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RESEARCH ARTICLE

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## Radical scavenging activity of lipids from seaweeds isolated by solid-liquid extraction and supercritical fluids

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**Abstract** – *In vitro* antioxidant activities of the lipid fractions from two selected seaweeds, *Solieria chordalis* and *Sargassum muticum* were investigated according to the extraction methods. The activity of neutral lipids, glycolipids and phospholipids, thanks to extraction by chloroform/methanol (1/1 v/v; CM) mixture, pure supercritical carbon dioxide (sc-CO<sub>2</sub>), supercritical carbon dioxide with 2% of ethanol (sc-CO<sub>2</sub> + EtOH 2%) and supercritical carbon dioxide with 8% of ethanol as co-solvent (sc-CO<sub>2</sub> + EtOH 8%), were studied using DPPH radical scavenging assays. All the lipid classes demonstrated a free radical scavenging activity at the concentration of 1 mg/ml. The best scavenging activity (86.6 ± 5.7%) was obtained when the neutral lipid fraction was extracted from *S. chordalis* with a CM mixture. The neutral lipid fraction extracted with sc-CO<sub>2</sub> showed a lower activity than those obtained with solvents. However, the addition of ethanol in sc-CO<sub>2</sub> did not affect the antioxidant activity of neutral lipids fixed at around 16% of radical scavenging. For *S. muticum*, the activity of glycolipids (50.9 ± 0.8%) and phospholipids (48.4 ± 1.6%) obtained with sc-CO<sub>2</sub> were twice as large as that of fractions obtained with CM, 29.6 ± 3.4% and 28.0 ± 4.2%, respectively. The activity of neutral lipids did not change with the extraction method with around 25% of radical scavenging. This is the first report of free radical scavenging activity of lipid classes obtained by supercritical carbon dioxide extraction from seaweeds.

**Keywords:** antioxidant / DPPH / supercritical carbon dioxide / green extraction / bio-refinery

**Résumé** – **Activité antiradicalaire des lipides de macroalgues isolés par extraction solide-liquide et fluides supercritiques.** L'activité antioxydante des fractions lipidiques de deux macroalgues, *Solieria chordalis* et *Sargassum muticum* a été étudiée *in vitro* suite à l'application de différentes méthodes d'extraction. L'activité des lipides neutres, des glycolipides et des phospholipides obtenus après extraction avec un mélange chloroforme/méthanol (1/1 v/v, CM), avec du dioxyde de carbone supercritique pur (sc-CO<sub>2</sub>), du dioxyde de carbone supercritique additionné de 2 ou 8 % d'éthanol comme co-solvant a été étudiée en utilisant le test de piégeage du radical DPPH. Toutes les classes de lipides montrent une activité de piégeage du radical à la concentration de 1 mg/ml. La meilleure activité est obtenue pour les lipides neutres (86,6 ± 5,7 %) extraits de *S. chordalis* par le mélange CM. Les lipides neutres obtenus par sc-CO<sub>2</sub> ont montré une activité plus faible que ceux extraits par le mélange de solvants. L'ajout d'éthanol au sc-CO<sub>2</sub> ne modifie pas l'activité antiradicalaire des lipides neutres qui reste autour de 16 % de piégeage. Pour *S. muticum*, les activités des glycolipides (50,9 ± 0,8 %) et des phospholipides (48,4 ± 1,6 %) obtenus avec sc-CO<sub>2</sub> sont deux fois plus grandes que celles des fractions obtenues avec CM, 29,6 ± 3,4 % et 28,0 ± 4,2 %, respectivement. L'activité des lipides neutres n'est pas modifiée par le changement de méthode d'extraction et reste au voisinage de 25 % de piégeage du radical DPPH. C'est la première fois que l'activité antiradicalaire des classes de lipides extraites par dioxyde de carbone supercritique à partir des algues est exposée.

**Mots clés :** antioxydant / DPPH / dioxyde de carbone supercritique / procédés verts / bioraffinerie

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## 1 Introduction

Macro-algae or seaweed are photosynthetic-like plants that form biomass in intertidal zones and at the seabed. More than 10 000 species are identified and classified in three phyla based on their pigmentation. The Rhodophyta phylum contains the red seaweeds, the Chlorophyta phylum corresponds to the green seaweeds and the Phaeophyta to the brown seaweeds. According to the literature (FAO, 2017), the total weight of commercial seaweeds and aquatic plants in the world was 30.5 million tons in 2015, representing a \$4 billion market. 96% of these species are harvested from aquaculture and are mainly used for the hydrocolloid extraction (FAO, 2016).

