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(RESEARCH ARTICLE)

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Antimicrobial compounds of one *Streptomyces celluloflavus* strain isolated from Can Gio mangrove soil, Vietnam

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Abstract

Marine actinobacteria have emerged as a well-known potential source of secondary metabolites which possess useful properties in many fields including medical and agricultural applications. The aim of this research was investigated the antimicrobial compounds of marine Streptomyces celluloflavus isolated from mangrove soil samples taken in Can Gio mangrove soil (Can Gio district), Ho Chi Minh City, Vietnam. Herein agar-well diffusion method was employed to measure antimicrobial activity of the culturing medium extract against four pathogenic bacteria strains. The compound harvested were then analysed by GC-MS method. Thirty-one bioactive secondary metabolism compounds were identified in the ethyl-acetate – hexane extract of *Streptomyces celluloflavus*. The identification of these bioactive secondary metabolites compounds is based on the peak area, retention time, molecular weight, molecular formula, and antimicrobial actions. GC-MS analysis result revealed the presence of the 7 major components including 2-pentanone, 4-hydroxy-4-methyl, Cycloheptasiloxane, tetradecamethyl, Cyclododecane, 1,1,1,3,5,7,7,7-Octamethyl-3,5-bis(trimethylsiloxy)tetrasiloxane, Benzoic acid, 2-hydroxy-, 1-methylethyl ester, 1-Hexadecene and Heptasiloxane, hexadecamethyl . Therefore, it could be deduced that the remedial potential of mangrove soil Streptomyces celluloflavus based on plentiful compounds existing in the extract was highly expected.

Keywords: Bioactive Compounds; Can Gio District; GC-MS; Mangrove Forest Soil; *Streptomyces celluloflavus*.

1. Introduction

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

Mangrove soil is 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 [4]. Actinobacteria are group of aerobic, branched, unicellular Gram-positive bacteria with high percentage of G+C (70%) in their genetic material. Among such microbial community, actinobacteria particularly *Streptomyces* are well known as major sources of secondary metabolites particularly antibiotics [5].

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Gas chromatography - Mass spectrometry (GC-MS) has been used as one of the technological platforms for fingerprint analysis of secondary metabolites in both plant and on-plant species [6]. In the extent of our study program, the EtOAc extract of a *Streptomyces* sp. from mangrove forest soil of Can Gio, Ho Chi Minh city, Vietnam exhibited an inhibition activity against *Staphylococcus aureus*, *Escherichia coli, Bacillus cereus* and *Vibrio haemoparalyticus*. In this article, we reported the isolation and structural elucidation of secondary metabolites from the culture's broth of *Streptomyces celluloflavus* ANTHOIDONG 4.1 in two kinds of organic solvent as well as screening the antimicrobial activities of these components.

2. Material and methods

2.1. Actinobacteria material

The location of samples was chosen carefully from many species of 3 years-old mangrove plants (e.g. *Bruguiera sexagula, Ceriops decandra, Sonnertia, Avicennia, Rhirophora*...) in plantation site, such as 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. 10° 68' 04" N; Long. 107° 02' 64" E).

The samples were collected on in December 2019, during the low tide period. Soil samples were collected by using a sterile spatula and stored in sterile polythene bags, and then were stored in icebox (5 °C) and transportation to Can Tho University as soon as possible; soil samples were stored in refrigerator (-10 °C) in Microbiology Lab. until isolation. The soil samples were removed adherent particles and were superficially disinfected [7].

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 was used for the isolation of actinobacteria [8]. 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 28 °C 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 Petri plates 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.

2.2. Fermentation and extraction

One of the best *Streptomyces* sp. strains 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 1.014 g. And then, the crude extract was tested the antibacterial activity with *Bacillus cereus* by the agar-well diffusion method and analyse compounds by the GC-MS method.

2.3. GC-MS analysis

Table 1 GC-MS temperature scheme

	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		

The sample was analysed 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: 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.

3. Results

3.1. Identification of isolated actinobacteria

The homology searches of 16S rRNA gene sequence of one selected strain (ANTHOIDONG 4.1) in GenBank by BLAST (which showed 99% similarity with *Streptomyces* sp. 2011 (GenBank Accession No. JF751041.1) revealed that it had similarity to the sequence of *Streptomyces celluloflavus.*

3.2. The compound recognition by GC-MS analysis

GC-MS analysis of acetate ethyl – hexane extract of *Streptomyces celluloflavus* ANTHOIDONG 4.1 strain revealed 31 peaks. Nonetheless, there was either some duplication of the substances or the compounds without antibiotic. Therefore, the results of interest were only 7 major peaks (Figure 1).

