

Short Communication

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Microalgae-based unsaponifiable matter as source of natural antioxidants and metal chelators to enhance the value of wet *Tetraselmis chuii* biomass

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Abstract: The present work aimed to determine the antioxidant, metal chelating and neuroprotective potential of the unsaponifiable matter (UM) of *Tetraselmis chuii* to be applied to a biorefinery setting. The UM obtained via saponification from crude lipids extracted from microalgal wet biomass showed a radical scavenging activity (RSA) towards the DPPH radical of $90.7 \pm 1.3\%$ and $57.1 \pm 1.2\%$ at a concentration of 10 and 5 mg/mL, respectively. The UM fraction also displayed metal chelating capacity at a concentration of 5 mg/mL: $58.5 \pm 1.4\%$ and $50.9 \pm 4.0\%$ for copper and iron, respectively. The chemical characterization of the UM revealed significant levels of total phenolics (TPC, 13.61 mg GAE/g) and carotenoids (2.45 mg/g of β -carotene, lutein and violaxanthin). Overall, the separation of the UM containing high value metabolites might significantly upgrade the total wet biomass value in a biorefinery, allowing the exploitation of a stream with relevant antioxidant and metal chelating activities.

Keywords: *Tetraselmis chuii*, Unsaponifiable Matter, Antioxidants, Metal Chelators, Phenolics

Abbreviations

AChE, acetylcholinesterase;
BHT, butylated hydroxytoluene;
BuChE, butyrylcholinesterase;
CCA, copper chelating activity;
CL, crude lipids;
DW, dry weight;
DHA, docosahexaenoic acid;
EPA, eicosapentaenoic acid;
EDTA, ethylenediaminetetraacetic acid;
GAE, gallic acid equivalents;
HVP, high value products;
ICA, iron chelating activity;
LDL, low density lipoproteins;
MBB, microalgal- based biodiesel;
PUFA, polyunsaturated fatty acids;
PV, pyrocatechol violet;
RSA, radical scavenging activity;
TPC, total phenolic content;
TAG, triacylglycerol;
UM, unsaponifiable matter.

1 Introduction

Microalgae offer great potential as lipid feedstocks for the exploitation of edible oils and biodiesel, being currently recognized as one of the most promising alternatives to land plant-based biomass to replace oil crops and petroleum-based fuels [1,2]. Microalgae possess several advantages over conventional feedstocks such as high growth rates and high productivity, being able to provide a steady supply of biomass for the whole year [3,4]. However, current production costs have hampered the

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commercialization of microalgal-based ventures due to the substantial investments in capital and energy for biomass production, harvesting and drying [5]. These high energy requirements are one of the main challenges to be overcome in order to turn microalgal bioproducts into economically feasible commodities [6,7].

Recent techno-economic studies have put forward that the economics of microalgae production are highly improved by avoiding biomass drying [8] and by coupling the exploitation of high value biochemicals with lipid production in a biorefinery [9,10]. However, a biorefinery will only be successful if all biochemical components of microalgal biomass are exploited through an optimized downstream procedure composed of different streams for the generation of commercial end-products [11]. To implement such a zero-waste process and decrease costs, improved procedures for processing wet biomass and extracting bioactive compounds must first be developed. For example, microalgae are well-known sources of ω 3-fatty acids (i.e., n-3 fatty acids like eicosapentaenoic, EPA, and docosahexaenoic, DHA, acids), phenolics and carotenoids. These added-value compounds play important roles in promoting human health by modulating the immune system and by providing novel avenues for treatment of human ailments: e.g., cancer, Alzheimer's and cardiovascular diseases [12-19]. The extraction of these and other high value biochemicals could offer new opportunities in a biorefinery approach to generate additional income, thus improving the odds for large-scale commercial production of microalgae biomass in the foreseeable future.

Microalgal crude lipids are comprised mainly of TAG, phospholipids, glycolipids and unsaponifiable matter (UM). The presence of unsaponifiable lipophilic compounds (e.g., carotenoids, phytol, phytosterols, phenolic compounds and hydrocarbons, among others) in oils used in the production of biofuels may lead to engine coking and carbon depositing. Therefore, these must be removed, being often seen as waste. However, considering that UM is a potential source of added-value compounds that could perform as antioxidants and thus retard the deterioration of food and cosmeceutical products, their recovery would be a significant benefit [20,21].