Seaweeds are a source of bioactives for cosmetics. Indeed, some seaweeds or seaweed extracts possess an INCI name and are used for their properties. Red seaweeds from *Chondrus*, *Palmaria* or *Gelidium* genera and green seaweeds like *Ulva* or *Enteromorpha* are used for emollient, humectant, masking, soothing, smoothing or for skin conditioning and skin protecting properties (Bedoux *et al.*, 2014). The main seaweeds used in cosmetics are brown seaweeds especially from the Laminariaceae and Fucaceae families.

Bioactive compounds from seaweeds are identified as polysaccharides, minerals, proteins, fibres, phenolic compounds, vitamins or lipids. The lipid family includes fats, waxes, sterols, fat-soluble vitamins, glycerolipids, phospholipids, glycolipids and others (Holdt and Kraan, 2011). Usually, the study of seaweed lipidome consists of a total lipid content determination and fatty acid profiling. The major lipid roles involve energy storage, including carbon storage (Reed *et al.*, 1999), structural composition of cellular and intracellular membranes, and cell signalling (Thompson, 1996). The seaweed total lipid content is lower compared to terrestrial plants (Darcy-Vrillon, 1993) or microalgae (Mubarak *et al.*, 2015). The total lipid content is generally established between 1 and 8% dry weight (dw) of seaweeds with a wide variation between species (Melo *et al.*, 2015). However, lipids from seaweeds have a vast array of activities and are possible sources of active compounds due to the large amount of seaweeds on the seaside, the possibility of aquaculture and the biodiversity (Miyashita *et al.*, 2013).

Seaweed lipids can be separated in different classes according to their chemical structures. Lipid classes consist of non-polar lipids, glycolipids and phospholipids as found in plants. Seaweeds contain also betaine and some unusual lipids. The main source of lipids in seaweeds is not well determined. Phospholipids or glycolipids are defined depending on studies as being the major sources of lipid fractions (LeTutour, 1990; Murata and Nakazoe, 2001; Bhaskar *et al.*, 2005; Khotimchenko, 2005). Specific activities have been described for some lipid classes or some lipid molecules. Non-polar lipids consist in hydrocarbons, carotenoids, sterols and glycerolipids. Carotenoids, mainly  $\beta$ -carotene, lutein and violaxanthin in red and green seaweeds and fucoxanthin in brown seaweeds (Holdt and Kraan, 2011), operate both as light energy harvesters and as antioxidants by inactivating reactive oxygen species formed by air and light exposures (von-Elbe and Schwartz, 1996). Sterols, mainly cholesterol in Rhodophyta, fucosterol in Phaeophyta, clionasterol and isofucosterol in Chlorophyta (Kumari *et al.*, 2013) are known to have nutritional benefits on health as they possess antioxidant or

anti-inflammatory activities (Kim and Van Ta, 2011) comparable to those obtained from nuts and seeds (Phillips *et al.*, 2005). Non-polar lipid fraction contributes to antioxidant activities through carotenoids, sterols and terpenoids (Okuzumi *et al.*, 1993; Yan *et al.*, 1999; Plaza *et al.*, 2008; Jassbi *et al.*, 2013). Glycolipids are glycosylated derivatives of glycerolipids and derivatives of ceramides. Glycolipids play an important role in cell protection against chemical stress, in membrane bilayer stabilization and they act as markers for cellular recognition (Holdt and Kraan, 2011; Boudière *et al.*, 2014). Phospholipids incorporate two main structures, glycerophospholipids and sphingophospholipids. Glycerolipids, glycolipids, phospholipids and betaine lipids are fatty acid providers. The chain length and degree of unsaturation in seaweeds are higher than those of plants (Kumari *et al.*, 2013). The lipid activity on bacteria, fungi, virus and parasites may be due to fatty acids (Desbois and Smith, 2010). Indeed, fatty acids inhibit enzyme activities, disrupt the electron transport chain and oxidative phosphorylation, and interfere with cellular energy production in microbes. They also damage nutrient uptake, generate peroxidation and auto-oxidation degradation products or direct lyse bacterial cells (Lee *et al.*, 2009; Vedhagiri *et al.*, 2009; Plouguerné *et al.*, 2013). Many fatty acids demonstrate good antioxidant activities (Henry *et al.*, 2002). Furthermore, pigments, polyunsaturated fatty acids, lutein and glycolipids isolated from seaweeds demonstrate activities on pro-inflammatory pathways by inhibition of nitric oxide or icosanoid production in cells (Ishihara *et al.*, 1998; Banskota *et al.*, 2014a, b; Lopes *et al.*, 2014). Thus, according to the literature, all the lipid classes demonstrate antioxidant activities.

