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Lipid classes, fatty acid content, and antibacterial characteristics of seaweed collected at Bach Long Vi island (*Lobophora tsengii* D. Tien & Z. Sun)

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Abstract. In this paper, the lipid classes, fatty acid composition, and antimicrobial activities of *L. tsengii* were described. The concentrations of arachidonic acid (AA) and eicosapentaenoic acid (EPA) were 8 % and 11.46 % of the unsaturated fatty acid content, respectively, which accounted for 53.11 % of the total fatty acid content. In terms of total lipid content, polar lipid (PL) had the greatest content (43.47 %). Among them, PE and PC contents are equivalent (over 30 %) and LPE content (18.86 %) is twice as high as PS content (9.29 %). The evaluation of microbial activities against seven microorganims strains indicated that the extract of *L. tsengii* showed a strong inhibitory effect against *Staphylococcus aureus* with an IC_{50} value of 54.95 ± 1.38 µg/mL.

Keywords: Lobophora tsengii, fatty acids, phospholipid class, antibacterial

Classification numbers: 1.1.1, 1.1.6.

1. INTRODUCTION

Brown algae (Phaeophyta) are primarily utilized in human nutrition, especially in several Asian countries because they contain many mineral elements, vitamins, proteins, and amino acids [1]. Phaetophyla are also documented as an important source of polysaccharides (fucoidans, and alginate), lipids, carotenoids, terpenoids and phenolic compounds [1-3]. Seaweed constitutes a good and large source of polyunsaturated fatty acids, with a healthy (n-6) FA : (n-3) FA ratio of about 1.0. N-3 FAs are known to have anti-inflammatory and antioxidant activities while conversely, the majority of n-6 fatty acids, which serve as precursors of arachidonic acid and prostaglandin E2, tend to stimulate inflammation and cell proliferation [4].

Recently, it has become evident that in addition to their role in preventing cardiovascular diseases, certain n-3 polyunsaturated fatty acids (PUFAs), particularly EPA and DHA, play a significant role as essential components of brain cells. These fatty acids are crucial for the proper development and functioning of the brain and nervous system [5]. The presence of multidrug-resistant pathogens is a global problem that causes great concern in healthcare institutions. Notably, infections caused by multidrug-resistant *Staphylococcus aureus* (MRSA) caused more than 20,000 deaths in the USA and UK alone in 2005 [6]. DHA and EPA have shown potential in effectively managing persistent *S. aureus* infections [7]. Numerous studies have highlighted the antibacterial properties found in cell lysates or extracts of various microalgae species, including diatoms [8, 9]. Furthermore, the identification of antibacterial compounds, such as fatty acids, has also been reported in these studies [10, 11].

Compounds and extracts of brown algae exhibit many biological activities, such as anti-cancer [2, 12], antiviral [2], anti-diabetic [13], cholesterol-lowering [14], anti-inflammatory [15], anticoagulant [15], antioxidant [16, 17], antifungal, antibacterial [16], hepatoprotective [17], anti-angiogenic [20], anti-cancer [21], anti-bone loss [22], and neuroprotective activities [23]. The genus Lobophora belongs to the family Dictyotaceae, order Dictyotales, phylum Phaeophyta and has about 71 species [24]. Most of the studies focus on *Lobophora variegata* species, with diverse biological activities such as antibacterial, antifungal, antiviral, antiparasitic, insecticidal, antithrombotic, antioxidant, anti-inflammatory, and anti-cancer activity [25]. Among them, *L. tsengii* D. Tien & Z. Sun [26] and *Lobophora* sp. are two new species which were discovered and reported in 2020. Therefore, the research and selection of natural antibacterial compounds from seaweed to replace antibiotics is of great interest to the world. In this paper, we focused on studying the lipid classes and fatty acid contents, and evaluating the antimicrobial effects of total lipids in brown algae.

2. MATERIALS AND METHODS

2.1. Plant materials

In July 2023, *Lobophora tsengii* D.Tien & Z.Sun, a species of brown algae, was collected from Bach Long Vy Island, Hai Phong, Viet Nam. Dr. Dam Duc Tien of the Institute of Marine Environment and Resources, Vietnam Academy of Science and Technology, recognized the voucher specimen (LO1), which was then kept at the Institute of Natural Products Chemistry, also under the Academy.

