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STUDY ON THE CHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY AGAINST TEST MICROORGANISMS OF TWO MARINE MICROALGAE SPECIES, *Thraustochytrium pachydermum* TSL10 AND *Aurantiochytrium* sp. SC145, FROM THE SPRATLY ISLANDS, VIETNAM

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ABSTRACT

In recent years, marine microalgae belonging to the family Thraustochytriaceae, particularly the genera *Thraustochytrium* and *Aurantiochytrium*, have attracted considerable attention due to their ability to biosynthesize biologically valuable compounds such as DHA, EPA, and various secondary metabolites with potential applications in pharmaceuticals, functional foods, and biotechnology. The Spratly Islands are a region with high marine biodiversity; however, studies on the chemical constituents and biological activities of marine microalgae from this area remain limited. Under the Ministry of National Defence project KCB-TS, two strains, *Thraustochytrium pachydermum* TSL10 and *Aurantiochytrium* sp. SC145, were selected for investigation in order to elucidate the chemical values and potential applications of these indigenous microalgal strains.

Objectives of the study:

To identify the chemical constituents of the two marine microalgae species, *Thraustochytrium pachydermum* TSL10 and *Aurantiochytrium* sp. SC145, collected from the Spratly Islands, Vietnam and to evaluate the biological activities of the isolated compounds.

Research contents:

- Isolation and structural elucidation of compounds from the two marine microalgae species, *Thraustochytrium pachydermum* TSL10 and *Aurantiochytrium* sp. SC145, collected from the Spratly Islands, Vietnam and evaluation of the antimicrobial activities of the isolated compounds from these two marine microalgae species.

The results of this study contribute to confirming the potential of the Spratly marine area in the research, conservation, and sustainable exploitation of marine biological resources, thereby supporting biodiversity conservation and promoting the development of the marine economy. In addition, these findings will enrich the database of Vietnamese marine microorganisms and open new directions for the sustainable exploitation and application of marine resources.

CHAPTER 1. INTRODUCTION

1.1. Overview of *Thraustochytrium*

1.1.1. Morphological Characteristics and Classification of Species Belonging to the Genus Thraustochytrium

Thraustochytrium is a genus of heterotrophic marine microorganisms belonging to the class Labyrinthulomycetes, capable of decomposing organic matter through extracellular enzymes. This group has undergone several taxonomic revisions before being recognized as distinct from fungi and Oomycetes based on rDNA analyses. Classification is mainly based on morphological characteristics and zoospore reproductive mechanisms. Recent molecular studies have shown that *Thraustochytrium* is not a monophyletic group, and many species have been reassigned to other genera such as *Schizochytrium* and *Aurantiochytrium*.

Table 1.1. Microalgae species in the genus Thraustochytrium

No.	Scientific name	Author, year of isolation
1	*T. aureum*	Goldstein (1963)
2	*T. motivum*	Goldstein (1963)
3	*T. rossii*	Bahnweg and Sparrow (1974)
4	*T. kerguelense*	Bahnweg and Sparrow (1974)
5	*T. antarcticum*	Bahnweg and Sparrow (1974)
6	*T. kinnei*	Gaertner (1967)
7	*T. benthicola*	Raghu Kumar (1980)
8	*T. proliferum*	Sparrow (1943)
9	*T. pachydermum*	Scholz (1958)
10	*T. arudimentale*	Artemchuk (1972)
11	*T. striatum*	Schneider (1967)
12	*T. roseum*	Goldstein (1963)
13	*T. aggregatum*	Ulken (1965)
14	*T. gaertnerium*	Bongiorni (2005)
15	*T. caudivorum*	Schärer (2007)

1.1.2. Chemical constituents of Thraustochytrium species

1.1.2.1. Steroids

Table 1.2. Steroids from Thraustochytrium species

No.	Compound	Species
1	cholesterol	*Thraustochytrium* sp. ATCC 26185/CHN-1
2	24-methyl-cholesta-5,22-dienol	*Thraustochytrium* sp. ATCC 26185

3	24-methylene cholesterol	*Thraustochytrium* sp. ATCC 26185 / CHN-1
4	24-ethyl-cholesta-5,22-dienol	*Thraustochytrium* sp. ATCC 26185
5	24-ethyl-cholesta-5,7,22-trienol	*Thraustochytrium* sp. ATCC 26185 / CHN-1
6	ergosterol	*T. aureum*
7	7-dehydroporiferasterol	*T. aureum*
8	(22*E*,24*R*)-ethylcholesta-7,22-dien-3*β*,5*α*,6*β*-triol	*T. aureum*
9	poriferasterol glucoside	*T. aureum*

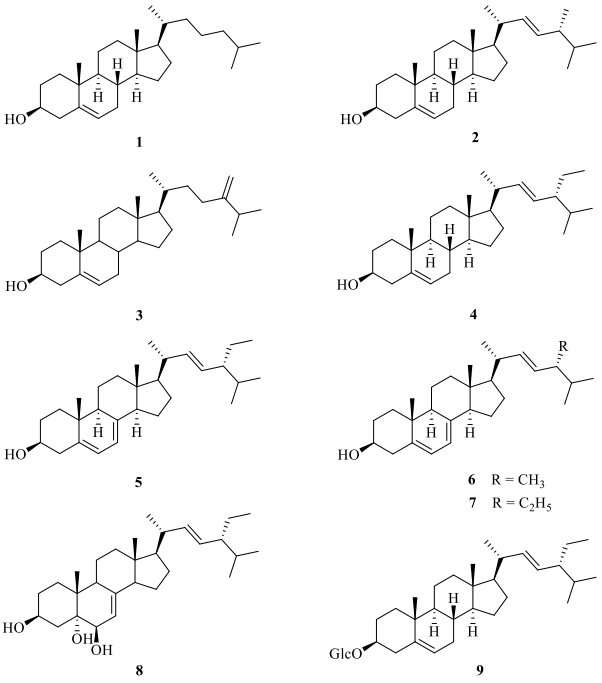

Figure 0.1. Structures of steroids from Thraustochytrium species

1.1.2.2. Nitrogen-containing compounds

Table 1.3. Nitrogen-containing compounds from Thraustochytrium

No.	Compound	Species
10	thraustochtroside A	*T. globosum*
11	thraustochtroside B	*T. globosum*
12	thraustochtroside C	*T. globosum*
13	pyrrolezanthine-6-methyl ether	*T. aureum*

14	3-(3-aminopropyl)-6-((4-hydroxyphenyl)methyl)-2,5-piperazinedione	*T. aureum*
15	5-methyluracil	*T. aureum*
16	indole-3-carboxylic acid	*T. aureum*
17	adenosine	*T. aureum*

