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Original Research Article

# Bioactive Secondary Metabolites from Marine Streptomyces Isolated from Mangrove Forest Soil of Can Gio, HoChiMinh City, Vietnam

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**Abstract**

The objective of this research was studying the bioactive secondary metabolites composition of marine *Streptomyces albogriseolus* and to evaluate the isolates for possible in vitro antifungal and antibacterial activities. The compound obtained were screened by GC-MS method. While agar-well diffusion method was employed to measure antimicrobial activity against four human pathogenic bacteria. Twenty-five bioactive secondary metabolites compounds were identified in the ethyl-acetate – hexane extract of *Streptomyces albogriseolus*. The identification of bioactive secondary metabolites compounds is based on the peak area, retention time, molecular weight, molecular formula, and antimicrobial actions. GC-MS analysis of *Streptomyces albogriseolus* revealed the existence of the Cyclohexasiloxane, dodecamethyl, Cyclohexasiloxane, tetradecamethyl, 2, 6 dihydroxybenzoic acid 3TMS, Heptasiloxane, hexadecamethyl, Octasiloxane, 1,1,3,3,5,5,7,7,9,9,1 1,11,13,13,15,15- Hexadec and Tetracosamethyl, cyclododecasiloxane. Thus, it could be inferred that the therapeutic potential of marine *Streptomyces albogriseolus* is because of different compounds present in the extract prepared.

**Keywords:** GC/MS, Bioactive compounds, Mangrove Forest soil, *Streptomyces albogriseolus*.



## Introduction

In the many years ago, marine microorganisms, such as bacteria, microalgae and fungi, have become increasingly important as sources for new bioactive natural products[1,2]. Nowadays marine microorganisms have been the important study because of production of novel metabolites which represent various biological properties such as antiviral, antitumor or antimicrobial activities. The studies of bioactive metabolites compounds produced by marine micro-organisms have received many significant achievements in the world[3]. From marine microorganisms, there are many compounds having interesting biological activities that should be useful to development for their pharmaceutical uses, as new drugs[4,5].

Mangrove soils have a unique character

for the growth of various kinds of microorganisms that play an important role in degrading the soil. Mangrove soils provide a unique ecological niche for the growth of diversified microorganisms which find use in recycling environmental nutrients and production of exclusive secondary metabolites of pharmaceutical importance. The total microbial community of tropical mangrove forest comprise 91% of bacteria and fungi, 7% of algae and 2% of protozoa[6]. Actinomycetes are group of aerobic, branched, unicellular Gram-positive bacteria with high percentage of G+C (70%) in their genetic material. Actinomycetes particularly Streptomyces are well known as major sources of secondary metabolites particularly antibiotics[7].

In the past few years, Gas chromatography Mass spectrometry (GC-MS) is used as one of the technological platforms for fingerprint analysis of secondary metabolites in both plant and on-plant species[8]. In the course of our screening program, the EtOAc extract of a *Streptomyces* sp. from mangrove forest soil of Can Gio, HoChiMinh city, Vietnam exhibited an inhibition activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* and *Vibrio haemoparalyticus*. In this paper, we reported the isolation and structural elucidation of secondary metabolites from the culture's broth of *Streptomyces albogriseolus* ANTHOIDONG 7.1 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. Your goal is to simulate the usual appearance of papers in the. We are requesting that you follow these guidelines as closely as possible.

## Materials and Methods

### 2.1. Actinomycete material

Plant soil samples were collected carefully from many species of mangroves viz. *Bruguiera sexagula, Ceriops decandra, Sonnertia, Avicennia, Rhirophora*…., from the 3 years-old plants in plantation site, Tam Thon Hiep – village, (soil pH = 4.22, salinity 10‰; Thanh An site, (soil pH= 6.18, salinity 7‰; An Thoi Đông village (soil pH= 4.16, salinity 8‰). (Lat. 10º 68' 04" N; Long. 107º 02' 64" E).

