The Pharmaceutical and Chemical Journal, 2018, 5(1):196-203

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Bioactive Compounds from Marine Streptomyces Sp. 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 *Streptomyces* sp. strain ND7a which isolated from sponges at Ha Tien Sea, Kien Giang province, Vietnam. Six bioactive compounds were identified in the organic solvent hexan-aceton and aceton-methanol. The identification of bioactive chemical compounds is based on the peak area, retention time, molecular weight and molecular formular. GC-MS analysis of *Streptomyces* sp. strain ND7a revealed the existence of the 2-Pentanone, 4-hydroxy-4-methyl, Cyclohexasiloxane, dodecamethyl, Cycloheptasiloxane, tetradecamethyl, Oxime-, methoxy-phenyl, Hexanedioic acid, bis(2-ethylhexyl) ester and Diisooctyl phthalate.

Keywords bioactive compounds, GC-MS, Ha Tien Sea, Marine sponge, *Streptomyces*

1. Introduction

In the last decade, marine microorganisms, such as bacteria, microalgae and fungi, have become increasingly important as sources for new bioactive natural products [1-5]. Marine microorganisms have been the important study in recent years because of production of novel metabolites which represent various biological properties such as antiviral, antitumor or antimicrobial activities. These secondary metabolites serve as model systems in discovery of new drugs [6-7]. The studies of the secondary substances produced by marine micro-organisms have obtained many significant achievements in the world [8]. Among the secondary metabolites from marine microorganisms, there are many compounds having interesting biological activities that should be useful to development for their pharmaceutical uses. Metabolites from microorganisms is a rapidly growing field, due, at least in part, to the suspicion that a number of metabolites obtained from algae and invertebrates may be produced by associated microorganisms [9]. Meanwhile, the search of bioactive secondary metabolites from marine microorganisms is not widely explored in Vietnam [10-11].

In the past few years, Gas chromatography Mass spectrometry (GC-MS) is used as one of the technological platform for finger print analysis of secondary metabolites in both plant and non-plant species [12]. Taking into consideration the medicinal importance of this plant, the ethyl acetate root extract of medical plant [13] and/or leaves as Neem (*Azadirachta Indica* A. Zuss) [14], flowers *Holarrhena antidysentrica* Wall [15] were analyzed using GC-MS. This work will help to identify the bioactive components. GC-MS is the best technique to identify bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitro compound etc.

In the course of our screening program, the EtOAc extract of a *Streptomyces* sp. from marine sponge of Ha Tien Sea, Kien Giang province, Vietnam exhibited an inhibition activity against *Salmonella typhymurium, Echerichia coli, Bacillus cereus* and *Candida albicans*. In this paper, we reported the isolation and structural elucidation of secondary metabolites from the cultures broth of *Streptomyces* sp. in two kinds of organic solvent. The present study

was aimed to identify the chemical constituents in ethyl acetate extract of marine actinomycetes were analyzed by the GC-MS technique.

2. Materials and methods

2.1. Actinomycete material

The marine sponge was collected in Ha Tien Bay – Kien Giang province in April 2016. The sponge sample (1 g) was added to the 10 mL of sterile sea water in a conical flask. The flask was agitated for about one hour. The marine sponge was filtered and the filtrate was serially diluted to obtain 10^{-1} to 10^{-7} dilutions using the sterilized sea water. An aliquot of 100 µL of each dilution was spread on the media. Different media like Starch Casein Agar (SCA) was used for isolation of actinomycetes. The media containing 50% of sterile sea water were supplemented with rifampicin (5 µg/mL) and nystatin (25 µg/mL) (Himedia Mumbai) to inhibit bacterial and fungal contamination, respectively. The petriplates were incubated up to 3 weeks at 28°C. The isolated discrete colonies were observed and used for identification.

The obtained strain *Streptomyces* sp. was identified by using 16S rRNA gene sequencing method. The universal primers including forward primer, 5'- AGA GTT TGA-TCA TGG CTC A-3', and reverse primer, 5'- AAG GAG GTG ATC CAG CC- 3', were used for amplifying nearly full length of 16S rRNA gene sequence (about 1500 bp.). The obtained sequence was analyzed by comparing with bacterial 16S rRNA sequences in GenBank by Blastn, which showed 99% similarity with *Streptomyces* sp. 2011 (GenBank Accession No. JF751041.1).