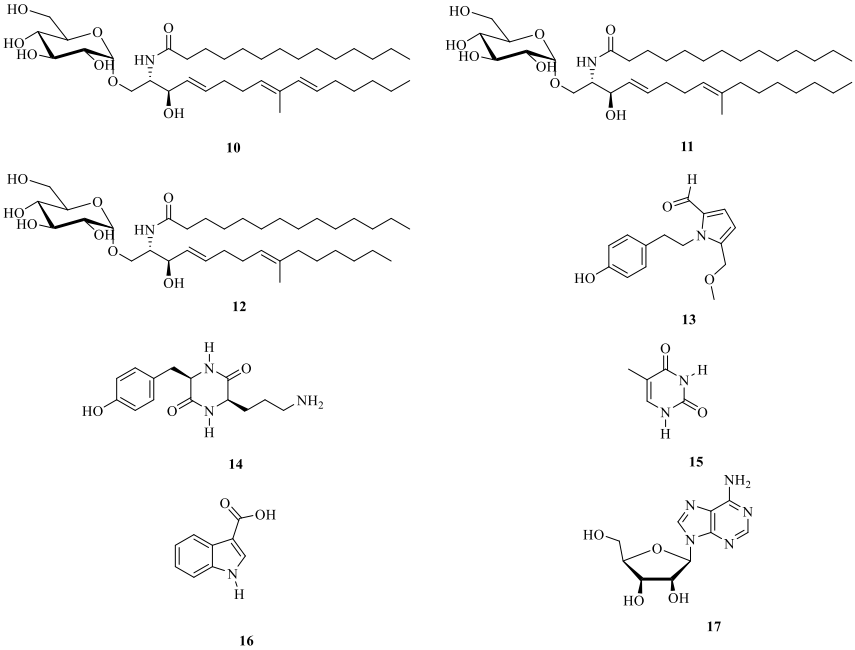

Figure 0.2. Structures of Nitrogen-containing compounds from Thraustochytrium

1.1.2.3. Fatty acids

Table 1.4. Fatty acids from Thraustochytrium

No.	Compound	Species
18	lauric acid	*Thraustochytrium* sp. ATCC 26185
19	myristic acid	*Thraustochytrium* sp. ATCC 26185, CHN-1
20	pentadecanoic acid	*Thraustochytrium* sp. ATCC 26185
21	9-pentadecenoic acid	*Thraustochytrium* sp. ATCC 26185
22	palmitic acid	*Thraustochytrium* sp. ATCC 26185, CHN-1
23	palmitoleic acid	*Thraustochytrium* sp. ATCC 26185, CHN-1
24	heptadecanoic acid	*Thraustochytrium* sp. ATCC 26185

25	stearic acid	*Thraustochytrium* sp. ATCC 26185, CHN-1
26	oleic acid	*Thraustochytrium* sp. ATCC 26185, CHN-1
27	linoleic acid	*Thraustochytrium* sp. ATCC 26185
28	linolenic acid	*Thraustochytrium* sp. ATCC 26185
29	stearidonic acid	*Thraustochytrium* sp. ATCC 26185
30	arachidic acid	*Thraustochytrium* sp. ATCC 26185
31	dihomo-γ-linolenic acid	*Thraustochytrium* sp. ATCC 26185
32	arachidonic acid	*Thraustochytrium* sp. ATCC 26185, CHN-1
33	omega-3 arachidonic acid	*Thraustochytrium* sp. ATCC 26185
34	eicosapentaenoic acid	*Thraustochytrium* sp. ATCC 26185, CHN-1
35	behenic acid	*Thraustochytrium* sp. ATCC 26185
36	adrenic acid	*Thraustochytrium* sp. ATCC 26185
37	osbond acid	*Thraustochytrium* sp. ATCC 26185, CHN-1
38	docosapentaenoic acid	*Thraustochytrium* sp. ATCC 26185
39	docosahexaenoic acid	*Thraustochytrium* sp. ATCC 26185, CHN-1

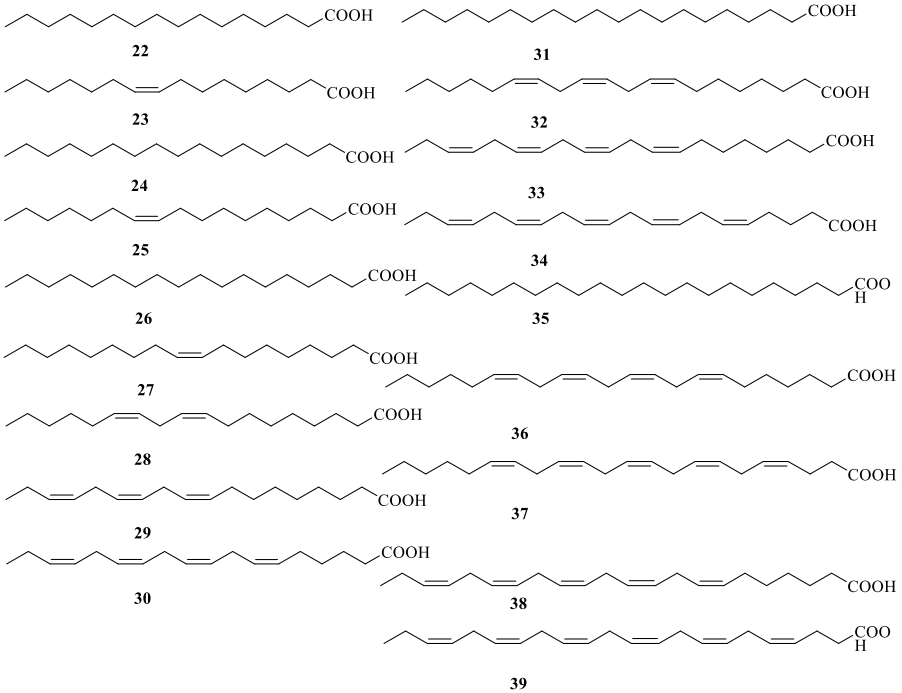

Figure 0.3. Structures of fatty acid from Thraustochytrium

1.1.2.4. Carotenoids and phosphatidyls

Table 1.5. Carotenoids from Thraustochytrium

No.	Compound	Species
40	*β*-carotene	*Thraustochytrium* sp. CHN-1
41	echinenone	*Thraustochytrium* sp. CHN-1
42	canthaxanthin	*Thraustochytrium* sp. CHN-1
43	phoenicoxanthin	*Thraustochytrium* sp. CHN-1
44	astaxanthin	*Thraustochytrium* sp. CHN-1
45	(3*S*,3′*R*)-*trans* astaxanthin	*Thraustochytrium* sp. CHN-1
46	(3*R*,3′*R*)-*trans* astaxanthin	*Thraustochytrium* sp. CHN-1
47	(3*R*,3′*R*)-*cis* astaxanthin	*Thraustochytrium* sp. CHN-1
48	(3*S*,3′*R*)-*cis* astaxanthin	*Thraustochytrium* sp. CHN-1
49	(3*R*,3′*R*)-*cis* astaxanthin	*Thraustochytrium* sp. CHN-1