2.2. Total lipid (TL) extraction

Total lipids were extracted using the methods of Bligh and Dyer [27] and Dinh *et al*. [28]. To put it briefly, a 20 mL Teflon-lined screw-capped glass tube was used to extract 400 mg of dried algal powder using 6 mL of methanol:chloroform mixture (2:1, v/v) (3 replicates). The samples were dried at 50 °C for one hour. After that, any remaining particulate matter was removed using a vacuum pump and a Whatman GF/A filter (Whatman Plc, Maid-stone, UK). Two extractions of the residue were made. Chloroform (4 mL) and distilled water (4 mL) were added to the combined filtrate and the organic phase was separated. After dryring over Na_2SO_4, the solution was filtred and evaporated under reduced pressure to obtain the total lipids which were stored in chloroform at –5 °C.

2.3. Lipid class analysis

After dissolving the whole lipid in $CHCl_3$ (10 mg/mL), it was separated on coated silica gel plates (6 cm × 6 cm). Sorbfil PTLC-AF-V (Sorbfil, Krasnodar, Russia) was used with a mixture of $CHCl_3/CH_3OH$ (2/1, v/v) for the second separation and a mixture of n-hexane/diethyl ether/acetic acid (85/15/1, v/v/v) for the first. The TLC was heated to 240 °C for 10 minutes after being sprayed with 10 % H_2SO_4/CH_3OH reagent. An Epson Perfection 2400 scanner from Japan was used to visualize the spots in grayscale mode. Lipid classes were quantitatively analyzed using a Sorbfil TLC Video Densitometer from Krasnodar, Russia [28].

2.4. Polar lipid analysis

10 × 10 cm glass-backed HPTLC Silica gel 60 plates (Merck, Germany) were used to separate polar lipids. The first separation was performed using $CHCl_3$/MeOH/C_6H_6/28 % NH_4OH (65 : 30 : 10 : 6, v/v/v/v) and the second was performed using $CHCl_3$/MeOH/AcOH/CH_3COCH_3/C_6H_6/H_2O (70:30:4:5:10:1, v/v/v/v/v). Standard phospholipids and a phosphormolybdate solution were employed to detect phospholipids on TLC plates [29]. After being digested with perchloric acid, the phospholipid concentration was measured using spectrophotometry [30].

2.5. Fatty acid analysis

Fatty acid methyl esters (FAMEs) were obtained by treating the total lipids with 2 % H_2SO_4 in CH_3OH at 80 °C for 2 hours in a screw-capped vial and then separated by TLC using a mixture of hexane:diethyl ether (95/5, v/v). FAMEs were analyzed on a Shimadzu GC-2010 gas chromatograph (Kyoto, Japan) using a flame ionization detector (FID) on Capillary Equity 5 (Merck, L × ID 30 m × 0.25 mm, df 0.25 µm). At a rate of 20 milliliters per minute, He was the carrier gas. For 20 minutes, the column temperature was raised from 160 °C to 240 °C at a rate of 2 °C per minute. The temperatures of the flame-ionization detector and sample injection port were kept at 250 °C. The Equivalent Chain Length, a common method that determines retention time values based on the fatty acid standards C16:0 and C18:0, was used to identify fatty acids. To obtain this, the use of a spectrum-standard substance library was required. Using specialized software, the ELC values of the capillary column were converted into equivalent retention times to identify fatty acids. Using the standard reagent system C16:0 and C18:0 on a C-R3A machine, specialized CP-Sil 88 is produced according to the following formula:

$$\boldsymbol{ECL = 16 + \frac{2(lgRT_x - lgRT_{16:0})}{lgRT_{18:0} - lgRT_{16:0}}}$$

2.6. Antimicrobial assay

Microorganisms: The microorganisms used in this study consisted of *Enterococcus faecalis* (ATCC29212), *Staphylococcus aureus* (ATCC13709), *Bacillus cereus* (ATCC13245), *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC15442), *Salmonella enterica* (ATCC13076), and *Candida albicans* (ATCC10231).