2.2. Fermentation, extraction and isolation

Streptomyces sp. strain ND7c was cultured in 250 ml flasks at 30^{o}C for 24 hours with shaking at 150 rpm. Fermentation was carried out in 100 L fermenter with 50 L medium 2216 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 (50 L) was extracted with ethyl acetate (25 L × 3 times). The combined organic solutions were then decanted, filtered and concentrated under reduced pressure to yield 5.2 g of crude extract which was chromatographed on a silica gel column using a gradient of 1 - 100% acetone in hexane to afford three fractions F1-3, after that it was continuously chromatographed on a silica gel column using a gradient of 1 - 100% acetone in methanol to afford three fractions F4-6. Therefore, six fractions were received from 2 kinds of organic solvent (hexane – acetone and acetone – methanol).

2.3. GC/MS analysis

The samples were analysed at GC/MS of Chemistry Laboratoty, Department of Chemistry, College of Natural Science, Can Tho University. GC-MS analysis of the sample was carried out using Shimadzu Thermo with column TG-SQC; 15m x 0.25mm x 0.25µm. Helium was used as the carrier gas and the temperature programming was set as follows:

	Speed (^{o}C/min)	Temperature (^{o}C)	Keep (min)
Initial		50	1.00
Ramp 1	2.00	70	2.00
Ramp 2	10.00	150	2.00
Ramp 3	10.00	250	10.00
Total time		43 minutes	

10 µl sample was injected with split less mode. Mass spectra was recorded over 35-400 amu range with electron impact ionization energy 70 eV, total running time for a sample was 43 min. Quantitative determination were made by relating respective peak areas to TIC areas from GC-MS.

3. Results and Discussion

GC-MS analysis of compounds from extract of Streptomyces sp. strain ND7a with organic solvent Hexan-Aceton as shown in Figure 1.

Figure 1: GC-MS chromatogram of extract of Streptomyces sp. strain ND7a in organic solvent Hexan-Aceton

Chromatogram GC-MS analysis of hexan-aceton extract of *Streptomyces* sp. strain ND7a showed the presense of three major peaks (Table 1) and the components corresponding to the peaks were dethermined as follows. The first set up peak was determined to be 2-Pentanone, 4-hydroxy-4-methyl (Figure 2).

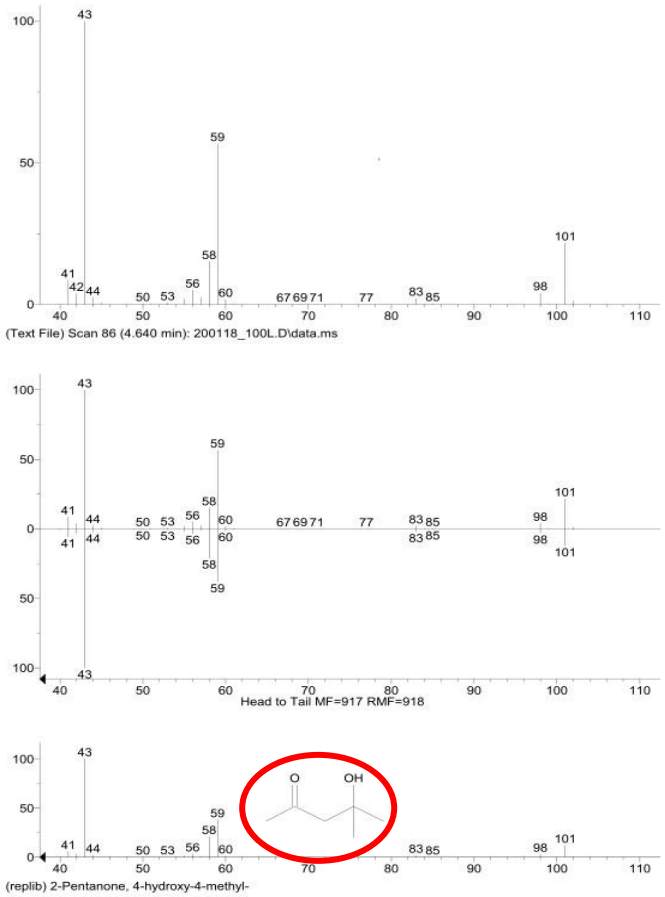

Figure 2: Mass spectrum of 2-Pentanone, 4-hydroxy-4-methyl with retention time (RT) = 4.64

The second peak indicated to be Cyclohexasiloxane, dodecamethyl (Figure 3) and the next peaks considered to be Cycloheptasiloxane, tetradecamethyl (Figure 4).