The samples were collected on in December 2019. For isolation of bacterial rhizosphere, samples were collected during the low tide. Soil samples were collected by using a sterile spatula and stored in sterile polythene bags, and then were stored in icebox (5ºC) and transportation to Can Tho University as soon as possible; soil samples were stored in refrigerator (-10ºC) in Microbiology Lab. until isolation. The soil samples were removed adherent particles and were superficially disinfected according to de Araújo et al.[9].

A known weight of soil (1 g) was aseptically weighed and transferred to a stoppered (150 mL) sterile conical flask containing 99 mL of sterile saline (0.9%) diluent. The sediment-diluent mixture was agitated by means of mechanical shaking for about 45 minutes. After the above time, the supernatant was collected and streaked on the Starch Casein Agar medium[10] was used for the isolation of actinobacteria. It was supplemented with Aginalxic (0.5 mg/L) and Nystatin (0.5 mg/L) to inhibit fungi and Gram-negative bacteria. The inoculated plates were incubated at 28ºC for 3–6 weeks. The colonies bearing distinct morphological characteristics were picked up and transferred to freshly prepared media until pure cultures were obtained.

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 Blast N, which showed 99% similarity with *Streptomyces* sp. 2011 (GenBank Accession No. JF751041.1).

### 2.2. Fermentation and extraction

One best *Streptomyces* sp. strain was cultured in 250 ml flasks at 30°C for 24 hours with shaking at 150 rpm. Fermentation was carried out in 100 L fermenter with 50 L Starch Casein medium and 10% bacterial inoculum at 30°C for 52 hours. Neutral pH was maintained automatically by NaOH or HCl 1N. The obtained culture broth (15 L) was extracted with ethyl acetate (25 L × 3 times). The combined organic solutions were then decanted, filtered and concentrated under reduced pressure at 50°C to yield 1.012 g. 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; L30m x ID 0.25mm x DF 0.25µm 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 (°C/min) | Temperature (°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, total running time for a sample was 33 min. Quantitative determination was made by relating respective peak areas to TIC areas from GC-MS.

## Results and Discussion

GC-MS analysis of compounds from extract of *Streptomyces albogriseolus* ANTHOIDONG 7.1 strain. Chromatogram GC-MS analysis of acetate ethyl – hexane extract of *Streptomyces albogriseolus* ANTHOIDONG 7.1 strain, presence of 25 major peaks (Figure 1).

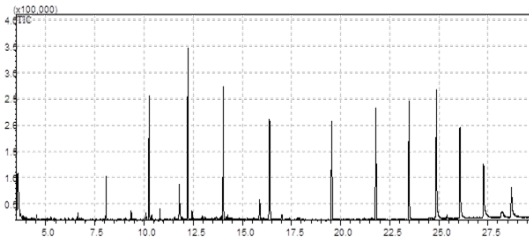


**Figure 1: GC-MS chromatogram of extract of *Streptomyces albogriseolus* ANTHOIDONG 7.1 strain in organic solvent ethyl acetate-Hexane**

However, number of peaks is either repeat or not antimicrobial only therefore some peaks really only six peaks (Figure 2 and Table 1).

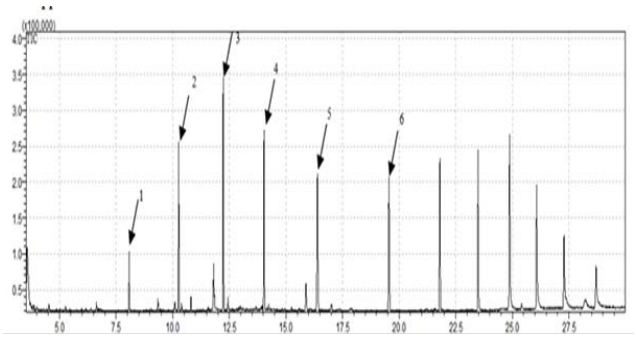
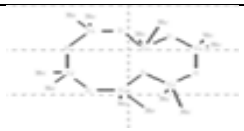
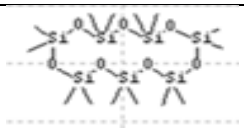
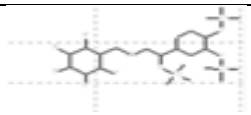
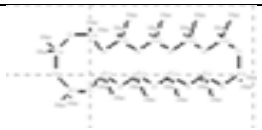