Culture media: MHB (Mueller-Hinton Broth), MHA (Mueller-Hinton Agar), TSB (tryptic Soy Broth), TSA (Tryptic Soy Agar) for bacteria; SDB (Sabourand – 2 % dextrose broth) and SA (Sabourand – 4 % dextrose agar) for yeast. Dried *L. tsengii* was extracted using a mixture of MeOH/CH_2Cl_2 (2/1). The extract was initially dissolved in 100 % DMSO and subsequently diluted with deionized water to create a series of concentrations (128.0, 32.0, 8.0, 2.0 and 0.5 µg/mL). 10 µL of tested solution with various concentrations were added to a 96-well plate. 200 mL of microorganisms (5 × 105 CFU/mL) were added to the plate and further incubated at 37 °C for 24 h. The experiment was triplicated. Ampicillin and cefotaxin were used as positive controls for (+) Gram and (-) Gram bacteria, respectively. Nystatin was used as a positive control for

yeast. The minimum inhibitory concentration (MIC) was determined at the well with the lowest concentration which inhibited the growth of microorganisms. The IC_{50} values were calculated based on the data obtained from TECAN spectrometry using Rawdata software [31].

Statistics: One-Way ANOVA analysis, especially Duncan's test, was used to assess the experimental results using SPSS software. A significant difference was defined as $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Lipid class composition

The total lipid content of the brown algae *L. tsengii* is 4.13 ± 0.2 % of its dry weight, which is similar to what has been found in other research on most brown algae [27, 32, 33]. Polar lipid (PL), sterol (ST), diacylglycerol (DG), free fatty acid (FFA), triacylglycerol (TG), monoalkyldiacylglycerol (MADG), and hydrocarbons and wax (HW) were the primary lipid classes found by TLC. Figures 1 and 2 display the contents of the lipid classes.

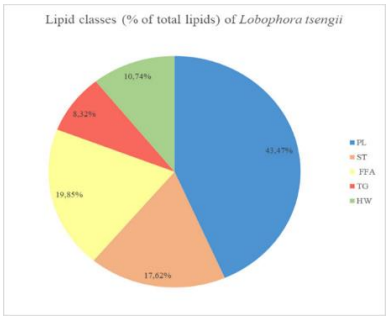

Figure 1. The brown algae *L. tsengii*'s lipid class composition.

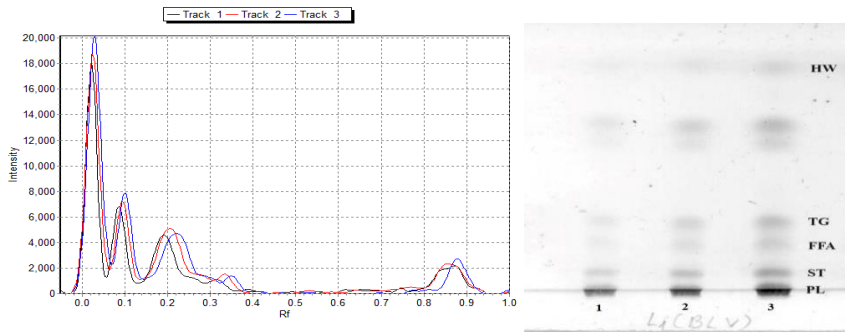

Figure 2. Chromatogram contents of the lipid classes in brown algae *L. tsengii*.

(*The sample concentration gradually increases from tract 1 to tract 3)*

When compared to the other lipid classes found, polar lipids made up the largest portion of the total lipids (43.47 %). This number was lower than that of another brown algae Sargassum species (46.55 - 68.16 %) [34], but it was noticeably greater than the PL concentration of another Lobophora species (26.8 %) [35]. Polar lipids, including phospholipids (PLs) and sphingolipids (SLs), are present in the human diet and are crucial parts of cellular membranes. These dietary polar lipids can alter the makeup of cellular membranes through interactions, which can impact a number of signaling pathways and enzymatic activities. The polar lipids have several positive

benefits, such as their capacity to reduce cholesterol, their anti-inflammatory and anti-cancer qualities, and their function in brain development [36]. FFA, ST, HW, and TG were the other main lipid classes identified in *L. tsengii*, with respective contents of 19.85 %, 17.62 %, 10.74 %, and 8.32 %. A small variation was seen in the distribution compared to the Lobophora species (25.9 %, 18.5 %, 2.7 %, and 26.0 %).