Figure 0.4. Structures of carotenoids from Thraustochytrium

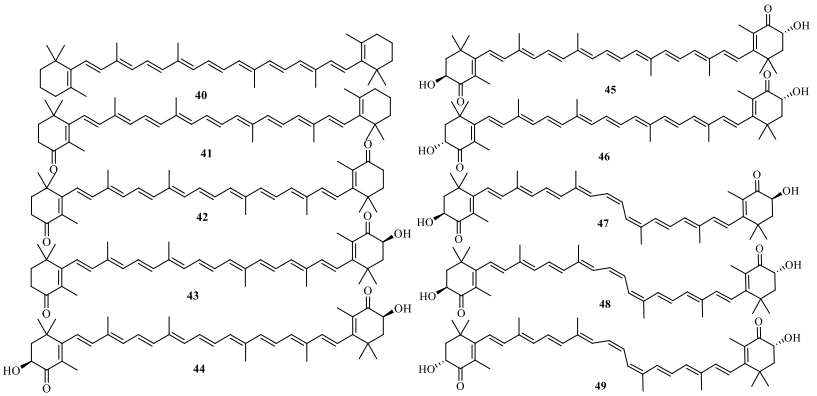

Table 1.6. Phosphatidyls from Thraustochytrium

No.	Compound	Species
50	1-oleoyl-2-palmitoyl-phosphatidylcholine	*Thraustochytrium* sp. ATCC 26185
51	phosphatidylethanolamine	*Thraustochytrium* sp. ATCC 26185
52	phosphatidylinositol	*Thraustochytrium* sp. ATCC 26185
53	phosphatidylglycerol	*Thraustochytrium* sp. ATCC 26185
54	diphosphatidylglycerol	*Thraustochytrium* sp. ATCC 26185

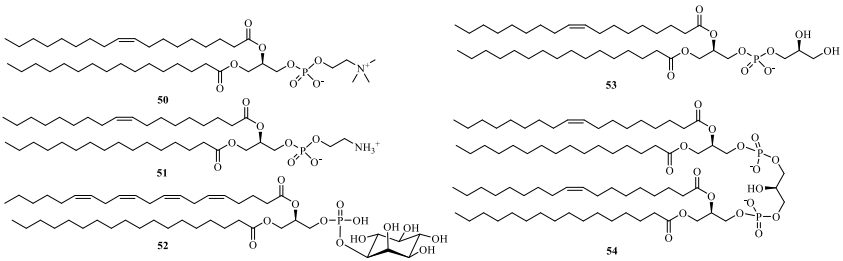

Figure 0.5. Structures of phosphatidyl from Thraustochytrium

1.1.2.5. Others

In addition to the compound classes mentioned above, studies on microalgae belonging to the genus *Thraustochytrium* have also identified several other compounds, such as squalene, 1→6-β-D-glucan, and *p*-hydroxybenzoic acid.

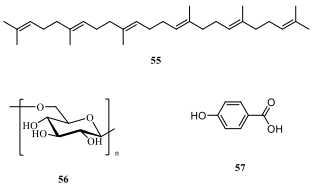

Figure 0.6. Structures of other compounds from Thraustochytrium

1.1.3. Bioactivities of microalgae species in the genus Thraustochytrium

1.1.3.1. Antioxidant

Species belonging to the genus Thraustochytrium exhibit significant antioxidant activity due to the presence of carotenoids, squalene, extracellular polysaccharides (EPS), and polyunsaturated fatty acids (PUFAs). Many studies have shown that extracts from this genus possess strong free radical scavenging activity and potential applications in cosmetics and functional foods.

1.1.3.2. Anti-inflammatory effect

Studies have shown that *Thraustochytrium* is capable of inhibiting the production of nitric oxide (NO) and inflammatory mediators. This activity is mainly associated with EPS and PUFAs such as DHA and EPA,

contributing to the regulation of immune responses and reduction of inflammatory reactions.

1.1.3.3. Antibacterial activity

Many species of the genus Thraustochytrium exhibit antibacterial activity against both Gram-positive and Gram-negative bacteria. Their extracts and EPS are capable of inhibiting various pathogenic bacterial strains, indicating potential applications in pharmaceuticals and biotechnology.

1.1.3.4. Anticancer activity

Bioactive compounds from *Thraustochytrium*, particularly EPS, carotenoids, squalene, and PUFAs, have been reported to inhibit the growth of various cancer cell lines through antioxidant mechanisms, immune regulation, and effects on the cell cycle.

1.1.3.5. Other biological activities

In addition to the activities mentioned above, *Thraustochytrium* is also considered a potential source of DHA and squalene for the pharmaceutical, functional food, and aquaculture industries. Some species are also capable of participating in environmental treatment through the biotransformation of organic wastes and the accumulation of biologically valuable compounds.

1.2. Introduction to the Genus *Aurantiochytrium*

1.2.1. Morphological characteristics and classification of species in the genus Aurantiochytrium

Previously, the genus *Aurantiochytrium* was grouped together with the genera *Oblongichytrium* and *Schizochytrium*. Cells of this genus grow through successive binary fission and are characterized by spherical thalli with thin cell walls. The released zoospores exhibit morphology similar to those of *Schizochytrium*, whereas vegetative cells usually exist as solitary cells. Currently, *Schizochytrium limacinum* and *S. mangrovei* have been renamed *Aurantiochytrium limacinum* and *A. mangrovei*, respectively. In these strains, under certain conditions, amoeboid cell discharge has been observed prior to zoospore division. Approximately 11 species of the genus *Aurantiochytrium* have been identified to date.