Tetraselmis microalgae are prime candidates for producing biomass for a microalgal-based biorefinery due to their robustness, high growth rates and lipid contents. In particular, *T. chuii* has been classified as a suitable species for a multi-product algal crop for biomass and biofuel production. Moreover, these species are easily observed and analysed for cell viability due to its relatively large cell size and high motility [22-25].

The main objective of the present study is to propose a novel procedure for separating and upgrading the value of *T. chuii* microalgal biomass-derived UM as feedstock for important bioactive compounds as value-added products. This approach not only minimizes dewatering/drying costs, but also improves the value of microalgae biomass. Evidence for the potential upgrade of the *T. chuii* biomass value by using UM as source of high value metabolites (e.g. carotenoids and phenolic compounds) with antioxidant and metal chelating activities is presented and discussed.

2 Materials and methods

2.1 Materials

Sulphuric acid, hydrochloric acid, potassium hydroxide (KOH), dimethyl sulphoxide (DMSO), acetonitrile, ethanol, methanol, hexane, ethyl acetate, ferrozine, copper sulphate pentahydrate, Folin-Ciocalteu (F-C), sodium carbonate (Na_2CO_3), EDTA, glycerol, lutein and additional reagents were purchased from VWR International (Leuven, Belgium). Butylated hydroxytoluene (BHT), acetylcholinesterase (AChE), butyrylcholinesterase (BChE), 1,1-diphenyl-2-picrylhydrazyl (DPPH), acetylthiocholine iodide (ATChI), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), pyrocatechol violet, galanthamine, β -carotene, zeaxanthin, canthaxanthin, astaxanthin, and fucoxanthin were purchased from Sigma-Aldrich (Steinheim, Germany). Neoxanthin and violaxanthin standards were kindly provided by Dr Monya Costa and Dr João Silva (ALGAE Research Group, CCMAR).

2.2 Microalgal biomass production

Tetraselmis chuii was supplied by Laboratório Experimental de Organismos Aquáticos (LEOA), University of Algarve, Portugal. Microalgal cells were grown in 100-L plastic bags in sterilized sea water (37 ppm), supplemented with modified ALGAL medium [26], under a photon flux density of $80 \mu\text{mol}/\text{m}^2/\text{s}$ at room temperature. The biomass obtained was harvested by centrifugation (Avanti J-25, Beckman Coulter, CA, USA) at 4000 g for 10 min, at room temperature (RT), and stored at -20°C until further analysis.

2.3 Extraction

Briefly, 73 g of *T. chuii* wet biomass was dispersed in ethanol (300 mL) and stirred at reflux temperature for 90 min. Subsequently, the mixture was immediately centrifuged (Beckman Coulter Avanti J-25) at 4000 g for 10 min (ET-1). The insoluble matter (i.e., residual biomass) underwent further extraction using ethanol twice, at reflux temperature, for 60 min (ET-2; 150 mL) and 30 min (ET-3; 150 mL). The extracts were afterwards pooled, centrifuged and filtered (Whatman n° 4). Ethanol and water were removed from the mixture using a rotatory evaporator, under reduced pressure, to obtain a crude extract.

2.4 Saponification of crude extract

The UM was separated from the crude lipid extract of *T. chuii* by saponification [27] with few modifications. Briefly, 40 mL of absolute ethanol and 10 mL of aqueous KOH (6 g/10 mL) solutions were added to crude extract (1.0 g), and the resulting mixture was stirred at reflux temperature for 90 min. Solvent was then evaporated to 2/3 of its volume, transferred into a separating funnel, and distilled water (250 mL) added. The UM was extracted with a mixture of hexane and ethyl acetate (85:15; v/v) 3 times, and the combined solvent layers were washed with distilled water until neutral pH. The solvent was dried over anhydrous sodium sulphate and filtered, and the solvent removed using a rotary evaporator; the UM was stored at -20 °C for further analysis.