Interest in employing natural antioxidants is encouraged by consumers mainly because of the potential toxic effects of some synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene or *tert*-butylhydroquinone (Safer, 1999). Free radical and reactive oxygen species induce cutaneous damages like early aging, inflammatory disorders or skin cancers. The use of antioxidant compounds in cosmetics aims to avert and control oxidative skin damages (Vertuani *et al.*, 2003). These compounds can act on aging processes by different mechanisms like reductive capacity, binding of transition metal ion catalysts or radical scavenging (Zubia *et al.*, 2007).

General procedures for lipid extraction correspond to solid/liquid methods and employ organic solvents. Generally, a chloroform/methanol/water mixture (2/2/1 v/v/v) is used (Bligh and Dyer, 1959) for lipid extraction. In this method, chloroform dissolves fat, and methanol breaks down lipid protein bonds and inactivates lipase in a monophasic system (Maciel *et al.*, 2016). Water is then added to produce a biphasic system and wash non-lipid compounds. To obtain a good extraction yield, the Bligh and Dyer method must be repeated twice or three times on the same seaweed sample lengthening extraction duration and exposing the lipids extracted to oxidation. The supercritical fluids demonstrate their ability to extract lipids from seaweeds (Grosso *et al.*, 2015). Supercritical carbon dioxide (sc-CO<sub>2</sub>) is the most common supercritical fluid used due to its low critical pressure and temperature (73.9 bar and 31.1 °C). Moreover, it is non-toxic, non-flammable, cheap, broadly available, chemically inert under numerous conditions, and gaseous at normal pressure and

temperature allowing to recover an extract without use of any organic solvent (Careri *et al.*, 2001; Mendes *et al.*, 2003; Macías-Sánchez *et al.*, 2008; Quitain *et al.*, 2013). CO<sub>2</sub> prevents the extract from degradation, giving a non-oxidizing atmosphere (Jaime *et al.*, 2007). Furthermore, sc-CO<sub>2</sub> has low viscosity and high diffusivity, allowing a faster and deeper penetration into the seaweed particles (Careri *et al.*, 2001; Ali-Nehari *et al.*, 2012). As the majority of CO<sub>2</sub> is recycled after extraction, the cost of each extract decreases. Moreover, the transition to the industrial scale is easily conceivable and allows a large scale production of bioactive compounds (Reverchon and De Marco, 2006). sc-CO<sub>2</sub> is convenient to extract non-polar compounds and its solubility properties can be modified by adding a safe and polar solvent like ethanol. In a green approach, the production of waste must be avoided (Buschmann *et al.*, 2017). With sc-CO<sub>2</sub> with or without ethanol as co-solvent, the solid residue after lipid extraction is not waste, it can be used for the extraction of interesting compounds such as proteins or polysaccharides by other green methods like microwaves, ultrasonic or enzymatic assisted extractions. Thus, the sequence of selective extraction of the compounds leads to total use of the algal biomass. It is the tenet of a bio-refinery approach (Trivedi *et al.*, 2015).

As this extraction method has been little applied to seaweeds (Crampon *et al.*, 2011), the free radical scavenging activity of lipid classes extracted from seaweeds by supercritical carbon dioxide has never been reported. The present study aimed to investigate the free radical scavenging properties of lipid classes extracted from two different seaweeds, *Solieria chordalis* and *Sargassum muticum*, from Brittany's coast according to conventional and eco-friendly methods.