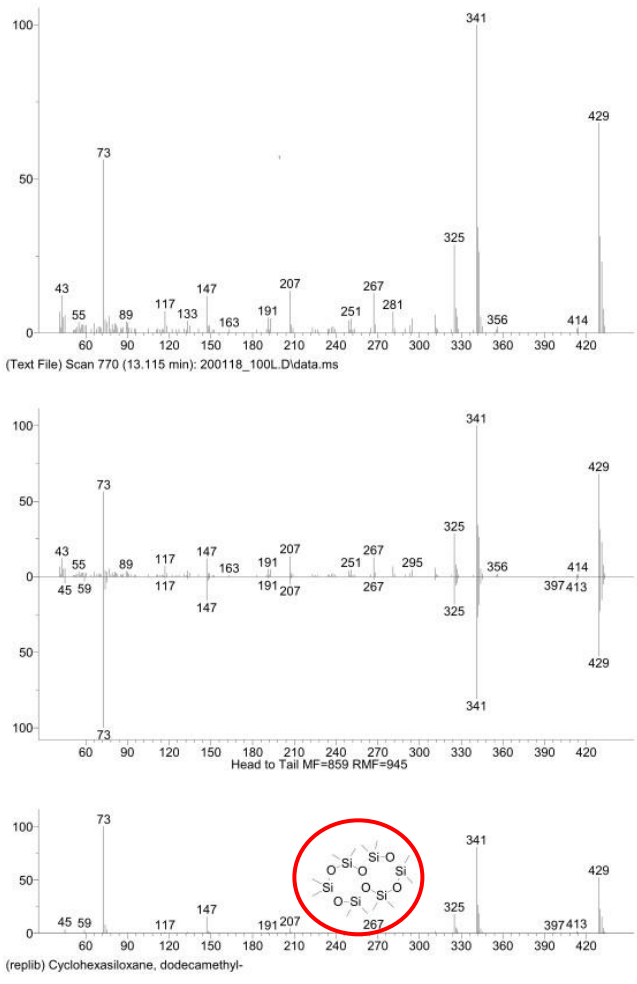

Figure 3: Mass spectrum of Cyclohexasiloxane, dodecamethyl with retention time (RT) = 13.115

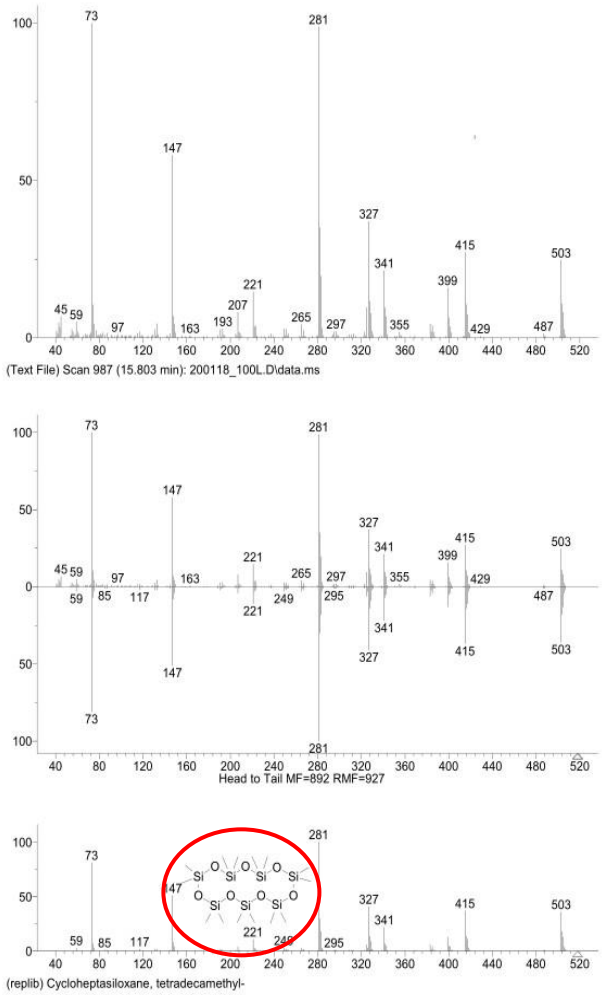

Figure 4: Mass spectrum of Cycloheptasiloxane, tetradecamethyl with retention time (RT) = 15.803