3.2. Compositions and contents of phospholipid classes

Phospholipids play important roles in a number of biological functions, such as intracellular signaling, cell proliferation, apoptosis, membrane protein control, and membrane trafficking. PC makes up 40 - 50 % of all phospholipids in mammalian cell membranes, making it the most prevalent phospholipid [37]. Cell membranes contain lesser amounts of PS, PA, PI, and PG, although PE is the second most prevalent phospholipid (20–50 %) [38,39]. Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylethanolamine (LPE) are the primary glycerophospholipid classes that TLC has identified in PL. Approximately 71.85 % of the total known phospholipids in *L. tsengii* are composed of PC and PE. PE is frequently the main lipid component in microbial membranes and is usually the second most abundant phospholipid in animal and plant lipids, behind PC. PC was discovered to have a high level in both PL (37.97 %) and LPE (18.86 %) in our study. Table 1 and Figure 3 display the lipid phospholipid class compositions and contents of brown algae *L. tsengii*.

Table 1. Phospholipid class compositions and contents of brown algae *L. tsengii*.

No	Compositions of phospholipid classes	Content (%)
1	PS	9.29
2	LPE	18.86
3	PC	37.97
4	PE	33.88

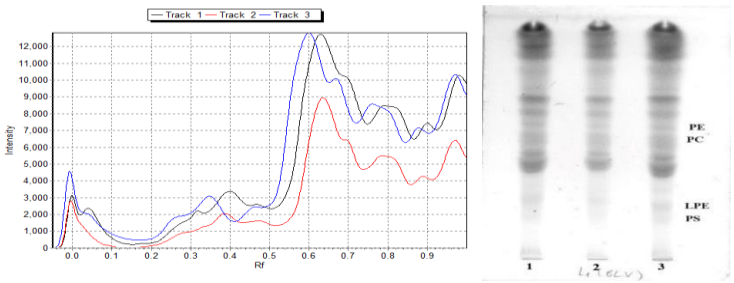

Figure 3. Chromatogram compositions and contents of phospholipid classes of brown algae *L. tsengii*.

(*The sample concentration gradually increases from tract 1 to tract 3)*

3.3. Fatty acid compositions

Table 2 displays the FA compositions of *L. tsengii*'s total lipids. The findings indicated that *L. tsengii* has a high amount of PUFA (32.98 % of total FA), with the highest content being eicosapentaenoic acid (EPA) (11.46 %). 8 % come from arachidonic acid (AA), 2.45 % from octadecatrienoic acid (ALA), and 1.84 % from stearidonic acid (SA). Amounts of less than 2 %

of other long-chain polyunsaturated fatty acids are also found. High amounts of omega-3 (20 : 5 n-3, 18:4n-3, and 18 : 3 n-3) and omega-6 (20 : 4 n-6 and 18 : 2 n-6) PUFAs are found in the majority of seaweeds' total lipids. In addition to their important nutritional qualities, PUFA have a number of positive health impacts. These benefits, which have significant pharmacological and nutraceutical uses, include anti-inflammatory, anti-cancer, anti-obesity, and anti-cardiovascular disease qualities [40].

Table 2. FA contents of *L. tsengii*'s total lipid.

Fatty acid	Name of fatty acids	Content (%)
∑ SAFAs		**46.03**
12:00	Acid dodecanoic	0.05
14:0	Acid tetradecanoic	14.01
15:0	Acid pentadecanoic	0.54
16:0	Acid hexadecenoic	30.23
17:0	Acid heptadecanoic	0.19
18:0	Acid octadecanoic	0.63
19:0	Acid nonadecanoic	0.25
20:0	Acid eicosanoic	0.15
∑ MUFAs		**20.13**
16:1n-7	Acid 9-hexadecenoic	0.18
16:1n-5	Acid 11-hexadecenoic	1.94
18:1n-9	Acid 9 octadecenoic	18.02
∑ PUFAs		**32.98**
18:3n-6	Acid 6,9,12-octadecatrienoic (GLA)	0.12
18:4n-3	Acid 6,9,12,15-octadecatetraenoic (SA)	1.84
18:2n-6	Acid octadecadienoic	5.48
18:3n-3	Acid 9,12,15-octadecatrienoic 9(ALA)	2.45
20:4n-6	Acid 5,8,11,14-eicosatetraenoic (AA)	8.00
20:5n-3	Acid 5,8,11,14 eicosapentaenoic (EPA)	11.46
20:4n-3	Acid 8,10,14,17 eicosatetraenoic	0.48
20:3n-9	Acid 5,8,11-eicosatrienoic	1.77
20:3n-6	Acid 8,11,14- eicosatrienoic	1.24
20:2n-6	Acid 11,14-eicosadienoic	0.13
PUFA/SFA		0.72
∑n-6 FA		14.97
∑n-3 FA		13.78
n-6/n-3		1.09
UI		146.68
AI		1.77
TI		0.75

