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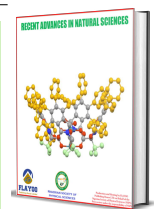

Antibiosis and GC-MS of secondary metabolites of rhizosphere bacteria from Manatee food plants in the humic freshwater ecosystem of Eniong river, Nigeria

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ABSTRACT

Microorganisms are able to synthesize secondary metabolites of various structures and bioactivities. These metabolites are produced to help the organism compete successfully with other organisms in their natural habitat and adapt with changing environmental milieu. The ability of rhizosphere bacteria (*Bacillus subtilis* NC_000964.3 and *Pseudomonas aeruginosa* NC_002516.2) isolated from the rhizospheric soil of Manatee food plants *Mimosa pygra, Ipomeoa aquatica* and *Pistia stratoites* to inhibit the growth of human pathogens (*P. aeruginosa, E. coli, S. aureus* and *B. subtilis*) was evaluated using standard methods. It was observed that the growth extracts of *B. subtilis* strains M_5, M_8 and P_7 and *P. aeruginosa* strains I_3 and M_9 contained useful bioactive compound. GC-MS analysis of the cell -free methanol extract of the antibiotic producing bacterial strains was also evaluated and the results showed that their inhibitory potentials against bacterial pathogens are due to the presence of phenylethyl alcohol, 2-ethyl-4-methyl-1,3-dioxolane, bicyclo [4.2.0] octa-1,3,5-triene and 4-amino-2-methyl-5,6-dimethyl pyrimidine for B. subtilis and 3,4-dimethyl tetrahydrofuran, 4,6-dimethyl-4-hydroxy-5-heptenoic acid and 2,4-dimethyl-4-heptanol for *Pseudomonas aeruginosa*. These strains of rhizosphere bacteria may be exploited to produce new antibiotics.

1. INTRODUCTION

A phenomenon known as antibiosis occurs when the growth of one or more organisms is hampered by the presence of other species or the release of their metabolites. Antibiotics are chemical or bioactive metabolites created by bacteria that, in small doses, have the power to prevent other organisms from growing, metabolizing, and even killing them. The role of natural antibiotics has been disputed for decades and has been the subject of much investigation [1]. Natural antibiotics are hypothesized to aid in microbial defense, fitness, interference, and competition. Antibiotic-producing organisms are widespread in microbial communities around the world [1–3]. It is well acknowledged that in terrestrial ecosystems, naturally occurring antibiotics accumulate at inhibiting concentrations largely in nutrient-rich environments, including those that antibiotic producers come across when colonizing the rhizosphere of plants or animal hosts [1].

Numerous crucial environmental tasks are carried out by the freshwater ecosystem, including nutrient recycling, water purifi-

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cation, flood mitigation, groundwater recharge, and the provision of habitat for species. Additionally, people utilize them for recreation, especially in coastal areas [4]. This environment supports a wide variety of hydrophytes, vertebrate and non-vertebrate species, and microbiomes because to its high biological diversity. *Pseudomonas, Bacillus, Azotobacter, Micrococcus, Enterococcus, Acromobacterium, Salmonella, Shigella, Citrobacter, Flavobacterium*, and *Escherichia species* are only a few of the known bacterial species. There are additional known instances of fungi from the genera *Penicillium*, *Aspergillus*, *Candida*, *Fusarium*, *Geotricum*, *Saccharomyces*, and others [5]. These microbes are crucial to the mineralization of the intricate organics and other substances found in freshwater sediment. Within plants rhizosphere, considerable microbial activity occurs. The microbes present modulate biogeochemical processes within this system [6]. Simulation of bacteria is due to release from plant roots of a vast majority of plant materials including carbohydrates, vitamins, amino acids, enzymes, mucilage, sloughed root cells and carbon allocated to root-associated symbionts [7, 8]. This in turn affects the number and diversity of microorganisms present within the rhizosphere, their composition and interactions [9].