Table 1.7. Microalgae species in the genus Aurantiochytrium

No.	Compound	Species
1	*Aurantiochytrium limacinum (Schizochytrium limacinum)*	Honda (1998)

2	*Aurantiochytrium mangrovei (Schizochytrium mangrovei)*	Raghukumar (1988)
3	*Aurantiochytrium acetophilum*	Ganuza (2019)
4	*Aurantiochytrium minutum*	Ganuza (2019)
5	*Aurantiochytrium* sp. KRS101	Ryu (2013)
6	*Aurantiochytrium* sp. KH105	Arafiles (2014)
7	*Aurantiochytrium* sp. TC20	Lee Chang (2013a)
8	*Aurantiochytrium* sp. t66	Jakobsen (2008)
9	*Aurantiochytrium* sp. TC22	Lee Chang (2014)
10	*Aurantiochytrium* sp. 6-2	Chi-Hei Ip (2024)
11	*Aurantiochytrium sp.* ICTFD5	V.P. Bagul (2021)

1.2.2. Chemical constituents of Aurantiochytrium species

1.2.2.1. Steroids

Table 1.8. Steroids from Aurantiochytrium

No.	Compound	Species
1	cholesterol	*Aurantiochytrium* spp.
55	4,4-dimethyl-5*α*-cholest-8-en-3*β*-ol (4,4-dimethylzymosterol)	*Aurantiochytrium* spp.
56	4-methylchondrillasterol	*Aurantiochytrium* spp. *A. mangrovei* BT3
57	chondrillasterol	*Aurantiochytrium* spp.
58	poriferasterol	*Aurantiochytrium* spp.
59	poriferasterol acetate	*Aurantiochytrium* spp.
60	lathosterol	*Aurantiochytrium* spp.
61	lanosterol	*Aurantiochytrium* spp.
62	3-*O*-*β*-D-glucopyranosyl-stigmasta-5,7,22-triene	*A. limacinum* mh0186
63	3-*O*-*β*-D-glucopyranosyl-4*α*-methylstigmasta-7,22-diene	*A.limacinum* mh0186

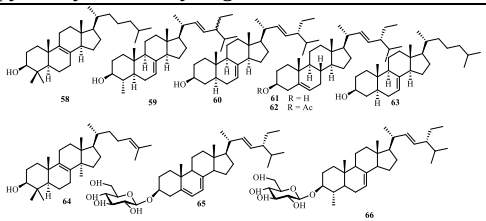

Figure 0.7. Structures of steroid from Aurantiochytrium

1.2.2.2. *Nitrogen-containing compounds*

Table 1.9. Nitrogen-containing compounds from Aurantiochytrium

No.	Compound	Species
64	tetradecanenitrile	*Aurantiochytrium* sp. KRS101
65	pentadecanenitrile	*Aurantiochytrium* sp. KRS101
66	octadecanenitrile	*Aurantiochytrium* sp. KRS101
67	hexadecanamide	*Aurantiochytrium* sp. KRS101
68	*N*,*N*-dimethylpalmitamide	*Aurantiochytrium* sp. KRS101
69	*O*-methyloxime hexanal	*Aurantiochytrium* sp. KRS101
70	acetylated m-glyceride	*Aurantiochytrium* sp. SYLR6#3
71	acetylated m-glyceride-hydrate	*Aurantiochytrium* sp. SYLR6#3

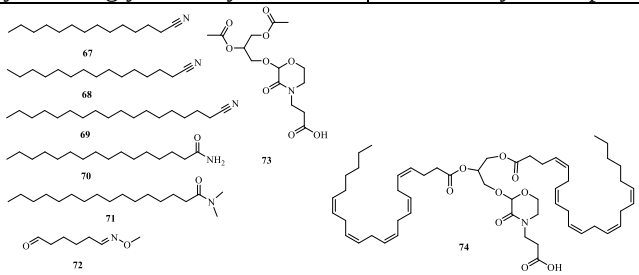

Figure 0.8. Structures of nitrogen-containing compounds from Aurantiochytrium

1.2.2.3. Fatty acid and carotenoid derivatives

Table 1.10. Fatty acids from Aurantiochytrium

No.	Compound	Species
72	α-linolenic acid	*Aurantiochytrium* sp. T7
73	stearidonic acid	*Aurantiochytrium* sp. T7
74	omega-3 arachidonic acid	*Aurantiochytrium* sp. T7
75	eicosapentaenoic acid	*Aurantiochytrium* sp. T7
76	docosapentaenoic acid	*Aurantiochytrium* sp. T7
77	docosahexaenoic acid	*Aurantiochytrium* sp. T7
78	methyl palmitate	*Aurantiochytrium sp. KRS101*
79	2-propenyl octadecanoate	*Aurantiochytrium sp. KRS101*
80	tridecanedioic acid	*Aurantiochytrium sp. KRS101*

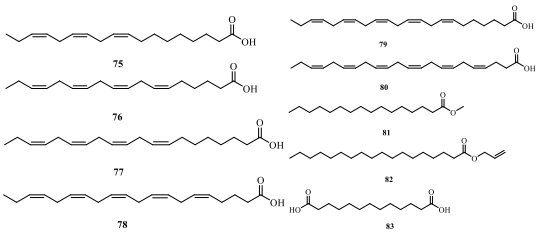

Figure 0.9. Structures of fatty acid and ester from Aurantiochytrium

In addition to fatty acids, several carotenoid compounds such as β-carotene **(40),** echinenone **(41),** canthaxanthin **(42),** and astaxanthin **(44)** have also been isolated from the strain *Aurantiochytrium C*h25 belonging to the genus *Aurantiochytrium.*

1.2.2.4. Others

In addition to the compound classes mentioned above, studies have also isolated several other compounds, such as 2-phenyl-2-tipyl-acenapthenone **(84)**, 2-pentanone **(85)**, 1,2-dimethylcyclohexane **(86)**, phenol **(87)**, and 4-methylphenol **(88)** from microalgal species belonging to the genus *Aurantiochytrium*.

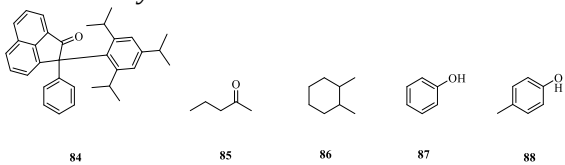

Figure 0.10. Structures of other compounds from Aurantiochytrium

1.2.3. Bioactivities of Aurantiochytrium species

1.2.3.1. Antibacterial activity

Species belonging to the genus *Aurantiochytrium* have been reported to exhibit antibacterial activity against various pathogenic bacterial strains. This activity depends on the extraction solvent and the bioactive compounds present in the microalgae.

1.2.3.2. Antioxidants

Extracts from *Aurantiochytrium* exhibit significant antioxidant activity through free radical scavenging and inhibition of lipid oxidation. This activity is mainly associated with carotenoids, phenolics, sterols, and polyunsaturated fatty acids.

1.2.3.3. Anti-inflammatory Activity

Some species of the genus *Aurantiochytrium* are capable of inhibiting the production of nitric oxide (NO) and pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, indicating their potential application in the development of natural anti-inflammatory agents.

1.2.3.4. Anticancer Activity.

DHA-rich oils and compounds from *Aurantiochytrium* have been demonstrated to inhibit the growth of several cancer cell lines. This activity is believed to be related to DHA and other bioactive lipid compounds.

1.2.3.5. Immunostimulatory Activity

Biomass and oils from *Aurantiochytrium* are capable of enhancing immunity and improving disease resistance in various aquaculture species due to their high contents of DHA and EPA.

