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ORIGINAL ARTICLE

# A successful biorefinery approach of macroalgal biomass as a promising sustainable source to produce bioactive nutraceutical and biodiesel

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## Abstract

This study aimed to utilize four types of macroalgal biomass with a zero-waste biorefining concept for co-production of bioactive polysaccharide and biodiesel. The polysaccharide of macroalgal biomass obtained from *Ulva* spp., *Sargassum* spp., *Cladophora* spp., and *Spirogyra* spp. was extracted and partially purified by water-alcohol precipitation. The partially purified polysaccharide showed high antioxidant activity by scavenging DPPH and ABTS with $IC_{50}$ values of 3.50–37.31 mg/mL and 0.86–8.91 mg/mL, respectively, and high antiproliferative activity on human colon cancer cell line Caco-2 with $IC_{50}$ values of 0.66–12.20 mg/mL, while the antityrosinase activity was observed only *Sargassum* spp. and *Cladophora* spp. at 60.59% and 14.16%, respectively, at 10 mg/mL of tested polysaccharide extract. Interestingly, rare sugar including tagatose, psicose, and allose in polysaccharide were found to be 0.88–28.69 mg in 1 g of polysaccharide extract. After polysaccharide extraction, the macroalgal biomass residue was used to extract lipid prior to biodiesel production by acid-catalyzed transesterification. The extracted lipids of 3.09–10.05% were mainly composed of C16–C18 (>84%), and their biodiesel qualities were also satisfactory according to international requirements of biodiesel. It is expected that biorefinery approach will contribute greatly to zero-waste industrialization of macroalgal biomass-based bioactive nutraceuticals and biofuels.

**Keywords** Polysaccharide · Biodiesel · Antioxidant · Macroalgae · Biorefinery

## 1 Introduction

Currently, food and energy are major and critical topics worldwide when developing new technologies toward sustainability of economic growth and global development. The acceleration of increasing human population and growing world economy has stimulated the search for sustainable alternative sources to supply food demand and energy reserves. Examples among the most attractive sources are macroalgae, which are also recognized as a viable commercial biomass. Macroalgal biomass has valuable applications in various industrial processes, such as food industry (due to the rich content of proteins, lipids, and carbohydrates) and pharmaceutical industry (due to abundant antioxidant activities) [1]. Moreover, macroalgae are already farmed in tropical countries on large-scale production, mainly for human consumption [2]. Consequently, we have put our efforts on researching the utilization of macroalgae to harvest valuable sources of bioactive nutraceuticals and biodiesels.

Despite the outstanding advantages of bioactive components available in macroalgae, few reports on integrated production of high-valued bioproduct from macroalgae, focusing on co-production of nutraceuticals and biodiesel, were performed. For example, Trivedi et al. [2] have attempted to fully

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utilize both valuably active polysaccharides and biodiesels from them. High bioactivity of macroalgal polysaccharide attracts researchers in nutraceutical and biomedical areas, and recent trends in natural drug research of macroalgae have revealed the favorable biomedical potential in the treatment of human diseases [3]. The biological activity of macroalgal polysaccharide holds many significant properties, such as antioxidant, antityrosinase, immunostimulatory, antiviral, and antiproliferative activities on cancer cells [4, 5]. Polysaccharides have many proton donors such as carboxyl and/or hydroxyl groups of rhamnose, xylose, galactose, glucose, tagatose, psicose, and allose which are responsible for antioxidant and anticancer activity [6]. Interestingly, polysaccharides containing tagatose, psicose, and allose belonging to rare sugar would contribute greatly to active nutraceutical food as low-calorie sweetener [7]. Several species of macroalgae, such as *Gracilaria* spp., *Sargassum* spp., *Ulva* spp., and *Gelidium* spp., have been investigated for evaluating the bioactivities and determining rare sugars. However, studies on bioactivities and rare sugar of macroalgal polysaccharides are still in an exploratory stage [8]. Moreover, after obtaining macroalgal polysaccharides, unavoidable biomass residue waste (namely, polysaccharide-extracted macroalgal biomass residues; PMBRs) is generated in the process that would be a pollutant to the environment and unfavorably increase the disposal cost of PMBRs. Therefore, it is necessary to utilize biomass residue waste. Advantageously, such zero-waste strategies would be useful, representing a renewable and sustainable source of energy because the macroalgal biomass contains lipids (>3%) that could be effectively used as a promising feedstock for biodiesel production [2]. Although macroalgal biomass has low lipid content, the fatty acid profiles of lipids are favorable as biodiesel feedstocks, especially that long-chain fatty acid C16–C18 could enhance biodiesel qualities [9]. The utilization of PMBRs not only helps the remediation or removal costs of PMBRs but also saves the overall production costs of macroalga-based industries. Thus, making bioactive polysaccharide profitable and competitive from macroalgal biomass by the integrated production of additional gainful value-added components that have a proven market value should be considered to utilize the macroalgae without generating waste. A zero-waste biorefining technology is a viable and suitable approach and also is indispensable that could maximize the macroalgal biomass utilization for the co-production of chemicals along with fuels [2, 10, 11]. Although there are several reports on the biorefinery of macroalgal biomass, none of them evaluate the use of macroalgal biomass for integrated production of active polysaccharide and biodiesel with desired characteristics.

Therefore, this research aimed to utilize the four types of Thai macroalgal biomasses with a zero-waste biorefining concept for the co-production of bioactive polysaccharide and biodiesel. Four macroalgal types include *Cladophora* spp., *Sargassum* spp., *Spirogyra* spp., and *Ulva spp.*; the key studies are the following sections. Firstly, the macroalgal biomass was hot-water extracted and partially purified by alcohol precipitation to obtain polysaccharide. The polysaccharide was characterized to evaluate the bioactivity potential including antioxidant, antityrosinase, and antiproliferative properties on human cancer cells. The PMBRs was used to extract lipid prior to acid-catalyzed transesterification for biodiesel production. The fatty acid composition and biodiesel qualities of the macroalgal lipids were also measured. Such processes and research outputs thus represent considerable promise for developing macroalgae-based nutraceutical and biofuel applications.