Recent studies have expanded our understanding of rhizosphere bacteria in aquatic systems, highlighting their unique roles and interactions within their environments. Findings by Pan *et al.* [10] & Lu *et al.* [11] demonstrated that aquatic rhizosphere bacteria, similar to their terrestrial counterparts, produce a diverse array of secondary metabolites that influence nutrient cycling and plant health. These findings underscore the complexity and dynamism of microbial interactions in semi-aquatic environments, where water flow and sediment composition add additional layers of ecological interaction not commonly observed in terrestrial habitats.

Furthermore, comparative analyses of secondary metabolite production in terrestrial and aquatic rhizospheres have revealed significant differences in the chemical profiles of produced antibiotics, likely due to the distinct selective pressures exerted by the aquatic environment [12, 13]. These studies suggest that aquatic rhizosphere bacteria might employ unique biochemical pathways to synthesize secondary metabolites, which could offer novel bioactive compounds for medical and agricultural applications.

The impact of these metabolites on microbial community dynamics and plant interactions in aquatic systems has also been explored in recent research. For example, a study by Etesami & Glick [14] in coastal marshlands showed that secondary metabolites from rhizosphere bacteria significantly affect the growth patterns of both plant hosts and neighboring microbial populations, potentially influencing the overall ecosystem stability and resilience to environmental stressors.

Moreover, the exploration of antibacterial activity in these environments has gained momentum, with studies such as those by Saeed *et al.* [15] identifying potent antimicrobial agents from bacteria isolated from the rhizosphere of aquatic plants. These agents not only offer potential for therapeutic uses but also play crucial ecological roles, mediating interactions within the microbial community and protecting plant hosts against pathogens.

Rhizosphere bacteria have been documented to serve as biocontrol and antibacterial agents in addition to influencing plant development. Raaijmakers [16], Weller *et al.* [17], and Mavrodi *et al.* [18] all found that rhizosphere-dwelling biocontrol bacteria may suppress plant diseases through the use of antibiotics. Examples of antibiosis that protects eukaryotic hosts and their symbionts from pathogens include associations between ants and *Pseudonocardia* species, which produce antibiotics that shield ant fungal gardens from the mycoparasite Escovopsis [19], and the southern pine beetle *Dendroctonus frontalis* and the fungus *Entocorticium* sp. A, which is shielded [20]. The genera *Pseudomonas* and *Baccilus* isolated from the rhizosphere of maize and potatoes plant showed antagonistic activity against *Aspergillus flavus, Fusarium verticilliodes* and phytopathogenic fungi such as *Fusarium osysporum*, *Fusarium solani*, *Rhizoctonia solani* and *Pythium ultimum* respectively [20, 21]. Rhizosphere bacteria isolated from Kochi, India, showed positive activity against *Enterococcus* sp and *Staphylococcus aureus* [22]. Also, Nair *et al.* [23] isolated antibacterial compound from *Baccilus horikoshii* found in the rhizosphere region of Alfalfa plant. In addition, it has been observed that the growth of *Micrococcus luteus* and *Staphylococcus aureus* is inhibited by other soil bacteria, including *Streptomyces* species, *Pseudomonas aeruginosa*, *Actinomyces* strain, and *Bacillus* species [24, 25]. These findings suggest that soil microbes are good sources of secondary metabolites with potent antimicrobial properties.