1.2.3.6. Other Biological Activities

In addition to the activities mentioned above, *Aurantiochytrium* is also considered a potential source of DHA, squalene, and carotenoids for the pharmaceutical, cosmetic, functional food, and biodiesel industries. Some strains are also capable of utilizing organic wastes to produce biomass and biologically valuable compounds.

1.3. Domestic studies related to the two marine microalgae species *Thraustochytrium pachydermum* TSL10 and *Aurantiochytrium* sp. SC145

Domestic studies on the two marine microalgae species *Thraustochytrium pachydermum* TSL10 and *Aurantiochytrium* sp. SC145 has mainly focused on isolation, identification, optimization of culture conditions, and evaluation of lipid and PUFA accumulation. However, in-depth studies on their chemical constituents, isolation of secondary metabolites, and evaluation of biological activities remain limited, indicating the need for further investigations to effectively exploit their biological potential.

CHAPTER 2. MATERIALS AND METHODS

2.1. Research Material

2.1.1. Thraustochytrium sp. TSL10

Seawater samples were collected from Truong Sa Lon Island (Spratly Islands) on May 26, 2021, at coordinates 08°38'30"N–111°55'55"E. After five days of cultivation, the TSL10 microalgal strain was obtained with a high cell density of $103.3 \pm 1.2 \times 10^6$ cells/mL.

2.1.2. Aurantiochytrium sp. SC145

Seawater samples were collected from Son Ca Island (Spratly Islands) on June 5, 2021, at coordinates 10°22'42"N–114°28'33"E. After five days of cultivation, the SC145 microalgal strain was obtained with a cell density of $108.1 \pm 1.6 \times 10^6$ cells/mL.

2.2. Methods

2.2.1. Isolation methods

Compounds were isolated using chromatographic techniques such as thin-layer chromatography (TLC), column chromatography (CC), and high-performance liquid chromatography (HPLC).

2.2.2. Structural elucidation methods

The structures of the isolated compounds were determined using modern spectroscopic methods, including ECD, HR-ESI-MS, and NMR.

2.2.3. Antimicrobial Assay

The antimicrobial activities of the compounds were evaluated by the minimum inhibitory concentration (MIC) method against Gram-positive bacteria, Gram-negative bacteria, and yeast strains.

2.3. Isolation of compounds

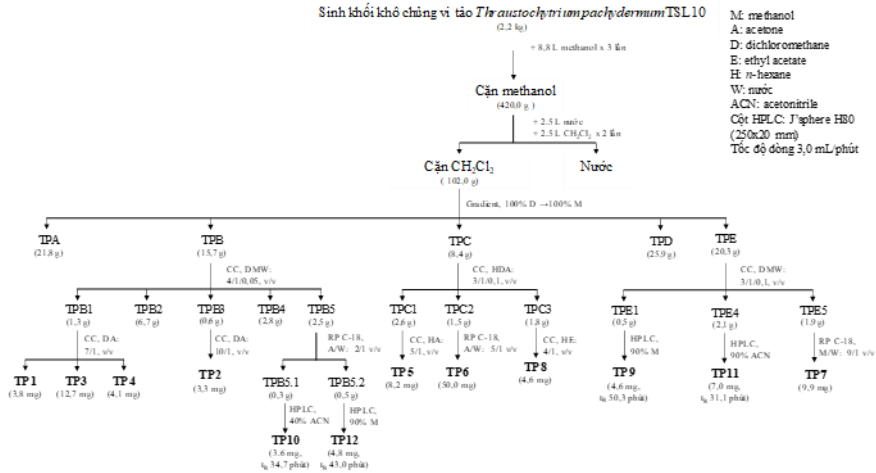

Figure 0.1. Isolation scheme for T. pachydermum TSL10

Figure 2.6. Isolation scheme for Aurantiochytrium sp. SC145

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Structural elucidation of the isolated compounds

3.1.1. From T. pachydermum TSL10

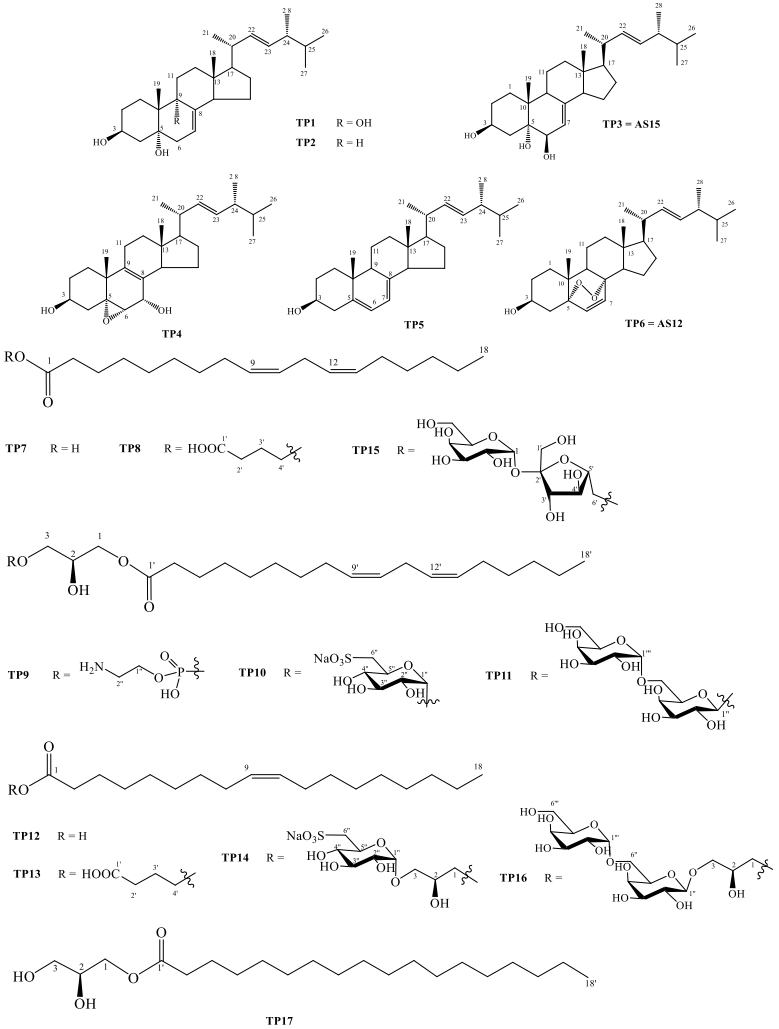

Figure 0.1. Isolated compounds from T. pachydermum

From the marine microalgal strain *T. pachydermum*, 12 compounds were isolated and structurally elucidated. These included one new compound, ergosta-7(8),22(23)-diene-3*β*,5*α*,9*α*-triol (**TP1**), and 11 known compounds: ergosta-7(8),22(23)-diene-3*β*,5*α*-diol (**TP2**), cerevisterol (**TP3 = AS15**), 5*α*,6*α*-epoxy-(22*E*)-ergosta-8,22-diene-3*β*,7*α*-diol (**TP4**), ergosterol (**TP5**), 5*α*,8*α*-*epi*-dioxyergosta-6,22-dien-3*β*-ol (**TP6 = AS12**), linoleic acid (**TP7**), 4-((9*Z*,12*Z*)-octadeca-9,12-dienoyloxy)butanoic acid (**TP8**), 1-linoleoyl-*sn*-glycero-3-phosphocholine (**TP9**), cibotiglycerol (**TP10**), gingerglycolipid B (**TP11**), and 6′-*O*-linoleylsucrose (**TP12**).