**Figure 2: GC-MS chromatogram of extract of *Streptomyces albogriseolus* ANTHOIDONG 7.1 strain in organic solvent ethyl acetate-Hexane**

**Table 1: Major bioactive metabolite compounds identified in hexane extract of *Streptomyces albogriseolus* ANTHOIDONG 7.1 strain**

| Peak | RT | Bioactive metabolite compound | Molecular Weight (g/mol) | Molecular Formula | Chemical structure | Bioactive |
|---|---|---|---|---|---|---|
| 1 | 8.082 | Cyclohexasiloxane, dodecamethyl | 444 | $C_{12}H_{36}O_6S_{16}$ |  | Antibacterial, Antifungal |
| 2 | 10.254 | Cyclohexasiloxane, tetradecamethyl | 518 | $C_{14}H_{42}O_7Si_7$ |  | Antifungal. |
| 3 | 12.230 | 2, 6 dihydroxybenzoic acid 3TMS | 563 | $C_{24}H_{34}F_5N_3Si_3$ |  | antimicrobial |
| 4 | 14.542 | Heptasiloxane, hexadecamethyl | 532 | $C_{15}H_{48}O_6Si_7$ |  | Antibacterial, |
| 5 | 16.394 | Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadec | 430 | $C_{16}H_{50}O_7Si_8$ |  | Antimicrobial |
| 6 | 19.554 | Tetracosamethyl, cyclododecasilo Xane | 888 | $C_{24}H_{72}O_{12}Si_{12}$ |  | Antimicrobial |

Gas chromatography and mass spectroscopy analysis of compounds was carried out in ethyl acetate-hexane extract of *Streptomyces albogriseolus* ANTHOIDONG 7.1 strain shown in Table 1 and the GC-MS chromatogram of the 6 peaks of the compounds detected was shown in Figure 2.

Six major peaks and the components corresponding to the peaks were determined as follows:

- The First set up peak Cyclohexasiloxane, dodecamethyl
- The second peak was Cyclohexasiloxane, tetradecamethyl The third peak was 2, 6 dihydroxybenzoic acid 3TMS
- The fourth peak was Heptasiloxane, hexadecamethyl
- The fifth peak was Octasiloxane, 1,1,3,3,5,5,7,7,9,9,1 1,11,13,13,15,15-Hexadec
- The sixth peak was Tetracosamethyl, cyclododecasiloxane

Mebude & Adeniyi[11] using GC-MS Analyzed Phyto Components from the Stem Bark of *Cola nitida* Schott & Endl and the result determined the extracts were Cycloheptasiloxane tetradeca-methyl (35.287%), Cyclohexasiloxane dodecamethyl (24.941%), Cyclooctasiloxane hexadecamethyl (17.574%), 1H-cycloprop (e) azulen-7-ol-decahydro-1,1,7- trimethyl-4-methylene (7.816%), Cycloconasiloxane octadecamethyl (6.995%), Benzimidazol-5-amine-1-4-ethoxyp (2.265%) and 5-acetyl-2-benzylsulfanyl-6-methyl-nicotinonitrile (1.467%). Dodecamethyl cyclohexasiloxane: they used Cyclohexasiloxane, dodecamethyl (D6) ($C_{12}H_{36}O_6Si_6$) in they used Cyclohexasiloxane, dodecamethyl (D6) ($C_{12}H_{36}O_6Si_6$) in personal care products such as hair/skin care products, antiperspirants and deodorants, antibacterial, antifungal.

