Bioactive glyceroglycolipids from marine macroalgae: Isolation, purification, and food preservation potential

Yingying Sun[1,2,3,4], Yu Zhang[1], Chenwei Mao[1], Yang Mu[1,5], Tianle Li[1], Xiujing Jiang[1], Yadong Hu[6], Mingxuan Pan[6], You Yu[1], Haonan Wang[1], Xin Wei[1*]

(1. *Jiangsu Key Laboratory of Marine Bioresources and Environment, Jiangsu Ocean University, Lianyungang 222005, Jiangsu, China*;
2. *Co-Innovation Center of Jiangsu Marine Bio-industry Technology, Lianyungang 222005, Jiangsu, China*;
3. *Jiangsu Institute of Marine Resources Development, Lianyungang 222005, Jiangsu, China*;
4. *Jiangsu Key Laboratory of Marine Genetic Resources and Breeding, Jiangsu Ocean University, Lianyungang 222005, Jiangsu, China*;
5. *Jiangsu Zhongjin Matai Medicinal Packaging Co., Ltd, Lianyungang 222002, Jiangsu, China*;
6. *Jiangsu Coast Development Group Co., Ltd, Nanjing 210095, China*)

Abstract: Macroalgae represent valuable marine resources, being rich in bioactive glyceroglycolipids with antioxidant and anti-inflammatory properties. In this study, the extraction processes for glyceroglycolipids from *Palmaria palmata*, *Chorda filum*, and *Enteromorpha clathrata* were systematically optimized. Using single-factor and response surface methodologies, optimal extraction conditions were established, achieving yields of 69.96 mg/g, 65.14 mg/g, and 85.73 mg/g, respectively. The resultant extracts were subsequently evaluated for their antioxidant and hygroscopic-moisturizing activities. It was observed that the glyceroglycolipid extracts exhibited a significant, concentration-dependent increase in the scavenging of DPPH, hydroxyl, and ABTS radicals, although their overall efficacy remained lower than that of vitamin C. In terms of hygroscopic and moisturizing properties, the extracts performed better than hyaluronic acid but were inferior to glycerol across varying humidity conditions. Further purification employing liquid-liquid extraction, thin-layer chromatography, and silica gel column chromatography enabled the isolation of specific glyceroglycolipid fractions. Additionally, treatment of *Channa argus* fillets with these glyceroglycolipids resulted in improved physicochemical indices compared to the control group, effectively delaying spoilage. These findings underscore the potential of marine macroalgae-derived glyceroglycolipids in food preservation, providing a solid foundation for further research and application within the food industry.

Keywords: macroalgae, glyceroglycolipids, response surface design, anti-oxidant activity, food preservation
DOI: 10.25165/j.ijabe.20261901.10113

Citation: Sun Y Y, Zhang Y, Mao C W, Mu Y, Li T L, Jiang X J, et al. Bioactive glyceroglycolipids from marine macroalgae: Isolation, purification, and food preservation potential. Int J Agric & Biol Eng, 2026; 19(1): 270–282.

1 Introduction

Macroalgae are multicellular algae inhabiting marine ecosystems, typically attached to rocks or the seabed, where they serve as important habitats and sources of nutrition for marine organisms[1]. They display a wide range of morphologies and are taxonomically classified into three main groups: red (Rhodophyta), green (Chlorophyta), and brown algae (Phaeophyta)[2]. Due to their structural complexity, macroalgae synthesize a variety of nutrients and bioactive compounds[3], and certain species possess not only nutritional value but also demonstrated medicinal properties[4-6]. As rich reservoirs of natural products, macroalgae frequently contain glyceroglycolipids—compounds whose presence and bioactivity are closely associated with macroalgal biodiversity[7].

Glyceroglycolipids are lipid–sugar conjugates that are ubiquitously present in cell membranes and exhibit a broad spectrum of bioactivities[8]. Structurally, they are characterized by a glycerol backbone linked to sugar moieties and fatty acids, which distinguishes them from other glycolipid classes such as sphingolipids or those with atypical lipid components. Common glyceroglycolipids identified in macroalgae include sulfoquinovosyl diacylglycerol (SQDG), digalactosyl diacylglycerol (DGDG), and monogalactosyl diacylglycerol (MGDG)[9,10], which vary in their fatty acid composition and the pattern of sugar substitution at the sn-3 position. Macroalgae constitute a significant natural source of diverse glyceroglycolipids. In recent years, multiple studies have successfully isolated glyceroglycolipids from various macroalgal species, including *Ahnfeltia tobuchiensis*[11], *Chondria armata*[9], *Gracilaria verrucosa*[12], and other red algae[13,14]; *Fucus vesiculosus*[15], *Laminaria gurjanovae*[16], *Sargassum wightii*[17], and other brown algae[18-20]; *Codium tomentosum*[21], *Ulva rigida*[22], *Ulva pertusa*[23], *Tydemania expeditionis*[24], and other green algae[25-27]. Further investigations have also identified glyceroglycolipids in *Caulerpa taxifolia*[28] and characterized complex lipids in other green algae using techniques such as thin-layer chromatography

Received date: 2025-08-19 **Accepted date:** 2025-12-22
Biographies: Yingying Sun, Professor, research interest: development and utilization of seaweed resources, Email: syy-999@163.com; **Yu Zhang**, MS, research interest: development and utilization of seaweed resources, Email: 1912024097@qq.com; **Chenwei Mao**, Mr, research interest: bioengineering, Email: 1839420286@qq.com; **Yang Mu**, Mr, research interest: bioengineering, Email: my159961@hotmail.com; **Tianle Li**, MS, research interest: food engineering, Email: 2022220839@jou.edu.cn; **Xiujing Jiang**, MS, research interest: bioengineering, Email: jxj20805256@163.com; **Yadong Hu**, PhD, research interest: seaweed bioactive substances, Email: huyadong@jsyhkf.com; **Mingxuan Pan**, PhD, research interest: seaweed bioactive substances, Email: panmingxuan@jsyhkf.com; **You Yu**, Mr, research interest: bioengineering, Email: y787351581227@163.com; **Haonan Wang**, Mr, research interest: bioengineering, Email: 18651250283@163.com.
***Corresponding author: Xin Wei**, PhD, research interest: development and utilization of seaweed resources, Address: No. 59, Cangwu Road, Haizhou District, Lianyungang City, Jiangsu Province. Email: weixin@jou.edu.cn.

(TLC) and high-performance liquid chromatography (HPLC)[28]. The bioactivity of these compounds is strongly influenced by the degree of fatty acid saturation and chain length[29], encompassing a wide range of effects such as anti-algal[30], antioxidant[31], antiviral[32], anti-inflammatory[33], and antibacterial[34], thereby highlighting their considerable potential for applications in medicine, food, and cosmetics.

P. palmata is an economically important macroalga distributed along the eastern coasts of the Atlantic and Pacific Oceans[35]. Rich in polysaccharides, phytosterols, and fatty acids[36], *P. palmata* has demonstrated a wide range of notable bioactivities. Existing studies have identified SQDG, DGDG, and MGDG in *P. palmata*, and these glyceroglycolipids exhibit antioxidant and anti-inflammatory properties[37]. *C. filum*, a brown alga of the Laminariaceae family distributed in temperate Northern Hemisphere waters[38], has been reported to contain glyceroglycolipids with antioxidant activity, although current research remains geographically limited and focused on preliminary characterization[39]. In contrast, studies on *E. clathrata* have mainly addressed its polysaccharides and physiological functions[40], while reports on glyceroglycolipid extraction or activity from this species are scarce both domestically and internationally, indicating a significant research gap. These edible, renewable macroalgae are abundant in temperate and cold-temperate seas, providing accessible raw materials for large-scale utilization. Our preliminary studies confirmed the presence of glyceroglycolipids in *P. palmata* and *C. filum*, supporting further exploration of their extraction and functional evaluation.

Although research on macroalgal glyceroglycolipids began in the last century[41], progress remains limited due to species diversity and structural complexity, and many glyceroglycolipids remain unidentified[33]. Structural variations, including fatty acid chain length and glycosyl composition, affect biosynthesis, metabolism, and bioactivity. Artificial synthesis, although offering controllable purity[42], is hindered by high costs, environmental concerns, and reduced biological activity, making naturally derived glyceroglycolipids more desirable. However, efficient separation and purification remain challenging because of their high structural similarity, restricting practical application.

Glyceroglycolipids exhibit a diverse range of bioactivities, encompassing antibacterial, antioxidant, and immunomodulatory effects. For instance, MGDG has demonstrated potent antibacterial activity against pathogens such as *Haemophilus influenzae*[43], and its efficacy is influenced by both acyl structure[44] and the degree of fatty acid chain unsaturation. Furthermore, galactosyl glyceroglycolipids have been reported to inhibit *Bacillus cereus*, while SQDG can suppress the proliferation of *Escherichia coli* JM190[45]. Their antioxidant properties are equally noteworthy, given that excessive free radicals contribute to cellular damage and aging[46-48]. Consistent with this, studies have shown that lipid extracts from algae possess free radical scavenging activity[49], and polar lipids from *Grateloupia turuturu* exhibit combined antioxidant and anti-inflammatory effects[50]. Additionally, MGDG, DGDG, and SQDG have demonstrated anti-inflammatory activity in mouse models[51]. Sulfated glyceroglycolipids have shown antiviral properties since 1989[52]. Despite the edible and economic value of *P. palmata*, *C. filum*, and *E. clathrata*, systematic studies on their glyceroglycolipid extraction, purification, chemical profiling, and multifunctional bioactivities remain limited, and no comprehensive evaluation integrating hygroscopicity, moisturizing ability, antioxidant activity, and food preservation has been reported.

Therefore, this study aims to address these research gaps by establishing an efficient extraction and purification strategy for glyceroglycolipids from the three macroalgae, characterizing their chemical profiles, and systematically evaluating their antioxidant, hygroscopic, moisturizing, and preservative activities. These efforts highlight the innovative aspect of this work and underscore its necessity for advancing macroalgal utilization and supporting their applications in natural preservatives and functional materials.

2 Materials and methods

2.1 Macroalgae and reagents

The dried samples of *P. palmata*, *C. filum*, and *E. clathrata* were purchased from Jiangsu Blue Ocean Marine Biotechnology Co., Ltd. After cleaning and oven-drying, the seaweed material was ground using a mechanical grinder and sieved through a 40-mesh screen for subsequent use. Glycolipid standards, including SQDG, DGDG, and MGDG, were obtained from Avant Polar Lipids (USA). *S. marcescens* was sourced from our laboratory collection and was cultured in LB medium at 37°C. Snakehead fish (*Channa argus*) were purchased from a seafood market in Lianyungang, Jiangsu Province.