3.1.2. From Aurantiochytrium sp. SC145

From the marine microalga *Aurantiochytrium* sp., twenty-three compounds were isolated and structurally elucidated. Among them, two new compounds, *N*-methoxymethyltryptophol (**AS1**) and 4-hydroxyphenylethyl 2-hydroxy-3-phenylpropanoate (**AS2**), together with twenty-one known compounds, namely 3-hydroxy-4-(4-hydrophenyl)-2-butanone (**AS3**), 3-indoleacetic acid (**AS4**), tryptophol (**AS5**), indole-3-carbaldehyde (**AS6**), methyl indole-3-acetate (**AS7**), (*erythro*)-2-hydroxy-3-methylpentanoic acid (**AS8**), 2-hydroxy-3-phenylpropanoic acid (**AS9**), methyl 2-hydroxy-3-phenylpropanoate (**AS10**), tyrosol (**AS11**), 5*α*,8*α*-epi-dioxyergosta-6,22-dien-3*β*-ol (**AS12**), 5*α*,8*α*-epi-dioxyergosta-6,9(11),22-trien-3*β*-ol (**AS13**), chaxine C (**AS14**), cerevisterol (**AS15**), ergosta-5,7,22-trien-3*β*-ol (**AS16**), fungisterol (**AS17**), cyclo-(*L*-Pro-*L*-Leu) (**AS18**), adenosine (**AS19**), (9*Z*,12*Z*)-octadeca-9,12-dienoic acid (**AS20**), (*S*)-2,3-dihydroxypropyl (9*Z*,12*Z*)-octadeca-9,12-dienoate (**AS21**), cibotiglycerol (**AS22**), and tyrosyl linoleate (**AS23**), were identified.

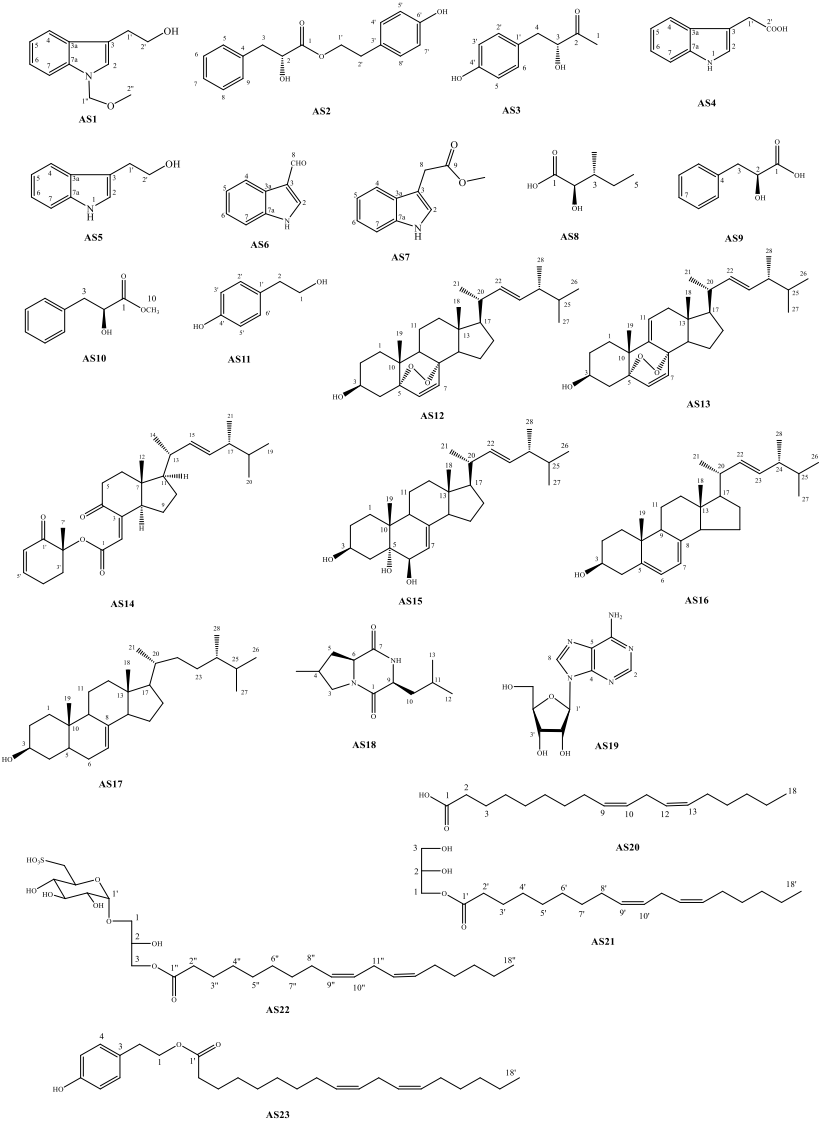

Figure 0.2. Isolated compounds from Aurantiochytrium sp.

3.2. Bioactivities of the isolated compounds

The isolated compounds were evaluated for antimicrobial activity against six microbial strains, including Gram-positive bacteria (*Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*), and the yeast *Candida albicans*.

3.2.1. Antimicrobial activity of compounds from T. pachydermum

Streptomycin and cycloheximide were used as positive controls. Streptomycin inhibited the Gram-positive bacterial strains (*E. faecalis*, *S. aureus*, and *B. cereus*) and Gram-negative bacterial strains (*E. coli*, *P. aeruginosa*, and *S. enterica*), with MIC values of 256, 256, 128, 32, 256, and 128 µg/mL, respectively. Cycloheximide inhibited the yeast *Candida albicans* with an MIC value of 32 µg/mL.

Among the compounds acting predominantly against Gram-positive bacteria, six sterol compounds (**TP1–TP6**) exhibited activity against all three Gram-positive strains, with MIC values ranging from 64 to 256 µg/mL. **TP3** showed the strongest activity against *S. aureus* (MIC 32 µg/mL), while **TP6** exhibited consistent activity against all three Gram-positive strains (MIC 64–128 µg/mL), surpassing that of streptomycin against *E. faecalis* and *S. aureus* (MIC 256 µg/mL). In contrast, **TP1, TP2, TP4,** and **TP5** exhibited weaker activity (MIC ≥ 64 µg/mL) and were inactive against Gram-negative bacteria.