2.5 Evaluation of biological activities in the UM fraction

2.5.1 DPPH radical scavenging assay

The RSA of the UM was evaluated according to a previously reported method [28], adapted to 96-well microplates [29]. Samples (22 µL at concentrations of 1, 5 and 10 mg/mL of microalgal UM) were mixed with 200 µL of DPPH solution (120 µM in methanol), in 96-well flat bottom microplates, and incubated in the dark at RT for 30 min. Upon incubation, their absorbance was measured at 515 nm using a microplate reader (Biotek Synergy 4) and the RSA was expressed as percentage of inhibition relative to a control containing ethanol instead of the sample. Butylated hydroxytoluene (BHT, 1 mg/mL) was used as positive control.

2.5.2 Copper chelating activity

Copper chelating activity (CCA) was determined using pyrocatechol violet (PV), as described in Megías et al. [30]. Briefly, samples (30 µL at 1, 5 and 10 mg/mL of microalgal UM) were placed in 96-well microplates, and 200 µL of sodium acetate buffer (50 mM, pH 6) were added. This was followed by the addition of 6 µL of PV (4 mM) and 100 µL of CuSO₄•5H₂O (50 µg/mL in water). The absorbance of the solution was measured at 632 nm using a microplate reader. EDTA was used as positive control at a concentration of 1 mg/mL.

2.5.3 Iron chelating activity

Iron chelating activity (ICA) was determined by measuring the formation of the Fe²⁺-ferrozine complex, as described elsewhere [30] with modifications. Samples (30 µL at concentrations of 1, 5 and 10 mg/mL) were mixed with 200 µL of dH₂O and 30 µL of a FeCl₂ solution (0.1 mg/mL in water) in 96-well microplates. After 30 min, 12.5 µL of ferrozine solution (40 mM in water) were added. The change in colour was measured in a microplate reader at 562 nm. The synthetic metal chelator EDTA was used as positive control (1 mg/mL).

2.6 Determination of carotenoid composition of the UM fraction

Carotenoid composition was determined in an HPLC (Knauer Advanced Scientific Instruments, Berlin, Germany) system comprising of a Knauer pump model 1000, diode-array-detector (DAD) model 2600 with a vacuum degasser, using a Luna 5u C18 100A (5 µm, 250 x 4.6 mm, Phenomenex®) column and ClarityChrom software. Carotenoids were eluted with a gradient mobile phase composed of acetonitrile (0-15 min), acetonitrile : ethyl acetate (50:50; 15-35 min) and acetonitrile (35-45 min), at a flow rate of 1 mL/min with an injection volume of 20 µL as described in our previous report [31]. Carotenoids were identified and quantified using individual calibration curves for each standard (β-carotene, lutein, violaxanthin, zeaxanthin, neoxanthin, canthaxanthin, astaxanthin, and fucoxanthin).

2.7 Determination of total phenolic contents (TPC)

TPC of the UM was determined by the Folin-Ciocalteu (F-C) assay according to Velioğlu *et al.*, [32]. Briefly, 5 μ L of UM fraction (at 10 mg/mL) were mixed with 10-fold diluted F-C reagent in distilled water (100 μ L), followed by incubation at RT for 5 min. Upon incubation, 100 μ L of Na_2CO_3 (75 g/L, w/v) were added, and the solution was further incubated at RT for 90 min. The absorbance of the solution was measured at 725 nm using a microplate reader (Biotek Synergy 4). Results were expressed as gallic acid equivalents (GAE), in milligrams per gram of extract (dry weight, DW), using a calibration curve with gallic acid standard solutions at concentrations ranging from 0.002 to 2 mg/mL ($r^2 = 0.999$).

2.8 GC-MS analysis of UM

Identification and quantification of the rest of the components present in the UM fraction was determined by GC-MS (Agilent 6890 Network GC System, 5973 Inert Mass Selective Detector), equipped with a DB5-MS capillary column (25 m \times 0.25 mm internal diameter, 0.25 μ m film thickness) using helium as carrier gas, using total ion mode and the MS spectra of the National Institute of Standards and Technology (NIST) library as reference.