2.2 Extraction, isolation, and purification

Five grams of dried macroalgae powder were placed in an Erlenmeyer flask and extracted under ultrasonic assistance. The single-factor experiments investigated four factors, including liquid-solid ratio, extraction temperature, methanol volume fraction, and extraction time. Each factor was tested at five levels, with ranges of 15-35 mL/g, 35-75°C, 50-90%, and 1-3 h, respectively. The extract was decanted, and the residue was subjected to a second extraction using an equal volume of methanol. The combined extracts were then centrifuged, and the supernatant was collected. The precipitate was rinsed with pure water, and the rinse solution was combined with the supernatant. Methanol was subsequently removed via filtration and rotary evaporation under reduced pressure to obtain the crude extracts. The methanol extract yield was calculated using the following formula, where m_1 was the mass of the methanol extract (g), and m_0 was the mass of the macroalgae dry powder (g).

$$Y(\%) = (m_1/m_0) \times 100\% \quad (1)$$

The liquid-solid ratio (A), extraction temperature (B), methanol volume fraction (C), and extraction time (D) were selected as variables, with glyceroglycolipid content as the response. A four-factor, three-level response surface design was employed to optimize the extraction conditions for the three macroalgae species.

The glyceroglycolipid content in the methanol extracts was estimated spectrophotometrically by quantifying the sugar moieties using a modified phenol-sulfuric acid method[53,54]. This method determines the total sugar content, which is expressed as glycolipid content based on the structural composition of common macroalgal glyceroglycolipids (e.g., MGDG, DGDG), where sugar units constitute a significant and relatively constant mass fraction. Galactose was selected as the standard because it is the primary monosaccharide constituent of the glycosyl headgroups in the most prevalent glyceroglycolipids found in macroalgae, such as MGDG and DGDG. Specifically, a calibration curve was established using galactose standards (0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL). For each assay, 1 mL of the standard or the sample solution (1 mg/mL in pure water) was mixed with 1 mL of a 5% (w/v) phenol aqueous solution. Then, 5 mL of concentrated sulfuric acid was added rapidly to the mixture. The solution was vortexed immediately and

incubated at 80°C for 20 min. After cooling to room temperature, the absorbance was measured at 490 nm against a pure water blank. The glyceroglycolipid content in the samples was calculated as follows: The total sugar content, expressed as galactose equivalents (GE), was directly obtained from the galactose standard curve. This value was then used to represent the glyceroglycolipid content in the sample, under the assumption that the detected sugar signal primarily originates from the glycosyl head groups of the glyceroglycolipids. The results are expressed as the mass of glycolipid per mass of the crude extract.

To isolate glyceroglycolipids via liquid-liquid extraction, 5 g of the crude extract was dissolved in 250 mL of deionized water and thoroughly shaken. The solution was then sequentially extracted three times with dichloromethane, ethyl acetate, and n-butanol, each using a volume equivalent to twice that of the aqueous phase. The organic phases were collected and evaporated under reduced pressure to obtain the corresponding fractions. Each fraction was analyzed by thin-layer chromatography (TLC) on silica gel G plates, using a mobile phase of chloroform: methanol: water (75:25:3, v/v/v). After development, the plates were sprayed with 50% ethanol-sulfuric acid, heated at 110°C for 10 min for visualization, and compared against SQDG, DGDG, and MGDG standards. For Fourier transform infrared (FTIR) spectroscopy analysis, dried fractions were mixed with KBr, ground, and pressed into pellets (400-4000 cm^{-1}). Further purification was achieved by silica gel column chromatography (100-200 mesh, 4.0×40.0 cm) at a flow rate of 1.0 mL/min. The column was eluted sequentially with the following solvents, each using two column volumes: A) petroleum ether, B) ethyl acetate, C) a 1:1 (v/v) mixture of ethyl acetate and methanol, and D) methanol. Target glyceroglycolipid spots were identified by TLC (iodine vapor visualization) and scraped for collection. Antioxidant activities, including DPPH, hydroxyl radical, and ABTS radical scavenging assays, were assessed according to the kit protocols.

2.3 Measurement of hygroscopic and moisturizing activity

Hygroscopicity was evaluated by accurately weighing 0.1 g of glyceroglycolipid extracts, glycerol, or hyaluronic acid into weighing bottles, which were then immediately sealed with lids. The samples were placed in desiccators maintained at controlled relative humidities (43% and 81%). After removing the lids, the desiccators were sealed. Sample mass was recorded at designated time intervals (4, 12, 24, 36, 48, and 60 h) at room temperature, with three replicates per treatment group. Moisture absorption rate (%) was calculated using the following formula:

$$\text{Moisture absorption rate}(\%) = ((M_1 - M_0)/M_0) \times 100\% \quad (2)$$

where, M_1 is the sample mass at a given time point, g, and M_0 is the initial sample mass before placement in the desiccator, g.

Aqueous solutions (10% w/v) of the glyceroglycolipid extracts, glycerol, and hyaluronic acid were prepared. For each weighing bottle, the mass was recorded before and after sample addition. The bottles were then placed in desiccators with their lids removed, and the desiccators were sealed. The samples were weighed at 4, 12, 24, 36, 48, and 60 h under room temperature conditions, with three replicates per group. The moisture retention rate (%) was calculated using the following formula:

$$\text{Moisture retention rate}(\%) = (1 - (M_2 - M_3)/M_2) \times 100\% \quad (3)$$

where, M_2 is the initial mass of the sample, g, and M_3 is the mass of the sample at a given time point, g.

2.4 Antibacterial activity and preservative property of glyceroglycolipids extracts

Antibacterial activity was evaluated by testing glyceroglycolipid extracts at concentrations of 4, 6, and 8 mg/mL against *S. marcescens*. For the fish preservation assay, fresh *C. argus* fish were prepared by removing the head, tail, scales, and internal organs, rinsed, drained, and sliced into portions of approximately 35 g each. The fish slices were then immersed for 20 min in 1% (w/v) solutions of glyceroglycolipid extracts derived from the three macroalgae species and their respective fractions; control slices were treated with distilled water. After draining and removing excess surface moisture, all samples were packed in polyethylene ziplock bags and stored at 4°C. Physicochemical parameters, including electrical conductivity, were measured at 48-hour intervals over a 10-day storage period. Conductivity was determined by homogenizing 2 g of minced fish tissue in 20 mL of deionized water, allowing the mixture to stand for 20 min, filtering the supernatant, and measuring its conductivity using a conductivity meter. For thiobarbituric acid (TBA) value analysis, 2.5 g of the sample was mixed with 10 mL of water and 12.5 mL of 20% (w/v) trichloroacetic acid, allowed to stand for 40 min, centrifuged at 8000 r/min for 10 min, filtered, and the filtrate was diluted to 25 mL. Subsequently, 5 mL of the diluted supernatant was reacted with 5 mL of 0.02 mol/L TBA solution in a boiling water bath for 20 min. After cooling, the absorbance was measured at 532 nm. Total volatile basic nitrogen (TVB-N) and total bacterial count were determined according to the Chinese National Standards GB 5009.228-2016 and GB 4789.2-2010, respectively.

2.5 Statistical analysis

All experiments were performed in triplicate, and results are expressed as mean ±SD (n=3). Data analysis and visualization were conducted using Origin 2021, GraphPad Prism 10, and SPSS 14.0. The optimization of extraction parameters was achieved through response surface methodology using Design-Expert 13.1 software. A second-order polynomial model was generated by multiple regression analysis of the Box–Behnken design data. The model's goodness of fit was evaluated using the coefficient of determination (R^2), adjusted R^2, and predicted R^2. Its validity and the significance of the regression terms were assessed by analysis of variance (ANOVA) at a 95% confidence level. Tukey's post hoc tests were applied where appropriate. Statistical significance was defined as $p<0.05$.

3 Results and discussion

3.1 Optimization of the extraction process of crude glyceroglycolipids from macroalgae

P. palmata, *C. filum*, and *E. clathrata* were utilized to optimize the extraction of crude glyceroglycolipids. As the optimal conditions for *P. palmata* had been previously established and published, the optimization efforts in this study concentrated on *C. filum* and *E. clathrata*. Based on prior research, the liquid–solid ratio, extraction temperature, methanol concentration, and extraction time were identified as key factors influencing extraction efficiency. As shown in Figure 1a, an increase in the liquid-solid ratio initially enhanced both the methanol extract yield and the glyceroglycolipid content. Maximum values were observed at a ratio of 25 mL/g for *C. filum* (yield: 13.53%; glyceroglycolipid content: 55.14 mg/g) and 20 mL/g for *E. clathrata* (yield: 8.47%; glyceroglycolipid content: 92.34 mg/g), beyond which both parameters declined. An appropriate solvent volume facilitates the dissolution of target compounds. However, an excess may reduce extraction efficiency and increase operational costs.

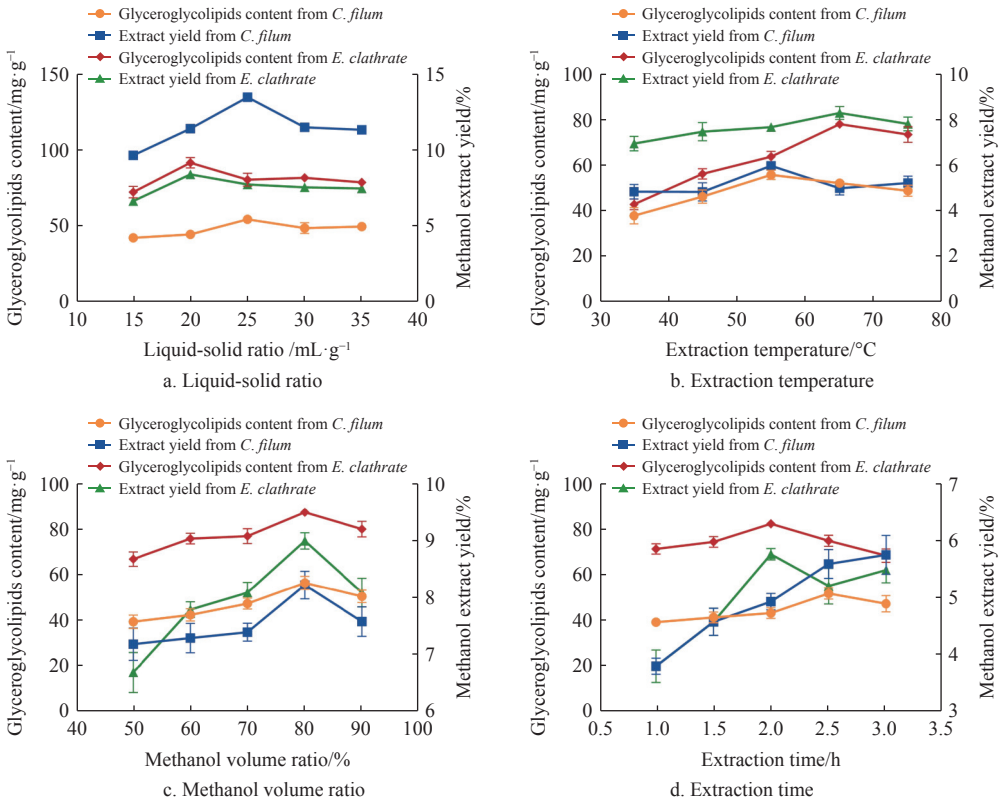


Figure 1 Effects of liquid-solid ratio, extraction temperature, methanol volume ratio, and extraction time on the extraction efficiency of glyceroglycolipids from *C. filum* and *E. clathrate*

Figure 1b indicates that extraction temperature positively influenced methanol extract yield and glyceroglycolipid content up to an optimum of 55°C for *C. filum* (yield: 6.01%; glyceroglycolipid content: 56.13 mg/g) and 65°C for *E. clathrata* (yield: 8.34%; glyceroglycolipid content: 78.43 mg/g). Further temperature increases led to a decline in both parameters, likely attributable to the degradation of heat-sensitive constituents. Elevated temperatures enhanced extraction by promoting cavitation bubble formation and improving solvent diffusion, although optimization was necessary to avoid thermal degradation of the target compounds. Figure 1c demonstrates that a methanol concentration of 80% yielded the highest extraction efficiency, with *C. filum* achieving a yield of 8.2% and a glyceroglycolipid content of 56.96 mg/g, and *E. clathrata* reaching 9.01% yield and 88 mg/g content. Figure 1d reveals that both methanol extract yield and glyceroglycolipid content initially increased with extraction time, peaking at 2.5 h for *C. filum* (yield: 5.6%; content: 52.13 mg/g) and 2 h for *E. clathrata* (yield: 5.77%; content: 82.51 mg/g). Beyond these optimal durations, a decline was observed, likely due to compound degradation and inefficient energy utilization.