## 2 Materials and methods

### 2.1 Macroalgae collection

Four strains of macroalgae, that are representatives of the genus of edible macroalgae, were studied. These were obtained from the Diversity of Algae and Plankton Research Unit, Science and Technology Research Institute, Chiang Mai University, Thailand, including *Ulva* spp. (collected from Pattani province, Thailand), *Sargassum* spp. (collected from Chonburi province, Thailand), *Cladophora* spp. (collected from Nan province, Thailand), and *Spirogyra* spp. (collected from Phrae province, Thailand). Fresh macroalgae were dried using a hot air oven with a temperature of 60 °C until a constant weight before use.

### 2.2 Polysaccharide extraction and purification

Dry macroalgae were firstly ground into powder, and then polysaccharides were extracted and purified as reported method with minor modifications [12, 13]. Briefly, dry macroalgal powder (30 g) was extracted with 1 L of distilled water at 98 °C for 1 h. The extracts were then concentrated and submitted to graded precipitation with ethanol (1:2 v/v), and the mixture solution was kept at 4 °C for 24 h, then centrifuged at 6000 rpm for 20 min, and dried at 55 °C to obtain crude polysaccharides (CPS). The CPS were partially purified through hot water extraction and acetone precipitation as the same extraction procedure. The pellets obtained after partial purification were partially purified polysaccharide (P-CPS). Total sugar of polysaccharide was determined by phenol-sulfuric acid method as described in Dubois et al. [14] with minor modifications. The purities of P-CPS were estimated using ion-exchange chromatography and the Bradford protein assay [15].

The sugar compositions of polysaccharides extract were determined by high-performance liquid chromatography (HPLC) (UFLC, Shimadzu, Japan) with Shodex VG-50 4E

column. The polysaccharide (1 g) was mixed with 1 M HCl and then heated at 97 °C for 1 h. After cooling, the supernatant was neutralized with 1 M NaOH, concentrated, and filtrated for sugar composition. It was then injected to HPLC, maintained at a temperature of 50 °C, eluted with distilled water at a flow rate of 0.6 mL/min, and detected by a refractive index detector (RID).

## 2.3 Biological activity of polysaccharide extract

### 2.3.1 Determination of antioxidant activity

DPPH and ABTS assay were used to determine the free radical scavenging activity of the polysaccharide extract described by Torres et al. [16] with minor modifications. Each well of microplate was filled with a mixture of 20 μL of polysaccharide extract, standard compounds, or negative controls and 280 μL of a methanolic solution of DPPH. The mixtures were incubated under continuous stirring at 25 °C for 20 min and were then measured at absorbance of 515 nm. The ABTS assay allowed the solution to be prepared in the dark conditions for 16 h at 25 °C with a mixing of 1 mL of 7 mM ABTS and 17.6 μL of 140 mM $K_2S_2O_8$. The mixtures were then diluted prior to an absorbance at 734 nm. The polysaccharide extract (20 μL) and 280 μL of ABTS solution were mixed and incubated in darkness for 20 min at room temperature. Measurements were then taken at 734 nm. The percentage of radical scavenging was established according to Eq. (1):

$$\%\text{radical scavenging} = \left[\left(\left(A_{\text{control}}-A_{\text{blank}}\right)-\left(A_{\text{sample}}-A_{\text{background}}\right)\right)/\left(A_{\text{control}}-A_{\text{blank}}\right)\right] \times 100 \quad (1)$$

where $A_{\text{control}}$ is the absorbance of radicals without sample, $A_{\text{blank}}$ is the absorbance of the deionized water, $A_{\text{sample}}$ is the absorbance of the sample with radicals, and $A_{\text{background}}$ is the absorbance of the sample. The half maximal inhibitory concentration ($IC_{50}$) was calculated as the concentration of the tested sample that inhibited 50% of radical scavenging.

### 2.3.2 Determination of anticancer activity

In order to evaluate the potential of polysaccharide extract for in vitro antiproliferative activity, the cancer cell line (Caco-2 cells) derived from human colon adenocarcinoma and normal cell line (Vero cells) were precultured under the condition following as temperature of 37 °C coupling with feeding 5%$CO_2$ in air and 10% fetal calf serum in DMEM as culture media. The precultured cells of $10^4$ cells were mixed with 100 μL culture media in microplate and then cultured under above conditions for 24 h. The cultured cells were then treated with the tested polysaccharide extract for 48 h by the addition of the polysaccharide extract dissolved in serum-free DMEM medium to give a final concentration of 20, 10, 5, 2.5, and 1.25 μg/ml. The treated cells were then mixed with 20 μl of 2 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and then were continuously incubated for 4 h. After incubation, DMSO (200 μl) was added to each well and mixed to ensure cell lysis and dissolving of the formasan crystals, before the absorbance at 540 nm was measured [15]. The percentage survival of the treated cancer and normal cultured cells were established according to Eq. (2):

$$\%\text{Survival} = \left(A_{\text{treated cancer}}/A_{\text{control}}\right) \times 100 \quad (2)$$

where $A_{\text{treated cancer}}$ is the absorbance of treating culture cells with sample and $A_{\text{control}}$ is the absorbance of culture cells. The half maximal inhibitory concentration ($IC_{50}$) was calculated as the concentration of the tested sample that inhibited 50% of culture cells.

### 2.3.3 Determination of antityrosinase activity

Inhibition of tyrosinase by polysaccharide extract was determined using L-DOPA as substrate following to Karkouch et al. [17] with minor modification. Briefly, a 20 μL of polysaccharide extract was mixed with 80 μL of 50 mM potassium phosphate buffer (pH 6.8) and 10 μL of tyrosinase (50 units/mL) in microplate. The mixtures were incubated for 5 min at 37 °C. After that, 90 μL of 2 M L-DOPA was added to mixtures and then incubated at 37 °C for 10 min. The absorbance of the resulting solution was measured at 475 nm. The percentage of tyrosinase inhibition was established according to Eq. ((3):

$$\%\text{Tyrosinase inhibition} = \left(A_{\text{sample}}/A_{\text{control}}\right) \times 100 \quad (3)$$

where $A_{\text{sample}}$ is the absorbance of the tyrosinase with sample and $A_{\text{control}}$ is the absorbance of tyrosinase without sample.

