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Research article

Metabolites and bioactivities of *Caulerpa lentillifera* waste and food grades

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Abstract

Importance of the work: *Caulerpa lentillifera* is widely consumed as human food, resulting in the production of large amounts of agricultural waste on a farming scale.

Objectives: To compare the bioactive compounds and biological activities of food and waste grades of fresh *C. lentillifera* and their crude extract.

Materials & Methods: A fresh sample of *C. lentillifera* was divided into two grades (commercial-grade A and waste-grade B) and two parts (fronds and stolons) for metabolite and biological activity analysis. Subsequently, the sample was further categorized into two grades (food and waste grade) for ethanol extraction and subsequent metabolite and biological activity analysis.

Results: The results for fresh *C. lentillifera* showed that, compared to other samples, the stolons of grade B contained higher amounts of chlorophyll, protein and sugar, while stolons of grades A and B had elevated levels of phenolic compounds and flavonoids. Stolons of grade A, as well as the fronds and stolons of grade B, had greater antioxidant and tyrosinase, α-amylase and α-glucosidase inhibitory activity levels than the fronds of grade A. The results for the crude extract showed that the waste grade had chlorophyll, phenolic compounds, flavonoids, ascorbic acid, antioxidants and anti-enzyme activities at higher levels than for the food grade. The crude extract of the waste grade showed inhibition of tyrosinase, α-amylase and α-glucosidase with half-maximal effective concentrations of 37.59 mg/mL, 13.34 mg/mL and 38.46 mg/mL, respectively.

Main finding: *C. lentillifera* waste could be a valuable source for the extraction of industrial chemicals for medicine, food production and the cosmetics industry.

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Introduction

Seaweeds are major coastal resources that are valuable for human consumption and important in the environment in many countries. For example, there are common seaweed species in the genus *Caulerpa* in tropical and subtropical waters that are widely consumed, with *C. lentillifera* J. Agardh (commonly called green caviar or sea grape) being a popular edible species because of its grass-green color and soft, succulent texture that is usually consumed in its fresh form (Rushdi et al., 2020). In Thailand, *C. lentillifera* is consumed fresh with traditional Thai sauce or salad dressing (Chaiklahan et al., 2020). Additionally, there is a modest production of *Caulerpa* juice made by blending fresh *Caulerpa* with a pinch of vanilla and sugar (Zubia et al., 2020). *C. lentillifera* has high nutritional value, making it an alternative food with health benefits for humans (Nagappan and Vairappan, 2014; Paul et al., 2014).

The thallus of *C. lentillifera* is composed of a horizontal stolon that is attached to a substrate by rhizoids, connecting upright photosynthetic and reproductive fronds (assimilators) which have spherical ramuli and are the edible parts of this species (Wichachucherd et al., 2019). The edible part of *C. lentillifera* is rich in carbohydrates, proteins and lipids, especially polyunsaturated fatty acids, minerals, vitamins and secondary metabolites (de Gaillande et al., 2017).

In Thailand, *C. lentillifera* is one of the commercial seaweed products and there are many farms in the southern region, with current market demand in Thailand being approximately 1 tonne/mth (Chaiklahan et al., 2020). According to the market demand for food-grade *C. lentillifera*—based on the physical qualities of fronds such as size, length, number of branches and color appearance—approximately 60–70% of the biomass is normally below acceptable levels and is discarded as waste (Chaiklahan et al., 2020), producing a large amount of agricultural waste on a commercial farming scale. In addition, *C. lentillifera* in the food market has a short shelf life (5–6 d), after which it is also discarded as waste (Srinorasing et al., 2021).

Research interests have focused on transforming natural waste into products of commercial utility by studying their bioactive compounds and bioactivity, with polyphenol-rich natural materials or extracts being of interest in cosmetic and bioremedial contexts (Kuppusamy et al., 2015). *C. lentillifera* waste from Thailand has been used as material for producing polysaccharide extract (Chaiklahan et al., 2020) and lipid extract (Srinorasing et al., 2021). To date, researchers have focused on the bioactive compounds and bioactivities of *C. lentillifera* for consumption as human food; however, there is only limited knowledge available concerning the bioactive compounds and biological activities of *C. lentillifera* waste. Although there have been comparisons of the nutritional values of *C. lentillifera* waste and food grade (Chaiklahan et al., 2020), there is a lack of information comparing the bioactive compounds and biological activities of this waste and food grade.