Regarding antifungal activity, six compounds (**TP1–TP6**) inhibited *C. albicans*, with MIC values ranging from 64 to 256 µg/mL. However, most of these compounds exhibited weaker activity than the positive control cycloheximide (MIC 32 µg/mL).

The lipid compounds **TP7–TP12** did not exhibit antimicrobial activity. Nevertheless, compounds belonging to this class have been reported to possess several other biological activities, including anti-inflammatory [99,100], antioxidant [101], anticancer [102], and cardioprotective activities [103]. Overall, the isolated compounds tended to exhibit greater activity against Gram-positive bacteria than against Gram-negative bacteria. Specifically, compounds **TP1–TP6** were predominantly active against Gram-positive bacteria, suggesting their potential for the development of selective antibacterial agents. The sterol compounds isolated from *T. pachydermum* exhibited stronger antimicrobial activity than the lipid compounds.

3.2.2. Antimicrobial activity of compounds from Aurantiochytrium sp. SC145

The antimicrobial activities of the isolated compounds were evaluated against Gram-positive bacteria (*Enterococcus faecalis* ATCC299212, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC14579), Gram-negative bacteria (*Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* ATCC13076), and the yeast *Candida albicans* ATCC10231. Streptomycin was used as the positive control against Gram-positive (*E. faecalis*, *S. aureus*, *B. cereus*) and Gram-negative (*E. coli*, *P. aeruginosa*, *S. enterica*) bacteria, with MIC values of 256, 256, 128, 32, 256, and 128 μg/mL, respectively, while cycloheximide was used as the positive control against *Candida albicans* with an MIC value of 32 μg/mL.

The results showed that four compounds (**AS2**, **AS3**, **AS11**, and **AS23**) exhibited activity against both Gram-positive and Gram-negative bacteria with MIC values ranging from 64 to 256 μg/mL. Among them, **AS2** exhibited strong inhibitory activity against all three Gram-positive strains with MIC values of 64 μg/mL, but weaker activity against two Gram-negative strains (*E. coli* and *P. aeruginosa*, MIC 128 μg/mL) and *S. enterica* (MIC 256 μg/mL). **AS3** showed strong inhibitory activity against the Gram-positive strain *E. faecalis* ATCC299212 with an MIC value of 64 μg/mL, but weaker activity against the remaining microbial strains. **AS11** exhibited relatively consistent activity against both bacterial groups with MIC values ranging from 64 to 128 μg/mL. Meanwhile, **AS23** showed weaker activity with MIC values ranging from 128 to 256 μg/mL, indicating limited antimicrobial potency compared to compounds with lower MIC values. Nevertheless, this compound still exhibited notable activity against *E. faecalis* with an MIC value of 128 μg/mL, stronger than streptomycin (256 μg/mL).

For compounds mainly active against Gram-positive bacteria, **AS13** exhibited activity against all three Gram-positive strains with MIC values ranging from 64 to 256 μg/mL. In particular, **AS13** displayed consistent activity against all three Gram-positive strains (MIC 64–128 μg/mL), stronger than streptomycin (MIC 256 μg/mL against *E. faecalis* and *S. aureus*). Notably, the two sterol compounds **AS15** and **AS17** exhibited the strongest activity against Gram-positive bacteria, with the lowest MIC value of 32 μg/mL. Specifically, **AS15** was active against *S.*

aureus, whereas **AS17** was active against *E. faecalis* and *B. cereus*, showing significantly stronger activity than streptomycin and indicating their potential as selective antibacterial agents against Gram-positive bacteria.

Regarding antifungal activity, eight compounds (**AS2**, **AS3**, **AS11**, **AS13**, **AS14**, **AS16**, **AS17**, and **AS23**) inhibited *Candida albicans* with MIC values ranging from 32 to 256 µg/mL. However, most compounds were less active than cycloheximide (MIC 32 µg/mL), except for **AS2**, **AS3**, and **AS11**, which showed MIC values of 32 µg/mL, equivalent to the positive control, indicating strong antifungal potential.

Overall, the isolated compounds tended to exhibit greater activity against Gram-positive bacteria than Gram-negative bacteria. Compounds **AS2**, **AS3**, and **AS11** displayed broad-spectrum activity against Gram-positive bacteria, Gram-negative bacteria, and yeast. Notably, **AS2** and **AS11** exhibited stronger antimicrobial effects than **AS3**, as reflected by their lower MIC values. **AS23** also showed broad-spectrum antibacterial activity, although its antimicrobial potency was limited. Compound **AS17** exhibited strong activity against Gram-positive strains, while **AS13** mainly showed selective activity against Gram-positive bacteria.

The indole-containing compounds **AS1** and **AS4–AS7** did not exhibit antimicrobial activity. However, indole compounds are known to possess potent biological activities such as anticancer [104,105], antioxidant, and anti-inflammatory activities [106].

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

1. From seawater samples collected from Son Ca Island (Spratly Islands) and Truong Sa Lon Island (Spratly Islands), two corresponding microalgal strains, *Aurantiochytrium* sp. and *Thraustochytrium pachydermum*, were successfully cultivated. After extraction, isolation using chromatographic techniques, and structural elucidation using modern spectroscopic methods combined with comparison of spectral data with related compounds, we obtained the following results:

- Twelve compounds were isolated and structurally elucidated from the marine microalgal strain *T. pachydermum* TSL10. These included one new compound, ergosta-7(8),22(23)-diene-3*β*,5*α*,9*α*-triol (**TP1**), and 11 known compounds: ergosta-7(8),22(23)-diene-3*β*,5*α*-diol (**TP2**), cerevisterol (**TP3**), 5*α*,6*α*-epoxy-(22*E*)-ergosta-8,22-diene-3*β*,7*α*-diol (**TP4**), ergosterol (**TP5**), (22*E*,24*R*)-5*α*,8*α*-*epi*-dioxyergosta-6,22-dien-3*β*-ol (**TP6**), linoleic acid (**TP7**), 4-((9*Z*,12*Z*)-octadeca-9,12-dienoyloxy)butanoic acid (**TP8**), 1-linoleoyl-*sn*-glycero-3-phosphocholine (**TP9**), cibotiglycerol (**TP10**), gingerglycolipid B (**TP11**), and 6′-*O*-linoleylsucrose (**TP12**).

