12N2O2	168	antimicrobial activity
39 .20	0.64	Dodecyl acrylate	C15H28O2	240	Polyemerization(Buback & Kowollik, 1999)
45 .84	0.56	3-Isobutylhexa Hydropyrrolo [1,2-A] Pyrazine1,4-Dione #	C11H18N2O2	210	anticancerous and antimicrobial activity(Naragani et al., 2016)
49 .11	3.93	n-Hexadecanoic acid	C16H32O2	256	Anti-inflammatory(Ragunathan et al., 2019)
54 .62	3.38	Oleic Acid	C18H34O2	282	Anticancer, anti-inflammatory, wound healing(Sales-Campos et al., 2013)
55 .35	0.95	Octadecanoic Acid	C18H36O2	284	antifungal and antibacterial properties(Akpuaka et al., 2013)
66 .39	34.25	Bis(2-ethylhexyl) phthalate	C24H38O4	390	Antimicrobial activity(Lotfy et al., 2018)
70 .20	0.74	9-Octadecenoic acid (Z)-, 2-hydroxy-1- (hydroxy methyl) ethyl ester	C21H40O4	356	Antimicrobial(Marimuthu et al., 2014)
71 .04	5.54	Octadecanoic acid, 2,3-dihydroxypropyl ester	C21H42O4	358	Antimicrobial and anticancer ,antimicrobial as hormone in animals and plants, antipyretic, antiulcer, hypolipidemic , antihyperglycemic and antidiabetic (AlAmery, 2020; Lakshmi & Nair, 2017; Sirikhansaeng et al., 2017)

Conclusion

The work highlights the significance of *Bacillus cereus* that was isolated from Gulf of Suez and was identified by MALDI-TOF to look into the possible antifungal. *Bacillus Cereus* demonstrated the largest zone of inhibition against *Candida Albicans*. *Bacillus Cereus* may produce Bis(2-ethylhexyl)phthalate, and Octadecanoic acid, 2,3 dihydroxypropyl ester as two major bioactive compound with antimicrobial activity.

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