Table 1: Major compounds identified in extract of *Streptomyces* sp. strain ND7a (hexan-aceton)

S/N	RT (min)	Name of the compound	Molecular weight (g/mol)	Molecular formula	Peak (%)	Bioactivity
1	4.64	2-Pentanone, 4-hydroxy-4-methyl	116.3	$C_6H_{12}O_2$	93.00	Antimicrobial
2	13.115	Cyclohexasiloxane, dodecamethyl	444	$C_{12}H_{36}O_6Si_6$	97.50	Antifungal properties
3	15.803	Cycloheptasiloxane, tetradecamethyl	518	$C_{14}H_{42}O_7Si_7$	97.40	Antiperspirants and deodorants. Antibacterial, Antifungal.

GC-MS analysis of compounds from extract of *Streptomyces* sp. strain ND7a with organic solvent Aceton-Methanol as shown in Figure 5.

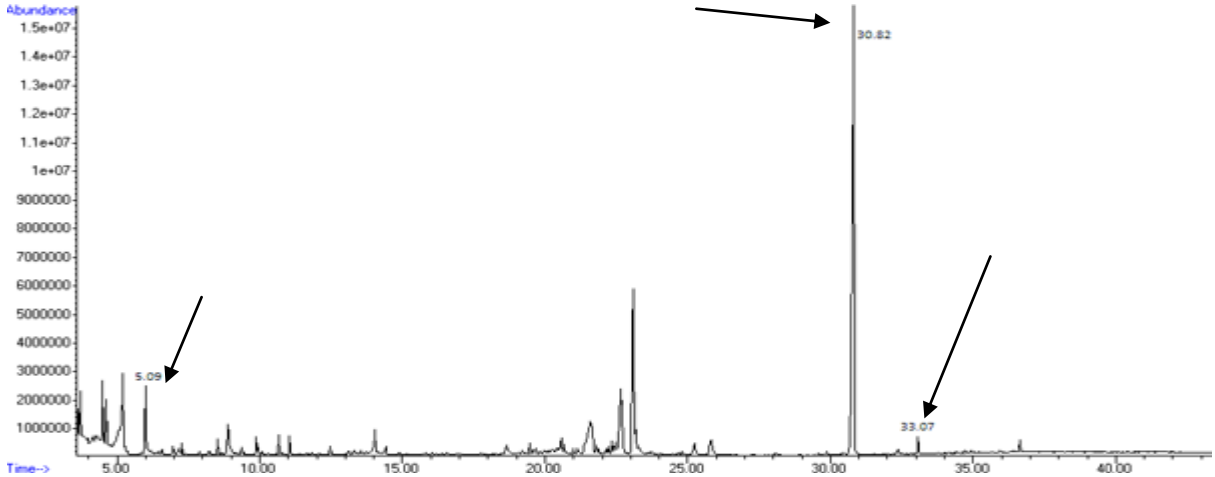

Figure 5: GC-MS chromatogram of extract of Streptomyces sp. strain ND7a in organic solvent Aceton-Methanol

Chromatogram GC-MS analysis of hexan-aceton extract of *Streptomyces* sp. strain ND7a showed the presence of three major peaks (Table 2) and the components corresponding to the peaks were determined as follows. The first set up peak was determined to be Oxime-, methoxy-phenyl (Figure 6).

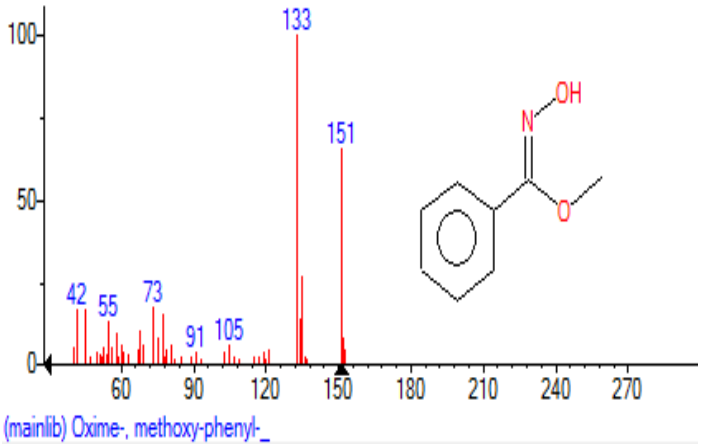

Figure 6: Mass spectrum of Oxime-, methoxy-phenyl with retention time (RT) = 5.99

The second peak indicated to be Hexanedioic acid, bis(2-ethylhexyl) ester (Figure 7) and the next peaks considered to be Diisooctyl phthalate (Figure 8).