Mammals get their gut bacteria mostly from the food they consume [26]. Recent research has revealed that metabolites isolated from commensal microorganisms of aquatic mammals possess a variety of antimicrobial activities. Studies on faecal specimens from terrestrial mammalian species have shown striking degrees of host specificity of microbiota, reflecting the influence of host phylogeny, gut anatomy, and diet [26]. It is well recognized that certain of these metabolites alter how the host behaves, which may have an impact on a person's susceptibility to illness and pathogen invasion. The West African manatee, *or Trichechus senegalensis*, is a big aquatic animal that is also known as a sea cow. *Ipomoea aquatica, Echinochloa stagnina, Echinochloa stagnina, Nymphaea lotus, Echinochloa cruspavonis, Pistia stratiotes, Vossia cuspidate, Mimosa pygra,* and *Nymphaea lotus* are noTable examples of the aquatic and emergent plants that they consume. Manatees have recently been wrongfully and illegally taken from the humic freshwater habitat of the Eniong River in Nigeria's Akwa Ibom State. When the animals were killed, the intestines were dominated by newly cropped specimens of the plants *Mimosa pygra*, *Ipomoea aquatica*, and *Pistia stratiotes*. Recent arguments contend that antibiotics serve less as defensive agents and more as signaling molecules crucial to bacterial physiology, communication, and gene regulation. Antibiotics are thought to seldom accumulate in natural environments in inhibitory quantities [27]. According to Mavrodi *et al.* [18], antibiotics are likely to serve as signaling chemicals as well as antagonists in natural environments. Our understanding of the function played by these substances in nature, however, has been significantly hampered by our knowledge of the quantities, spatial and temporal patterns of antibiotic synthesis in the field. Natural antibiotics are notoriously difficult to detect and measure *in situ* because of microbial destruction, chemical breakdown, and/or binding to soil and organic materials, according to Bonsall [28] and Mavrodi *et al.* [18].

Although the mechanisms that affect the form and function of the microbiotas of terrestrial and aquatic animals are becoming more understood, relatively little is known about the factors that affect aquatic species that live in humic habitats. There are increasing concerns about the rising levels of antibiotic resistance, high cost and toxicity, the need to look for new therapeutic agents from soil microbiome has been on the increase. This is because soil microbiomes represent a promising habitat for the discovery and isolation of highly effective antibiotics as less than 1% of soil bacterial species are currently known. Information on the antibiotic potentials of bacterial isolates from the Niger Delta region of Nigeria is scanty, this prompted our search for new antibiotics from the rhizosphere of manatee food plants. Therefore, we report in this study the antibacterial activity and GC-MS analysis of methanolic extracts of bacteria from the rhizosphere of manatee food plants.

2. MATERIALS AND METHODS

The research area is a humic environment of the Eniong River, a tributary of the Cross River's middle course, and is situated on Nigeria's southern coast in the Niger Delta. The river lies between latitude 5^o12'N – 5^o22'N and longitude 7^o54'E – 8^o20'E. Rhizosphere samples (plant root and associated sediment) of three commonly encountered Manatee food plants (*Mimosa pygra* (Figure 1a), *Ipomeoa* aquatica (Figure 1b) and *Pistia stratoites* (Figure 1c) detected in the stomach of dead manatee were collected manually from 10 different locations within the study area. All rhizosphere samples collected were placed in sterile tubes, transported to the laboratory and stored at 4°C for no more than 24 h before processing. Due to the presence of humic chemicals and soluble iron complexes, the river has a vivid coloration. Additionally, it supports a variety of aquatic animal species, including the critically endangered manatee (*Trichechus senegalensis* (Figure 2). Isolation of bacteria was carried out using serial dilution technique as described by Willey *et al.* [29]. Bacterial isolates were obtained on nutrient agar slant (NA) using the pour plate technique [30].