Thus, the main objective of the current study was to enhance the value of *C. lentillifera* waste. Therefore, the first aim was to determine the metabolite content and biological activities—antioxidant activity and tyrosinase, α-glucosidase and α-amylase inhibitory activity levels—of fresh *C. lentillifera* for its commercial and waste grades. To do this, the thallus of *C. lentillifera* was divided into fronds and stolons, to assess whether there was a difference between parts. The second aim was to evaluate the metabolite content and biological activities of food grade (only fronds of commercial grade) and waste of *C. lentillifera* crude extracts using ethanol as the solvent. The results should inform the suitability of the seaweed as a valuable source for the extraction of industrial chemicals for medicine, food production and the cosmetics industry

Materials and Methods

Seaweed sample and extract preparation

Samples of the seaweed *C. lentillifera* were collected from Ban Laem, Phetchaburi province (13°02’14.4”N 100°05’11.6”E), along the coastline of the Gulf of Thailand in January 2022, where commercial seaweed culture is conducted in open ponds. Seawater is pumped from the sea through each pond, which is approximately 50 m × 50 m and 1–2 m in depth. The fresh *C. lentillifera* was cleaned and graded to be sold to the market as commercial grade (grade A) or as below grade (grade B), as shown in Fig. 1. After that samples of *C. lentillifera* (grades A and B) were separated into their frond and stolon parts for metabolite, antioxidant and anti-enzyme determination.

For *C. lentillifera* crude extract preparation, *C. lentillifera* was divided into food grade (fronds of grade A) and waste (stolons of grade A, fronds and stolons of grade B). Fresh thalli were cleaned and dried in a hot-air oven at 70°C for 5 d. Dried *C. lentillifera* was processed in a milling machine (2 mm sieve; Polymix; Kinematica, Switzerland) and extracted using

the maceration method. Dried seaweed (30 g) was soaked in 500 mL of absolute ethanol and shaken in an orbital shaker at 100 revolutions per minute (rpm) and 25°C in the dark for 72 hr. Ethanol was selected as the solvent since it is considered safe for further use or application. After passing through Whatman no. 1 filter paper, the solvent was evaporated in a rotary evaporator to yield the crude extract (about 10% of the starting dry material). Then, each dried extract sample was dissolved in absolute ethyl alcohol to make a stock solution of 30 mg/mL and stored at 4°C until used for further analysis.

Metabolite determination of fresh C. lentillifera

Chlorophyll content

Fresh *C. lentillifera* (0.5 g) was soaked in 2 mL of dimethyl sulfoxide (DMSO) and incubated in the dark for 24 hr (Barnes et al., 1992). The green solution was measured at 440 nm, 645 nm and 663 nm using a spectrophotometer (UV 1800; Shimadzu) and calculation of the chlorophyll and carotenoid contents was performed using the equation of Arnon (1949).

Protein content

Fresh *C. lentillifera* (1 g) was extracted with 10 mL of 25 mM potassium phosphate (KP) buffer at pH 7.8 in an ice bath for 5 min and centrifuged at 15,000 rpm and 4°C for 20 min. The supernatant was used to determine the total protein content based on a Bradford protein assay (Bradford, 1976) and bovine serum albumin was used as a standard.

Total sugar content

Fresh *C. lentillifera* (1 g) was extracted with 10 mL of distilled water for 5 min and centrifuged at 5,000 rpm for 20 min. The supernatant was used to determine the total sugar content using the phenol-sulfuric acid method (Dubois et al., 1956) and glucose was used as a standard.

Phenolic compound and flavonoid content

Fresh *C. lentillifera* (1 g) was extracted with 10 mL of absolute ethyl alcohol for 5 min and centrifuged at 5,000 rpm for 15 min at room temperature. The supernatant was used as a sample for phenolic compound determination based on the Folin-Ciocalteu method (Singleton et al., 1999) and the flavonoid content was determination using the aluminum chloride colorimetric method (Zhishen et al., 1999). Gallic acid and quercetin were used as standards for the phenolic compound and flavonoid analyses, respectively.