## 2.4 Fatty acid and biodiesel property analysis

Extraction of lipid from polysaccharide-extracted macroalgal biomass residues (PMBRs) was performed according to Cequier-Sánchez et al. [18] with minor modification. Dried PMBRs (100 mg) were extracted with 10 mL $CH_2Cl_2$/MeOH (2:1 v/v) and sonicated with sonicator (40 kHz) at room temperature with extracted twice times. The extracted lipids were centrifuged to obtain a clear supernatant, and the solvent was removed by feeding nitrogen gas stream. The extracted lipid was dried and weighed. The lipid content was calculated as percentage of lipid to PMBRs. The extracted lipid was converted to fatty acid methyl esters (FAMEs) by acid-catalyzed transesterification [19]. The extracted lipid sample (10 mg) was mixed with 0.5 mL toluene, 1.5 mL of methanol, and 50 μl of 35% conc. HCl and the mixtures were then incubated at 98 °C for 2 h. After cooling, 1 mL of hexane was added and vortexed. The hexane layer

(FAME) was collected prior to analyzing the FAMEs composition using a 7890B Gas Chromatograph equipped with a cross-linked capillary HP-5 column (length 30 m, 0.32 mm I.D, 0.25-μm film thickness) and a flame ionization detector. Operating conditions were as follows: inlet temperature at 230 °C, initial oven temperature at 45 °C held for 2 min, then ramped to 100 °C at 25 °C/min, held for 4 min, then ramped to 200 °C at 5 °C/min, held for 8 min, then ramped to 250 °C at 5 °C/min, held for 6 min, and the detector temperature at 230 °C. The fatty acids were identified by comparing their retention times with known pure standards. The fatty acid profiling was used to calculate the biodiesel properties of macroalgae such as saponification value (SV), iodine value (IV), cetane number (CN), unsaturation degree (DU), long-chain saturated factor (LCSF), cold filter plugging point (CFPP), and high heating value (HHV) using empirical Eqs. ((3), (4), (5), (6), (7), (8), and (9) reported by Srinuanpan et al. [19].

$$\mathrm{SV} = \sum[(560 \times \mathrm{F})/\mathrm{MW}] \tag{3}$$

$$\mathrm{IV} = \sum[(254 \times \mathrm{F} \times \mathrm{D})/\mathrm{MW}] \tag{4}$$

$$\mathrm{CN} = [46.3 + (5458/\mathrm{SV})]-(0.225 \times \mathrm{IV}) \tag{5}$$

$$\mathrm{DU} = \%\mathrm{MUFA} + (2 \times \%\mathrm{PUFA}) \tag{6}$$

$$\mathrm{LCSF} = (0.1 \times \mathrm{C16}) + (0.5 \times \mathrm{C18}) + (1 \times \mathrm{C20}) + (1.5 \times \mathrm{C22}) + (2 \times \mathrm{C24}) \tag{7}$$

$$\mathrm{CFPP} = (3.1417 \times \mathrm{LCSF})-16.477 \tag{8}$$

$$\mathrm{HHV} = 49.43-(0.041 \times \mathrm{SV})-(0.015 \times \mathrm{IV}) \tag{9}$$

where $D$ is the number of double bonds, $F$ is the % of each type of fatty acid, and MW is the molecular weight of corresponding fatty acid. MUFA is the weight percentage of the monounsaturated fatty acids (wt%), and PUFA is the weight percentage of the polyunsaturated fatty acids (wt%).

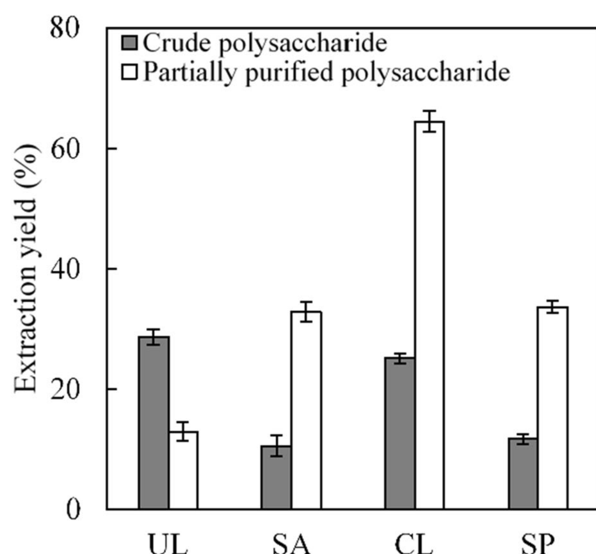
### 2.5 Statistical analysis

All experiments were performed in triplicates. The results are expressed as mean plus standard deviations. Analysis of variance was performed to evaluate significant differences in treatment means, and the least significant difference was used to separate means, using SPSS software.

## 3 Results and discussion

### 3.1 Polysaccharide extraction and purification

Polysaccharide extracts (PS) from four macroalgae biomass including *Ulva* spp., *Sargassum* spp., *Cladophora* spp., and *Spirogyra* spp. were extracted using hot water and then partially purified by alcohol precipitation (resulting in crude polysaccharide extract; CPS). The resulting CPS was re-extracted and re-purified by hot water-alcohol precipitation (resulting in partially purified polysaccharide extract; P-CPS). As shown in Fig. 1a, the extraction yields of CPS were 10.57–28.69%w/w, while extraction yields of P-CPS were in range of 12.96–64.52%w/w of original CPS. The highest P-CPS was observed in *Cladophora* spp. biomass (64.52%w/w) followed by *Sargassum* spp., *Spirogyra* spp., and *Ulva* spp., respective-