A sensitivity assay was carried out to determine the antibiotic producing ability of the bacterial isolates against pure and clinical isolates of *Pseudomonas aeruginosa*, S*taphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* using the crowded technique. Assay plates were incubated for 24hrs at 37^{o}C. The method of Maniatis *et al.* [31] and Kraft *et al.* [32] were employed to extract and cure the plasmid from the antibiotic – producing isolates. Preliminary identification of the antibiotic producing bacterial strain revealed a Gram positive, cocci in pairs ($EHSA_4$) as the best producer [33, 34]. Confirmation was done by the 16S rRNA gene sequence analysis. DNA was extracted from the pure culture by a salting –out procedure [35] and amplified by polymerase chain reaction using primers and PCR conditions adapted from Tuleva *et al.* [36] with forward primer 12F1 (5'CGTGCTTAACACATGCAA3') and the reverse primer 1390 (5'GCCACCGGCTTCGGGTGTTTA 3'). The amplified products were electrophoresed using 2% agarose gel and stained with ethidium bromide. The Sanger sequencing approach was used to do DNA sequencing utilizing an automated PCR cycle - Sanger Sequencer^{TM} 3730/3730XL DNA analyser (Applied Systems). Direct blasting on http://blast.ncbi.nlm.nih.gov yielded the nucleotide sequence. Using the agar well diffusion technique, antibiotic susceptibility of clinical isolates of *Pseudomonas aeruginosa, Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* to methanolic growth extracts of antibiotic manufacturers was assessed [37]. The zones of inhibition were measured and recorded. Ciprofloxacin (100µg/mL) was used as the positive control. Whereas, bioactive compounds present in potent antibiotic-producing bacterial isolates were characterized and identified in accordance with the method of Elleuch [37]. GC-MS was performed on the methanolic extract of the crude culture medium (3mg/0.2mL), which had passed through a 0.45µm syringe filter to remove bacterial cells. A Hewlett-Packard 5890 gas chromatograph coupled with an AutoSpec mass spectrometer was used. Identification of individual compounds was done by comparison with the mass spectra of authentic reference compounds and reference libraries.

3. RESULTS

Thirteen (13) bacterial species including *Bacillis subtilitis, Streptococcus* sp*, Proteus* sp*, Clostridium* sp*, Micrococcus* sp*, Serratia* sp*, Staphylococcus aureus, Pseudomonas aeruginosa, Chromatium* sp, *Klebsiella* sp, *Flavobacterium* sp, *Actinomyetes* and *Enterobacter* sp were isolated from the rhizosphere of Manatee food plants. Their Gram staining characteristics is given in Table 1. Extracts from *B. subtilis* and *P. aeruginosa* were active against clinical isolates of *E. coli* and *S. aureus*, with zones of inhibition greater than 10mm (Table 2). Isolates M_5, M_8 and P_7 were found to be strains of *B. subtilis*, while isolates I_3 and M_9 were strains of *P. aeruginosa* based on their genomic properties (Table 3). GC-MS analysis of methanol extracts from broth cultures of *B. subtilis* and *P. aeruginosa* strains revealed variations in compounds elaborated (Table 4). Nine compounds were detected in broth culture of isolates M_5, M_8 and P_7 strains from *B. subtilis* while seven bioactive compounds were detected in isolates I_3 and M_9 from *P. aeruginosa*. The chromatograms of the bioactive compounds are given in Figures 3 and 4 while the elucidated structure of the compounds are presented in Figure 5.

4. DISCUSSION

Gram staining characteristics of the bacterial strains from the root zone of manatee food plants showed the presence of thirteen (13) isolates, of which ten (10) were rod shaped, two (2) cocci, and one (1) filamentous with many having the ability for spore formation. They were identified as *P. aeruginosa*, *E. coli*, *B. subtilis* and species of *Streptococcus, Clostridium, Micrococcus, Serratia, Chromatium, Klebsiella, Flavobacterium, Proteus, Actinomycetes,* and *Enterobacter*.

The ability of rhizosphere bacteria isolates to inhibit the growth of human pathogens (*P. aeruginosa, E. coli, S. aureus* and *B. subtilis*) was also evaluated. Methanol extracts of isolates from *B. subtilis*(P_7) exhibited promising antibacterial activity against clinical isolates of *E. coli* and *S. aureus* (inhibition zone 10 - 14mm), but was bacteriostatic to *P. aeruginosa* (inhibition zone 6- 8mm). Similarly, the methanol extract of *P. aeruginosa* isolates (I_3 and M_9) was most active against clinical strains of *E. coli* and *S. aureus* (inhibition zone = 10-14mm), but bacteriostatic against *B. subtilis* (inhibition zone <8mm). These results corroborate with report in literature. *Bacillus* species