Ascorbic acid content

Fresh *C. lentillifera* (1 g) was extracted with 5 mL of 5% trichloroacetic acid (TCA) for 5 min and centrifuged at 15,000 rpm and 4°C for 10 min. The supernatant was used to determine the ascorbic acid content based on the ferric chloride in acidic medium method (Li et al., 2012) and ascorbic acid was used as the standard.

Antioxidant properties of fresh C. lentillifera

Sample preparation

Fresh *C. lentillifera* samples (0.5, 1, 2 and 4 g) were extracted with 10 mL of absolute ethanol for 5 min (making the concentrations of the extracts 50 mg/mL, 100 mg/mL, 200 mg/mL and 400 mg/mL, respectively) and centrifuged at 5,000 rpm for 15 min at room temperature. The supernatant was used for the different antioxidant activity assays.

Radical scavenging assay

Radical scavenging of fresh *C. lentillifera* was carried out using the 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) method (Brand-Williams et al., 1995). In brief, 1.9 mL of the supernatant was mixed with 100 µL of 1 mM DPPH and incubated in the dark at room temperature for 30 min. The solution was measured at 517 nm using the spectrophotometer. The percentage of DPPH radical scavenging was calculated from the equation: $[(A_0 - A_1) / A_0] \times 100$, where A_0 is the absorbance of the blank solution and A_1 is the absorbance of the extract solution. The result was reported as the half-maximal effective concentration (EC_{50}). Trolox was used as the standard.

Reducing power assay

The reducing power assay of fresh *C. lentillifera* was performed according to Su et al. (2012), whereby 0.2 mL of the supernatant in 0.5 mL of 0.2 M KP buffer (pH 6.8) was reacted with 1% potassium ferricyanide and incubated at 50°C for 20 min. After adding 0.5 mL of 10% TCA, the solution was mixed with 1.5 mL of distilled water plus 0.1 mL of 0.1% (weight per volume) ferric chloride and incubated for 10 min at room temperature. The absorbance was measured at 700 nm using the spectrophotometer and the result was reported as an EC_{50} value (the effective concentration of the extract at which the absorbance was 0.5). Trolox was used as the standard.

Enzyme inhibition of fresh C. lentillifera

Sample preparation

Fresh *C. lentillifera* samples (0.5, 1, 2 and 4 g) were extracted with 10 mL of distilled water for 5 min (making the concentrations of the extracts 50 mg/mL, 100 mg/mL, 200 mg/mL and 400 mg/mL, respectively) and centrifuged at 5,000 rpm for 15 min at room temperature. The supernatant was used for the different enzyme inhibition assays.

Tyrosinase inhibition assay

The tyrosinase inhibition assay was measured using 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA) as a substrate (Liang et al., 2012). The supernatant (100 µL) was mixed with 100 µL of the mushroom tyrosinase solution (500 U/mL) and 1,800 µL of KP buffer (0.2 M, pH 7.0). After incubation in a water bath at 30°C for 15 min, 50 µL of 10 mM L-DOPA solution was added and the absorbance at 475 nm was immediately monitored using the spectrophotometer at 0 and 6 min. The percentage inhibition of tyrosinase activity was calculated using the equation: $[(\Delta A_{control} - \Delta A_{sample}) / \Delta A_{control}] \times 100$, where $\Delta A_{control}$ is the change in absorbance at 475 nm of the control (distilled water) and ΔA_{sample} is the change in absorbance at 475 nm with the extract. The EC_{50} value was also reported. Arbutin was used as the standard.

α-Amylase inhibition assay

The aqueous sample (80 µL) was mixed with 100 µL of α-amylase from a porcine pancreas (5 U/mL in 40 mM KP buffer, pH 6.9). After incubation at 37°C for 10 min, 20 µL of 2-chloro-4-nitrophenyl-α-D-maltotrioside (6.6 mg/mL) was added and the absorbance was immediately measured at 405 nm using the spectrophotometer at 0 and 5 min (Kumar et al., 2011). The percentage inhibition of α-amylase activity was calculated using the equation $[(\Delta A_{control} - \Delta A_{sample}) / \Delta A_{control}] \times 100$, where $\Delta A_{control}$ is the change in absorbance at 405 nm of the control (distilled water) and ΔA_{sample} is the change in absorbance at 405 nm with the extract. The EC_{50} value was also reported. Acarbose was used as the standard.