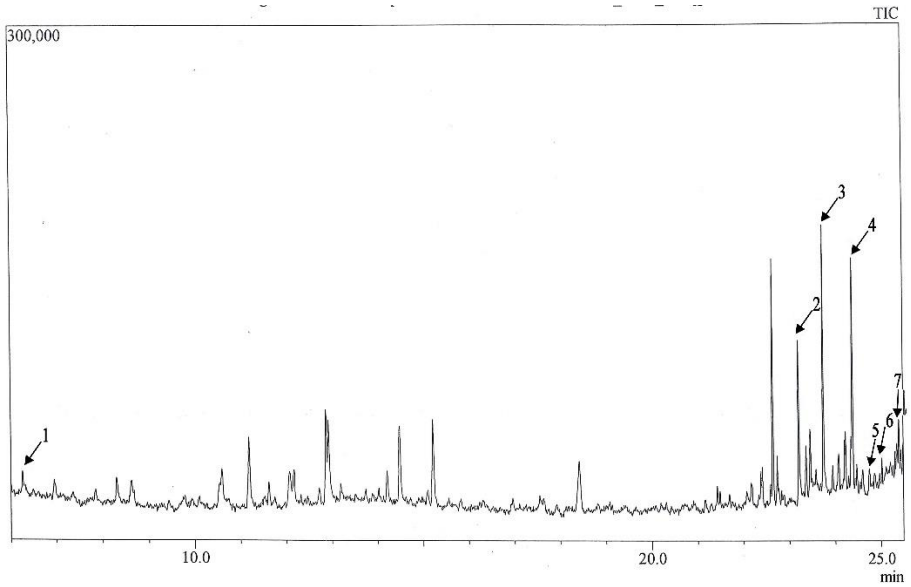
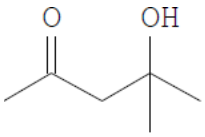
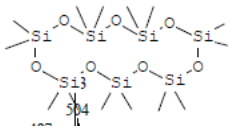
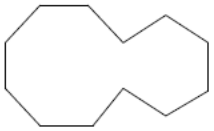
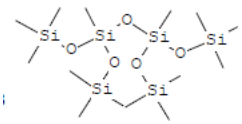
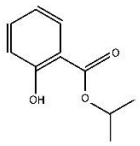
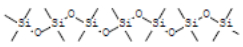


Figure 1 GC-MS chromatogram of extract of *Streptomyces celluloflavus* ANTHOIDONG 4.1 in organic solvent acetate ethyl – hexane

Seven major peaks and the components corresponding to the peaks were determined as follows (Table 2):

1. 2-pentanone, 4-hydroxy-4-methyl
2. Cycloheptasiloxane, tetradecamethyl
3. Cyclododecane
4. 1,1,1,3,5,7,7,7-Octamethyl-3,5-bis(trimethylsiloxy)tetrasiloxane
5. Benzoic acid, 2-hydroxy-, 1-methylethyl ester
6. 1- Hexadecene
7. Heptasiloxane, hexadecamethyl

Table 2 Major bioactive components identified in the extract of *S.celluloflavus* ANTHOIDONG 4.1 strain

Peak	RT	Compound name	Mol. weight	Bioactive
1	5.485	2-pentanone, 4-hydroxy-4-methyl $C_6H_{12}O_2$	116 	Antimicrobial, antifungal, antioxidant
2	23.365	Cycloheptasiloxane, tetradecamethyl $C_{14}H_{42}O_7Si_7$	518 	Antifungal
3	23.451	Cyclododecane $C_{12}H_{24}$	168 	Antimicrobial antitumor
4	24.595	1,1,1,3,5,7,7,7-Octamethyl-3,5-bis(trimethylsiloxy)tetrasiloxane $C_{14}H_{42}O_5Si_6$	458 	Antimicrobial
5	25.020	Benzoic acid, 2-hydroxy-, 1-methylethyl ester $C_{10}H_{12}O_3$	180 	Antimicrobial, anti-inflammatory
6	25.661	1-Hexadecene $C_{16}H_{32}$	224 	Antimicrobial
7	25.780	Heptasiloxane, hexadecamethyl $C_{16}H_{48}O_6Si_7$	532 	Antimicrobial

4. Discussion

4.1. Compound 1

2-pentanone, 4-hydroxy-4-methyl. This is also called diacetone alcohol. It plays role in plant metabolism and has been isolated from *Achnatherum robustum*. Diacetone alcohol has been used in products used to control fungi pests in the garden or home [9] (PubChem-NCBI, 2022). Seddek et al. [10] showed in research that the most bioactive compounds produced in major amounts by *Oscillatoria* sp. was diacetone alcohol and the extract exhibited appreciable antimicrobial, antioxidant and cytotoxic activity.