Using liquid–solid ratio (*A*), extraction temperature (*B*), methanol volume ratio (*C*), and extraction time (*D*) as variables and glyceroglycolipid content from *C. filum* as the response, a Box–Behnken design was implemented with Design-Expert 13 software. Multiple regression analysis yielded the quadratic model:

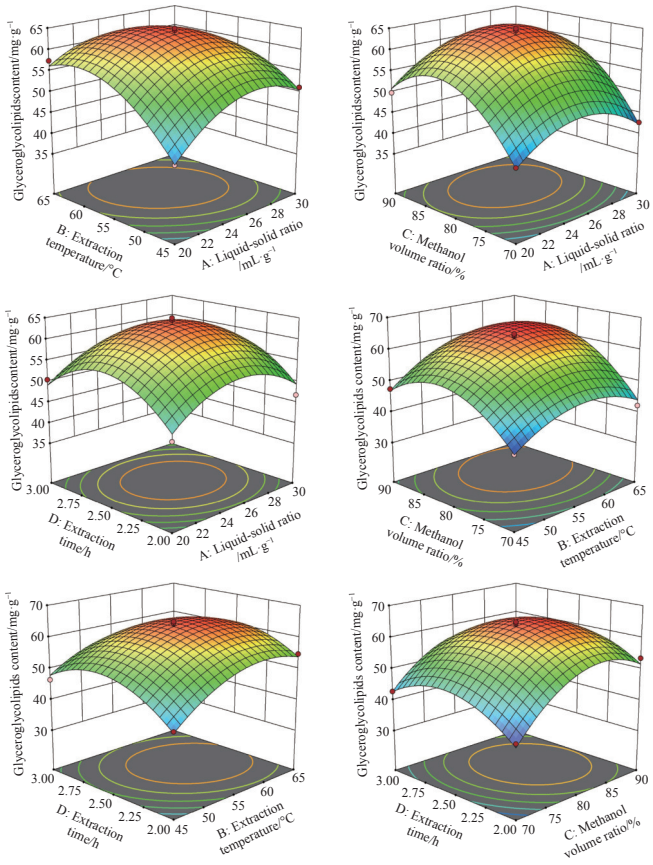
$$Y = 63.46 + 1.19A + 4.02B + 5.49C + 1.06D - 2.78AB + 0.88AC - 0.08AD + 1.89BC - 1.75BD - 1.20CD - 6.40A^2 - 6.50B^2 - 9.61C^2 - 7.84D^2 \quad (4)$$

Analysis of variance (ANOVA) revealed that the model was highly significant ($p<0.0001$), while the lack-of-fit was not significant ($p>0.05$), confirming a good model fit. The model exhibited a high coefficient of determination (R^2=0.9769), with an adjusted R^2 of 0.9538 and a predicted R^2 of 0.8806, indicating strong agreement between the predicted and experimental values. The relationship between the independent factors and the response was quadratic, enabling the prediction of optimal extraction conditions (Figure 2). The relative influence of the factors was ranked as follows: extraction time>extraction temperature>liquid–solid ratio> methanol volume fraction. The model predicted a maximum glyceroglycolipid content of 65.03 mg/g under the following optimal parameters: a liquid-solid ratio of 25.34 mL/g, an extraction temperature of 58.37°C, a methanol volume fraction of 83.23%, and an extraction time of 2.53 h. For practical application, these conditions were adjusted to 25 mL/g, 58°C, 83%, and 2.5 h. Extraction under these adjusted conditions yielded 65.14±1.11 mg/g of glyceroglycolipids from *C. filum*, a value closely aligned with the predicted optimum, thereby validating the model's reliability.

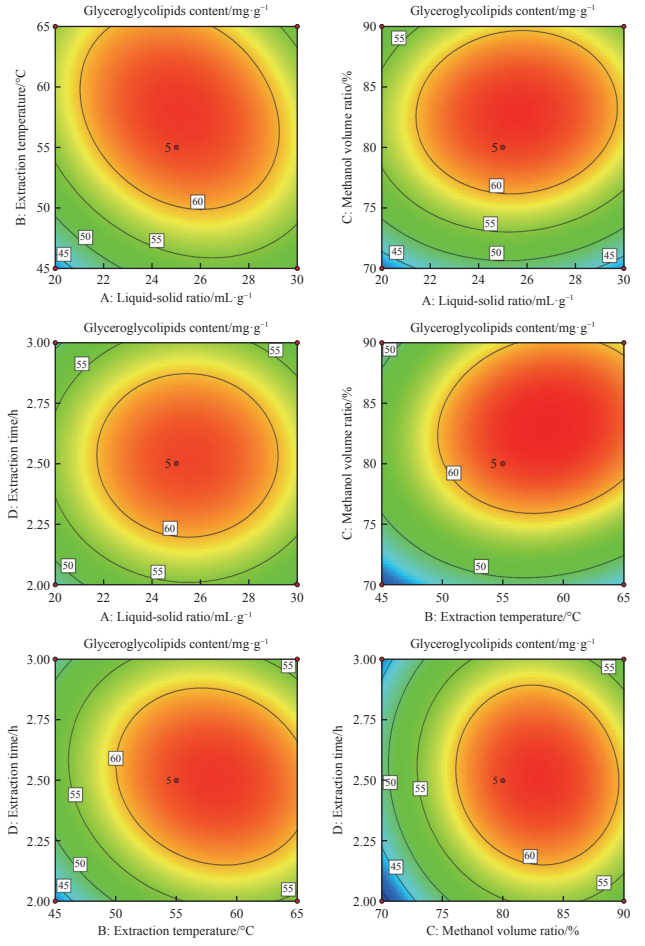
A four-factor, three-level response surface experiment was designed using Design-Expert 13 software to investigate the effects of liquid–solid ratio (*A*), extraction temperature (*B*), methanol volume ratio (*C*), and extraction time (*D*) on glyceroglycolipid content from *E. clathrate*. Multiple regression analysis produced the quadratic equation:

$$Y = 85.88 + 1.35A + 0.88B + 1.51C + 1.00D + 3.24AB - 3.44AC - 0.20AD - 1.68BC - 0.63BD - 1.71CD - 4.59A^2 - 2.69B^2 - 3.09C^2 - 3.13D^2 \quad (5)$$

ANOVA revealed the model was highly significant ($p<0.0001$) and that the lack-of-fit was non-significant ($p>0.05$), confirming a good model fit. The model's coefficient of determination (R^2) was 0.9535, with an adjusted R^2 of 0.9071 and a predicted R^2 of 0.7506, indicating a strong correlation between the observed and predicted values. A quadratic relationship existed between the independent



a. 3D Response surfaces



b. Contours

Figure 2 3D Response surfaces (a) and contours (b) of Box-Behnken design model of glyceroglycolipids content from *C. filum*

factors and the response, allowing for the prediction of optimal extraction parameters. The relative influence of the factors on glyceroglycolipid content was ranked as follows: extraction time>liquid–solid ratio>methanol volume ratio>extraction temperature.

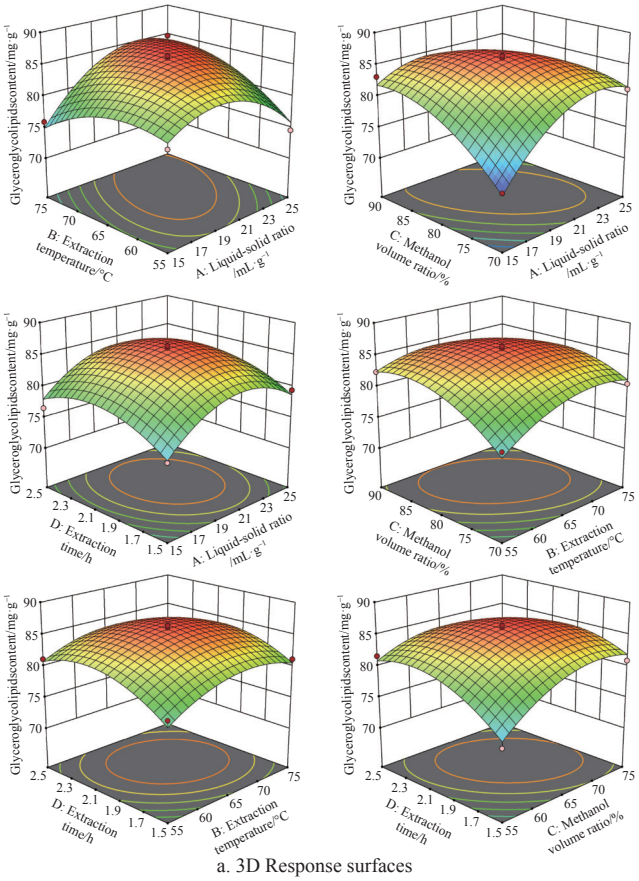
As detailed in Figure 3, significant interactive effects were observed between the liquid-solid ratio and extraction temperature, as well as between the liquid–solid ratio and extraction time (p<0.0001). The model predicted a maximum glyceroglycolipid content of 86.25 mg/g for *E. clathrata* at a liquid-solid ratio of 21.32 mL/g, extraction temperature of 68.16°C, methanol volume ratio of 79.79%, and extraction time of 2.06 h. For practical application, these parameters were adjusted to 21 mL/g, 68°C, 80%, and 2 h for practicality. Extraction under these optimized conditions yielded 85.73±1.51 mg/g, which closely matched the predicted value, thereby confirming the accuracy of the model.