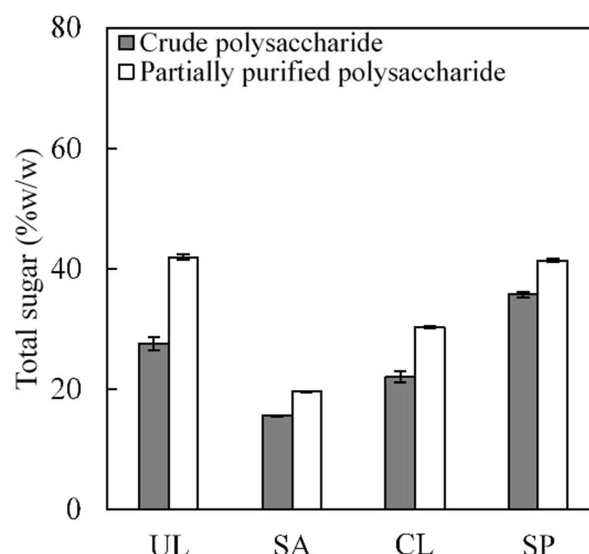




**Fig. 1** The extraction yield (%) and total sugar (%) of polysaccharide extract from four macroalgae. UL, *Ulva* spp.; SA, *Sargassum* spp.; CL, *Cladophora* spp.; SP: *Spirogyra* spp. Data are means of triplicates

ly. This indicated that the repeated both hot-water extraction and alcohol precipitation could be used to obtain a better purity of PS, but the extraction yields decreased from original ones as the hot water extraction would possibly denature all protein and remove them by centrifugation, resulting in higher purity of PS [8]. Hot water extraction and alcohol precipitation are traditional and effective processes and wildly used for extraction and purification of polysaccharide even in industrial scales [8, 20]. The results obtained from PS fractionation by ion-exchange column chromatography and Bradford protein assay confirmed that the proteins were not detectable, indicating the higher purity of PS (data not shown).

Considering total sugar contents and monosaccharide compositions of PS, the total sugar in P-CPS (19.63–41.87%w/w) was also higher than those of CPS (15.60–35.73%w/w) (Fig. 1b). The high total sugar content (>40%w/w) was observed in P-CPS extracted from *Ulva* spp. and *Spirogyra* spp. Based on Table 1, the P-CPS extracted from *Sargassum* spp., *Spirogyra* spp., and *Ulva* spp. had the highest content of rhamnose at 63.62, 42.17, and 35.35 mg/g PS, respectively. However, in P-CPS extracted from *Cladophora* spp., the most abundant monosaccharide was psicose as a rare sugar (28.69 mg/g PS). Xylose was the second most abundant monosaccharide in P-CPS extracted from *Cladophora* spp. (22.16 mg/g PS), *Spirogyra* spp. (12.55 mg/g PS), and *Ulva* spp. (11.10 mg/g PS) but was observed in very low concentration in *Sargassum* spp. (0.79 mg/g PS). In addition, galactose was completely absent in P-CPS extracted from *Ulva* spp. but was found in those of other macroalgae, ranging from 3.89–13.96 mg/g PS. Interestingly, tagatose as a rare sugar was lowly found in only P-CPS extracted from *Ulva* sp. (1.84 mg/g PS) and *Spirogyra* spp. (4.12 mg/g PS). Psicose are also a rare sugar and was found to be in range of 7.12–28.69 mg/g PS but was not observed in P-CPS extracted from *Ulva* spp. Moreover, all P-CPS extract did not observe the allose as a one type of rare sugar. Sugar acids, such as galacturonic acid and glucuronic acid, were also not detectable. It should be noted that the monosaccharide compositions of PS were important differences observed between species. Similar results were also found by He et al. [21] and Robin et al. [22]. Chen et al. [23] and Guerrero-Wyss et al. [24] reported that rare sugar-containing polysaccharide, particularly psicose and tagatose, could be used as a next-generation sugar substitute with low calorie value while also providing desirable sweetness but does not induce lipid metabolism disorder or substantial weight gain.

## 3.2 Bioactivity of partially purified polysaccharide extract

### 3.2.1 Antioxidant activity

P-CPS was selected to evaluate their bioactivity due to higher purity. Antioxidant activity of P-CPS was investigated including their DPPH radical scavenging activity (namely, anti-DPPH) and ABTS radical scavenging activity (namely, anti-ABTS), whereupon assessments were made to determine the $IC_{50}$ value, and the lower $IC_{50}$ value indicates higher effectiveness. As shown in Fig. 2a, all P-CPS extracts exhibited antioxidant activities and also displayed higher anti-ABTS than anti-DPPH. The highest activities of anti-DPPH and anti-ABTS were observed in P-CPS extracted from *Sargassum* spp. biomass with $IC_{50}$ values of 3.50 and 0.86 mg/mL, respectively, followed by P-CPS extracted from *Spirogyra* spp., *Cladophora* spp., and *Ulva* spp., respectively.

**Table 1** Sugar composition of polysaccharide extract from four macroalgae

| Sugar composition (mg/g biomass) | Crude polysaccharide | | | | Partially purified polysaccharide | | | |
|---|---|---|---|---|---|---|---|---|
| | *Ulva* spp. | *Sargassum* spp. | *Cladophora* spp. | *Spirogyra* spp. | *Ulva* spp. | *Sargassum* spp. | *Cladophora* spp. | *Spirogyra* spp. |
| Rhamnose | $11.17^{b}\pm0.12$ | $15.27^{a}\pm0.19$ | N.D. | $9.91^{c}\pm0.01$ | $35.35^{c}\pm0.25$ | $63.62^{a}\pm0.27$ | N.D. | $42.17^{b}\pm0.12$ |
| Xylose | $2.02^{b}\pm0.01$ | N.D. | $16.10^{a}\pm0.07$ | $2.44^{b}\pm0.03$ | $11.10^{b}\pm0.07$ | $0.79^{c}\pm0.01$ | $22.16^{a}\pm0.11$ | $12.55^{b}\pm0.32$ |
| Galactose | N.D. | N.D. | $9.81^{a}\pm0.01$ | N.D. | N.D. | $10.09^{b}\pm0.01$ | $13.96^{a}\pm0.03$ | $3.89^{c}\pm0.01$ |
| Glucose | N.D. | $3.13^{a}\pm0.02$ | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Tagatose | $5.46^{b}\pm0.03$ | N.D. | $4.86^{b}\pm0.03$ | $7.30^{a}\pm0.00$ | $1.84^{b}\pm0.03$ | N.D. | N.D. | $4.12^{a}\pm0.01$ |
| Psicose | N.D. | N.D. | $5.40^{b}\pm0.00$ | $11.40^{a}\pm0.00$ | N.D. | $7.18^{c}\pm0.01$ | $28.69^{a}\pm0.22$ | $12.77^{b}\pm0.16$ |
| Allose | N.D. | N.D. | $0.88^{a}\pm0.06$ | N.D. | N.D. | N.D. | N.D. | N.D. |
| Galacturonic acid | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Glucuronic acid | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |

*N.D.* not detected

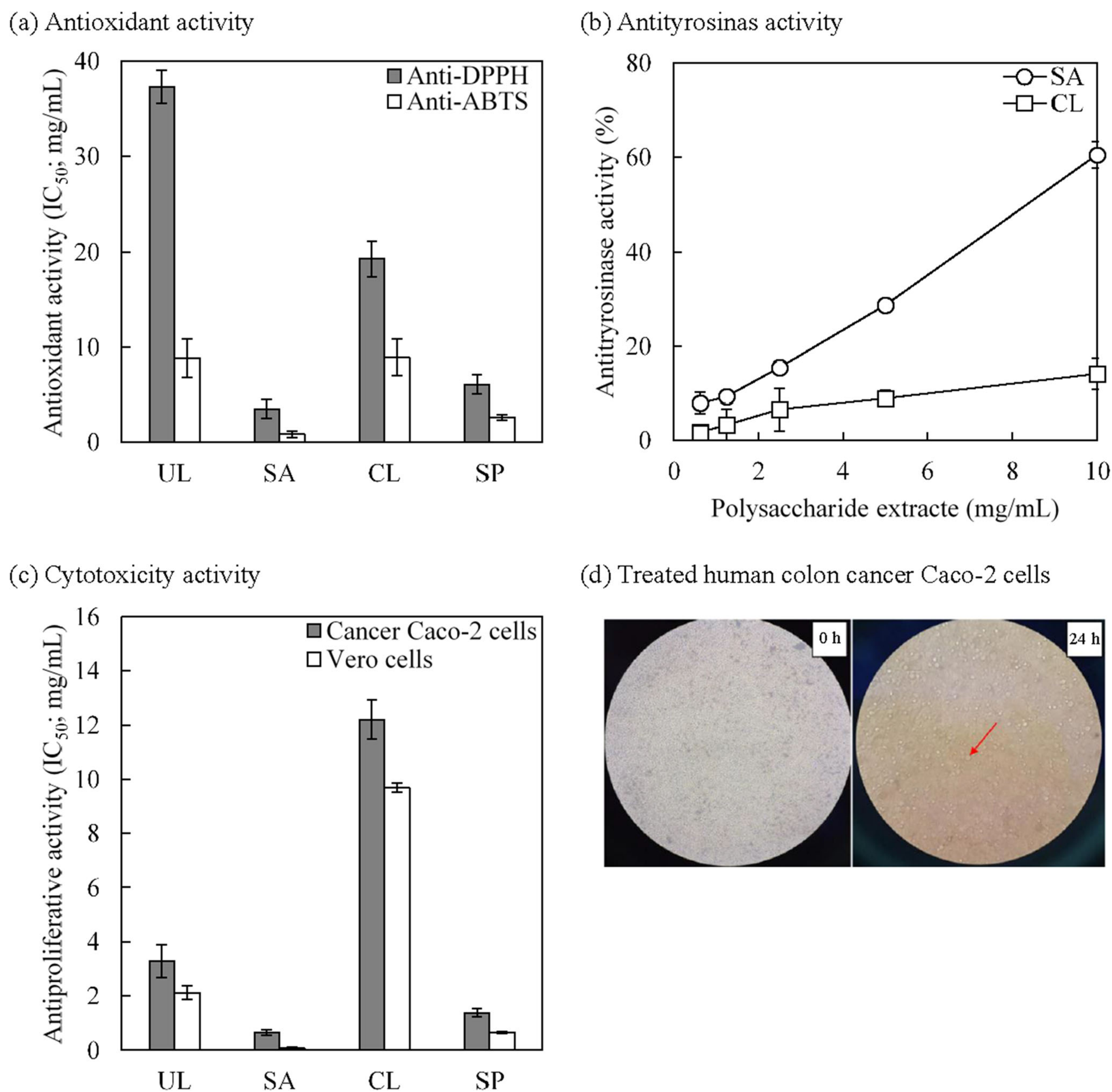



**Fig. 2** Antioxidant activity (**a**), antityrosinase activity (**b**), and cytotoxicity activity (**c**) of partially purified polysaccharide from four macroalgae. (**d**) Image of human colon cancer Caco-2cells before and after 24 h of treating polysaccharide extract from *Sargassum* spp. UL, *Ulva* spp.; SA, *Sargassum* spp.; CL, *Cladophora* spp.; SP, *Spirogyra* spp. Red arrow indicates the bubble in cell morphology. Data are means of triplicates

This result indicated that all P-CPS extracts possibly contain effective proton donors (hydroxyl groups) in sugar, such as rhamnose and xylose, that could react with unstable free radicals of ABTS and DPPH to convert them to more stable products, thereby terminating the radical chain reaction [6]. The solubility of polysaccharide in hydrophilic, lipophilic, and/or hydrophobic systems of DPPH and ABTS assay affects the scavenging of both radicals and resulted in the difference between antioxidant activities. A similar result was observed in Floegel et al. [25].