- Twenty-three compounds were isolated and structurally elucidated from the marine microalga *Aurantiochytrium* sp. Among them, two new compounds, *N*-methoxymethyltryptophol (**AS1**) and 4-hydroxyphenylethyl 2-hydroxy-3-phenylpropanoate (**AS2**), together with twenty-one known compounds, namely 3-hydroxy-4-(4-hydrophenyl)-2-butanone (**AS3**), 3-indoleacetic acid (**AS4**), tryptophol (**AS5**), indole-3-carbaldehyde (**AS6**), methyl indole-3-acetate (**AS7**), (*erythro*)-2-hydroxy-3-methylpentanoic acid (**AS8**), 2-hydroxy-3-phenylpropanoic acid (**AS9**), methyl 2-hydroxy-3-phenylpropanoate (**AS10**), tyrosol (**AS11**), 5*α*,8*α*-epi-dioxyergosta-6,22-dien-3*β*-ol (**AS12**), 5*α*,8*α*-epi-dioxyergosta-6,9(11),22-trien-3*β*-ol (**AS13**), chaxine C (**AS14**), cerevisterol (**AS15**), ergosta-5,7,22-trien-3*β*-ol (**AS16**), fungisterol (**AS17**), cyclo-(*L*-Pro-*L*-Leu) (**AS18**), adenosine (**AS19**), (9*Z*,12*Z*)-octadeca-9,12-dienoic acid (**AS20**), (*S*)-2,3-dihydroxypropyl (9*Z*,12*Z*)-octadeca-9,12-dienoate (**AS21**), cibotiglycerol (**AS22**), and tyrosyl linoleate (**AS23**), were identified.

2. The antimicrobial activities of compounds isolated from the marine microalgae *Aurantiochytrium* sp. and *Thraustochytrium pachydermum* were evaluated. The results showed that:

- For the marine microalgal strain *T. pachydermum* TSL10, eight compounds—**TP1–TP6, TP9,** and **TP10**—exhibited activity against several tested bacterial strains and yeast. Among them, **TP3** showed the strongest activity against *S. aureus*, with an MIC value of 32 µg/mL. Compounds **TP1, TP4,** and **TP6** exhibited strong activity against two Gram-positive bacterial strains, *S. aureus* and *B. cereus*, with MIC values of 64 µg/mL.
- For *Aurantiochytrium* sp., ten compounds (**AS2**, **AS3**, **AS11–AS17**, and **AS23**) exhibited inhibitory activity against several tested bacteria and yeast strains. Among them, the two compounds showing the strongest activity were **AS15** (against *S. aureus*, MIC 32 µg/mL) and **AS17** (against *E. faecalis* and *B. cereus*, MIC 32 µg/mL). Compounds **AS2**, **AS3**, **AS11**, and **AS12** exhibited strong activity against three Gram-positive bacterial strains with MIC values of 64 µg/mL, stronger than the positive control streptomycin (MIC 256 and 128 µg/mL). Compounds isolated from *Aurantiochytrium* sp. showed high selectivity, mainly against Gram-positive bacteria. Against the yeast *C. albicans* (MIC 32 µg/mL), three compounds (**AS2**, **AS3**, and **AS11**) exhibited strong activity with MIC values of 64 µg/mL, comparable to the positive control cycloheximide.

RECOMMENDATIONS

Studies on the chemical constituents and biological activities of the marine microalgae *Aurantiochytrium* sp. and *Thraustochytrium pachydermum* have contributed new compounds to their known chemical profiles. In particular, biological activity assays of compounds **AS2**, **AS3**, **AS11**, **AS12**, **AS13**, **AS15**, **AS17**, **TP1**, and **TP4** demonstrated antimicrobial activities comparable to those of the positive controls. Therefore, further biological evaluations should be conducted to better assess their potential applications.

NOVEL CONTRIBUTIONS OF THE THESIS

1. From the dried biomass of the marine microalgal strain *T. pachydermum* TSL10, one new compound, ergosta-7(8),22(23)-diene-3*β*,5*α*,9*α*-triol (**TP1**), together with 11 known compounds, was isolated and structurally elucidated. Of the 11 known compounds isolated from *T. pachydermum* TSL10, nine were isolated for the first time from marine microalgae of the genus *Thraustochytrium*: ergosta-7(8),22(23)-diene-3*β*,5*α*-diol (**TP2**), cerevisterol (**TP3**), 5,6-epoxy-(22*E*)-ergosta-8,22-diene-3,7-diol (**TP4**), (22*E*,24*R*)-5*α*,8*α*-epidioxyergosta-6,22-dien-3*β*-ol (**TP6**), 4-((9*Z*,12*Z*)-octadeca-9,12-dienoyloxy)butanoic acid (**TP8**), 1-linoleoyl-*sn*-glycero-3-phosphocholine (**TP9**), cibotiglycerol (**TP10**), gingerglycolipid B (**TP11**), and 6′-*O*-linoleylsucrose (**TP12**). Notably, the new compound **TP1** exhibited potent antimicrobial activity against the Gram-positive bacterium *S. aureus*, with an MIC value of 64 µg/mL, which was stronger than that of the positive control streptomycin (MIC = 256 µg/mL).

2. From the dried biomass of the marine microalgal strain *Aurantiochytrium* sp. SC145, two new compounds, *N*-methoxymethyltryptophol (**AS1**) and 4-hydroxyphenylethyl 2-hydroxy-3-phenylpropanoate (**AS2**), together with 21 known compounds, were isolated. Of the 21 known compounds, 16 were isolated for the first time from marine microalgae of the genus *Aurantiochytrium*: 3-hydroxy-4-(4-hydroxyphenyl)-2-butanone (**AS3**), 3-indoleacetic acid (**AS4**), tryptophol (**AS5**), indole-3-carbaldehyde (**AS6**), methyl indole-3-acetate (**AS7**), (*erythro*)-2-hydroxy-3-methylpentanoic acid (**AS8**), 2-hydroxy-3-phenylpropanoic acid (**AS9**), methyl 2-hydroxy-3-phenylpropanoate (**AS10**), tyrosol (**AS11**), 5*α*,8*α*-epidioxyergosta-6,9(11),22*E*-trien-3*β*-ol (**AS13**), chaxine C (**AS14**), fungisterol (**AS17**), cyclo-(L-Pro-L-Leu) (**AS18**), (*S*)-2,3-dihydroxypropyl (9*Z*,12*Z*)-octadeca-9,12-dienoate (**AS21**), cibotiglycerol (**AS22**), and tyrosyl linoleate (**AS23**).

Compound **AS2** exhibited potent inhibitory activity against all three Gram-positive bacterial strains *E. faecalis*, *S. aureus*, and *B. cereus* with an MIC value of 64 µg/mL for each strain. It also inhibited three Gram-negative bacterial strains, *E. coli*, *P. aeruginosa*, and *S. enterica*, with MIC values of 128, 128, and 256 µg/mL, respectively. Furthermore, **AS2** inhibited the yeast *C. albicans* with an MIC value of 32 µg/mL, quivalent to that of the positive control cycloheximide (MIC = 32 µg/mL).

PUBLICATION

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