3.2 Antioxidant activity of glyceroglycolipids extracts from macroalgae

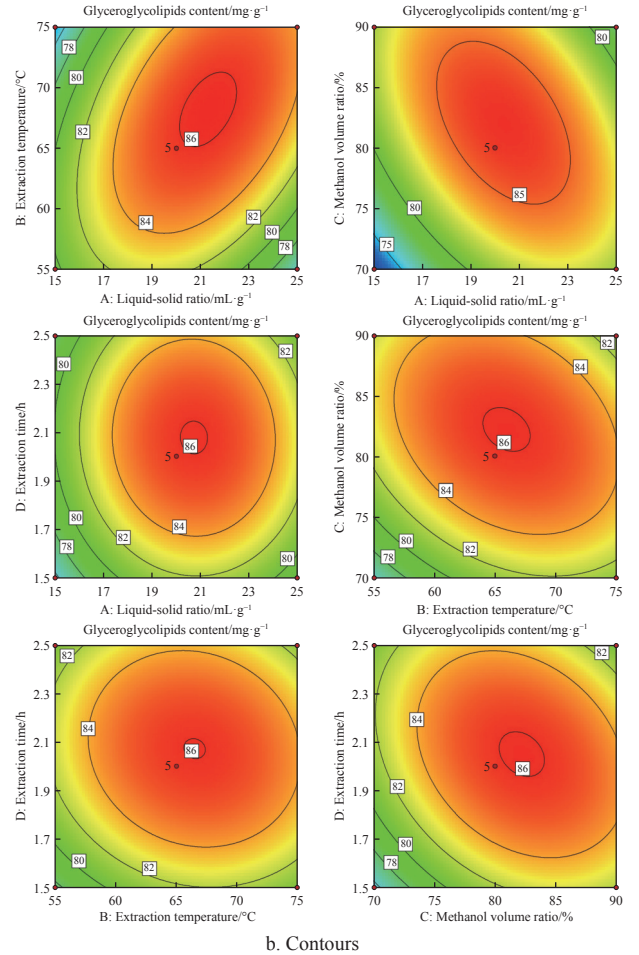
Oxygen readily forms reactive oxygen species (ROS), which induce cellular damage and contribute to food spoilage via oxidative processes, resulting in discoloration and nutrient degradation. Antioxidants can retard lipid peroxidation, thereby extending the shelf life and maintaining the quality of food products. Given their safety and diverse bioactivities, natural antioxidants derived from plant sources—particularly macroalgae—have garnered increasing interest[55]. Accordingly, this study assessed the antioxidant activity of glyceroglycolipid extracts from *P. palmata*, *C. filum*, and *E. clathrata* as potential natural preservatives.

As shown in Figure 4a, all three extracts exhibited dose-dependent DPPH radical scavenging activity, with *E. clathrata* reaching 58.12% at 8 mg/mL, followed by *P. palmata* (54.26%) and *C. filum* (40.16%). Notably, the activities of *E. clathrata* and *P. palmata* exceeded those previously reported for glyceroglycolipids extracted from *E. kurome* and *U. lactuca*, which showed 42.59% and 27.14% scavenging activity, respectively, at the same concentration[55]. Furthermore, compared to seaweed-derived polysaccharides, glyceroglycolipids demonstrate significantly enhanced antioxidant potential. For instance, these values surpassed the 30% scavenging activity reported for *Sargassum muticum* polysaccharides at 4 mg/mL[56].

Hydroxyl radical scavenging (Figure 4b) likewise exhibited a concentration-dependent increase. *E. clathrata* extract reached 52.11% at 4 mg/mL, whereas *P. palmata* and *C. filum* achieved 53.12% and 56.2% at 16 mg/mL, respectively. The overall order of efficacy was: Vitamin C (Vc)>*E. clathrata*>*C. filum*>*P. palmata*. For ABTS radicals (Figure 4c), scavenging also rose with concentration. *C. filum* showed the highest activity among extracts (58.7% at 4 mg/mL), followed by *P. palmata* (56.2%) and *E. clathrata* (55.48% at 8 mg/mL). These values also surpassed those previously reported for glyceroglycolipids extracted from *E. kurome* (20.3%) and *U. lactuca* (9.7%). Moreover, all tested glyceroglycolipid extracts outperformed the approximately 10% scavenging activity reported for *Gracilaria lemaneiformis* polysaccharides at 10 mg/mL[57]. This marked contrast highlights the distinct advantage of the polar lipid-based glyceroglycolipids over carbohydrate-dominated polysaccharides in neutralizing cationic radicals. The superior scavenging efficiency at significantly lower concentrations further demonstrates the potential of glyceroglycolipids as a more potent and economically viable class of natural antioxidants. In summary, the antioxidant activities of all extracts increased with concentration, and their relative



a. 3D Response surfaces



b. Contours

Figure 3 3D Response surfaces (a) and contours (b) of Box-Behnken design model of glyceroglycolipids content from *E. clathrate*

effectiveness varied depending on the specific assay. However, all glyceroglycolipid extracts demonstrated lower antioxidant capacity compared to vitamin C, which is likely attributable to the presence of non-active components in the crude extracts. Unlike vitamin C, which is prone to degradation over time, the amphiphilic structure of glyceroglycolipids confers greater stability and enhanced membrane permeability, thereby offering the potential for more sustained antioxidant protection. These findings suggest that macroalgal glyceroglycolipids represent promising natural antioxidants with broad application potential.

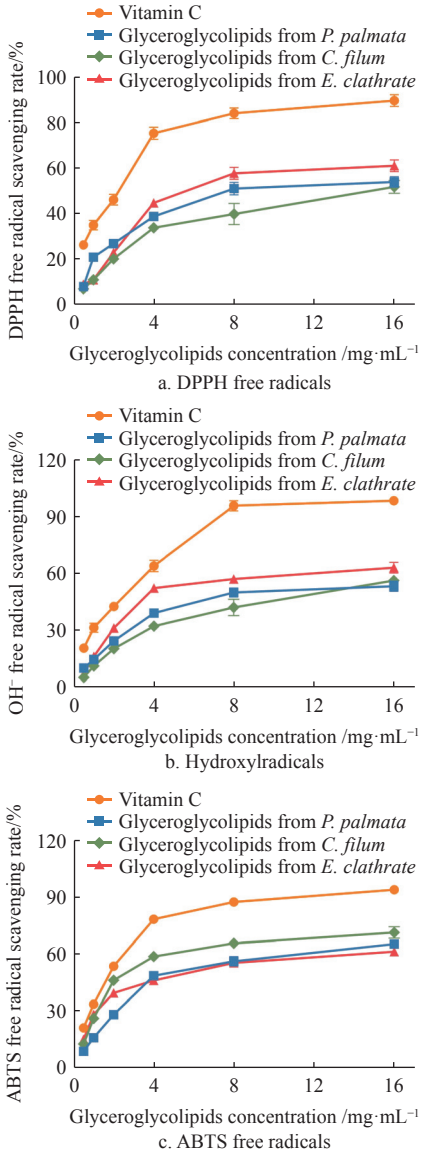


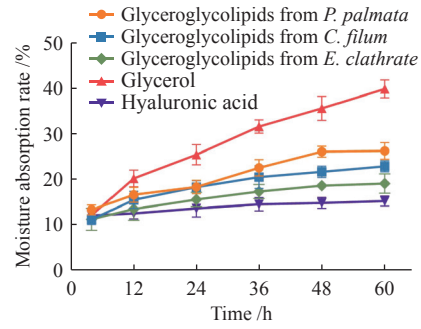
Figure 4 Scavenging activity of glyceroglycolipids extracts from three marine macroalgae on DPPH free radicals (a), hydroxyl radicals (b), and ABTS free radicals (c)

3.3 Hygroscopic and moisturizing activity of glyceroglycolipids extracts from seaweed macroalgae

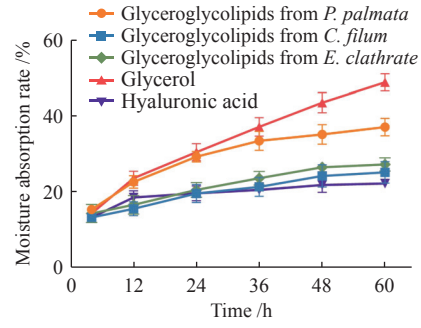
Humectants are substances capable of absorbing and retaining moisture across a wide range of relative humidity conditions and over extended periods, widely utilized in the food and cosmetic industries to enhance texture and hydration. Currently, most commercially available humectants are synthetic, which has raised safety concerns and consequently driven the demand for natural, non-toxic alternatives. Algae—including both macroalgae and microalgae—are rich sources of natural bioactive compounds, positioning their extracts as promising candidates for next-

generation natural humectants. For example, extracts from marine macroalgae have shown moisturizing abilities often surpassing hyaluronic acid[55].

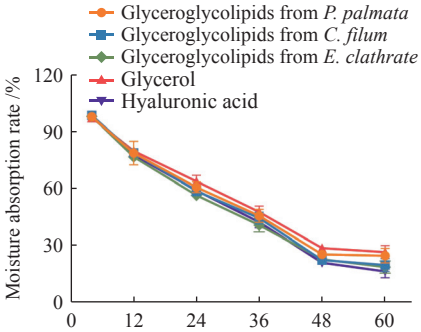
This study assessed the moisture absorption and retention of glyceroglycolipid extracts from *P. palmata*, *C. filum*, and *E. clathrata* to evaluate their potential as natural preservatives and cosmetic ingredients. At 43% relative humidity (RH), the extracts exhibited progressive moisture absorption over 48 h before reaching a plateau (Figure 5a). The order of moisture absorption capacity was: glycerol>*P. palmata*>*C. filum*>*E. clathrata*>hyaluronic acid. The maximum absorption rates reached 26.14% for *P. palmata*, 22.75% for *C. filum*, and 18.96% for *E. clathrata*—all exceeding the moisture absorption reported for crude fucoidan from *Sargassum fusiforme*. At 81% RH, moisture absorption increased markedly within the first 48 h (Figure 5b). *C. filum* and *E. clathrata* extracts plateaued at 25.07% and 27.19%, respectively, while *P. palmata* continued increasing to 37.07%. The order of effectiveness was glycerol, *P. palmata*, *E. clathrata*, *C. filum*, then hyaluronic acid. These absorption rates surpassed those reported for polysaccharides from *Fucus vesiculosus* (19.07%)[58]. Although glycerol demonstrated superior efficacy, the natural, non-toxic, and eco-friendly glyceroglycolipid extracts outperformed hyaluronic acid.



a. Moisture absorbing activity (RH=43%)



b. Moisture absorbing activity (RH=81%)



c. Moisture retention rate

Figure 5 Moisture absorbing activity (a: RH=43%; b: RH=81%) and moisture retention rate (c) of glyceroglycolipids extracts from three species of macroalgae

All three extracts exhibited significant moisturizing effects, characterized by a rapid decline in moisture retention within the initial 48 h. After 60 h, the retention rates were 24.41% for *P. palmata*, 19.54% for *C. filum*, and 18.45% for *E. clathrata*, compared to 26.39% for glycerol and 16.13% for hyaluronic acid (Figure 5c). The order of moisturizing efficacy was: glycerol>*P. palmata*>*C. filum*>*E. clathrata*>hyaluronic acid, exceeding the performance reported for *Dendrobium candidum* extracts (~50% at 6 h)[59]. The observed moisturizing activity is likely attributed to the amphiphilic structure of glyceroglycolipids, wherein their hydrophilic sugar chains effectively bind water molecules, thereby helping to maintain skin or substrate hydration.