Figure 1. a = *Mimosa pygra;* b = *Ipomeoa* aquatica; c = *Pistia stratoites*.

from terrestrial and marine environment are known to be good sources of antibiotic compounds [38–40]. Similarly, the genera *Pseudomonas* has been reported to show biological activity [40]. Also, Gislin *et al.* [22] reported the antibacterial activity of *B. amyloliquefaciens* from the rhizosphere and diverse cultivation at Kochi, India against *Enterococcus* sp and *S. aureus*. Nair *et al.* [23] reported antibacterial activity of *Bacillus horikoshii* isolated from the rhizosphere of Alfalfa plant against *Klebsiella* and *S. aureus*. Similar reports exist for *actinomycetes*, *streptomycetes*, *Pseudomonads* and other genera from rhizospheric soils [41, 42]. Therefore, our results suggest that *P. aeruginosa* and *B. subtilis* from the rhizosphere of manatee food plants could serve as potent sources of novel antibiotic compounds.

Molecular analysis of the antibiotic producing bacterial isolates was done using the 16S rRNA gene amplification and sequencing, followed by BLAST analysis using the mega blast tool of GenBank (http://blast.ncbi.nlm.nih.gov). This method is generally accepted as a better method of bacterial identification in comparison to other phenotypic methods [22]. Using this method, gene sequence comparison of P_7, M_5 and M_8 demonstrated 99% similarities to *B. subtilis* NC_000964.3. Similarly, sequence comparison of I_3 and M_9 demonstrated 99% similarities to *P. aeruginosa* NC_002516.2, confirming the identity of the five bacterial strains (P_7, M_5, M_8, I_3 and M_9).

GC-MS analysis of the cell -free methanol extract of the antibiotic producing bacterial strains was also evaluated. *B. subtilis* produced a total of nine bioactive compounds from its culture broth. Similarly, *P. aeruginosa* synthesized seven

Table 1. Gram staining characteristics of isolated bacterial species.

Isolate code	Gram staining reaction	Shape	Probable organism
P_7, M_5, M_8	+	Thick rod	*Bacillis subtilus*
P_1	+	Cocci in chain	*Streptococcus* sp
I_6, M_1	-	Rod	*Proteus* sp
M_6	+	Thick rod	*Clostridium* sp
M_3, M_7	+	Tiny cocci	*Micrococcus* sp
P_4, M_4	-	Rod	*Serratia* sp
P_3	-	Short rod	*Escheriichia coli*
I_3, M_9	-	Rod	*Pseudomonas aeruginosa*
P_5, M_4	-	Rod	*Chromatium* sp
I_2, I_4	-	Rod	*Klebsiella* sp
P_2, P_6	-	Rod	*Flavobacterium* sp
I_1	+	Filamentous	*Actinomycetes*
I_5, M_2	-	Rod	*Enterobacter* sp

Table 2. Gram staining characteristics of isolated bacterial species.

		Test clinical isolates			
Code	Isolates	*P. aeruginosa*	*E. coli*	*S. aureus*	*B. subtilis*
$P_7M_5M_8$	*B. subtilis*	+ - -	+++--	+++ - -	- - -
P_1	*Streptococcus* sp	-	-	-	-
I_6, M_1	*Proteus* sp	-	-	-	-
M_6	*Clostridium* sp	-	-	-	-
M_3, M_7	*Micrococcus* sp	-	-	-	-
P_4, M_4	*Serratia* sp	-	-	-	-
P_3	*E. coli*	-	-	-	-
I_3, M_9	*P. aeruginosa*	-	+++	+++	+
P_5, M_4	*Chromatium* sp	-	-	-	-
I_2, I_4	*Klebsiella* sp	-	-	-	-
P_2,P_6	*Flavobacterium* sp	-	-	-	-
P_1	*Actinomycetes*	-	-	-	-
I_5, M_2	*Enterobacter* sp	-	-	-	-

Key: 0-6mm = -, 6-8mm = +, 8-10mm = ++, 10-14mm = +++.

