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Bioactive Compounds from Marine *Microbacterium tumbae* ND2.7c Strain by Gas Chromatography-Mass Spectrometry

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Abstract The objectives of this study were analysis of the secondary metabolite products from extract of marine *Microbacterium tumbae* ND2.7c strain which isolated from sponges at Ha Tien Sea, Kien Giang province, Vietnam. Eleven bioactive compounds were identified in the organic solvent ethyl acetate-hexane. The identification of bioactive chemical compounds based on the peak area, retention time, molecular weight, and molecular formula. GC-MS analysis of *Microbacterium tumbae* ND2.7c strain revealed the existence of the 11 bioactive compounds had antimicrobial activity as cyclopentasiloxanedecamethyl-, tetrasiloxane 3.5-diethoxy-1.1.1.7.7.7-hexamethyl-3.5-bis(trimethylsiloxy), 1-dodecene, 3-isopropoxy-1.1.1.7.7.7-hexamethyl-3.5.5-tris(trimethylsiloxy) tetrasiloxane, 3,5-di-t-butylphenol, phthalic acid, 3.4-dihydroxymandelic acid 4TMS, 1.6-dioxacyclododecane-7-12-dione, 2-Propyl-1-pentanol, 2-(2'.4'.4'.6'.6'.8'.8'-heptamethyltetrasiloxan-2'yloxy)-2.4.4.6.6.8.8.10.10-nonamethyl cyclopentasiloxane and phthalic acid monoethyl ester. These compounds are bioactive secondary metabolites having antimicrobial activity, antifungal, antioxidant, against human pathogenic bacteria.

Keywords Bioactive compounds, GC-MS, Ha Tien Sea, *Microbacterium tumbae*, sponge

1. Introduction

The look for drugs from natural sources has been on the rise in ethnomedicinal research. And the plantatae kingdom has served as an inexhaustible source of use of medicine, foods, additives, flavoring agents, lubricants, coloring agents, and gums from time out of mind [1]. Marine organisms produce an outsized diversity of bizarre, often highly complex, natural products. The number of new chemical structures reported from marine biology has been continuously increasing over the past decade. With over 200 novel chemical structures described per annum, corals (Cnidaria, Anthozoa) are the second most prolific source of natural products retrieved from marine animals after sponges [2].

Sponges are well known to be hosts for a large community of microorganisms, which comprise a significant percentage (up to 50–60%) of the biomass of the sponge host [3-4]. The role of those diverse microbes in sponge biology varies from source of nutrition to mutualistic symbiosis with the sponge [5]. Marine actinomycetes were a group of aerobic, branched, unicellular Gram-positive bacteria with a high percentage of G+C (70%) in their genetic material.

Genus *Streptomyces* are used widely in industry because of their ability to produce numerous chemical compounds, including enzymes, antitumor agents, and (in the main) antibiotics [6]. Another genus/species in actinobacteria also are a rare genus in actinobacteria had the ability of bioactive metabolites as the species in genus *Streptomyces* as *Microbacterium* sp. [7], biosynthesis glucosylmannosyl-glycerolipid using cosmetics [8].

Abdelmohsen *et al.* [9] recognized *Microbacterium* sp. and *Rhodococcus* sp. isolated from sponges that had antibacterial activity to *Staphylococcus aureus* and *Enterococcus faecalis.*

In the past few years, Using Gas chromatography Mass spectrometry (GC-MS) was one of the technological platforms for fingerprint analysis of secondary metabolites in both plant and non-plant species [10]. Taking into consideration the medicinal importance of plant, the ethyl acetate root extract of the medical plant and leaves as Neem (*Azadirachta indica* A. Zuss) [11], flowers *Holarrhenaanti dysentrica* Wall [12] were analyzed by using GC-MS. This work will help to spot the bioactive components. GC-MS is that the best technique to spot bioactive constituents of long-chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitro compounds, etc. [13].

2. Materials and Methods

2.1. Materials

The *Microbacterium tumbae* ND2.7c strain with high antibacterial activity against reference bacteria tested were *Bacillus cereus, Escherichia coli, Salmonella typhimurium,* and *Candida albicans* isolated from a sponge at Ha Tien Sea, KienGiang province, Vietnam [14]. Using *Bacillus cereus* (ATCC 11778) tested the antibacterial activity of the crude extract from *Microbacterium tumbae* ND2.7c by the agar-well diffusion method.