3.4 Purification and preparation of glyceroglycolipids from crude extracts

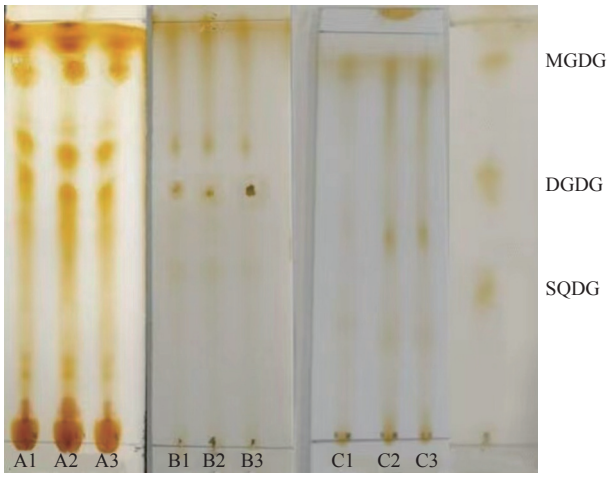
3.4.1 Liquid-liquid extraction and FTIR analysis

Liquid-liquid extraction of glyceroglycolipid extracts from three macroalgae was conducted using dichloromethane, ethyl acetate, and n-butanol, and the yields are summarized in Table 1. The highest yields were obtained from the n-butanol extracts, followed by the dichloromethane and ethyl acetate. Chromatographic analysis (Figure 6) revealed that the extracts from *P. palmata*—dichloromethane (A1), ethyl acetate (A2), and n-butanol (A3)—contained DGDG and MGDG, with SQDG being less prominent. The extracts from *C. filum*—dichloromethane (B1), ethyl acetate (B2), and n-butanol (B3)—showed the presence of DGDG, while the extracts from *E. clathrata*—dichloromethane (C1), ethyl acetate (C2), and n-butanol (C3)—contained MGDG.

Table 1 The yield and extraction rate of glyceroglycolipids analogues compounds from three species of macroalgae

Macroalgae	Yield/%	Extraction rate/%		
		Dichloromethane extracts	Ethyl acetate extracts	N-butanol extracts
P. palmata	11.49±0.95	2.55±0.39 (A1)	2.41±0.29 (A2)	3.56±0.37 (A3)
C. filum	12.33±0.35	2.46±0.31 (B1)	2.25±0.16 (B2)	4.40±0.40 (B3)
E. clathrate	11.40±0.34	2.37±0.22 (C1)	3.28±0.26 (C2)	4.34±0.27 (C3)

Note: Data are presented as mean±SD, n=3.



Note: A1, A2, and A3 correspond to the dichloromethane, ethyl acetate, and n-butanol extracts from *P. palmata*, respectively. Similarly, B1–B3 and C1–C3 represent the corresponding extracts from *C. filum* and *E. clathrata*, respectively.

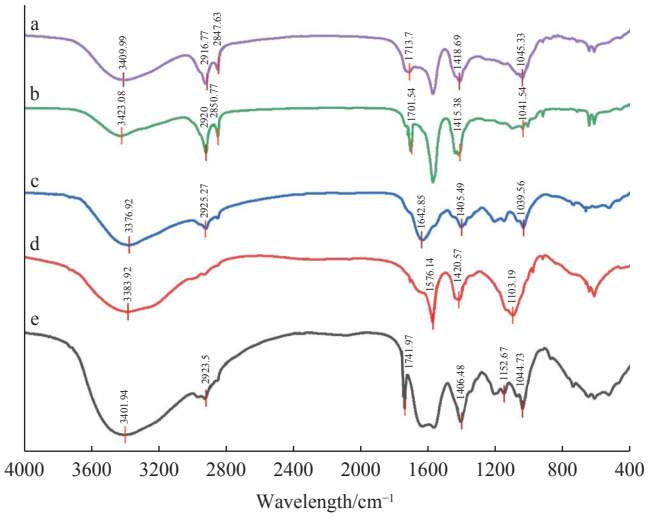
Figure 6 TLC analysis of the glyceroglycolipids extracts by liquid-liquid extraction from three macroalgae

FTIR spectroscopy provides insights into the chemical structure of molecules by detecting the characteristic absorption of specific bonds or functional groups. Glyceroglycolipids, as amphiphilic

lipids composed of a glycerol backbone linked to sugar headgroups and fatty acid tails, exhibit a distinct FTIR fingerprint. To further characterize the key fractions identified by TLC (Figure 6), FTIR analysis was performed on the ethyl acetate and n-butanol fractions from *P. palmata*, the dichloromethane and ethyl acetate fractions from *C. filum*, and the dichloromethane fraction from *E. clathrata*. The acquired spectra (Figure 7) revealed characteristic absorptions consistent with glyceroglycolipid structures across all five samples. Critically, all spectra showed a strong, complex absorption envelope in the 1000–1200 cm^{-1} region, which is characteristic of C–O stretching vibrations within carbohydrate moieties. Within this region, the prominent and sharp bands centered between 1030 and 1100 cm^{-1} can be specifically assigned to the C–O–C stretching vibrations of the glycosidic bonds linking the sugar units to the glycerol backbone, a key diagnostic feature for this compound class[60]. Absorptions around 1400 cm^{-1} may result from bending vibrations of methyl/methylene groups, free carboxyl groups, or C–C stretching vibrations. Further confirming the lipidic nature, several fractions—specifically the ethyl acetate fractions of *P. palmata* and *C. filum*, and the dichloromethane fraction of *E. clathrata*—displayed sharp carbonyl (C=O) stretching bands between 1702 and 1742 cm^{-1}, indicative of ester linkages from the acyl chains. The presence of hydroxyl groups, another essential component, was evidenced by broad absorptions in the 3200–3600 cm^{-1} range. Additionally, weak but discernible bands near 2920 and 2850 cm^{-1} were observed, corresponding to the asymmetric and symmetric C–H stretching vibrations of methylene groups in the fatty acid chains. Collectively, this set of spectral features strongly supports the successful enrichment of glyceroglycolipids in the selected fractions.

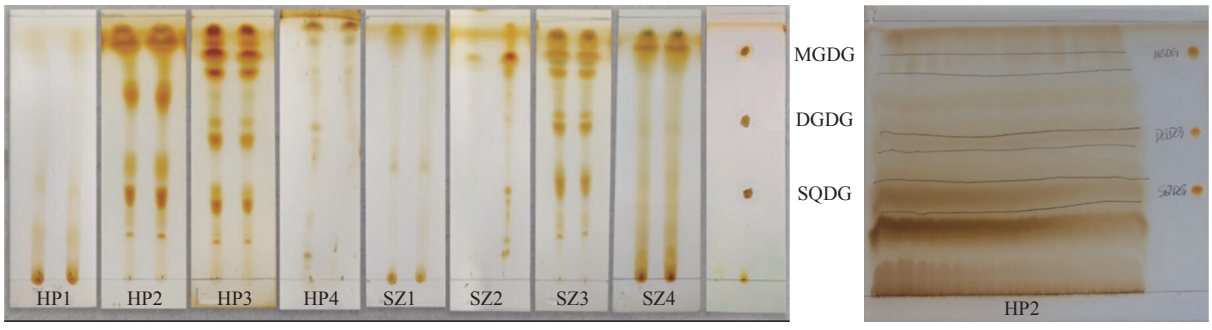
3.4.2 Silica gel column chromatography separation and thin layer chromatography preparation

To enable detailed characterization, glyceroglycolipids were enriched and purified from the crude extracts. Following preliminary screening by TLC and HPLC, the ethyl acetate fractions of *P. palmata* and *C. filum* were selected for further separation via silica gel column chromatography (100-200 mesh, 4.0×40 cm). This step generated four distinct sub-fractions from each algal extract: HP1–HP4 from *P. palmata* with masses of 0.431, 0.528, 0.537, and 0.477 g, respectively; and SZ1–SZ4 from *C. filum* with masses of 0.422, 0.498, 0.505, and 0.394 g, respectively. TLC analysis against authentic standards elucidated the glyceroglycolipid composition of the principal sub-fractions (Figure 8a): HP3 contained MGDG, SQDG, and MGDG3; HP2 contained SQDG and MGDG; SZ3 contained DGDG and MGDG; and SZ4 contained MGDG. Quantitative HPLC indicated that the target glyceroglycolipids collectively accounted for more than 50% of the peak area in these enriched sub-fractions. To isolate individual compounds for structural and bioactivity assessment, the most compositionally diverse sub-fraction, HP3, was further purified by preparative TLC. This yielded three discrete glyceroglycolipids: SQDG (11.9 mg), DGDG (9.3 mg), and MGDG (7.1 mg) (Figure 8b). The isolation yield aligns with that reported by Katsuoka et al.[61], confirming the effective purification of three major glyceroglycolipids from *P. palmata*.



Note: a: dichloromethane extracts from *E. clathrate*; b: ethyl acetate extracts from *C. filum*; c: dichloromethane extracts from *C. filum*; d: n-butanol extracts from *P. palmata*; e: ethyl acetate extracts from *P. palmata*

Figure 7 Infrared spectroscopy analysis of different extracts



a. Thin layer chromatography analysis

b. Preparation of glyceroglycolipids by TLC

Note: HP1–HP4 and SZ1–SZ4 represent the purified glyceroglycolipid sub-fractions obtained from the ethyl acetate extracts of *P. palmata* and *C. filum*, respectively, via silica gel column chromatography, with their specific compositions (e.g., MGDG, DGDG, SQDG) as identified by comparison with standards.

Figure 8 Thin layer chromatography analysis (a) and preparation of glyceroglycolipids by TLC (b)

A preliminary cost analysis was conducted based on processing 250 g of *P. palmata*. The estimated unit costs were 704 RMB/mg for SQDG, 450 RMB/mg for DGDG, and 613 RMB/mg for MGDG. These costs are lower than current market prices for commercial glyceroglycolipid standards (approximately 788 RMB/mg for SQDG, 500 RMB/mg for DGDG, and 680 RMB/mg for MGDG), indicating the economic feasibility of the extraction and purification process developed in this study.

3.5 Antibacterial activity and preservative property of glyceroglycolipids extracts from macroalgae in *C. argus* fillet

During meat storage and spoilage, various microorganisms—such as *Lactobacillus* spp., *Pseudomonas* spp., *Enterobacteriaceae*, and *Serratia marcescens*—contributed to degradation. Notably, *S. marcescens*, which is ubiquitous in soil, water, and on plant and

animal surfaces, is a known contaminant of dairy, meat, and seafood products. It produces red pigments that cause undesirable discoloration in these foods. To evaluate the preservative potential of glyceroglycolipids extracts, their inhibitory effects against *S. marcescens* were tested. The results showed that extracts from *P. palmata* and *C. filum* inhibited *S. marcescens* at 8 mg/mL, whereas *E. clathrate* extracts showed no significant activity at this or lower concentrations (data not shown). Preservation performance was further assessed using *C. argus* fillets during refrigerated storage. The effects of *C. filum* and *E. clathrata* extracts on key spoilage indicators are presented in Figure 9; corresponding data for *P. palmata* extracts have been published previously.