## 2 Materials and methods

### 2.1 Lipid class fractions

The lipid class fractions were prepared according to Terme *et al.* (2017). Briefly, *S. chordalis* (Rhodophyta, Gigartinales, Solieriaceae) and *S. muticum* (Phaeophyceae, Fucales, Sargassaceae) were collected on the littoral area of Saint Gildas de Rhuys (47° 29' 34.0" N 2° 49' 51.0" W, Atlantic coast, France) in October 2017 and in July 2014, respectively. The fresh algae were sorted and cleaned with tap water to remove epiphytes, sediments, organic debris, and macro fauna. The fresh seaweeds were ground to pieces of about 3 mm with a hammer mill, stored at -25 °C and freeze-dried. The lipids of *S. chordalis* were extracted using chloroform/methanol (1/1 v/v) or supercritical carbon dioxide pure or with 2% or 8% of ethanol. Chloroform/methanol (1/1) and pure supercritical carbon dioxide were applied to *S. muticum* in order to extract the total lipids. For chloroform/methanol extraction, dry algae were extracted three times at room temperature with the solvent mixture. Extract was then washed with brine, dried over sodium sulphate and evaporated under reduced pressure. For sc-CO<sub>2</sub> extraction, in the pilot scale system, the fluid was heated (45 °C) and pressurized (290 bar) to achieve the supercritical state. The supercritical fluid flow was fixed at 10 kg.h<sup>-1</sup>. The lipid fractionation was conducted onto a silica gel column (silica 60 Å-particle size 20–45 µm – Fisher

Scientific, UK) eluted with dichloromethane to isolate neutral lipids, acetone for glycolipids and finally methanol for phospholipids. Fractions were collected and evaporated to dryness.

### 2.2 Free-radical scavenging activities

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined according to Boulho *et al.* (2017) with slight modifications. Briefly, 100 µl of each lipid fraction at different concentrations between 1 and 10 mg/ml in methanol was mixed in a 96-well plate with 100 µl of a DPPH solution (0.1 g/l) in methanol prepared just before use. Due to the colour intensity of each fraction, it was necessary to prepare a blank of 100 µl of each sample at the same concentration tested in methanol mixed with 100 µl of methanol. A solution of BHA (butylated hydroxyanisole) at different concentrations range from 1 to 20 µg/ml in methanol (final concentration) was also tested as a positive control. After stirring, the microplate was incubated at 37 °C for 30 min (stationary state). Finally, the absorbance was read at 517 nm by a ThermoScientific Multiscan Go UV-vis apparatus. All the tested solutions (sample and standard) were made in triplicate and the results obtained were expressed as a mean of the percentage of DPPH radical scavenging. The ability to scavenge the DPPH radical was calculated using the equation:

$$\% \text{scavenging} = \frac{A_{\text{control}} - (A_{\text{sample}} - A_{\text{sampleblank}})}{A_{\text{control}}} \times 100,$$

where  $A_{\text{control}}$  is the absorbance of the DPPH solution,  $A_{\text{sample}}$  is the absorbance of the tested sample (sample + DPPH) and  $A_{\text{sample blank}}$  is the absorbance of the sample (sample + methanol).

### 2.3 Statistical analysis

All measurements were made in triplicate. All data are reported as mean ± standard deviation (s.d.). Statistical analyses were by one-way analysis of variance ANOVA and Tukey's pairwise *a posteriori* test. All statistical analyses were performed using Past 3.12 (Hammer *et al.*, 2001) at  $p < 0.05$  level.

## 3 Results and discussion

DPPH reagent has been widely used for measuring the free radical scavenging activities of compounds or extracts. DPPH reagent is scavenged by antioxidant molecules through the donation of a hydrogen forming the reduced diphenylpicrylhydrazine (DPPH-H). The changes in the wavelength of the maximum absorption permit to quantify the scavenging of the radical and it is visually noticeable as a colour change from purple to yellow.

Table 1 indicates the percentage of DPPH radical scavenging activity of lipid classes from *S. chordalis* according to the method used for lipid extraction at a concentration of 1 mg/ml of lipid class in methanol.



**Table 1.** DPPH radical scavenging activity (%) at 1 mg/ml and EC<sub>50</sub> (mg/ml) of the lipid classes obtained from *Solieria chordalis* according to the extraction method and DPPH radical scavenging activity (%) at 10 µg/ml and EC<sub>50</sub> (µg/ml) obtained from BHA.