Table 3. Genomic properties of bioactive strains.

	Isolate number	Sample type	DNA type	Gene bank accession number	ID of organism
1	P_7	DNA	Genomic	NC_000964.3	*B. subtilis*
2	M_8	DNA	Genomic	NC_000964.3	*B. subtilis*
3	I_3	DNA	Genomic	NC_002516.2	*P. aeruginosa*
4	M_9	DNA	Genomic	NC_002516.2	*P. aeruginosa*
5	M_5	DNA	Genomic	NC_000964.3	*B. subtilis*

bioactive components. For extract of *B. subtilis* strains, 2-ethyl-2-pentanal (22.25%) and 2-ethyl-2-hexenal (21.49%) were the major compounds detected. Others were 2-ethyl-4-methyl-1,3-dioxalane (17.91%), 3-octanol (11.91%), trimethyl silanol (7.25%), 4-amino-2-methyl-5,6-dimethyl pyrimidine (5.77%), cyclohexane carboxylic acid (4.97%), bicyclo [4.2.0] octa-1,3,5-triene(4.51%) and 4-methyl-1,3-dioxane (3.95%). For *P. aeruginosa* extract, the major compound detected was 4-methyl-4-octanol (35.31%), followed by 2-ethyl-2-hexenal (16.77%), 2-ethyl-2-pentenal (15.05%), 2-methyl-2-hexanol (12.67%), 1,1-dimethoxy-2-methylpropane (8.26%), 2-methoxy quinoline (6.57%), and isothiocyanato ethane (5.37%). The antimicrobial activity of phenolic compounds and aromatic alcohols such as phenylethyl alcohol, have been reported [43]. Their bactericidal action was related to their physicochemical properties, and involved alterations in membrane function, resulting in cell death, particularly for Gram – negative bacteria. The antibacterial activity of fatty alcohols has been documented. Alcohols with carbon length between 8 and 12 showed activity against *S. aureus* and *P. acnes* [44]. These alcohols disrupt cell membrane fluidity by infiltrating the membrane's molecular structure, with the polar hydroxyl group hydrogen-bonded into the aqueous phase and the nonpolar carbon chain aligned into the lipid phase by dispersion forces. The antibacterial strains' extracts included cyclohexane carboxaldehyde, 2-ethyl-2-pentenal, and 2-ethyl-2-hexenal. Antibacterial activity has been observed for aliphatic

Table 4. Bioactive compounds from methanol extract of broth cultures from rhizosphere bacterial isolates.

Isolate	RT	Area (%)	Compound
B. subtilis strains M_5, M_8 and P_7.	3.297	4.51	Bicyclo [4.2.0] octa-1,3,5-triene
	3.387	4.97	Cyclohexane carboxaldehyde
	3.600	22.25	2-ethyl-2-pentenal
	3.657	11.91	3-octanol
	3.773	17.91	2-ethyl-4-methyl-1,3-dioxolane
	4.144	21.49	2-ethyl-2-hexenal
	4.271	3.95	4-methyl-1,3dioxane
	4.942	7.25	Trimethyl silanol
	6.667	5.77	4-amino-2-methyl-5,6-dimethyl pyrimidine
P. aeruginosa strains I_3 and M_9.	3.660	15.05	2-ethyl-2-pentenal
	3.709	12.67	2-methyl-2-hexanol
	3.818	5.37	Isothiocyanato-ethane
	4.178	16.77	2-ethyl-2-hexenal
	4.369	35.31	4-methyl-4-octanol
	4.950	8.26	1,1-dimethoxy-2-methyl-propane
	5.880	6.57	2-methoxy-quinoline

Figure 2. Manatee (*Trichechus senegalensis)* encountered during the study.