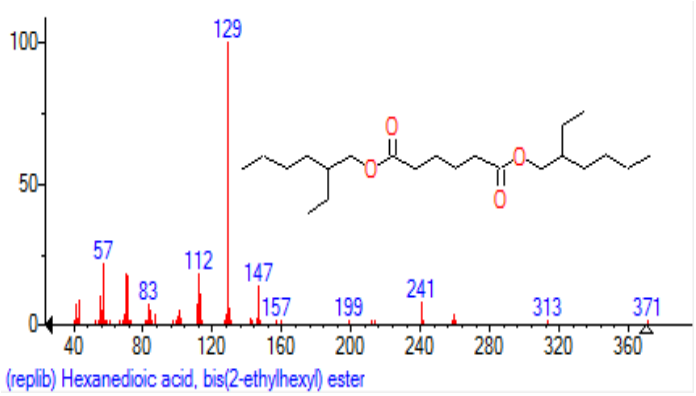

Figure 7: Mass spectrum of Hexanedioic acid, bis(2-ethylhexyl) ester with retention time (RT) = 30.82

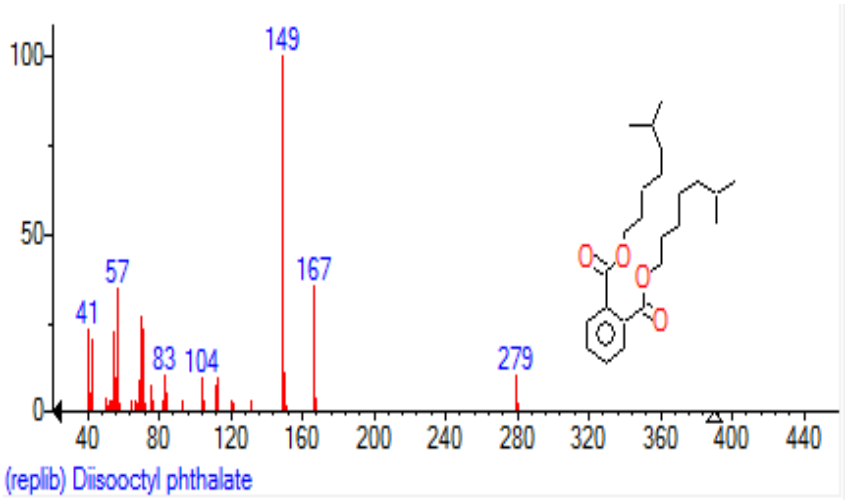

Figure 8: Mass spectrum of Diisooctyl phthalate with retention time (RT) = 33.07

Table 2: Major compounds identified in extract of *Streptomyces* sp. strain ND7a (aceton-methanol)

S/N	RT (min)	Name of the compound	Molecular weight (g/mol)	Molecular formula	Peak (%)	Bioactivity
1	5.99	Oxime-, methoxy-phenyl	151	$C_8H_9NO_2$	84.5	Antifungal, Antibacterial, Anticancer and Antitumor
2	30.82	Hexanedioic acid, bis(2-ethylhexyl) ester	370	$C_{22}H_{42}O_4$	52.1	Antimicrobial activity Antioxidant, antiproliferative
3	33.07	Diisooctyl phthalate	390	$C_{24}H_{38}O_4$	20.0	Antifungal, Antibacterial, Antiviral and Antioxidant activities