### 3.2.2 Antityrosinase activity

It is well known that tyrosinase is a key copper-containing enzyme that can catalyze the hydroxylation of L-tyrosine to L-DOPA and subsequently oxidation of DOPA to dopaquinone in melanin synthesis. Inducing melanin formation could be occurred due to prolonged exposure to sunlight and then resulting in too high melanin synthesis that could damage on human skin [6]. Therefore, it is essential to explore the source of natural compounds that exhibit rich antityrosinase activity. As shown

in Fig. 2b, within the range of 0.625 to 10 mg P-CPS/mL, tyrosinase inhibition activity varies between 7.97 and 60.59% for P-CPS extracted from *Sargassum* spp. and 1.74 and 14.16% for P-CPS extracted from *Cladophora* spp., indicating that P-CPS could inhibit the tyrosinase enzyme-binding L-OPDA substrate complex. However, P-CPS-extracted *Spirogyra* spp. and *Ulva* spp. displayed no inhibition of the tyrosinase enzyme. Chen et al. [26] reported that polysaccharide containing effective hydroxyl groups could form hydrogen bonds to a site on the enzyme, leading to steric hindrance or conformational change. Therefore, the P-CPS extracted from *Sargassum* spp. and *Cladophora* spp. could be used as an active ingredient in skin protection due to its higher tyrosinase inhibition in conjunction with antioxidant activity.

### 3.2.3 In vitro antiproliferative activity on human cancer Caco-2 cells

In addition to the antioxidant and antityrosinase capabilities obtained from the studies macroalgae, the antiproliferative of P-CPS was carefully essayed against human colon cancer Caco-2 cells, compared to normal Vero cells. The results are shown in Fig. 2c. All P-CPS exhibited antiproliferative activity on Caco-2 cells with $IC_{50}$ of 0.66–12.20 mg/mL and Vero cells with $IC_{50}$ of 0.07–9.69 mg/mL. The highest antiproliferative activities on Caco-2 cells and Vero cells were observed in P-CPS extracted from *Sargassum* spp. biomass with $IC_{50}$ of 0.66 and 0.07 mg/mL, respectively, followed by *Spirogyra* spp., *Ulva* spp., and *Cladophora* spp., respectively. The microscopic pictures in Fig. 2d show the significant bubble in cell morphology after 24 h of treating Caco-2 cancer cells with P-CPS extracted from *Sargassum* spp. In addition, Vero cells were more sensitive than Caco-2 cells. This indicated that P-CPS could be potentially used as an inhibitor compound to treat the cancer cells, but it would possibly damage the growth of normal cells in the human body. Similarly, Umthong et al. [15] demonstrated that polysaccharide extracted from *Trigona laeviceps* can inhibit the two normal cell lines (namely CH-liver cells and HS27 fibroblast cells). They suggested that adding polysaccharide purification step using size exclusion chromatography gave the highest inhibition effect on cancer cell lines but the lowest inhibition on normal cell. In addition, Trabelsi et al. [4] reported that the low molecular weight of polysaccharides from *Porphyridium cruentum* had stronger antiproliferative activity than the others. Thus, the P-CPS needs to be further minimized, purified, and examined to determine their potential function before acting as the template for future drug design.

## 3.3 Fatty acid profiles of macroalgae lipids extracted from residue biomass and their biodiesel properties

The polysaccharide-extracted macroalgal biomass residues (PMBRs) were used to extract the macroalgal lipid and then were acid transesterified to fatty acid methyl esters (FAMEs). Lipid content and fatty acids (FAs) profiles of PMBRs are summarized in Table 2. The highest lipid content (10.05%) was observed in PMBRs of *Spirogyra* spp. followed by *Ulva* spp. (8.76%), *Cladophora* spp. (4.50%), and *Sargassum* spp. (3.09%), respectively. Similar results were reported by Trivedi et al. [2] and Baghel et al. [27]. They found that the macroalgae lipid extracted from biomass residues could be used as an effective feedstock of biodiesel and nutraceutical with lipid content over 1% of biomass residues. The main FAs compositions of the macroalgal lipid in this study were C16–C18 (>84%). The highest long-chain FA C16-C18 was observed in macroalgal lipid extracted from PMBRs of *Spirogyra* spp. (>96% with high C16:0 of >65%) followed by *Cladophora* spp. (>92%), *Ulva* spp. (>86%), and *Sargassum* spp. (>84%), respectively, which are preferred compositions for biodiesel production [19]. The FAs profiles are composed mostly of palmitic acid (C16:0), stearic acid (C18:0), and elaidic acid (C18:1n9t). The total percentage of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) in all macroalgal lipids were found to be over 90% that higher contents of SFAs and MUFAs both play an important role in terms of biodiesel properties with higher oxidative stability [19]. The higher polyunsaturated fatty acids (PUFAs) content may be regarded as a value-added product for the nutraceutical economy. This study found that the PUFAs in macroalgal lipid were observed between 4 and 12% and the macroalgal lipid extracted from PMBRs of *Spirogyra* spp. gave the highest PUFAs content. Moreover, this study found that the macroalgal lipid extracted from PMBRs of *Sargassum* spp. had the highest ratio of SFAs/UFAs of 9.13 followed by *Cladophora* spp., *Ulva* spp., and *Spirogyra* spp., respectively. Talebi et al. [28] suggested that a higher SFAs/UFAs ratio could improve the oxidative stability of biodiesel with generating high efficiency of combustion due to good values for density and viscosity.