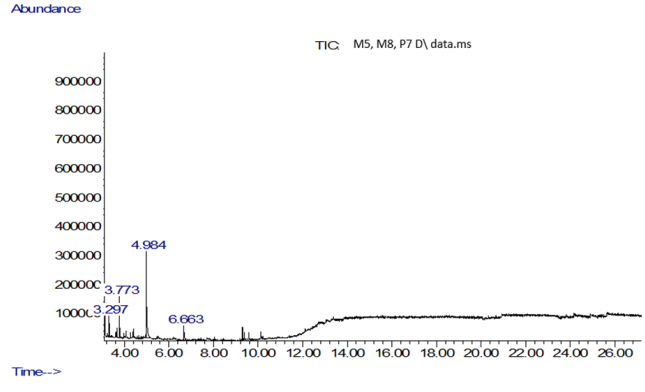

Figure 3. Profile of bioactive compounds elaborated by *B. subtilis* strains M_5, M_8 and P_7 .

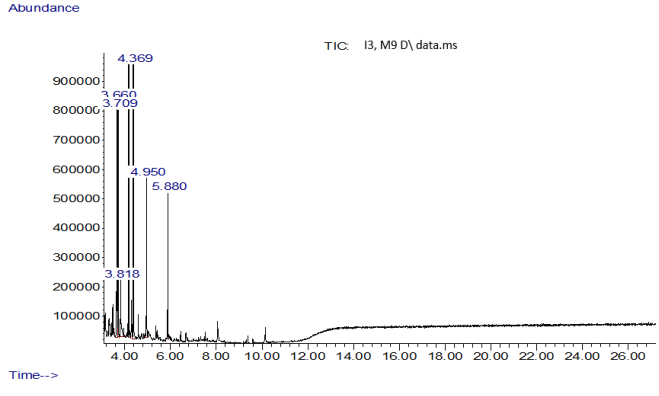

Figure 4. Profile of bioactive compounds elaborated by *Pseudomonas aeruginosa* strains I_3 and M_9.

aldehydes [45]. These aldehydes work by changing the function of membrane-associated proteins, interacting with the cell membrane's nucleophilic groups and producing considerable disruption in the lipidic bilayer. This action is more noticeable for the α, β unsaturated aldehydes. According to Kim *et al.* [46], silanols are a novel family of antibacterial drugs that appear to be more effective than their comparable alcohols. The authors examined the antibacterial activities of silanols, alcohols, and phenols on E. coli C3000 (ATCC15597), a laboratory strain of *S. aureus, P. aeruginosa,* and *E. faecalis* and discovered that silanols with stronger antibacterial activity had lower MIC values. Quinolones, on the other hand, have been shown to exhibit a wide range of biological actions, including antibacterial [47], antimalarial [48], anti-inflammatory [49], cytotoxicity [50], and so on. These compounds may be responsible for the extracts' antibacterial effect, either alone or in combination. In contrast to other species, the bioactive *B. subtilis* and *P. aeruginosa* strains