Microbial proliferation under refrigeration conditions leads to the decomposition of fats and proteins in *C. argus* fillets, resulting in spoilage. The total bacterial count serves as a key hygiene indicator, with a safety threshold of 6 lg CFU/g established for aquatic products[62]. As shown in Figure 9a, bacterial counts increased in both control and treated fillets. However, the control group exceeded the safety threshold by day 6. Treatment with glyceroglycolipid extracts from the three macroalgae effectively delayed microbial growth. By day 10, total bacterial counts remained below the 6 lg CFU/g limit in groups treated with ethyl acetate extracts of *C. filum* (5.94 lg CFU/g) and *P. palmata* (5.96 lg CFU/g), n-butanol extracts of *P. palmata* (5.98 lg CFU/g), and dichloromethane extracts of *E. clathrata* (5.99 lg CFU/g), thereby extending the shelf life by four days compared to the control. Microbial degradation generates ionic small molecules from proteins and lipids, which increases electrical conductivity—an indicator of freshness loss. Figure 9b illustrates that conductivity increased progressively during refrigerated storage. Among the *C. filum* treatments, the ethyl acetate extract resulted in the lowest conductivity, whereas the crude glyceroglycolipid extract exhibited the highest. For *E. clathrata*, the dichloromethane extract yielded the lowest conductivity. Although the crude extracts displayed slightly weaker activity than the control, the treated groups with *C. filum* and *E. clathrata* extracts generally exhibited reduced conductivity, indicating better preservation of freshness.

Fish freshness is closely related to pH, which typically decreases initially due to postmortem lactic acid accumulation and subsequently increases as microbial protein breakdown generates alkaline compounds[63]. Figure 9c illustrates an initial decline in pH followed by a gradual rise during refrigerated storage. By day 10, control fillets reached a pH of 7.43, whereas samples treated with various glyceroglycolipid extract fractions maintained significantly lower pH values (dichloromethane extracts: 7.03–7.23; ethyl acetate extracts: 7.01–7.11; n-butanol extracts: 7.12–7.32), demonstrating the preservative efficacy of the glyceroglycolipids. Total Volatile Basic Nitrogen (TVB-N), which reflects the degradation of proteins into ammonia and amines, serves as another key freshness indicator, with higher values indicating advanced spoilage. As shown in Figure 9d, TVB-N levels increased substantially from day 2 onward. The control group exceeded the Level II freshness limit of 20 mg/100 g[64] by day 6, rendering the fillets inedible. In contrast, several treatment groups did not surpass this threshold until day 8 or 10. Specifically, on day 10, the ethyl acetate and n-butanol extracts of *P. palmata* exhibited TVB-N values of 20.66 and 21.28 mg/100 g, respectively, while the ethyl acetate extract of *C. filum* and the dichloromethane extract of *E. clathrata* showed values of 20.96 and 20.94 mg/100 g. These findings indicate that glyceroglycolipid extracts effectively retard protein degradation and spoilage progression.

Lipid oxidation is a primary cause of meat quality deterioration, leading to the formation of malondialdehyde (MDA), which is quantified via thiobarbituric acid (TBA) assays[65]. A TBA value exceeding 1.0 mg/kg is generally considered indicative of spoilage[66]. Figure 9e shows that TBA values increased in all groups during refrigeration. However, by day 10, the control group surpassed the 1.0 mg/kg threshold, whereas several treated groups remained below this limit. Among the effective treatments, TBA values (from lowest to highest) were as follows: butanol extract from *P. palmata* (0.824 mg/kg)<ethyl acetate extract from *P. palmata* (0.812 mg/kg)<dichloromethane extract from *E. clathrate* (0.913 mg/kg), among others. These results confirmed the antioxidant capacity of glyceroglycolipids in inhibiting lipid oxidation. In summary, glyceroglycolipid extracts from the three macroalgae exhibited significant antimicrobial, antioxidant, and preservative properties, extended the shelf life of fish fillets, and demonstrated considerable potential as natural food preservatives.

Building upon the confirmation of the preservative efficacy of glyceroglycolipid extracts through the aforementioned key chemical indicators, this study further evaluated their overall quality-preserving effects on fish fillets during storage via direct visual assessment. Selected high-performing extract treatments—ethyl acetate and n-butanol extracts from *P. palmata*, ethyl acetate extract from *C. filum*, and dichloromethane extract from *E. clathrata*—were subjected to systematic observation and documentation of macro-morphological changes over time. A comprehensive sensory evaluation was also conducted, covering color, texture (Figure 10), and odor (detailed sensory data are provided in Table 2). These results demonstrated that treatments with ethyl acetate extracts from *C. filum* and dichloromethane extracts from *E. clathrate* exhibited superior preservative performance, significantly extending the shelf life of the fish fillets.

Table 2 Sensory evaluation indicators of *C. argus* fillets during storage

Time/d	Control	Ethyl acetate extracts from *P. palmata*	N-butanol extracts from *P. palmata*	Ethyl acetate extracts from *C. filum*	Dichloromethane extracts from *E. clathrate*
0	The fish meat is firm and elastic, with depressions caused by finger pressure disappearing immediately. The cross-section of the muscle is glossy and free of any off-odor.				
6	The meat shows weakened elasticity, with depressions recovering slowly after pressing and leaving slight finger marks. A faint fishy odor is detectable.	The meat has turned yellow in color, but the texture remains firm and springy (rebounds after pressing). A slight fishy odor is detectable.	The meat has turned yellow in color, but the texture remains firm and springy (rebounds after pressing). A slight fishy odor is detectable.	The meat shows slight yellowing in color. However, the texture remains firm and resilient (rebounds upon pressing), and no fishy odor is detected.	The meat shows slight yellowing in color. However, the texture remains firm and resilient (rebounds upon pressing), and no fishy odor is detected.
10	The meat has turned yellowish in color, the surface has become slimy, and it emits a strong putrid odor.	The meat has become soft and loose, the surface is free of mucus, it recovers slowly after pressing, and emits a putrid odor.	The meat has become soft and loose, the surface is free of mucus, it recovers slowly after pressing, and emits a putrid odor.	The meat has become slightly soft and loose, the surface shows no mucus, and a slight acidic odor is detectable.	The meat has become slightly soft and loose, the surface shows no mucus, and a slight acidic odor is detectable.

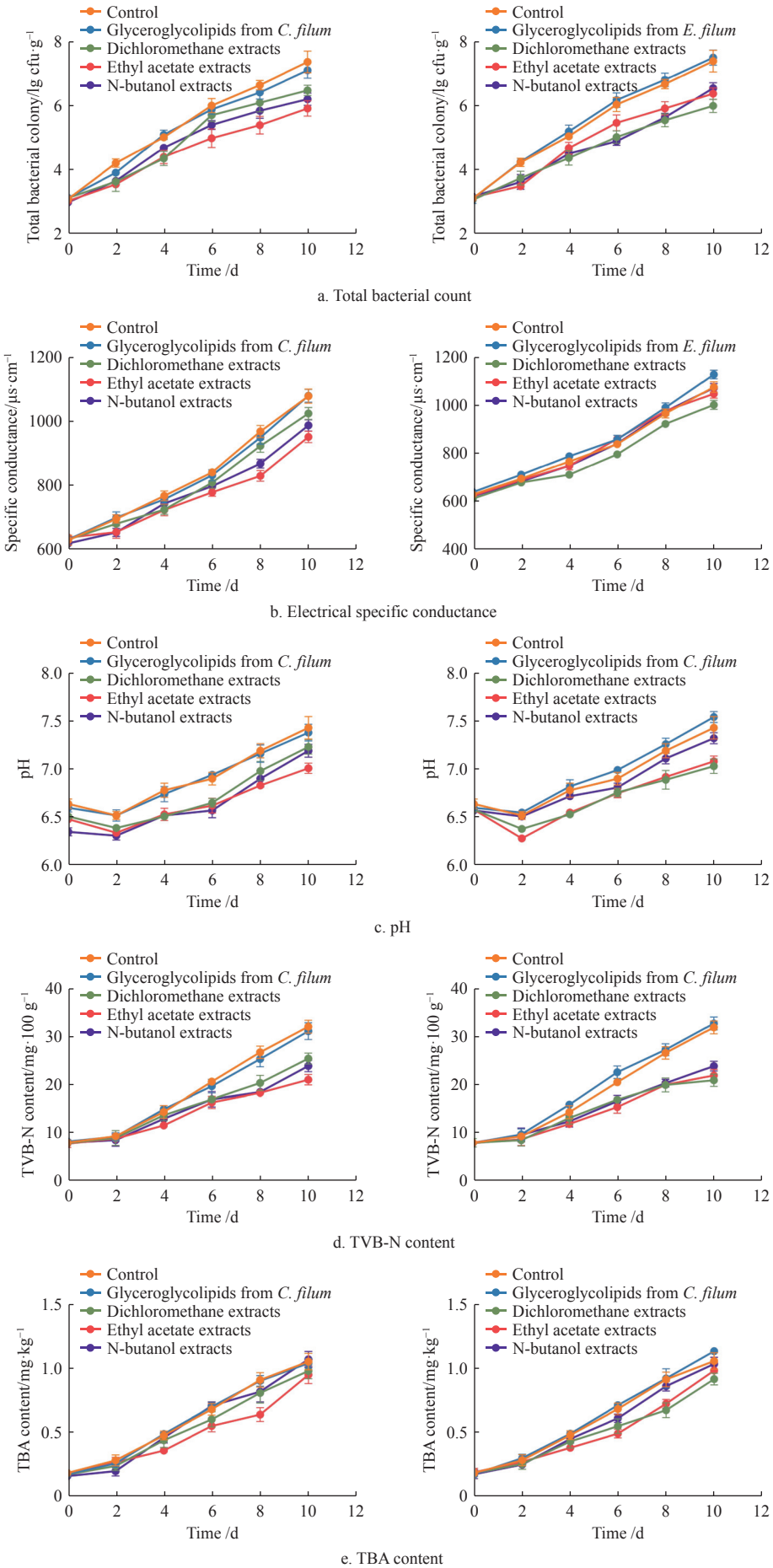


Figure 9 Effects of glyceroglycolipids extracts on total bacterial count (a), electrical specific conductance (b), pH (c), TVB-N content (d), and TBA content (e) of *C. argus* fillets during storage

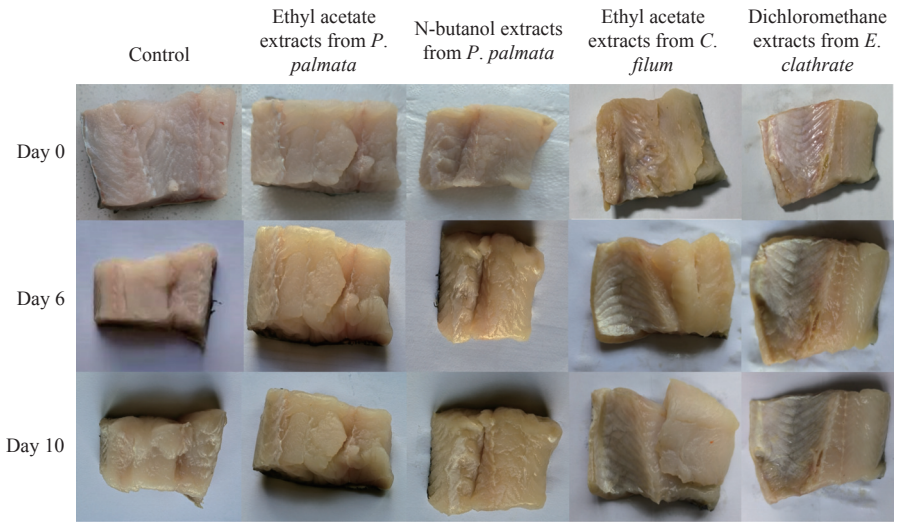


Figure 10 The influence of different extracts on the preservation effect of *C. argus* fillets during storage