UI (unsaturation index) was calculated by multiplying the percentage of each fatty acid by the number of double bonds, followed by summing up these contributions;

*AI (atherogenic index): AI = [C12:0 + (4*C14:0) + C16:0]/(∑MUFAs + n-3 PUFAs + n-6 PUFAs);*

*TI (thrombogenic index): TI = (C14:0 + C16:0 + C18:0)/ [(0.5 *∑ MUFAs) + (0.5 * n-6 PUFAs) + (3 * n-3 PUFAs) + (n-3 PUFAs/n-6 PUFAs)].*

The study by Pham Quoc Long *et al*. demonstrates the notable variation in the concentration of the four fatty acids - ALA, AA, EPA, and DHA - in nine species of seaweed belonging to the Dictyotales family in Viet Nam. While ALA makes up less than 5 % of brown agar, it makes up 18 % in *Padina australis* Hauck. Five brown agar species (*Dictyopteris jamaicensis*, *Sptoglossum vietnamense* Pham-Hoang Ho 1969, the EPA acid concentration of W.R. Taylor, *Spatoglossum pacificum* Yendo, *Dictyota indica* Sonder, and *Padina australis* Hauck) account for less than 3 % [35]. According to some sources, there is also docosahexaenoic acid (DHA, 22:6n-3). According to previous reports, *Padina australis* contains 18.54 % DHA, *Lobophora* sp. contains 14.26 %, *Undaria pinnatifida* contains 8.55 %, *Sargassum muticum* contains 7.33 %, and *Colpomenia peregrine* has 5.80 % [41]. Nevertheless, DHA was not found in our investigation.

One crucial index for evaluating the quality of lipids is the PUFA/SFA ratio. For this ratio, the British Department of Health suggests a minimum value of 0.45 [42]. The PUFA/SFA ratio of *L. tsengii* in this investigation was 0.71, which is higher than the suggested minimum value. The low ratio of n-6 to n-3 polyunsaturated fatty acids (PUFA) is a distinctive feature of the lipid compositions of marine algae [43]. Numerous studies have demonstrated that improvements in glucose tolerance, obesity, inflammation, and other metabolic dysfunctions are linked to maintaining a balanced ratio of n-6 to n-3 polyunsaturated fatty acids [38]. The n-6 PUFA/n-3 PUFA ratio shouldn't be more than 10 [44], according WHO guidelines. Furthermore, in order to prevent neurological, cardiovascular, and inflammatory diseases, the European Nutritional Societies recommend that this ratio should not surpass 5 [45]. According to this study, *L. tsengii*'s n-6 PUFA/n-3 PUFA ratio was 1.09. In particular, it is suggested that indices of atherogenicity and thrombogenicity be used in place of the polyunsaturated/saturated ratio as a measure of how diet affects the incidence of coronary heart disease. Ulbricht proposed the thrombosis index (TI) and angiogenesis index (AI) to evaluate risk variables linked to the onset of coronary heart disease [46]. According to the results of our investigation, the low n-6/n-3 ratio and high PUFA n-3 content cause both indices to be less than 2 (AI = 1.77; TI = 0.75). These two mild indexes, however, are 2.72 greater than the findings we reported. According to the findings, *L. tsengii* might be good for heart health. Eight saturated fatty acids were found in *L. tsengii*, making up 46.03 % of the total SFA concentration. According to earlier research, palmitic acid (C16:0) made up 20 - 40 % of the total fatty acids in marine algae, which is a large amount [27]. The percentage of palmitic acid in our study was 30.23 %. At 14.01 %, myristic acid (C14:0), another saturated fatty acid, was found. Less than 0.5 % of the remaining saturated fatty acids, including the long-chain saturated fatty acid C20:0, were present.