Obaseki et al.[12] investigates the anti-inflammatory properties of *Hydrocotyle bonariensis* Comm. Ex Lam, a medicinal plant used by indigenous traditional healers to manage chronic inflammatory diseases especially rheumatism and arthritis. The extract was finally subjected to GC/MS analysis, phytochemical analysis of the extract revealed the presence of saponine, phenol, flavonoid, tannin, terpenoid and sterol. The compounds that have been reported to exert anti-inflammatory effects were three of the major compounds suggested by the GC/MS analysis to be present in the hexane extract of H. bonariensis leaves, occurrence of Cycloheptasiloxane, tetradecamethyl-(Siloxane) which Mahmoud et al.[13] using GC-MC method to analyze the extract of Argemon eochroleuca Sweet which people often used to against some pathogenic fungi. The result determined Cyclohexasiloxane, tetradecamethyl compound in the extract.

Rizwana, et al.[14] using The GC-MS of fruit extracts showed some important oxygenated mono and diterpenes, esters, and phenols. from *Camellia oleifera* seed cake and stems of *Cola nitida*[15,16]. Compounds, such as tetracosamethyl-cyclododecasiloxane, cyclosiloxane, and hexadecamethyl have shown antibacterial, antifungal, and antioxidant properties[17-20].

Al bratty et al.[21] investigated the phytochemical profile of aqueous decoction of gerger leaves of the Saudi origin by GC-MS assay. Twenty-seven chemical compounds belonging to seven classes constituted the gerger decoction: organic siloxanes (39.75%), shows the structures of the seven organic siloxanes isolated from gerger leaves. Octasiloxane,1,1,3, 3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-(20.49%) contributed to a higher proportion than other siloxanes. A study indicated the antimicrobial activity

of octasiloxane and also confirmed its presence in herbs[22]. Siloxanes generally were reported to exhibit significant antimicrobial and antioxidant properties.

Shunmugapriya et al. [23] using Gas Chromatography–Mass Spectrometry determined 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 glycidyl oleate (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 antioxidant compound were identified.

Momin & Thomas[24] used GC-MS analysis of antioxidant compounds present in different extracts of an endemic plant Dillenis scabrella (Dilleniaceae) and barks. The result of GC-MS analysis was carried out on four crude extracts of leaves and bark and Octasiloxane, 1,1,3,3,5,5,7,7,9,9,1 1,11,13,13,15,15-Hexadec presence having anti-microbial agent[22, 25-26] in the extracts.

Helal et al.[27] used GC-MS analysis revealed the occurrence of ten bioactive phytocomponents including cyclotrisiloxane, hexamethyl-, cyclotetrasiloxane, octamethyl-, cyclopentasiloxane, decamethyl, dodecanoic acid, methyl ester, tetrafluorophthalonitrile, gamma-Sitosterol, nonacosane, 4,4,6a,6b,8a,11,11,14b-Octamethyl,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one, thiazolo[4,5-f]quinoline and 7-methyl among Cyclotetrasiloxane, octamethyl- C8H24O4Si4 had antimicrobial activity[28].

In general, *Streptomyces albogriseolus* had the ability of more neomycine production when it meets stress condition[29]. Besides, *Streptomyces albogriseolus* can utilize polyethylene as carbon source for growth[30].

Six compounds presence in the extract of *Streptomyces albogriseolus* ANTHOIDONG 7.1 strain which were demonstrated their antimicrobial activity by many the scientists in the world and we hope these compounds will be applied for take care of human healthy in the future.

Thus, it could be inferred from the present study that the presence of various compounds as revealed through biochemical and GC-MS analyses may be responsible for antioxidative and medicinal potential of *Streptomyces albogriseolus* ANTHOIDONG 7.1 strain. However, the advanced analysis is required to further harness the medicinal potential of *Streptomyces* sp.

## Conclusion

In the present study six compounds from the ethyl acetate – hexane of *Streptomyces albogriseolus* ANTHOIDONG 7.1 strain were identified by Gas-chromatography–Mass spectrometry (GC-MS) analysis in two kinds of organic solvent (hexane-acetone). The biological activities of each of the identified components range from antimicrobial, antioxidant and anti-tumoral activities. The nature of the identified compounds are mostly organic acids. The research findings have shown that is extensively rich in secondary metabolites and they have been reported as bioactive compounds and they have been used in the world.

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