In recent years, research on the application of glycolipids in the food industry has gradually increased. Ferdouse et al.[67] found that adding glycolipids from *Aspergillus oryzae* altered the flavor and metabolic profile of sake yeast, while incorporating glycolipids into milk and cheese inhibited the growth of *Listeria monocytogenes*[68]. The ability of glyceroglycolipids to preserve the quality of fish fillets may be attributed to their inherent properties. Due to their unique amphiphilic structure, glyceroglycolipids are both hydrophilic and lipophilic. When the lipophilic end penetrates the fish tissue, the hydrophilic long-chain end is exposed to air, where it can bind with water molecules in the atmosphere, thereby maintaining the moisture content of snakehead fish fillets and exerting a humectant effect. Meanwhile, the antioxidant and antibacterial activities of glyceroglycolipids help inhibit lipid oxidation and bacterial proliferation in the fish, contributing to effective preservation. Other researchers have also found that glycolipids in ethyl acetate extracts of macroalgae exhibit inhibitory effects against pathogens such as *Clostridium difficile*[34]. Given their antioxidant, moisturizing, and antibacterial properties, macroalgae-derived glyceroglycolipids show promising potential as novel preservatives. Moreover, naturally sourced and safe macroalgae undoubtedly represent an ideal material for developing new natural preservation agents.

4 Conclusions

This study successfully established a systematic workflow for the extraction and characterization of glyceroglycolipids from three underutilized macroalgae (*P. palmata*, *C. filum,* and *E. clathrate*). By optimizing extraction parameters, substantial yields of bioactive compounds were achieved. Comprehensive in vitro evaluations further confirmed that these glyceroglycolipid extracts exhibit significant antioxidant, hygroscopic, and preservative capacities, which are comparable or even superior to some existing natural alternatives. The findings strongly indicate that these marine-derived glyceroglycolipids are promising, sustainable candidates to replace synthetic additives in the food, cosmetic, and pharmaceutical industries, potentially offering new pathways for the high-value utilization of macroalgal resources.

However, this work has several limitations that should be acknowledged. First, the study focused on crude or semi-purified glyceroglycolipid extracts. While bioactive, these mixtures contain co-extracted compounds, making it difficult to unequivocally attribute the observed effects solely to specific glyceroglycolipid molecular species. Second, the bioactivity evaluations were conducted *in vitro*. The efficacy, metabolic fate, and potential toxicity of these extracts in *in vivo* systems remain unknown and are crucial for assessing their practical applicability. Finally, the economic feasibility and scalability of the proposed extraction and purification process were not evaluated, which is a critical factor for industrial adoption.

Based on these limitations, the following specific directions are proposed for future research: 1) Employ advanced purification techniques (preparative HPLC or counter-current chromatography) to isolate individual, highly pure glyceroglycolipids (MGDG, DGDG, SQDG) for structure-specific bioactivity assessment. 2) Validate the promising *in vitro* activities, particularly the antioxidant and preservative effects, through *in vivo* studies using suitable animal models. 3) Conduct a comprehensive techno-economic analysis to assess the scalability and cost-effectiveness of the production pipeline, from algal biomass to purified product. Addressing these points will be essential to translate these promising natural compounds from the laboratory to commercial applications.

Acknowledgements

We are thankful for the support from institutions such as Jiangsu Provincial Department of Finance, Jiangsu Provincial Agricultural Science and Technology Independent Innovation Fund Project, and Science and Technology Bureau of Lianyungang City. This research was supported by Jiangsu Province Agricultural Independent Innovation Project (Grant No. CX (24)3063), Lianyungang "521 Project" Scientific Research Project (Grant No. LYG065212024015), Lianyungang Key Research and Development Program (Grant No. CG2415), Project of Jiangsu Province Graduate Research and Practical Innovation Program, Innovation Training Program for College Students of Jiangsu Ocean University, and The "Haizhou Bay Talent" Innovation Program Project of Jiangsu Ocean University.

[References]

[1] Van Alstyne K L, Puglisi M P. DMSP in marine macroalgae and macroinvertebrates: Distribution, function, and ecological impacts. Aquatic Sciences, 2007; 69(3): 394–402.

[2] Wan A H L, Davies S J, Soler-Vila A, Fitzgerald R, Johnson M P. Macroalgae as a sustainable aquafeed ingredient. Reviews in Aquaculture, 2019; 11(3): 458–492.

[3] Verland M, Mydland L T, Skrede A. Marine macroalgae as sources of protein and bioactive compounds in feed for monogastric animals. Journal of the Science of Food and Agriculture, 2019; 99(1): 13–24.
[4] Leandro A, Pereira L, Gonçalves A M M. Diverse applications of marine macroalgae. Marine Drugs, 2019; 18(1): 17.
[5] Vicente T, Lemos M F L, Félix R, Valentão P, Félix C. Marine macroalgae, a source of natural inhibitors of fungal phytopathogens. Journal of Fungi (Basel), 2021; 7(12): 1006.
[6] Guo J Y, Qi M H, Chen H W, Zhou C, Ruan R, Yan X J, et al. Macroalgae-derived multifunctional bioactive substances: The potential applications for food and pharmaceuticals. Foods, 2022; 11(21): 3455.
[7] Sun Y Y, Dong S S, Zhang N S, Zhou J, Long Z K. Screening and isolation of glyceroglycolipids with antialgal activity from several marine macroalgae. Journal of Applied Phycology, 2021; 33(4): 2609–2616.
[8] Iván C, Francisco S. Chemistry and biology of bioactive glycolipids of marine origin. Marine Drugs, 2018; 16(9): 294.
[9] Al-Fadhli A. Glycolipids from the red alga *Chondria* armata (Kutz.) Okamura. Glycobiology, 2006; 16(10): 902–911.
[10] Xu X, Miao X. Glyceroglycolipid metabolism regulations under phosphate starvation revealed by transcriptome analysis in *Synechococcus elongatus* PCC 7942. Marine Drugs, 2020; 18(7): 360.
[11] Sanina N M, Kostetsky E Y, Shnyrov V L, Tsybulsky A V, Novikova O D, Portniagina O Y, et al. The influence of monogalactosyldiacylglycerols from different marine macrophytes on immunogenicity and conformation of protein antigen of tubular immunostimulating complex. Biochimie, 2012; 94(4): 1048–1056.
[12] Khotimchenko S V. Lipids from the marine alga *Gracilaria verrucosa*. Chemistry of Natural Compounds, 2005; 41(3): 285–288.
[13] Song Y Y, Li M, Wu Z Z, Chen J J, Yan X J. Rapid identification of lipid compositions from *Porphyra haitanensis* by UPLC-Q-TOF MS. Journal of Chinese Mass Spectrometry Society, 2015; 36(6): 528–534.
[14] Guo W J, Li G L, Hou Y X, Wang R R, Liu Y, Liu X H, et al. Chemical constituents from the red alga *Symphyocladia latiuscula*. Journal of Chinese Pharmaceutical Sciences, 2017; 26(10): 754–762.
[15] Deal M S, Hay M E, Wilson D, Fenical W. Galactolipids rather than phlorotannins as herbivore deterrents in the brown seaweed *Fucus vesiculosus*. Oecologia, 2003; 136: 107–114.
[16] Shevchenko N M, Anastiuk S D, Gerasimenko N I, Dmitrenok P S, Zviagintseva T N. Polysaccharide and lipid composition of the brown seaweed *Laminaria gurjanovae*. Russian Journal of Bioorganic Chemistry, 2007; 33: 88–98.
[17] Arunkumar K, Selvapalam N, Rengasamy R. The antibacterial compound sulphoglycerolipid 1-0 palmitoyl-3-0(6 ′-sulpho-α-quinovopyranosyl)-glycerol from *Sargassum wightii* Greville (Phaeophyceae). Botanica Marina, 2005; 48(5): 445–451.
[18] Sanina N M, Kostetsky E Y, Goncharova S N. Thermotropic behaviour of membrane lipids from brown marine alga *Laminaria japonica*. Biochemical Society Transactions, 2000; 28(6): 894–897.
[19] Kim Y H, Kim E H, Lee C, Kim M H, Rho J R. Two new monogalactosyl diacylglycerols from brown alga *Sargassum thunbergii*. Lipids, 2007; 42(4): 395–399.
[20] Nunes N, Rosa G P, Ferraz S, Barreto M C, Carvalho M A A P D. Fatty acid composition, TLC screening, ATR-FTIR analysis, anti-cholinesterase activity, and in vitro cytotoxicity to A549 tumor cell line of extracts of 3 macroalgae collected in Madeira. Journal of Applied Phycology, 2020; 32(2): 759–771.
[21] Rey F, Cartaxana P, Melo T, Calado R, Pereira R, Abreu H, et al. Domesticated populations of *Codium tomentosum* display lipid extracts with lower seasonal shifts than conspecifics from the wild—Relevance for biotechnological applications of this green seaweed. Marine Drugs, 2020; 18(4): 188.
[22] Lopes D, Moreira A S P, Rey F, da Costa E, Melo T, Maciel E, et al. Lipidomic signature of the green macroalgae *Ulva rigida* farmed in a sustainable integrated multi-trophic aquaculture. Journal of Applied Phycology, 2019; 31(2): 1369–1381.
[23] Fang X Y, Zhou S, Liu Y, Gao H, Liu X H, Liu X X, et al. Chemical constituents from green alga *Ulva pertusa*. Chinese Traditional and Herbal Drugs, 2017; 48(22): 4626–4631.
[24] Jiang R W, Hay M E, Fairchild C R, Prudhomme J, Roch K L, Aalbersberg W, et al. Antineoplastic unsaturated fatty acids from Fijian macroalgae. Phytochemistry, 2008; 69(13): 2495–2500.
[25] Williams D E, Sturgeon C M, Roberge M, Andersen R J. Nigricanosides A and B, antimitotic glycolipids isolated from the green alga *Avrainvillea nigricans* collected in Dominica. Journal of the American Chemical Society, 2007; 129(18): 5822–5823.
[26] Kostetsky E, Chopenko N, Barkina M, Velansky P, Sanina N. Fatty acid composition and thermotropic behavior of glycolipids and other membrane lipids of *Ulva lactuca* (Chlorophyta) inhabiting different climatic zones. Marine Drugs, 2018; 16(12): 494.
[27] Sun Y Y, Dong S S, Guo G, Guo L, Pu Y F. Antialgal activity of glycoglycerolipids derived from a green macroalgae *Ulva prolifera* on six species of red tide microalgae. IOP Conference Series: Materials Science and Engineering, 2019; 484: 012057.
[28] Thanh L T, Anh N V T, Hue P T. Molecular species of glycolipid and anti-inflammation activity of lipid fractions in the green algae *Halimeda incrassata Lamx*. collected from Truong Sa, Viet Nam. Vietnam Journal of Chemistry, 2021; 59(5): 639–647.
[29] Hoyo J, Guaus E, Torrent-Burgués J. Monogalactosyldiacylglycerol and digalactosyldiacylglycerol role, physical states, applications and biomimetic monolayer films. European Physical Journal E: Soft Matter, 2016; 39(3): 39.
[30] Slattery M, Lesser M P, Dunton K. Allelopathy in the tropical alga *Lobophora variegata* (Phaeophyceae): mechanistic basis for a phase shift on mesophotic coral reefs. Journal of Phycology, 2014; 50(3): 493–505.
[31] Nova P, Pimenta-Martins A, Maricato É, Nunes C, Abreu H, Coimbra M A, et al. Chemical composition and antioxidant potential of five algae cultivated in fully controlled closed systems. Molecules, 2023; 28(12): 4588.
[32] Plouguerné E, de Souza L M, Sassaki G L, Cavalcanti J F, Villela Romanos M T, da Gama B A, et al. Antiviral sulfoquinovosyldiacylglycerols (SQDGs) from the Brazilian brown seaweed *Sargassum vulgare*. Marine Drugs, 2013; 11(11): 4628–4640.
[33] Guo S S, Wang Z G. Glyceroglycolipids in marine algae: A review of their pharmacological activity. Frontiers in Pharmacology, 2022; 13: 1008797.
[34] Treyvaud Amiguet V, Jewell L E, Mao H, Sharma M, Hudson J B, Durst T, et al. Antibacterial properties of a glycolipid-rich extract and active principle from Nunavik collections of the macroalgae *Fucus evanescens C. Agardh* (Fucaceae). Canadian Journal of Microbiology, 2011; 57(9): 745–749.
[35] Le Strat Y, Mandin M, Ruiz N, Robiou du Pont T, Ragueneau E, Barnett A, et al. Quantification of xylanolytic and cellulolytic activities of fungal strains isolated from *Palmaria palmata* to enhance R-phycoerythrin extraction of *Palmaria palmata*: From seaweed to seaweed. Marine Drugs, 2023; 21(7): 393.
[36] Pierrick S, Søndergaard S P, Lindegaard G L, Susse W, Justine D, Céline R. Concise review of the red macroalga dulse, *Palmaria palmata* (L.) Weber & Mohr. Journal of Applied Phycology, 2023; 35(2): 523–550. doi: 10.1007/s10811-022-02899-5.
[37] Banskota A H, Stefanova R, Sperker S, Lall S P, Craigie J S, Hafting J T, et al. Polar lipids from the marine macroalga *Palmaria palmata* inhibit lipopolysaccharide-induced nitric oxide production in RAW264.7 macrophage cells. Phytochemistry, 2014; 101: 101–108.
[38] Chizhov A O, Dell A, Morris H R, Haslam S M, McDowell R A, Shashkov A S, et al. A study of fucoidan from the brown seaweed *Chorda filum*. Carbohydrate Research, 1999; 320(1-2): 108–119.
[39] Gerasimenko N, Menchinskaya E, Esipov A, Aminin D, Pislyagin E. Application of glyceroglycolipids, photosynthetic pigments and extracts of brown algae for suppression ROS. Open Journal of Marine Science. 2016; 6(3): 371–385. doi: 10.4236/ojms.2016.63031.
[40] Ma M F, Fu T Y, Wang Y M, Zhang A J, Gao P Y, Shang Q S, et al. Polysaccharide from edible alga *Enteromorpha clathrata* improves ulcerative colitis in association with increased abundance of *Parabacteroides* spp. in the gut microbiota of dextran sulfate sodium-fed mice. Marine Drugs, 2022; 20(12): 764.
[41] Pohl P, Zurheide F. Fatty acids and lipids of marine algae and the control of their biosynthesis by environmental factors. Marine Algae in Pharmaceutical Science. 1979; 2: 473–523.
[42] Pagano D, Cutignano A, Manzo E, Tinto F, Fontana A. Glycolipids synthesis: improved hydrazinolysis conditions for preparation of 1, 2-polyunsaturated fatty acyl-β-monogalactosyl-glycerols. Carbohydrate Research, 2016; 424: 21–23.
[43] Erwan P, Da G B A P, Pereira R C, Eliana B B. Glycolipids from seaweeds and their potential biotechnological applications. Frontiers in Cellular and Infection Microbiology, 2014; 4: 174.