α-Glucosidase inhibition assay

The aqueous sample (50 µL) was incubated with 100 µL of the α-glucosidase solution (1 U/mL in 20 mM KP buffer, pH 6.9) at 37°C for 10 min. Then, 50 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside in the same buffer was added and the absorbance at 405 nm was immediately monitored using the spectrophotometer at 0 and 6 min (Apostolidis et al., 2007). The percentage inhibition of α-glucosidase activity was calculated using the equation $[(\Delta A_{control} - \Delta A_{sample}) / \Delta A_{control}] \times 100$, where $\Delta A_{control}$ is the change in absorbance at 405 nm of control (distilled water) and ΔA_{sample} is the change in absorbance at 405 nm with the extract. The EC_{50} value was also reported. Acarbose was used as the standard.

Metabolite determination of C. lentillifera ethanolic crude extract

The ethanolic crude extract stock solution (30 mg/mL) was used for the determination of chlorophyll, carotenoids (Arnon, 1949; Barnes et al., 1992), total phenolic compounds (Singleton et al., 1999), flavonoids (Zhishen et al., 1999) and ascorbic acid (Li et al., 2012) content.

Antioxidant property of C. lentillifera ethanolic crude extract

Concentrations of the ethanolic crude extract (0, 2.5, 5, 10 and 20 mg/mL) were prepared from the stock solution and used for the determination of antioxidant activity based on DPPH assay (Brand-Williams et al., 1995), reducing power assay (Su et al., 2012) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay (Re et al., 1999). The antioxidant activity of the ethanolic crude extract was reported as an EC_{50} value.

Enzyme inhibition of C. lentillifera ethanolic crude extract

The *C. lentillifera* crude extract samples (0, 5, 10, 20 and 40 mg/mL) were prepared in 10% DMSO and used for the determination of enzyme tyrosinase inhibition (Liang et al., 2012). The crude extract (0, 2.5, 5, 10 and 20 mg/mL) was also prepared in 10% DMSO for the determination of α-amylase inhibition (Kumar et al., 2011) and α-glucosidase inhibition (Apostolidis et al., 2007). The 10% DMSO was used as the control for percentage inhibition calculation. The enzyme inhibition activity of the crude extract was reported as an EC_{50} value.

Statistical analysis

All tests were carried out in triplicate and the R software program package (R Core Team, 2015) was used for statistical analysis. Data were subjected to analysis of variance. Multiple comparison tests and two comparison tests were performed (Duncan's multiple range test and a t test) with $p < 0.05$ considered as a statistically significant difference.

Results and Discussion

Fresh C. lentillifera analysis

The highest chlorophyll contents were recorded in the stolons of grade B and the highest carotenoids contents were recorded in the stolons of grade A. These result agreed with the difference in color levels of the *C. lentillifera* thallus for fronds and stolons (Fig. 1). Chloroplasts of *Caulerpa* can be transported between different thallus parts, due to the siphonous structure of the seaweed, possibly resulting in different chlorophyll and carotenoid contents and consequently different color levels in the fronds and stolons (Stuthmunn et al., 2022). Additionally, the chlorophyll content in the current study was higher than that of *C. lentillifera* cultured in tropical Australia, as reported by Paul et al. (2014).

Fig. 1 *Caulerpa lentillifera* grades: (A) and (B) commercial grade or grade A; (C) and (D) waste grade or grade B

Stolons of grade B had the highest total protein and total sugar contents, while both grade A and grade B stolon parts had significantly higher amounts of phenolic compounds and flavonoids compared to their respective frond parts (Table 1). Conversely, stolons of grade A had the lowest ascorbic acid content, whereas other samples had ascorbic acid levels in the range 0.15–0.17 mg/g fresh weight (FW; equivalent to 15–17 mg/100 g FW), as shown in Table 1. These findings regarding the ascorbic acid content aligned with de Gaillande et al. (2017), who reported substantial quantities of vitamin C in *C. lentillifera*, reaching up to 34.70 mg/100 g FW.