Extraction method	Lipid class						Control	
	Neutral lipids		Glycolipids		Phospholipids		BHA	
	% Scavenging	EC <sub>50</sub>	% Scavenging	EC <sub>50</sub>	% Scavenging	EC <sub>50</sub>	% Scavenging	EC <sub>50</sub>
CHCl <sub>3</sub> /MeOH	86.6 ± 5.7 <sup>a-α</sup>	< 0.5	49.3 ± 1.6 <sup>a-β</sup>	0.9 ± 0.1	30.9 ± 1.9 <sup>a-γ</sup>	4.9 ± 0.5	82.9 ± 2.9	5.5 ± 0.3
sc-CO <sub>2</sub>	16.9 ± 2.2 <sup>b-α</sup>	> 5	24.7 ± 1.1 <sup>b-β</sup>	4.8 ± 0.1	27.0 ± 0.5 <sup>a-β</sup>	2.1 ± 0.1		
sc-CO <sub>2</sub> + EtOH 2%	16.0 ± 0.6 <sup>b-α</sup>	> 5	11.4 ± 1.4 <sup>c-α</sup>	> 5	11.1 ± 0.7 <sup>b-α</sup>	> 5		
sc-CO <sub>2</sub> + EtOH 8%	15.3 ± 0.3 <sup>b-α</sup>	> 5	15.8 ± 3.3 <sup>c-α</sup>	> 5	46.2 ± 1.0 <sup>c-β</sup>	1.1 ± 0.1		

Values are the mean of three replicates (mean (% dw) ± s.d.). s.d., standard deviation. sc-CO<sub>2</sub>, supercritical carbon dioxide, BHA, butylated hydroxyanisole, EC<sub>50</sub> refers to the quantity of extract required to reduce DPPH radical by 50%. a-c, letters in the same column represent significant differences according to Tukey's pairwise a posteriori test, considering  $p < 0.05$ . α-γ, letters in the same row represent significant differences according to Tukey's pairwise a posteriori test, considering  $p < 0.05$ .

**Table 2.** DPPH radical scavenging activity (%) at 1 mg/ml and EC<sub>50</sub> (mg/ml) of lipid classes obtained from chloroform/methanol and supercritical carbon dioxide extraction from *Sargassum muticum* according to the extraction method and DPPH radical scavenging activity (%) at 10 µg/ml and EC<sub>50</sub> (µg/ml) obtained from BHA.

Extraction method	Lipid class						Control	
	Neutral lipids		Glycolipids		Phospholipids		BHA	
	% Scavenging	EC <sub>50</sub>	% Scavenging	EC <sub>50</sub>	% Scavenging	EC <sub>50</sub>	% Scavenging	EC <sub>50</sub>
CHCl <sub>3</sub> /MeOH	23.6 ± 0.5 <sup>a-α</sup>	> 5	29.6 ± 3.4 <sup>a-β</sup>	4.1 ± 0.3	28.0 ± 1.2 <sup>a-α, β</sup>	4.8 ± 0.1	83.3 ± 2.9	5.7 ± 0.3
sc-CO <sub>2</sub>	26.2 ± 1.2 <sup>a-α</sup>	3.8 ± 0.5	50.9 ± 0.8 <sup>b-β</sup>	0.9 ± 0.1	48.4 ± 1.6 <sup>b-β</sup>	1.0 ± 0.1		

Values are the mean of three replicates (mean (% dw) ± s.d.). s.d., standard deviation. sc-CO<sub>2</sub>, supercritical carbon dioxide, BHA, butylated hydroxyanisole, EC<sub>50</sub> refers to the quantity of extract required to reduce DPPH radical by 50%. a-b, letters in the same column represent significant differences according to Tukey's pairwise a posteriori test, considering  $p < 0.05$ . α-β, letters in the same row represent significant differences according to Tukey's pairwise a posteriori test, considering  $p < 0.05$ .