3.4. Antimicrobial effects of *L. tsengii* extract

Certain lipids possess high value-added properties, including antitumoral, antimicrobial, and anti-inflammatory activities and also important nutritional significance. Some reports suggest that the abundance of PUFA in seaweed constituents is associated with exhibiting anti-inflammatory activity [47]. Echeverria reported that n-3 PUFA (especially EPA and DHA) partially reduced proinflammatory indices such as NF-κB and Nrf2, which suggests that lipid fractions containing n-3 PUFA may inhibit NO inhibition through the regulation of NF-κB and Nrf2 factors [47]. *Lobophora* sp. lipid fractions have shown potent NO inhibitory activity [31]. Sun *et al*. looked into the bactericidal effects of EPA and DHA on *Streptococcus mutans* in another study [48]. Myristic acid, linoleic and oleic acids, and lauric acid have been observed to act synergistically to increase antibiacterial activity against *S. aureus* [49]. It's interesting to note that different levels of synergistic antibacterial effects were shown when tobramycin and FAs

were administered together. When used against methicillin-resistant S. aureus (MRSA, MW2), six active FAs, namely unecanoic, lauric, myristic, myristoleic, palmitoleic, and arachidonic acids, also showed synergistic antimicrobial effects, reducing cell survival by more than 1-3 logarithmic units in comparison to tobramycin alone [49]. According to Amel Ismail's research, FAs are ineffective at inhibiting Gram-negative bacteria like *E. coli*, whereas PUFAs C20 : 4 n-3, C20 : 5 w3, and C22 : 5n-3 have antibacterial qualities [50]. Our own research supports these findings, demonstrating both antibacterial activity and a significant presence of C20:5n-3 in the total lipid extract. Antibacterial efficacy against *A. salmonicida, A. hydrophila, S. typhimurium, S. agalactiae, S. aureus*, and *E. feacalis* was demonstrated by an extract from *U. rigida*. Differences in nitrate, ammonium, total phosphorus, and chlorophyll A concentrations between the two collecting locations may be the cause of the variation in *U. rigida*'s antibacterial activity [50]. This study assessed *L. tsengii*'s antibacterial qualities. By using the disk diffusion method, the antibacterial activity of the crude extract of *L. tsengii* using a $MeOH/CH_2Cl_2$ mixture (2/1, v/v) was assessed against seven strains of microorganisms, including *Salmonella enterica*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella pneumonia*, and *Candida albican*. With an IC_{50} value of 54.95 ± 1.38 µg/mL, the results demonstrated that the *L. tsengii* extract displayed antibacterial activity against *Staphylococcus yellow*, while showing no action against other strains at the tested dosages (0.5-128 µg/mL).

Table 3. MIC and IC_{50} values of *L. tsengii* extract against *S. aureus*.

Sample	*Staphylococcus aureus*	
	MIC (*µg/mL)*	IC_{50} (*µg/mL)*
L. tsengii extract	> 256	54.95 ± 1.38
Ampicillin	0.125 ± 0,0	0.02 ± 0.005

4. CONCLUSIONS

For the first time, the fatty acid profile and lipid classes of the newly discovered brown algae *L. tsengii* D. Tien & Z. Sun, which was obtained from Bai Tu Long Island, were examined. With 43.4 % of the content, the PL was determined to be the most prevalent class from TL. Four major glycerophospholipid classes were identified by quantitative TLC analysis of phospholipid classes: PC (37.97 %), PE (33.88 %), PS (9.29 %), and LPE (18.86 %). PUFA (32.98 % of total FA), EPA (11.46 % of total FA), and AA (8 % of total FA) were abundant in the TL of *L. tsengii*. The extract of *L. tsengii* demonstrated a moderate inhibition against *Staphylococcus aureus* with an IC_{50} value of 54.95 ± 1.38 µg/mL, according to the first attempt to investigate the antibacterial activity of the plant. Therefore, there are encouraging research opportunities for using fatty acids from *L. tsengii* to create new antibacterial medicines that combat diseases in both people and marine organisms. Their chemical makeup, other biological activities, the presence of active chemicals, their pharmacological characteristics, and possible uses in contemporary medicine will all be further investigated.

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CRediT authorship contribution statement. Dam Duc Tien: Methodology, Formal analysis, Investigation, Conceptualization. Dao Thi Kim Dung: Software, Formal analysis. Pham Quoc Long: Supervision. Dang

Thi Tuyet, Tran Thi Thu Thuy, Hoang Thi Minh Nguyet: Methodology, Data curation, Formal analysis. Doan Lan Phuong: Methodology, Writing-Review and Editing.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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