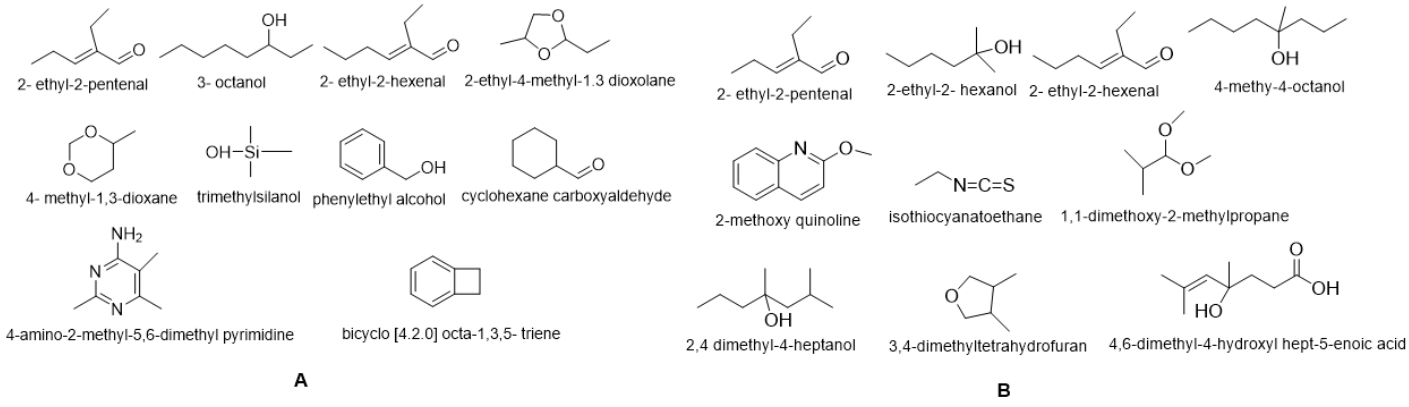

Figure 5. Bioactive compounds from methanolic extracts of A (*Bacillus subtilis* NC_000964.3) and the bioactive compounds from methanolic extracts B (*Pseudomonas aeruginosa* NC_002516.2).

support previous findings. Gislin *et al.* [22] discovered that *Bacillus* species are rich in antibiotic substances. This is consistent with the findings of this study. Sharma and Kaur [51] investigated the antibacterial activity of *Pseudomonas* and *Bacillus* rhizobacterial strains obtained from carnation rhizosphere soil. *Bacillus* sp isolated from the Amazon basin has also been shown to have antibacterial properties [52]. Iqbal *et al.* [53] identified antibacterial peptides from *B. safensis* strain MK12 waste dump soil in Pakistan and screened, characterized, and optimized them.

The bioactive compounds identified in our study, derived from the rhizosphere bacteria of manatee food plants, show promising antibacterial activity. To understand the potential mechanisms through which these compounds exert their effects, it is crucial to consider their molecular interactions with bacterial cells. This understanding is pivotal for leveraging their therapeutic potential and ecological significance. The primary mode of action of many natural bioactive compounds involves disrupting the bacterial cell membrane integrity. This disruption can lead to increased cell permeability, resulting in the leakage of vital cellular contents and eventual cell death. For instance, compounds such as lipopeptides and polyketides, commonly produced by soil bacteria, are known to insert themselves into the lipid bilayer of bacterial membranes, distorting the membrane structure and causing functional disruptions [54, 55].

Another potential mechanism is the inhibition of protein synthesis. Some bioactive compounds bind to bacterial ribosomes, blocking the ribosomal tunnel used during protein translation. This action effectively halts the protein production necessary for bacterial growth and replication, a mechanism employed by several known antibiotics like tetracyclines and macrolides [56]. Given the diverse microbial origin of the compounds studied, similar interactions could be involved in their antibacterial activity. Furthermore, these compounds might interfere with the synthesis or function of essential bacterial enzymes. Enzymatic inhibition can occur through the binding of bioactive compounds to the active sites of key bacterial enzymes, altering their conformation and thereby inhibiting their catalytic activity. This type of inhibition is crucial as it affects bacterial metabolic pathways and can lead to the buildup of toxic intermediates or the depletion of essential metabolites necessary for cell survival [57]. Bioactive compounds may also affect bacterial DNA replication. Certain compounds are capable of intercalating into DNA or binding to DNA gyrase, an enzyme critical for DNA replication. By disrupting these processes, the compounds prevent the bacteria from reproducing and maintaining their genomic integrity [58].

5. CONCLUSION

Secondary metabolites with a range of structures and bioactivities can be produced by microorganisms. Nutrients, growth rate, enzyme induction, and enzyme inactivation all affect how they are produced. The production of these metabolites, which have been crucial in the discovery and development of several antibiotics, helps the organism compete successfully with other species in its natural habitat and adapt to changing environmental conditions. As a result, a significant portion of commercially available antimicrobial medicines have microbial origins. The results of the current investigation have demonstrated that bacteria from the rhizosphere of manatee food plants can be powerful sources of new antibiotics and other bioactive substances.

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