2.9 Statistical analysis

Results were expressed as mean \pm SEM, and analysed by ANOVA to reveal differences and interactions between treatments or using the Tukey HSD test when parametricity of data did not prevail, using SigmaPlot software for Windows (Version 11.0, Systat Software, Inc.). Differences at $p < 0.05$ were considered significant. All measurements were carried out in triplicate.

3 Results and Discussion

The procedure developed for downstream processing of wet biomass of *T. chuii* is represented in Figure 1. The first stage of the process relies on the extraction of the crude lipids directly from wet biomass paste (containing 78% of water) with ethanol at reflux temperature, which resulted in a 24.4% lipid yield from raw biomass. The total lipid content obtained from *T. chuii* was comparable to that of

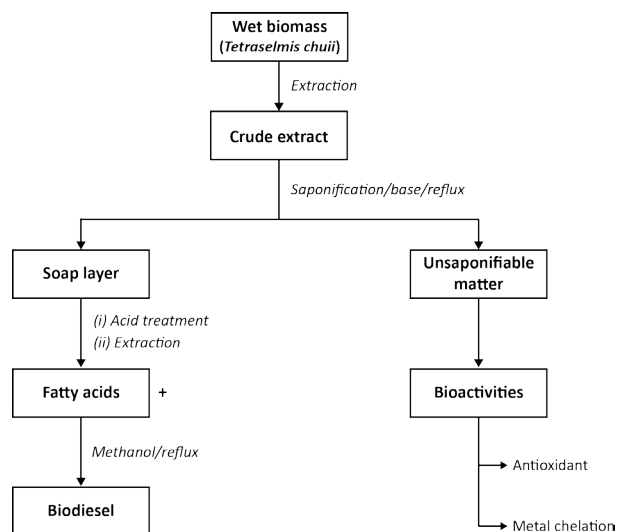


Figure 1: Schematic diagram of biodiesel production and evaluation bioactivities studies of UM from microalgae.

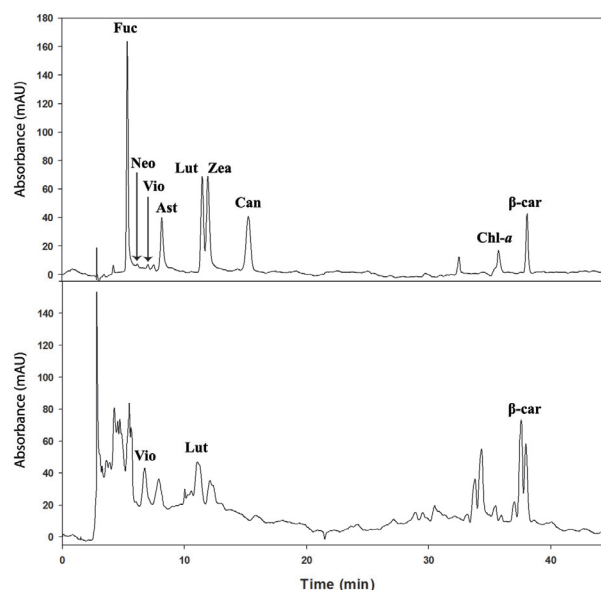


Figure 2: Chromatogram of HPLC-DAD (446 nm) assessment of pigments. Top: chromatogram of the standard containing fucoxanthin (Fuc), neoxanthin (Neo), violaxanthin (Vio), astaxanthin (Ast), lutein (Lut), zeaxanthin (Zea), canthaxanthin (Can), chlorophyll *a* (Chl-*a*) and β -carotene (β -car); Bottom: chromatogram of the UM showing the detected pigments.

the Bligh-Dyer method [33] widely used in the literature. The yield obtained showed that extraction from *T. chuii* at reflux temperature is an efficient method to extract the total lipids without any pre-treatment to break the cell wall. The main advantage of using ethanol for lipid extraction is that the drying step prior to extraction is minimized, thus allowing a significant reduction of the energy required

Table 1: Radical scavenging activity (RSA), metal chelating activity towards copper (CCA) and iron (ICA) ions and acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition activities of UM.