The antioxidant activity levels in the different parts and grades of fresh *C. lentillifera* were investigated based on the DPPH and reducing power methods. The antioxidant activity levels in both DPPH radical scavenging and reducing power of all samples increased with concentration (Figs. 2A and 2B). The stolons of grades A and B proved effective in DPPH radical scavenging, whereas the fronds and stolons of grade B proved effective in reducing power, with low EC_{50} values (Table 2). There was a correlation between the phenolics (phenolic compounds and flavonoids) and the EC_{50} values of *C. lentillifera*. When the phenol content was high, the EC_{50} was low, resulting in high antioxidant activity. Being exposed to the environment of light and oxygen that favors the production of free radicals and oxidizing agents, seaweeds may have strong antioxidant systems to protect themselves from oxidative damage (Nguyen et al., 2011).

Tyrosinase or polyphenol oxidase is responsible for the catalyzing reactions of melanin synthesis resulting in the epidermal hyperpigmentation of human skin that can lead to various dermatological disorders (Kim and Uyama, 2005). The tyrosinase inhibition of fresh *C. lentillifera* of all the tested samples increased with increasing concentrations of sample (Fig. 2C). The stolons of grade A and the fronds and stolons of grade B were efficient in inhibiting enzyme tyrosinase to a significantly higher extent than the fronds of grade A (Table 2).

Table 1 Metabolite contents of fresh *Caulerpa lentillifera* in different parts and grades

Sample	Chlorophyll a (µg/g FW)	Chlorophyll b (µg/g FW)	Total chlorophyll (µg/g FW)	Carotenoid (µg/g FW)	Protein (mg/g FW)	Total sugar (mg/g FW)	Phenolic (mg GAE/g FW)	Flavonoid (mg QE/g FW)	Ascorbic acid (mg/g FW)
Frond A	4.95±0.33^{b}	3.98±0.41^{b}	9.45±0.61^{b}	2.82±0.22^{b}	4.09±0.06^{b}	1.38±0.05^{b}	2.82±0.62^{b}	2.67±0.22^{b}	0.15±0.01^{a}
Stolon A	4.67±0.23^{b}	3.87±0.19^{b}	9.37±0.89^{b}	4.46±0.52^{a}	4.10±0.06^{b}	1.58±0.03^{b}	5.48±0.57^{a}	3.68±0.05^{a}	0.09±0.01^{b}
Frond B	5.77±0.28^{b}	4.85±0.44^{b}	10.70±0.61^{b}	1.88±0.21^{b}	3.99±0.05^{b}	1.58±0.10^{b}	2.65±0.17^{b}	1.36±0.05^{c}	0.17±0.02^{a}
Stolon B	12.98±0.82^{a}	14.62±1.75^{a}	25.12±0.55^{a}	2.78±0.25^{b}	4.41±0.03^{a}	1.91±0.10^{a}	5.35±0.64^{a}	3.31±0.22^{a}	0.15±0.00^{a}

FW = fresh weight

Values (mean ± SD) with different lowercase superscripts in same column are significantly ($p < 0.05$) different based on Duncan's multiple range test.

Table 2 Half-maximal effective concentration (EC_{50}) values of antioxidant activity and enzyme inhibition of fresh *Caulerpa lentillifera*

Sample	DPPH (mg/mL)*	Reducing power (g/mL)*	Tyrosinase inhibition (mg/mL)†	α-Amylase inhibition (mg/mL)‡	α- Glucosidase inhibition (mg/mL)‡
Frond A	398.53±19.79[a]	6.46±0.30[a]	204.86±1.92[a]	521.67±7.71[a]	511.55±12.70[a]
Stolon A	197.85±20.92[c]	2.48±0.04[b]	159.28±6.37[b]	230.24±9.96[b]	411.33±41.94[c]
Frond B	281.42±6.00[b]	1.65±0.04[c]	162.90±5.82[b]	216.61±1.45[b]	453.55±25.37[b]
Stolon B	216.03±3.61[c]	1.65±0.04[c]	173.16±3.05[b]	160.76±4.80[c]	359.61±11.90[c]

Values (mean ± SD) with different lowercase superscripts in same column are significantly ($p < 0.05$) different based on Duncan's multiple range test.
*EC_{50} DPPH and reducing power of Trolox were 9.58 µg/mL and 25.75 µg/mL, respectively.
†EC_{50} tyrosinase of arbutin was 81.44 µg/mL.
‡EC_{50} α-amylase and α-glucosidase of acarbose were 46.20 µg/mL and 0.15 µg/mL, respectively.