Based on its FAMEs profiles, the properties of biodiesel derived from macroalgal lipids were determined as shown in Table 3. These include saponification value (SV), iodine value (IV), cetane number (CN), unsaturation degree (DU), long-chain saturated factor (LCSF), cold filter plugging point (CFPP), and high heating value (HHV). The SV was found to be 198.15–201.29 mg KOH/g oil that is a calculation of the total molecular weight of all FAs available. A low SV suggests the involvement of long-chain FAs in the lipids. Consequently, IV is an indicator of overall unsaturation of biodiesel and is used to reflect the oxidation stability. A high IV indicated lower oxidative stability [29]. The IV was 21.71–47.92, which corresponded to the regular rate of IV with ≤120 g Iodine/100 g of oil. The CN obtained as over 62 guarantees the better ignition efficiency of the biodiesel and reasonable engine efficiency as well as mitigating engine white

**Table 2** Lipid content and fatty acid profiling of macroalgae lipid extracted from polysaccharide-extracted macroalgae biomass residues (PMBRs) of four macroalgae

| Fatty acids | Relative content (%) | | | |
|---|---|---|---|---|
| | *Cladophora* spp. | *Sargassum* spp. | *Spirogyra* spp. | *Ulva* spp. |
| C14:0 | 3.12$^{c}$±0.14 | 5.00$^{b}$±0.00 | 1.00$^{d}$±0.00 | 9.63$^{a}$±0.21 |
| C14:1 | N.D. | N.D. | N.D. | 0.14$^{a}$±0.28 |
| C15:0 | N.D. | N.D. | 0.32$^{a}$±0.14 | 0.14$^{a}$±0.42 |
| C16:0 | 65.92$^{a}$±0.57 | 59.76$^{a}$±1.70 | 48.29$^{b}$±2.05 | 47.78$^{b}$±1.56 |
| C16:1 | N.D. | N.D. | 1.91$^{b}$±0.07 | 2.65$^{a}$±0.35 |
| C17:0 | 0.47$^{a}$±0.21 | N.D. | 0.54$^{a}$±0.28 | N.D. |
| C17:1 | N.D. | N.D. | 0.34$^{a}$±0.28 | N.D. |
| C18:0 | 17.32$^{b}$2.26 | 24.61$^{a}$±2.76 | 8.17$^{c}$±0.21 | 6.20$^{c}$±0.00 |
| C18:1n9c | 2.91$^{b}$±0.07 | N.D. | N.D. | 4.13$^{a}$±0.21 |
| C18:1n9t | 3.72c±0.14 | N.D. | 26.04$^{a}$±0.28 | 18.99$^{b}$±0.07 |
| C18:2n6c | 2.39$^{b}$±0.07 | N.D. | 8.87$^{a}$±0.21 | 7.09$^{a}$±0.07 |
| C18:3n6 | N.D. | N.D. | 2.05$^{a}$±0.35 | N.D. |
| C20:0 | N.D. | N.D. | 1.12$^{a}$±0.14 | N.D. |
| C20:4n6 | N.D. | N.D. | 0.79$^{a}$±0.07 | 0.37$^{a}$±0.21 |
| C20:5n3 | N.D. | N.D. | N.D. | 0.44$^{a}$±0.28 |
| C22:0 | 0.61$^{a}$±0.07 | 0.42$^{a}$±0.14 | N.D. | N.D. |
| C22:1n9 | 1.03$^{a}$±0.21 | 1.26$^{a}$±0.28 | N.D. | N.D. |
| C22:6n3 | 2.51$^{b}$±0.07 | 8.61$^{a}$±0.07 | N.D. | 0.98$^{c}$±0.14 |
| C24:0 | N.D. | 0.34$^{b}$±0.28 | 0.56$^{b}$±0.28 | 1.46$^{a}$±0.28 |
| C16-C18 | 92.73$^{ab}$±1.91 | 84.37$^{c}$±2.62 | 96.21$^{a}$±1.48 | 86.84$^{bc}$±1.13 |
| SFA | 87.44$^{a}$±3.11 | 90.13$^{a}$±0.92 | 60.00$^{b}$±0.00 | 65.21$^{b}$±1.48 |
| UFA | 12.56$^{b}$±3.11 | 9.87$^{b}$±0.21 | 40.00$^{a}$±0.00 | 34.79$^{a}$±1.48 |
| MUFA | 7.66$^{b}$±0.28 | 1.26$^{c}$±0.28 | 28.29$^{a}$±2.05 | 25.91$^{a}$±0.64 |
| PUFA | 4.90$^{c}$±0.71 | 8.61$^{b}$±0.07 | 11.71$^{a}$±2.05 | 8.88$^{b}$±0.14 |
| SFA/UFA | 6.96$^{a}$±0.28 | 9.13$^{a}$±0.21 | 1.50$^{b}$±0.71 | 1.87$^{b}$±0.21 |
| Lipid content | 4.50$^{b}$±0.30 | 3.09$^{b}$±0.19 | 10.05$^{a}$±1.07 | 8.76$^{a}$±1.41 |

*N.D.* not detected, *SFA* saturated fatty acid, *UFA* unsaturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid

smoke fabrication, since the requirement for CN approved globally is ≥47. The DU was 17.46–51.71, showing biodiesel as a long-term storage stability. The biodiesel flow behavior consisting LCSF and CFPP were 10.80–19.59 °C and 17.45–45.07 °C, respectively, demonstrating inadequate quality in cold regions but is convenient to use in tropical countries. The HHV is the amount of fuel generating thermal after complete burning. The HHV was >40 MJ kg$^{-1}$. The biodiesel with a high carbon content was reported by Knothe [29] to

**Table 3** Estimated properties of biodiesel fuel from macroalgae lipid extracted from polysaccharide-extracted macroalgae biomass residues (PMBRs) of four macroalgae

| Macroalgae | SV | IV | CN | DU | LCSF | CFPP | HHV |
|---|---|---|---|---|---|---|---|
| *Cladophora* spp. | 200.89$^{a}$±0.63 | 21.71$^{c}$±1.21 | 68.73$^{a}$±0.19 | 17.46$^{c}$±0.33 | 16.17$^{ab}$±0.12 | 34.31$^{b}$±0.22 | 40.87$^{a}$±0.09 |
| *Sargassum* spp. | 198.70$^{a}$±0.92 | 39.22$^{b}$±0.55 | 65.10$^{ab}$±0.07 | 18.48$^{c}$±0.34 | 19.59$^{a}$±0.29 | 45.07$^{a}$±0.25 | 40.70$^{a}$±0.21 |
| *Spirogyra* spp. | 198.15$^{a}$±1.31 | 47.92$^{a}$±0.06 | 63.21$^{b}$±0.15 | 51.71$^{a}$±0.21 | 11.71$^{bc}$±0.21 | 20.32$^{c}$±0.23 | 40.59$^{a}$±0.29 |
| *Ulva* spp. | 201.29$^{a}$±0.91 | 47.81$^{a}$±0.13 | 62.81$^{b}$±0.13 | 43.67$^{b}$±0.23 | 10.80$^{c}$±0.14 | 17.45$^{c}$±0.32 | 40.46$^{a}$±0.33 |