UM (mg/mL)	RSA (%)	CCA (%)	ICA (%)	AChE (%)	BuChE (%)
1	23.9 ± 0.5 ^d	25.1 ± 3.1 ^d	24.9 ± 6.2 ^c	n.d.	n.d.
5	57.1 ± 1.2 ^c	58.5 ± 1.4 ^c	50.9 ± 4.0 ^b	n.d.	n.d.
10	90.7 ± 1.3 ^b	107.5 ± 1.7 ^a	88.9 ± 2.7 ^a	n.d.	n.d.
BHT*	89.2 ± 0.4 ^a	-	-	-	-
EDTA*	-	81.3 ± 0.6 ^b	93.1 ± 0.2 ^a	-	-
Galanthamine*	-	-	-	87.8 ± 0.1	75.6 ± 1.2

Values represent the mean ± standard error of the mean (SEM) of at least three experiments performed in triplicate ($n = 9$). In the same column, values followed by the different letter are significantly different at $P \geq 0.05$ (Tukey's HSD test). n.d. - not detected; *1 mg/mL.

for the downstream processing of biomass. Moreover, ethanol extraction presents other significant advantages: i) ethanol is a food-grade solvent allowing further food/feed applications for the residual biomass, which may improve the overall economics of the production process; ii) unlike non-polar solvents (e.g., hexane, diethyl ether or chloroform), ethanol is appropriate for wet biomass due to its miscibility with water and significant mass transfer rates of constituents even in high moisture feedstocks; and iii) lower alcohols (e.g. methanol and ethanol) can also inactivate enzymes and preclude degradation of the extracted compounds [34].

Upon lipid extraction, crude lipids were saponified with alcoholic KOH, and the UM was recovered from the soap layer containing FA. After saponification, an orange-red organic layer (i.e., upper layer) and a lower dark-green water layer containing the fatty acids in the form of potassium salts were generated. The conversion of the crude lipids into saponifiable lipids (i.e., potassium salt of fatty acids) and UM favours an effective separation of both fractions. In the present work, the UM was extracted with a mixture of hexane and ethyl acetate by liquid-liquid extraction. The resulting UM accounted for ca. 23% of the total crude ethanol extract. In comparison, Wang and Wang [35] reported a UM yield of 12.5 and 4.5% for *Nannochloropsis* sp. and *Schizochytrium limacinum*, respectively, using ultrasonication followed by a hot ethanol extraction. Interestingly, the UM content of microalgal oils is approximately 10-fold higher than that of commonly obtained from plants [36].

3.1 Evaluation of bioactive properties of UM

Upon its separation from the soap layer, UM was assayed for the presence of antioxidants and metal chelators in order to determine the biomass upgrade potential of this

fraction. UM displayed radical scavenging activity (RSA) towards the DPPH radical of $90.7 \pm 1.3\%$ at 10 mg/mL (Table 1), which was similar to that of the positive control (BHT) (89.2 ± 0.4 at 1 mg/mL). The obtained scavenging activity is very promising, considering that UM is a complex mixture of compounds (e.g. hydrocarbons, sterols, carotenoids and phenolic compounds). Molecules preventing or delaying the formation of free radicals are essential for the stabilization of food products during storage [37]. They can also play a vital role in the development of cosmeceuticals products for the health care and cosmetic industries [38]. In addition, previous reports have also proposed that a combination of phenolic compounds and carotenoids can synergistically prevent the oxidation of low density lipoproteins (LDL) [39]. Such property might contribute to the future design of functional foods or ingredients based on their antioxidant activity and their synergistic interactions. In addition, the demand by the consumer for natural sources of antioxidants has been growing, as common synthetic antioxidants (e.g. butylated hydroxytoluene, BHT, and butylated hydroxyanisole, BHA) have important drawbacks such as toxicity and carcinogenicity [40]. The high unsaponifiable content may also recommend the exploitation of microalgae biomass as a low cost renewable source of phytosterols for industrial processing in the fields of cosmetics and nutraceuticals.