Shettima *et al*. [16] discovered 2 – pentanone – 4 – hydroxy–4–methyl acid diethyl phthalate in *Guiera senegalensis* J.F. Gmel which is used in West African, Ethnomedicine for treating diarrhoea, dysentery, malaria, cough and microbial infections; and 2 – pentanone - 4–hydroxy–4–methyl acid diethyl phthalate is one in nine compounds from the ethyl acetate root extract of *Guiera senegalensis* were identified by Gas-chromatography–Mass spectrometry (GC-MS) analysis. The biological activities of each of the identified phytocomponents range from antimicrobial, antioxidant and antitumoral activities. Cyclohexasiloxane dodecamethyl and Cycloheptasiloxane tetradeca-methyl were analysed by GC-MS technique from the ethanol extract of stem bark of *Cola nitida* from the University of Ibadan, Nigeria. Cola has been reported to have very high medicinal values; it has been attributed to the treatment of ringworm, scabies, gonorrhea, dysentery and opthalmia [17]. Worthy of note also is the reports of Kim [18] and Jayeola for soft drinks production [19] that alluded to Cola used as a remedy for whooping cough and asthma. *Cola nitida* possess antifungal properties against dermatophytes (*Trichophyton rubrum*, *Trichophyton tonsurans*) and *Candida albicans* as reported by Sewanu *et al.* [20]. The antimicrobial activities of n-Hexane extract and 5 Column Chromatography fractions, of *Azadirachta indica* A. juss (Neem) leaves, showed antimicrobial activities against human pathogenic bacteria (*Salmonella typhi*) and yeast fungus (*Candida albicans*). Akpuaka *et al.* [14] used GC/MS identified 45 bioactive compounds in the n-Hexane extract of *Azadirachta indica* leaves out of which 33 have antifungal activity and Oxime-, methoxy-phenyl- and Diisooctyl phthalate were identified were two in 33 bioactive compounds. Both of compounds are Antifungal, Antibacterial, Anticancer, Antitumor [19] and Antifungal, Antibacterial, Antiviral and Antioxidant activities [21]. Paramanantham and Murugesan [15] and Kadhim *et al*. [22] identified Hexanedioic acid, bis(2-ethylhexyl) ester, is bioactive compound, antimicrobial activity, it was extracted from flower of *Holarrhena antidysentrica* Wall and *Vitis vinifera* tree.

4. Conclusion

In the present study six compounds from the ethyl acetate root extract of *Streptomyces* sp. strain ND7a were identified by Gas-chromatography–Mass spectrometry (GC-MS) analysis in two kinds of organic solvent (hexan-aceton and aceton-methanol). The biological activities of each of the identified components range from antimicrobial, antioxidant and antitumoral activities. The nature of the identified compounds are mostly organic acids. The research findings have shown that the is extensively rich in secondary metabolites and they have been reported as bioactive compounds and they have been used in the world.

Acknowledgements

The authors would like to acknowledge the technical assistance of Dr. Nguyen Trong Tuan, Department of Chemistry, College of Natural Science, Can Tho University, Viet nam for the GC-MS analysis and Chemistry BSc. Students for helpness to us. This study was done by fund from Ministry of Education and Training of Viet nam.