Biodiesel fuel parameters included saponification value (SV), iodine value (IV), cetane number (CN), degree of unsaturation (DU), long-chain saturation factor (LCSF), and cold filter plugging point (CFPP)

have a high HHV. Therefore, the macroalgal lipids extracted from PMBRs could be used as a potential biodiesel feedstock, which has favorable biodiesel characteristics that conform with the American Society for Research and Materials (ASTM) and European (EN) international biodiesel requirements.

### 3.4 Proposed biorefinery approach of macroalgal biomass

Presently a viable and sustainable biorefining technology has been used to maximize the valorization of macroalgal biomass for the integrated production of additional useful biochemicals that have a proven market value [2, 27]. According to Trivedi et al. [2], they suggested that the biorefinery process uses fewer reagents than if the biochemical component were extracted individually. In this study, the zero-waste biorefining process for co-production of bioactive nutraceutical and biodiesel from four macroalgal biomass is composed of two main steps of water-alcohol precipitation for preparing bioactive polysaccharide and lipid extraction prior to transesterification of PMBRs to produce macroalgal biodiesel, following the scheme displayed in Fig. 3. The major biomolecule components in microalgal biomass are total carbohydrate (TC) 31.97–56.07%, total protein (TP) 9.13–25.09%, and total lipids (TL) 3.1–10.1%. The bioactive polysaccharide and lipid yield were 105.7–286.9 g/kg-biomass and 27.6–88.7 g/kg-biomass (30.9–100.5 g/kg-PMBRs), respectively. The theoretical biodiesel yield was estimated at 26.5–85.1 g/kg-biomass (29.7–96.5 g/kg-PMBRs), which was calculated based on literature conversion factors [30]. The solvents used in the process are subjected to be easily recovered and reused, which reduces reagent demand resulting in the lower overall production cost of the process. After lipid extraction and acid-catalyzed transesterification, the lipid-free macroalgal biomass residues (LMBRs) were recovered at 650.6–886.7 g/kg-biomass (containing protein in a range of 9.13–25.09%) which could be used as an effective sustainable source for eco-friendly biofertilizer [31, 32] and economically animal feed [33, 34]. It should be noted that the developed biorefinery process in this study could save the production cost of value-added byproducts from macroalgal biomass without generating waste. Thus, these biorefinery processes of macroalgal biomass make it effective approach toward sustainable development of macroalgae-based industries.

## 4 Conclusion

This study showed that the successful biorefinery processes for macroalgal biomass would powerfully enhance the completeness of the macroalgae-based industries. Polysaccharide extracted by water extraction and alcohol precipitation could be used as an effective and potentially bioactive nutraceutical with high antioxidant and anticancer properties. Interestingly, the rare sugars could be found from polysaccharide extract. The study of antityrosinase activity obtained from extracted macroalgal compounds also reveals the high inhibition potential on tyrosinase enzyme which could be used in the skin whitening formulation. In vitro studies of antiproliferative activity on human cancer cells also confirm that the natural components extracted from macroalgal biomass exhibit excellent antiproliferative capability. In addition, we have demonstrated that the polysaccharide-extracted macroalgae biomass residues (PMBRs) are a suitable source of biodiesel feedstocks with generating desirable biofuel properties. This biorefinery process represents a promising zero-waste technology for economically and industrially viable production of food, feed, energy, and chemicals.

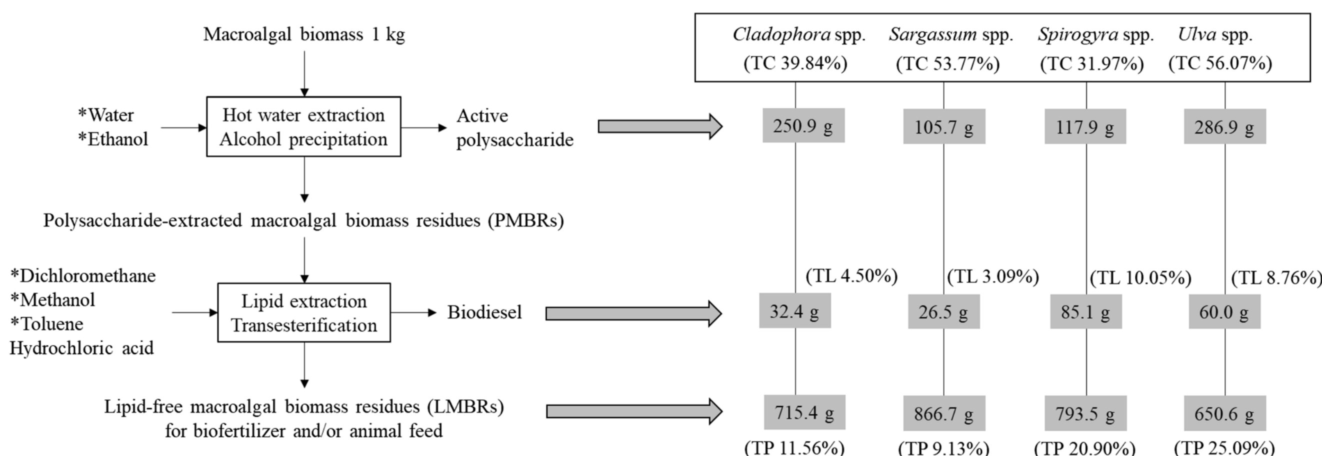



**Fig. 3** Zero-waste biorefinery processes for macroalgal biomass

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## Declarations

**Conflict of interest** The authors declare no competing interests.

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