[44] Parveez Ahamed A A, Rasheed M U, Peer Muhamed Noorani K, Reehana N, Santhoshkumar S, Mohamed Imran Y M, et al. In vitro antibacterial activity of MGDG-palmitoyl from *Oscillatoria acuminata* NTAPC05 against extended-spectrum β-lactamase producers. The Journal of Antibiotics, 2017; 70(6): 754–762.

[45] Furukawa T, Nishida M, Hada T, Kuramochi K, Sugawara F, Kobayashi S, et al. Inhibitory effect of sulfoquinovosyl diacylglycerol on prokaryotic DNA polymerase I activity and cell growth of *Escherichia coli*. Journal of Oleo Science, 2007; 56(1): 43–47.

[46] Schieber M, Chandel N S. ROS function in redox signaling and oxidative stress. Current Biology, 2014; 24(10): R453–R462.

[47] Nakai K, Tsuruta D. What are reactive oxygen species, free radicals, and oxidative stress in skin diseases. International Journal of Molecular Sciences, 2021; 22(19): 10799.

[48] Chaudhary P, Janmeda P, Docea A O, Yeskaliyeva B, Abdull Razis A F, Modu B, et al. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. Frontiers in Chemistry, 2023; 11: 1158198.

[49] Terme N, Boulho R, Kucma J P, Bourgougnon N, Bedoux G. Radical scavenging activity of lipids from seaweeds isolated by solid-liquid extraction and supercritical fluids. OCL, 2018; 25(5): D505.

[50] da Costa E, Melo T, Reis M, Domingues P, Calado R, Abreu M H, et al. Polar lipids composition, antioxidant and anti-inflammatory activities of the Atlantic red seaweed *Grateloupia turuturu*. Marine Drugs, 2021; 19(8): 414.

[51] Bruno A, Rossi C, Marcolongo G, Di Lena A, Venzo A, Berrie C P, et al. Selective in vivo anti-inflammatory action of the galactolipid monogalactosyldiacylglycerol. European Journal of Pharmacology, 2005; 524(1-3): 159–168.

[52] Gustafson K R, Cardellina J H, Fuller R W, Weislow O S, Kiser R F, Snader K M, et al. AIDS-antiviral sulfolipids from cyanobacteria (blue-green algae). Journal of the National Cancer Institute, 1989; 81(16): 1254–1258.

[53] Wei X, Jiang X X, Wang X, Li H, Yu Y, Sun Y Y. Marine macroalgae–derived glyceroglycolipid composite films for enhanced strawberry preservation with multifunctional bioactivities. LWT-Food Science and Technology, 2025; 211: 118803.

[54] Sun Y Y, Mu Y, Li T H, Wang S Y, Li Y X, Liu J, et al. Extraction, isolation and biological activity of two glycolipids from *Bangia fusco-purpurea*. Marine Drugs, 2024; 22(4): 144.

[55] Wei X, Hu X Q, Li T H, Li Y X, Yu Y, Jiang X J, et al. Comprehensive extraction and biological activities of mycosporine-like amino acids and glyceroglycolipids extracts from two macroalgae *Ecklonia kurome* and *Ulva lactuca*. Foods, 2025; 14(3): 440.

[56] Hou N N, Wang J, Zhang Q B. Isolation and evaluation of physicochemical properties of polysaccharides from *Sargassum moelleri*. Marine Sciences, 2017; 41(9): 102–109. (in Chinese)

[57] Cui M X, Wang X C, Wang Y, Wu J W, Liu K H. Ultrasonic-assisted extraction and anti-oxidation of polysaccharides from *Gelidium pacifium Okam*. Journal of Shanghai Ocean University, 2018; 27(5): 797–804. (in Chinese)

[58] Gao L W, Zhao X J, Zhao X Z. Optimization of extraction process of *euglena gracilis* polysaccharides and their moisture absorption and retention capacities. Food Research and Development, 2022; 43(20): 110–116. (in Chinese)

[59] Chen M, Sun Y, Zhao Y. Study on moisturizing effect of *Dendrobium candidum* extracts. Academic Journal of Shanghai University of Traditional Chinese Medicine, 2015; 29(6): 70–73. (in Chinese) doi: 10.16306/j.1008-861x.2015.06.018.

[60] Menikh A, Fragata M. Fourier transform infrared spectroscopic study of ion binding and intramolecular interactions in the polar head of digalactosyldiacylglycerol. European Biophysics Journal, 1993; 22(4): 249–258.

[61] Katsuoka M, Ogura C, Etoh H, Sakata K, Ina K. Galactosyl-and sulfoquinovosyldiacylglycerols isolated from the brown algae, *Undaria pinnatifida* and *Costaria costata* as repellents of the blue mussel, Mytilus edulis. Agricultural and Biological Chemistry, 1990; 54(11): 3043–3044.

[62] Guan Y, Lan W Q, Sun Y Q, Liu L, Zhou D P, Xie J. Effect of ultrasound-caffeic acid combined treatment on quality changes of sea bass during refrigeration. Food Science, 2022; 43(9): 207–214. (in Chinese)

[63] Abbas K A, Mohamed A, Jamilah B, Ebrahimian M. A review on correlations between fish freshness and pH during cold storage. American Journal of Biochemistry and Biotechnology, 2008; 4(4): 416–421.

[64] Zou J H, Yang H G, Tang D B, Cheng J R, Wang X P. Research progress on fresh-keeping technology of livestock and poultry meat. Meat Research, 2020; 34(10): 96–102. (in Chinese)

[65] Raharjo S, Sofos J N. Methodology for measuring malonaldehyde as a product of lipid peroxidation in muscle tissues: A review. Meat Science, 1993; 35(2): 145–169.

[66] Zhang Y L, Xiang J F, Zhu Y J, Sang Y F, Jiang S T, Lu J F, et al. Comparison of quality characteristics of live and dead crayfish during cold storage. Food Science, 2022; 43(1): 206–212. (in Chinese)

[67] Ferdouse J, Yamamoto Y, Taguchi S, Yoshizaki Y, Takamine K, Kitagaki H. Glycosylceramide modifies the flavor and metabolic characteristics of sake yeast. PeerJ, 2018; 6: e4768.

[68] Sun L, Forauer E C, Brown S, D'Amico D J. Application of bioactive glycolipids to control *Listeria monocytogenes* biofilms and as post-lethality contaminants in milk and cheese. Food Microbiology, 2021; 95: 103683.