4.2. Compound 2

Cycloheptasiloxane, tetradecamethyl. Mebude & Adeniyi 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%) [11]. Obaseki et al. [12] studied the anti-inflammatory properties of *Hydrocotyle bonariensis* Comm. Ex Lam, a medicinal plant resembling gotu kola leaves, is used by indigenous African healers to treat chronic inflammatory diseases, especially rheumatism and arthritis. This leaf extract shows the presence of saponins, phenols, flavonoids, tannins, terpenoids and sterols, which generally have anti-inflammatory effects. Among the major compounds analyzed by GC-MS present in the hexane extract of the leaves of *H. bonariensis*, also appeared Cyclohexasiloxane dodecamethyl, Cycloheptasiloxane, tetradecamethy.

In addition, Mustafa et al. also used the GC-MC method to analyze chemicals from the sweet latex of *Argemon eochroleuca*, which is resistant to some pathogenic fungi. It was determined that Cyclohexasiloxane and tetradecamethyl were also present in the extract [13].

4.3. Compound 3

Cyclododecane. This substance was in the extract of medicinal plant *Holarrhena antidysenterica* Wall, which has been used as remedy for bronchitis, hematuria, spermatorrhoea, epilepsy, asthma, piles, leprosy, eczema, diarrhea, fever and jaundice. It was revealed by GC-MS with 1.47% peak area [14]. Moreover, in thesis of Wongjuk [15], cyclododecane was present in the extract of another plant (*Dendrobium* sp.) and was supposed to have the ability of antioxidant and cancer treatment.

4.4. Compound 4

1,1,1,3,5,7,7,7-Octamethyl-3,5-bis(trimethylsiloxy)tetrasiloxane. The plant *Capparis spinosa* has been used for medicinal purposes due to antibiotic, anti-inflammatory, anti-oxidative and anti-hypertensive effects. The GC-MS of its extract revealed similar components to trimethylsiloxy tetrasiloxane [16].

4.5. Compound 5

Benzoic acid, 2-hydroxy-, 1-methylethyl ester. Du and Klessig studied that benzoic acids in general were involved in the inhibition of protein binding to salysilic acid, thereby increasing the resistance of tobacco and cucumber for leaf rot diseases caused by virus and fungus *Colletotrichum lagenarium* [17]. Wang et al. found Benzoic acid, 2-hydroxy,1-methylethyl ester along with 13 anti-inflammatory bioactive compounds isolated from *Inula wissmanniana*. The GC-MS analysis of the extract of *Euscaphis japonica* bark indicated different pharmaceutical ingredients, including benzoic acid ester. Antioxidant and antimicribial activity of *Curculigo orchioides* plant was demonstrated that was due to such constituents as benzoic acid ester [18-20].

4.6. Compund 6

1- Hexadecene. The compound was identified by GC-MS the extraxt of many actinomyces isolated from soil, with 8.94% peak area [21]. It was said to be antimicrobial against six bacteria strain and five fungi. In addition, 1-hexadecene was also recognised in the extract of fungus *Monochaetia kansensis*, with 22.7% of the content of components possessing antimicrobial and antioxidant activity [22].

4.7. Compound 7

Heptasiloxane, hexadecamethyl presenting in Egyptian red seaweed has anti-bacterial and anti-oxidative effects [23]. Next, Hassan et al. used GC-MS to analyze the composition of active ingredients obtained from the culture fluid of *Bacillus cereus* S1 strain isolated from Egyptian seawater, showing that Heptasiloxane, hexadecamethyl and some other substances, have anticoagulant, anti-inflammatory and inhibitory activities against both Gram-negative and Gram-positive bacteria [24]. In particular, Kawuri and Darmayasa obtained and analyzed bioactive compounds from a strain of the genus *Streptomyces* that inhibited pathogenic bacteria to giant freshwater prawn larvae, including the presence of Heptasiloxane, hexadecamethyl [25].

In general, the compounds in the siloxane group are antibacterial [26, 27]. Siloxane compounds are also synthesized by actinobacteria [28], and our results are not different to those of other research.

5. Conclusion

In our research, 7 compounds from the ethyl acetate – hexane of *Streptomyces celluloflavus* ANTHOIDONG 4.1 strain were identified by GC-MS analysis in two kinds of organic solvent (hexane- acetone) including 2-pentanone, 4-hydroxy-4-methyl, Cycloheptasiloxane, tetradecamethyl, Cyclododecane, 1,1,1,3,5,7,7,7-Octamethyl-3,5-bis(trimethylsiloxy)tetrasiloxane, Benzoic acid, 2-hydroxy-, 1-methylethyl ester, 1-Hexadecene and Heptasiloxane, hexadecamethyl. The biological activities of each of the identified substances range from antimicrobial, antioxidant and anti-tumoral properties. The nature of the identified compounds are hydrocarbons, siloxane and organic acids. The research results have demonstrated that the actinobacteria strain is extensively rich in secondary metabolites and they could be used as a potential source for pharmaceutical production in future.

Abbreviations

1. EtOAc: Ethyl acetate
2. GC-MS: gas chromatography – massive spectrometry

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no competing financial interest.

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