References

1. Ebel, R. (2006). Secondary metabolites from marine-derived fungi. In: (P. Proksch and W.E.G. Muller, eds) Frontiers in marine biotechnology. *Horizon Bioscience*, Norfolk. pp. 73–144.
2. Konig, G.M., Kehraus, S., Seibert, S.F., Abdel-Lateff, A. & Muller, D. (2006). Natural products from marine organisms and their associated microbes. *Chem Bio News* 7,229–238.
3. Laatsch, H. (2006). Marine bacterial metabolites. In: (P. Proksch and W.E.G. Muller, eds) Frontiers in marine biotechnology *Horizon Bioscience*, Norfolk. pp. 225–288.
4. Ramaswamy, A.V., Flatt, P.M., Edwards, D.J., Simmons, T.L., Han, B., & Gerwick, W.H. (2006). The secondary metabolites and biosynthetic gene clusters of marine cyanobacteria. Applications in biotechnology. In: (P. Proksch and W.E.G. Muller, eds) Frontiers in marine biotechnology. *Horizon Bioscience*, Norfolk. pp. 175–224.
5. Shimizu, Y. & Li, B. (2006). Microalgae as a source of bioactive molecules: special problems and methodology. In: (P. Proksch and W.E.G. Muller, eds) Frontiers in marine biotechnology. *Horizon Bioscience*, Norfolk. pp. 145–176.
6. Bernan, V.S., Greenstein, M. & Maiese, W.M. (1997). Marine microorganisms as a source of new natural products, *Adv. Appl. Microbiol*., 43, 57-90.
7. Fenical, W. (1997). New pharmaceuticals from marine organisms, *Trends Biotechnol*., 15,339-341.
8. Debbab, A, Aly, H., Lin, W.H & Proksch, W. (2010). Bioactive compounds from marine bacteria and fungi, *Microb. Biotechnol*., 3(5),544-563.
9. Chau, V.M., Phan, V.K., Nguyen, V.H., Nguyen, H.N., & Pham, V.C. (2012). Dược liệu biển Việt Nam: Thực trạng và Cơ hội phát triển, *Nxb. Khoa học tự nhiên và Công nghệ, Hà Nội*, p. 3 (Vietnamese).
10. Do, T.T., Le, D.Q., Quyen, D.T, & Pham, V.C. (2012). Isolation and identification of marine bacteria from marine mud in Vietnam with antimicrobial activity, *Journal of Vietnamese Environment*, 3(2), 71-75.
11. Quyen, V. T., Hieu, T.V., Vinh, L.C. Mai Huong, D.T., Hong Minh, L.T. Murphy, B., Minh, C.V. & Pham, P.V. (2015). Secondary metabolites by produced by Marime bacterium *Micromospora* sp. G019. *Vietnam J. of Chemistry*. 53(2e), 150-153. DOI: 10.15625/0866-7144.2015-2e-03.
12. KellD. B, Brown, M., Davey, H.M, Dunn, W.B., Spasic, I, & Oliver, S.G. (2005). Comprehensive natural products II. *Nat Rev Microbiol*. 3,557-565.
13. Muthulakshmi A, Jothibai Margret, R., & Moham, V.R. (2012). GC-MS Analysis of Bioactive components of *Feronia ephantum correa* (Rutaceae). *Journal of Applied Pharmaceutical Science*. 02(02), 69 – 74.
14. Akpuaka, A., Ekwenchi, M.M., Dashak, D.A. & Dildar, A. (2013). Biological Activities of Characterized Isolates of n-Hexane Extract of *Azadirachta Indica* A. Juss (Neem) Leaves. *Nature and Science*. 11(5), 141-147.
15. Paramanantham, M., & Murugesan, A. (2014). GC-MS analysis of *Holarrhena antidysentrica* Wall Flower. *International Journal of Science, Engineering and Technology Research (IJSETR)*, 3(3), 631-639.

16. Shettima, A.Y., Karumi, Y., Sodipo, O.A., Usman, H. & Tijjani, M.A. (2013). Gas Chromatography–Mass Spectrometry (GC-MS) Analysis of Bioactive Components of Ethyl acetate Root Extract of *Guiera senegalensis* J.F. Gmel. *Journal of Applied Pharmaceutical Science.* 3(03), 146-150.
17. Okeie, W., Ogunlesi, M., Ofor, E., & Osibote, E.A.S. (2009). Analysis of essential oil constituents in hydro-distillates of calotropis procera (oil). *R.Br. Res. Journal of phytochestry*. 3, 44-53.
18. Yogeswari, S., Ramalakshmi, S.N., & Muthu, J.M. (2012). Identification and comparative studies of different volatile fractions from *Monochaetia kansensis* by GC-MS. *Global Journal of Pharmacology*, 6(2), 65-71.
19. Rahdary, A.A. & Sobati, Z. (2012). Chemical composition of essential oil, antibacterial activity and brine shrimp lethality of ethanol extracts from *Sedum pallidum*. *Journal of Medicinal Plants Research*, 6(16), 3105-3109.
20. Sewanu, S.O., Opoku, A.R., & Oyedeji, A.O. (2012). The chemical Composition, antimicrobial and antioxidant properties of the essential oils of *Tulbaghia violacea* Hary and *Eucalyptus grandis* W. Hill ex Maiden. En US. *University of Zululand South Africa*.
21. Yanping, H., Suhua, H., Jincheng, W.U., & Shunquan, L. (2010). Identification of essential oils from the leaves of 11 species of *Eriobotrya*, 4379-4382.
22. Kadhim, M. J., Al-Rubaye, A. B, & Hameed, I. H. (2017). Determination of Bioactive Compounds of Methanolic Extract of *Vitis vinifera* Using GC-MS. *International Journal of Toxicological and Pharmacological Research*. 9(2), 113-126.