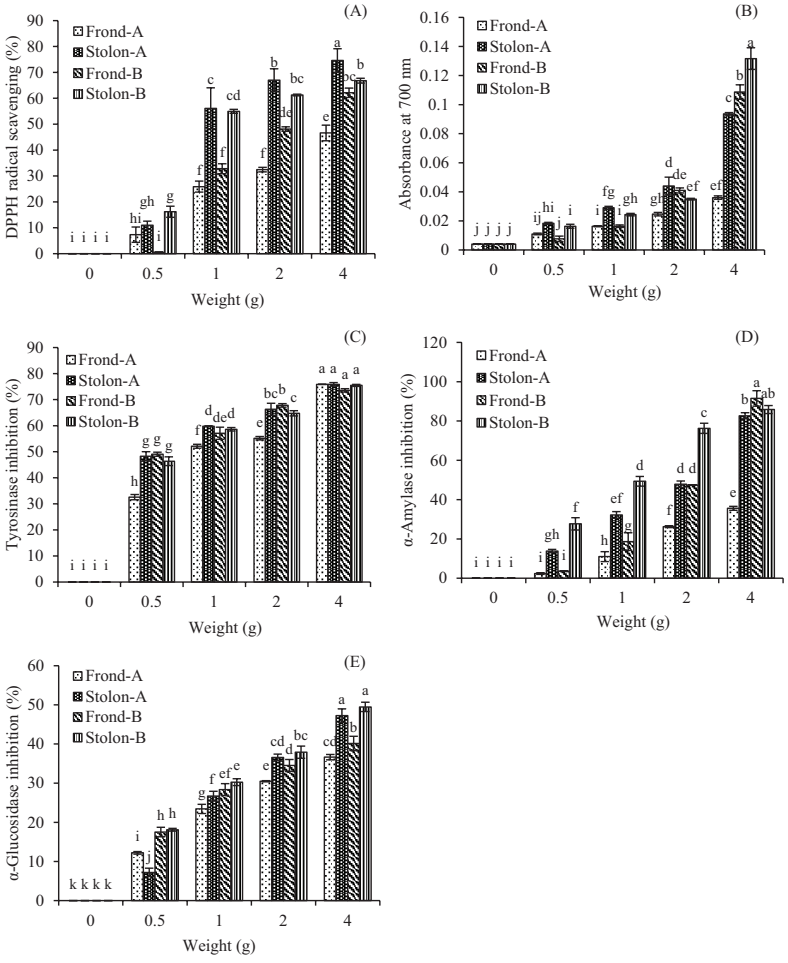

Fig. 2 Biological activities of fresh *Caulerpa lentillifera* based on: (A) DPPH radical scavenging; (B) reducing power; (C) tyrosinase inhibition; (D) α-amylase inhibition; (E) α-glucosidase inhibition, where bars and error bars represent means and ± SD, respectively. Different lowercase letters above bars denote significant ($p < 0.05$) difference among means.

The enzyme α-amylase is responsible for the breakdown of long chain carbohydrates, while α-glucosidase breaks down oligosaccharides and disaccharides to release glucose (Yang et al., 2019). The inhibition of these two enzymes can effectively control the postprandial increase of blood glucose in type 2 diabetes mellitus patients. The effects of fresh *C. lentillifera* on the activities of carbohydrate-hydrolyzing enzymes increased in a concentration-dependent manner (Figs. 2D and 2E). The stolons of grade B had the highest inhibition of α-amylase and α-glucosidase activity with the lowest EC_{50} (Table 2).

Overall, stolon B had a higher metabolite content than the other samples, leading to increased biological activity. This suggested that metabolites, such as chlorophyll, phenolic compounds and flavonoids, may contribute to its biological activities. Similarly, stolon A had higher levels of carotenoids, phenolic compounds and flavonoids compared to frond B, correlating with its elevated biological activity. Conversely, frond B had greater effectiveness in biological activities than frond A, despite no significant difference in their metabolite content. This could be attributed to the presence of other bioactive compounds in frond B, such as monosaccharide or fatty acid (Chaiklahan et al., 2020; Srinorasing et al., 2021), or the synergistic effects of the metabolites within the frond.