2.2. Fermentation, extraction

The *Microbacterium tumbae* ND2.7c strain was cultured at 30^{o}C for 24 hours with shaking at 150rpm. Fermentation was carried out in the fermenter with 5 L Starch Casein medium and 10% bacterial inoculum at 30^{o}C for 52 hours. Neutral pH was maintained automatically by NaOH or HCl 1N. The obtained culture broth (5 L) was extracted with ethyl acetate three times, with a ratio of 2:1. The combined organic solutions were then decanted, filtered, and concentrated under reduced pressure at 50^{o}C. And then, the crude extract was tested the antibacterial activity with *Bacillus cereus* by the agar-well diffusion method and analyze compounds by the GC-MS method.

2.3. GC/MS analysis

The sample was analyzed GC-MS using Shimadzu Thermo (GCMS-QP2010 Plus) with Shimadzu column SH-Rxi-5Sil MS; L30 m x ID 0.25 mm x DF 0.25 µm at the Department of Environmental Sciences, College of Environment and Natural Resources, Can Tho University. Using helium as the carrier gas, and the temperature programming set was as follows:

	Speed (^{o}C/min)	Temperature (^{o}C)	Keep (min)
Initial		50	1.0
Ramp 1	10.0	160	3.0
Ramp 2	20.0	300	10.0
Total time		33 minutes	

One µl sample was injected with split less mode. Mass spectra were recorded over 35-400 amu range with electron impact ionization energy 70 eV, the total running time for a sample was 33 min. Quantitative determination was made by relating respective peak areas to TIC areas from GC-MS at 0.1 ppm.

3. Results & Discussion

From 5 L obtained culture broth of *Microbacterium tumbae* strain ND2.7c, the crude extract yield was 0.9731 g. The extract was evaluated the antibacterial activity together with control (ampicillin) on the reference bacteria (*Bacillus cereus*). The result showed that the diameter of the sterile ring of crude extract (20 mm) was higher than the control (8 mm). GC-MS analysis of compounds from the extract of *Microbacterium tumbae* ND2.7c with organic solvent ethyl acetate as shown in Figure 1.

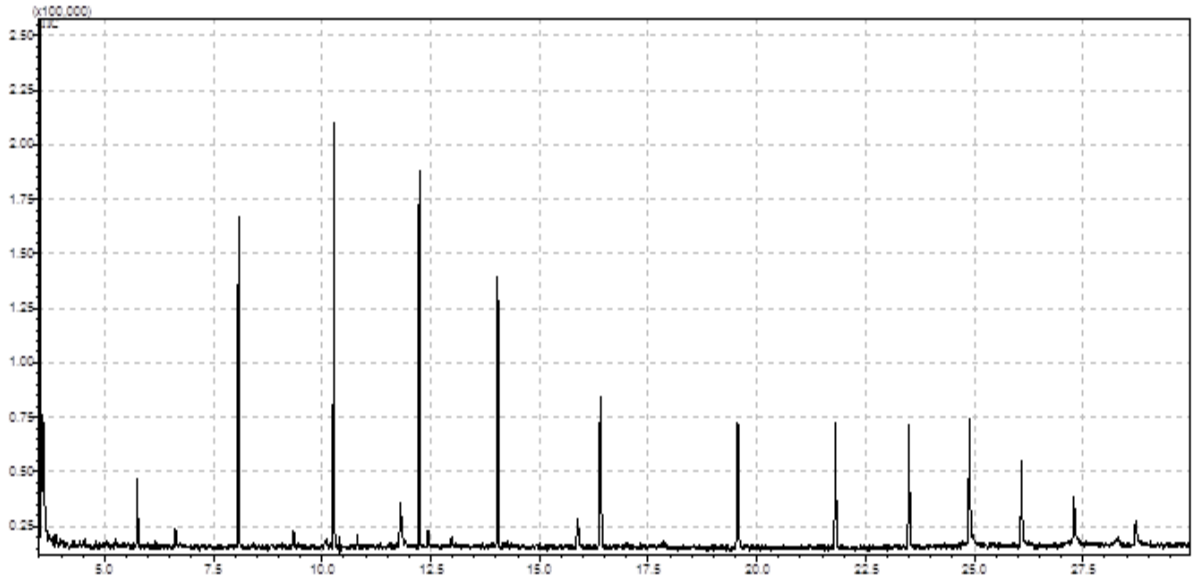

Figure 1: GC-MS chromatogram of extract of Microbacterium tumbae ND2.7c organic solvent ethyl acetate

Chromatogram GC-MS analysis of ethyl acetate extract of *Microbacterium tumbae* strain ND2.7c showed the presence of eleven major peaks (Table 1) and the components corresponding to the peaks were determined.