All lipid classes from *S. chordalis* showed free radical scavenging activities at 1 mg/ml. These activities are lower compared to BHA at 10 µg/ml. The neutral lipids from the chloroform/methanol (CM) extraction exhibited the highest activity (86.6 ± 5.7% of scavenging). This high activity might be due to α-tocopherol, fucosterol or squalene which have been identified in this fraction previously (Kendel *et al.*, 2015) and have been known as antioxidant compounds (Zubia *et al.*, 2007). The activity of neutral lipids was lower for the supercritical carbon dioxide (sc-CO<sub>2</sub>) extraction with or without co-solvent. However, the scavenging was not significantly different for each extract and was established around 16%. This result suggests that the composition of the neutral lipid fractions is the same whatever the extraction method. As the quantity of neutral lipid extracted increases with the addition of ethanol (Terme *et al.*, 2017), the combination of sc-CO<sub>2</sub> and ethanol seems to be the best way to obtain antiradical compounds from the neutral lipid fraction. For glycolipids, the best activity was obtained with the CM extraction (49.3 ± 1.6% of scavenging). The activity observed with the pure supercritical carbon dioxide extraction was twice lower (24.7 ± 1.1% of scavenging) than the one observed with CM extraction. In this case, the addition of ethanol in sc-CO<sub>2</sub> produced a less active fraction. However, no significant difference was observed by increasing the amount of ethanol despite an increase of the glycolipid quantity (Terme *et al.*, 2017). This result suggests that sc-CO<sub>2</sub> did not efficiently

extract antioxidant glycolipids from *S. chordalis*. The phospholipids extracted by sc-CO<sub>2</sub> demonstrated an equivalent activity than the one noticed for the phospholipids extracted using CM. The most active fraction was obtained with sc-CO<sub>2</sub> + ethanol 8% with a half more activity than the lipids extracted with CM. As the content of phospholipids decreased when ethanol was added to sc-CO<sub>2</sub> (Terme *et al.*, 2017), 8% ethanol in sc-CO<sub>2</sub> might extract phospholipids with a greater free radical scavenging activity. Phospholipids with polyunsaturated fatty acids were probably extracted with selectivity as they are known to have antioxidant properties (Henry *et al.*, 2002).

Table 2 indicates the percentage of DPPH radical scavenging activity of lipid classes from *S. muticum* according to the method used for lipid extraction at a concentration of 1 mg/ml of lipid classes in methanol.

Each lipid class from *S. muticum* showed a free radical scavenging activity at the 1 mg/ml. The activities are lower compared to BHA at 10 µg/ml. Glycolipids and phospholipids exhibited the best activities for sc-CO<sub>2</sub> extraction and the results obtained for these two classes were not significantly different. The activities of glycolipids (50.9 ± 0.8%) and phospholipids (48.4 ± 1.6%) obtained with sc-CO<sub>2</sub> were twice as large as that of fractions obtained with CM, 29.6 ± 3.4% and 28.0 ± 4.2%, respectively. Concerning neutral lipids, the results were not significantly different for the two extraction methods and the neutral lipid fraction obtained with sc-CO<sub>2</sub> is

near than twice less of those obtained for glycolipids and phospholipids. According to our previous results (Terme *et al.*, 2017), the glycolipid and phospholipids fractions represented up to 90% of the lipid fraction from this seaweed. Therefore, the supercritical carbon dioxide is a good method to extract a free radical scavenging fraction from the seaweed *S. muticum*.

Because of a lack of free radical scavenging data on lipid classes from seaweeds our results cannot be evaluated by comparing with other species or with other extraction methods. However, the results obtained for the lipid classes were in the same range than those obtained from methanol/chloroform extract from other *Sargassum* species (Matsukawa *et al.*, 1997; Yan *et al.*, 1998; Zubia *et al.*, 2007; Lu *et al.*, 2010). The results obtained for both seaweeds are comparable or greater than those obtained generally for brown or red seaweeds (Zhang *et al.*, 2007; de Alencar *et al.*, 2016). sc-CO<sub>2</sub> extracts from *Chlorella pyrenoidosa* demonstrated free radical scavenging activities between 17.35% and 54.16% at 10 mg/ml (Hu *et al.*, 2007). The DPPH radical scavenging activity increased with the increasing amount of ethanol in supercritical carbon dioxide as we observed for *S. chordalis*.

## 4 Conclusion

It can be concluded that supercritical carbon dioxide can be used to extract antioxidant lipids from *S. chordalis* and *S. muticum*. The results indicated that each of the lipid fractions, *i.e.*, neutral lipids, glycolipids and phospholipids, exhibited free radical scavenging compounds. The findings of this work demonstrated that non-polar lipids are the most active fraction when they are extracted with solvent mixture and polar lipids either glycolipids or phospholipids are the most active lipid class when the extraction was conducted with supercritical carbon dioxide. This work is useful to partially validate the bio-refinery approach and to up-grade the seaweed regarding antioxidant compounds in the cosmetic field.

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**Conflicts of interest.** The authors declare that they have no conflict of interest in relation to this article.

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