The metal chelating capacity of the UM fraction was slightly lower for iron (ICA) than for copper (CCA). ICA ranged between 24.9 ± 6.2 and $88.9 \pm 2.7\%$, whereas the highest concentration tested (10 mg/mL) was able to chelate all copper from the solution (Table 1). Conversely, UM had lower ICA and CCA than the positive control at 1 mg/mL. Most crude extracts contain different components and some of them may not be responsible for the activity of interest and thus decrease the effect observed per unit mass or unit volume. Therefore, taken together, these results suggest that UM contains compounds able to

chelate iron and copper effectively, which can be used to upgrade the overall biomass value. Interestingly, metal chelators can act as antioxidants, not only by preventing chain initiation through radical scavenging, but also by binding to metal ions, which can operate as catalysts of radical generation. Indeed, there has been increasing interest in recent years in the use of biodegradable metal chelating agents to replace traditional non-biodegradable agents. For example, ethylenediaminetetraacetic (EDTA) and diethylenetriaminepentaacetic (DTPA) acids have been largely used in several industrial, agricultural, domestic (e.g. chelator in detergents) and biotechnological applications [41]. However, these aminopolycarboxylates are hardly biodegradable, leading to the accumulation of considerable amounts in industrial effluents as well as in aquatic systems, which poses serious environmental concerns [42]. Thus, non-toxic metal chelators present in unsaponifiable matter separated from microalgal biomass could be used for industrial applications not only in the food industry, but also in the formulation of nutraceuticals and cosmetics [42,43].

3.2 Chemical characterization of UM

Analysis and chemical characterization of the bioactive compounds present in the unsaponifiable matter (UM) is a crucial approach in developing an efficient microalgae-based biorefinery platform. In this sense, the UM-fraction was characterized in terms of total phenolic contents (TPC), which was found to be equal to 13.6 mg gallic acid equivalents (GAE)/g DW. Therefore, the saponification method could be an efficient procedure to separate phenolic compounds from the lipids present in the microalgal biomass. Phenolic compounds often display antioxidant activity by donating a hydrogen atom of the phenolic hydroxyl groups, chelating metals, and scavenging free radicals and inhibiting oxidoreductases (e.g. lipoxygenase) [44].

Upon RP-HPLC analysis, three carotenoids, namely β -carotene, lutein and violaxanthin were identified at concentrations between 0.14-2.15 mg/g of the total UM (Table 2). Similar results have been obtained in the eustigmatophyte *Nannochloropsis gaditana*, where the UM obtained also contained carotenoids, neoxanthin in particular [20]. These pigments are present in most green microalgae, being essential not only for light harvesting, but also to protect the photosynthetic apparatus from the deleterious effects of reactive oxygen species [45]. Carotenoids are known to be important sources of antioxidants, and are associated with various human

Table 2: Carotenoid contents in the UM obtained from *T. chuii* wet biomass.

Components	Concentration (mg/g)
β -carotene	2.15 ± 0.13
Lutein	0.14 ± 0.01
Violaxanthin	0.16 ± 0.02
Total	2.45 ± 0.13

health benefits, such as prevention of age-related macular degeneration, cataracts, tumoural and cardiovascular conditions, and rheumatoid arthritis and muscular dystrophy ailments [45,46]. Because of this, carotenoids have become increasingly popular as dietary supplements, food colorants and ingredients in cosmetic, nutraceutical and pharmaceutical formulations [47].

The chemical composition of the UM was further analysed by GC-MS and a tentative identification of the compounds present on this fraction was performed (Table 3). The hydrocarbon fraction was composed of saturated, unsaturated and cyclic compounds, with a number of carbons ranging from 12 to 26. Among those, the main component was 1-hexadecene, which corresponded to 17.9% of total hydrocarbons (TH). Other major hydrocarbons, namely octadecane, tetradecane, eicosane, docosane, tetracosane and hexacosane were also detected with relative contents ranging from 4.4 to 14.5% of TH. Some of the minor hydrocarbons were 1-nonadecene, cyclotetradecane, dodecane and 5-eicosane. A combination or mixture of hydrocarbons is well known as mineral oil or liquid paraffin, which is a common ingredient in lipsticks, baby lotions, cold creams, ointments, cosmetics and emollients as well [48]. Rawlings and Lombard [49] reported that mineral oil could improve the skin softness compared to fatty acids, triacylglycerols and wax esters. Hence, the natural biodegradable products in the form of UM of the microalgae can be exploited as ingredients in cosmeceutical industry. Although most of the mineral oils are generally obtained from petroleum products, nowadays there is increasing consumer pressure for the use of natural or marine natural products rather than petroleum-based products.