Table 1: Major bioactive metabolite compounds identified in ethyl acetate extract of *Microbacterium tumbae* strain ND2.7c

No.	Bioactive metabolite compound	RT (min)	Molecular weight(g/mol)	Molecular formula	Pharmacological actions
1	Cyclopentasiloxane, decamethyl-	5.752	370	$C_{10}H_{30}O_5Si_5$	antimicrobial
2	Tetrasiloxane, 3.5-diethoxy-1.1.1.7.7.7-hexamethyl-3.5-bis(trimethylsiloxy)	8.080	518	$C_{16}H_{46}O_7Si_6$	antimicrobial
3	1-dodecene	9.344	168	$C_{12}H_{24}$	Antimicrobial, antifungal, antioxidant
4	3-isopropoxy-1.1.1.7.7.7-hexamethyl-3.5.5-tris(trimethylsiloxy)tetrasiloxane	10.267	567	$C_{18}H_{52}O_7Si_7$	Antimicrobial,
5	3, 5 di-t-butyl phenol	10.813	206	$C_{14}H_{22}O$	Antimicrobial,
6	Phthalic acid	11.804	222	$C_{12}H_{14}O_4$	Antimicrobial, antifungal, antioxidant
7	3.4-dihydroxymandelic acid 4TMS	12.234	472	$C_{20}H_{40}O_5Si_4$	Antimicrobial, antioxidant
8	1,6 dioxacyclododecane-7,12-dione	12.439	200	$C_{10}H_{16}O_4$	Antimicrobial, antioxidant
9	2-propyl-1-pentanol	12.984	130	$C_8H_{18}O$	Antifungal
10	2-(2’.4’.4’.6’.6’.8’.8’-heptamethyltetrasiloxan-2’yloxy)-2.4.4.6.6.8.8.10.10-nonamethylcyclopentasiloxane	14.038	652	$C_{16}H_{48}O_{10}Si_9$	Antimicrobial,
11	Phthisic acid, monoethyl ester	15.886	194	$C_{10}H_{10}O_4$	Antimicrobial, antifungal, antioxidant

Keskın et al. [15] recognized that the extract of olive leaves remarkably inhibited the growth of all tested Gram-positive and Gram-negative bacteria except for *Bacillus cereus, Enterobacter aerogenes,* and *Enterobacter cloacae*. The extract was analyzed by GC-MS method resulted in the identification of fifteen constituents, representing 99.68% of the extracts: cyclotrisiloxanehexamethyl (36.98%), cyclotetrasiloxaneoctamethyl (15.18%), and cyclopentasiloxanedecamethyl (14.59%) being the main components. This study's results also discovered cyclopentasiloxane, decamethyl- and demonstrated antibacterial activity. Mahmud et al. [16] used GC-MS analysis of the basic fraction of the leaves of *Polyalthiacinnamomea* identified four majors cyclosiloxanes of octamethylcyclotetrasiloxane (18.1%), decamethylcyclopentasiloxane (16.4%), dodecamethylcyclohexasiloxane (14.1%), and tetradecamethylcycloheptasiloxane (6.0%), and knew the bioactivity and phytochemistry of *Polyalthia cinnamomea* remarkably that inhibited the growth of ten bacteria tested. In another study, the alkaloid compounds of *Solanum nigrum* also were investigated using the GC-MS that revealed the existence of the cyclopentasiloxane. decamethyl, phthalic acid, and other compounds, the extract of the leaf of *Solanum nigrum* were assayed for in vitro antibacterial activity against *Escherichia coli, Proteus mirabilis, Staphylococcus aureus, Pseudomonas aerogenosa,* and *Klebsiella pneumonia* by using the diffusion method in agar [17].

Azadirachta indica is a common plant used frequently due to its medicinal significance at 0.01 mg/mL of the plant extract determined against different pathogenic bacteria. The plant extracts were analyzed for characterization by GC-MS. Many antibacterial compounds such as azulene, tetrasiloxane, phthalic acid, cyclopentasiloxane, hexadecanoic acid, spiropentane, dioctyl phthalate were detected in the plant extract among the phthalic acid, cyclopentasiloxane, dioctyl phthalate [18].