Besides hydrocarbons, the UM contained others components, including a sterol (campesterol), 2,4-bis(1,1-dimethylethyl) phenol, phytol and phthalate derivatives (Table 3). Sterols, sometimes collectively referred to as “phytosterols”, have been found in various plants and marine organisms, being used in food and feed formulations [50]. Interestingly, these hydroxylated steroids seem to play a major role in preventing coronary

Table 3: GC-MS chemical characterization of the UM.

Components	Relative abundance (%)
<i>Hydrocarbons</i>	
1-Hexadecene	17.9
5-Eicosene	1.7
Cyclotetracosane	2.3
Dococene/1-nonadecene	2.6
Docosane	7.8
Dodecane	2.0
Eicosane	10.0
Hexacosane	4.4
Octadecane	14.5
Tetracosane	4.5
Tetradecane	12.8
<i>Sub-total</i>	<i>80.5</i>
<i>Others</i>	
Campesterol	2.4
Diethyl phthalate	1.9
Mono (2-ethyl hexyl) phthalate	2.4
Phenol, 2,4-bis (1,1-dimethylethyl)	5.8
Phytol	7.0
<i>Sub-total</i>	<i>19.5</i>
<i>Total</i>	<i>100.0</i>

heart disease, displaying also a hypocholesterolemic effect [14,51]. Phytosterols like campesterol, stigmasterol and β -sitosterol have been reported to possess antioxidant properties that may be crucial to prevent lipid peroxidation [50,52]. On the other hand, phenol derivatives, such as 2,4-di-*tert*-butylphenol, have been reported as UV stabilizers, antifungal, cytotoxic, biopreservatives and dietary antioxidants. Moreover, they are often used as intermediates for synthesis and formulation of pharmaceuticals and perfumes [53-55]. Phthalate esters, including diethyl- and mono-(2-ethyl hexyl) phthalates, accounted for 1.9 and 2.4% of the UM, respectively. The GC-MS phthalate fragment pattern of mono-(2-ethyl hexyl) found in *T. chuii* was identical to that in a previous report. In addition, as phthalates are commonly used as plasticizers to impart flexibility and durability, the UM of *T. chuii* could therefore be used in the production of bioplastics. These have the advantage of being

biodegradable and thus an alternative to those manufactured using fossil-based resources. Moreover, phthalic acid ester has recently been reported as inhibitor of the hydrolytic activity of trypsin [56,57]. Phytol is an acyclic diterpene alcohol and was found to be a major component of UM (Table 3). It is a constituent of chlorophyll, and is used as a precursor for the synthesis of vitamin E. Phytol is considered to be a safe and cost-effective food supplement and has several possible pharmaceutical applications, since it is endowed with antioxidant, antinociceptive, antiallergic and anti-inflammatory activities as well as showed *in vitro* and *in vivo* schistosomicidal activity against *Schistosoma mansoni* [58].

4 Conclusions

The exploitation of UM containing high value metabolites with free radical scavenging and metal chelating activities will be an important step towards the enhancement of the total value of the produced biomass without significantly increasing production costs. In recent years, there has been mounting interest in cosmeceuticals produced from marine sources. The results presented here strongly suggest that extracts from marine microalgae contain chemical compounds that could be used in the formulation of novel products due to their antioxidant properties. Indeed, the exploitation of high value products can be key to lowering the current production costs of microalgae ventures in a biorefinery setting, in particular when large-scale production is considered. Last but not least, the final costs of production will be further improved with the procedure here proposed, since the steps of drying and cell wall disruption are avoided.

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Conflicts of interest: The authors declare that there are no conflicts of interest.

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