Essential oils from the leaves of *Cinnamomum pubescens* Kochummen (CP) and the whole plant of *Etlingeraelatior* (EE) were used popularly in antioxidant agents and antibacterial activity. GC-MS studies resulted in the identification of 79 and 73 compounds in CP and EE, respectively. The most abundant components of EE included β-pinene (24.92%) and 1-dodecene (24.31%). While the main compound in CP was 1,6-octadien-3-ol,3,7-dimethyl (11.55%), cinnamaldehyde (56.15%), and 1-phenyl-propane-2,2-diol diethanoate (11.38%), and they were suggested to use as a new source of natural antioxidant and antibacterial in the food and pharmaceutical industries, in our experiment also found 1-dodecene, so that it will use as a natural antioxidant and antibacterial [19].

According to Saravanakumar et al. ([20], the extract of *Clathria frondifera* [a marine sponge species] was examined for in vitro antimicrobial potency against three human pathogens. The chemical constituents of the *Clathria frontiera* fractioned extract analyzed by GC-MS revealed the presence of major compounds such as 1-Dodecene, 2-tert-Butyl-4-isopropyl-5-methylphenol, E-15-Heptadecenal, 1-Heptadecanol, n-Nonadecanol-1, Cholesterol, 26,26-Dimethyl-5,24(28)-ergostadien-3a-ol that might have a functional role in the chemical defense against microbial invasion effectively represses the bacterial and fungal.

Phyllanthus amarus belongs to the family of Euphorbiaceae, and it is well known in the traditional Nigerian system for its traditional uses. Using GC-MS analysis extract of leaves of *P. amarus,* the prevailing components in the ethanolic extract of leaves were 5-di-t-butyl-phenol, methyl 14-methyl pentadecanoat, palmitic acid (hexadecanoic acid), 10-octadecanoate, 9-hexadecenal, glycerol 1, 3-dipalmitate, 2, 13-octadecadiene-1-ol, dioctyl ester, and heptanoic acid (9-dece-1-yl ester 3,5-di-t-butyl phenol). The presence of various bioactive compounds confirms the antioxidant, antifungal, and antibacterial activity as palmitic acid, hexadecanoic acid, and 3,5-di-t-butyl phenol [21].

Recently, Manigundan *et al*. [22] isolated *Streptomyces* sp. strain UC1A-3 in rhizosphere soil of *Capsicum annum* (Chilli) from the agricultural fields in Udhagamandalam, Nilgiris, Tamil Nadu, India as PGPR and against some fungal pathogens. Using GC-MS technique analyzed the *Streptomyces* sp. strain UC1A-3, the results showed that twenty-nine compounds were detected, in particular, Phthalic acid ($C_8H_6O_4$), Pentadecanoic acid ($C_{15}H_{30}O_2$), i-Propyl 12-methyl-tetra decanoate ($C_{18}H_{36}O_2$), l-(+)-Ascorbic acid 2,6- dihexadecanoate ($C_{38}H_{68}O_8$), 1-Nonadecene ($C_{19}H_{38}$), and 1-Heptacosanol ($C_{27}H_{56}O$) reported as antimicrobial properties. And these compounds were also found out in our results from the extract of *Microbacterium tumbae* strain ND2.7c.

Moringaoleifera fruit and leaf are used often in traditional medicine in many countries in Asia. Shunmugapriya et al. [23] used GC-MS to determine the chemical compositions of the hexane extract of *Moringa oleifera* fruit. The extract of *Moringa oleifera* fruit contained 28 compounds of which the maximum quantum was 2,6-

dihydroxybenzoic acid, 3TMS derivative (38.8%) followed by tetrapentacontane (20.6%), hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11- dodecamethyl (8%), 2-propenoic acid, pentadecyl ester (5.97%), 3,4-dihydroxymandelic acid, 4TMS derivative (5.29%), bis(2-ethylhexyl) phthalate (2.56%), 1-dodecanol (2.53%) and glycidyloleate (2.51%) among the compounds as tetrapentacontane, hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11- dodecamethyl, 2-propenoic acid, pentadecyl ester, 3,4-dihydroxymandelic acid, 4TMS derivative, bis(2-ethylhexyl) phthalate had antimicrobial activity, and the antioxidant compound identified and they also present in the extract of *Microbacterium tumbae* strain ND2.7c.

Meshram et al. [24] described two new fungi species, *Muscodor ghoomensis* and *Muscodor indica,* living within the internal tissues of *Cinnamomum camphora* stems growing in Ghoom Monastery, Darjeeling, West Bengal, India. These fungi possess a fruity smell, lack reproductive structures, form pale yellow colored colonies with radial crevices on potato dextrose agar. The smell characteristic of *M. ghoomensis* and *M. indica* was attributed to a mix of 16 and 21 volatile organic compounds produced by the fungi. The predominant volatiles produced by these fungi were 4-octadecylmorpholine, 1, 6-dioxacyclododecane-7, 12-dione, 1,4-dimethyl-7-prop-1-en-2-yl-2,3,3a,5,6,7,8,8a-octahydro-1H-azulen-4-ol, and squalene identified using GC/MS method. The volatiles produced by these fungi had potential application as fumigants against plant and human pathogens. These compounds were also found out in our result so that they are the potential to produce bio-fungicide for humans and crops.

Dangang et al. [25] used the GC-MS method to identify the bioactive compounds present in formulated fermented diet made from 20% red kidney bean (*Phaseolus vulgaris* L.), 60% mung bean (*Vigna radiata*), 10% Irish potato (*Solanum tuberosum*), and 10% ripe fresh papaya (*Carica papaya*) fruits inoculated with different concentrations of *Lactococcus lactis* sp. (*Lc. lactis* sp.). The results of the GC-MS revealed the presence of twenty volatile compounds in each diet. Five compounds namely Benzyl Alcohol, 2-propyl-1-pentanol, 1,3-diethyl benzene, 1-Tridecyn-4-ol, Phthalic acid, and cyclobutyl isobutyl ester were identified in all diets. Using the GC-MS method to analyze the antifungal active compounds in *Bovistella radicata* alcohol extract, after the alcohol extract and purified by column chromatography (macroporous resin D-101) and the active compounds were named as SPAF-1 (the spore powder active fraction). The results showed that Alcohol extracts and SPAF-1 with two peaks. By comparing in NIST, the compound was 2-propyl-1-pentanol corresponding to peak1, and 2-Propyl-1-pentanol (13.6%) in the main constituents with MIC value was 250 µg/ml against *Trichophyton rubrum.* The antifungal effect of SPAF-1 (2-Propyl-1-pentanol is one-third compounds) is similar to the positive control anti-tineapedis effect of them was obvious [26]. 2-Propyl-1-pentanol also was found in the extract of *Microbacterium tumbae* strain ND2.7c.

Gadhiet al. [27] studied marine algae *Halimeda* sp., at Red Sea, they recognized the extract from this algae had the ability of microbial activity. Using GC-MS to analyze the extract of the algae, the result collected as 9-octadecenoic acid, 6,9,12-Octadecatrienoic acid, phenylmethyl ester, phenol, 2,6-bis (1,1-dimethylethyl), Diethyl phthalate, hexadecanol, heptadecanol, 2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy) -2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane, benzenemethanolbutanamide, hexanedioic acid dioctyl ester…with many bioactive compounds as phenol, 2,6-bis (1,1-dimethylethyl), Diethyl phthalate, 2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy) -2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane, and these compounds also found out in the extract of *Microbacterium tumbae* strain ND2.7c

When the scientists [28] studied the extract of marine sponge (*Dysidea herbacea*) by GC-MS method, they discovered many bioactive compounds such as 3-isopropoxy-1,1,1,7,7,7 hexamethyl 3,5,5-tris (trimethylsiloxy) tetrasiloxane, cyclohexasiloxanedodecamethyl, 3-isopropoxy-1,1,1,7,7,7 hexamethyl 3,5,5-tris(trimethylsiloxy) tetrasiloxane. These compounds were the same as the result of this study. Perhaps, when crushed sponges to get the extract, microbes living in sponges release these compounds.

4. Conclusion

In the present study, eleven compounds were identified by the GC-MS method from the ethyl acetate extract of *Microbacterium tumbae* strain ND2.7c. The biological activities of each of the components were range from antimicrobial, antioxidant, and antitumoral activities. The research findings have shown that there were extensively rich in secondary metabolites, and they have been reported as bioactive compounds and used in the world.

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