In the current study, variations were observed in the metabolites and biological activities of *C. lentillifera* across different parts and grades. Conversely, Chaiklahan et al. (2020) found no significant differences in the nutritional values—moisture, ash, total sugar, protein, lipids, chlorophyll a, chlorophyll b and carotenoids—between food-grade *C. lentillifera* and its waste. Additionally, the proportion of fronds to stolons in *C. lentillifera* was reported to be approximately 68% (Paul et al., 2014). An evaluation of the different parts of *C. lentillifera* describing the presence of metabolites and their biological activities would be an important step to better understand their importance as materials used in industry. The results of the current study highlight the variations in metabolite content and biological activities among different parts and grades of *C. lentillifera*. Generally, the stolons of grade A and the stolons and fronds of grade B samples had higher metabolite contents and biological activities compared to the fronds of grade A, which are the edible part. Given that fronds of grade A are consumed as food, while stolons of grade A and stolons and fronds of grade B are considered waste, the *C. lentillifera* was categorized for the subsequent extraction step. Fresh *C. lentillifera*-derived natural compounds and antioxidants could be used to extract bioactive substances for commercial benefits.

C. lentillifera crude extract analysis

Based on the above results, the fresh *C. lentillifera* contained metabolites and antioxidant molecules, along with antioxidant activity, as well as properties of tyrosinase, α-glucosidase and α-amylase inhibition. To replicate these metabolites and bioactivities for food grade (fronds of grade A) and waste grade (stolons of grade A, fronds and stolons of grade B), crude extract of *C. lentillifera* was prepared using absolute ethanol as the solvent extraction method.

The findings from the study on the crude extract of *C. lentillifera* presented a consistent trend with those from the study on fresh *C. lentillifera*. Waste grade *C. lentillifera* had higher contents of chlorophyll, phenolic compounds, flavonoids and ascorbic acid, along with demonstrating greater activity in antioxidants and enzyme inhibition compared to the food grade samples (Table 3).

The total phenolic compound content of *C. lentillifera* obtained in the current study (4.33–8.37 mg GAE/g dry weight (DW), equivalent to 43.31–71.78 mg GAE/g extract) was higher than that of the *C. lentillifera* ethanolic extract from Taiwan (1.30 mg GAE/g DW; Nguyen et al., 2011) and the *C. lentillifera* methanolic extract from Borneo (42.85 mg phloroglucinol equivalent/g extract; Matanjun et al., 2008), suggesting that the location affects the phenolic compound content in *C. lentillifera*. In addition, Chaiklahan et al. (2020) reported that polysaccharide extract from *C. lentillifera* waste contained 1.23 mg GAE/g sample of total phenolic compounds, whereas Srinorasing et al. (2021)

Table 3 Metabolite contents, antioxidant activity and some enzyme inhibition of ethanolic crude extract of *Caulerpa lentillifera*

Characteristic	*C. lentillifera* component	
	Food grade	Waste
Metabolite content		
Chlorophyll a (mg/g DW)	0.17±0.00^{a}	0.14±0.00^{b}
Chlorophyll b (mg/g DW)	0.09±0.00^{b}	0.14±0.00^{a}
Total chlorophyll (mg/g DW)	0.27±0.00^{b}	0.28±0.00^{a}
Carotenoid (mg/g DW)	Nd	Nd
Phenolic compound (mg GAE/g DW)	4.33±0.06^{b}	8.37±0.11^{a}
Flavonoid (mg QE/g DW)	0.92±0.03^{b}	1.91±0.28^{a}
Ascorbic acid (mg/g DW)	0.08±0.00^{b}	0.14±0.00^{a}
Antioxidant activity		
EC_{50} of DPPH (mg/mL)†	20.48±0.57^{a}	16.58±0.74^{b}
EC_{50} of reducing power (mg/mL)†	34.07±0.30^{b}	35.59±0.29^{a}
EC_{50} of ABTS (mg/mL)†	8.70±0.14^{a}	4.36±0.18^{b}
Enzyme inhibition		
EC_{50} of tyrosinase inhibition (mg/mL)‡	91.30±6.70^{a}	37.59±1.55^{b}
EC_{50} of α-amylase inhibition (mg/mL)§	55.29±1.26^{a}	13.34±0.42^{b}
EC_{50} of α-glucosidase inhibition (mg/mL)§	55.59±1.27^{a}	38.46±3.07^{b}

DW = dry weight; Nd = not detected.

Values (mean ± SD) with different lowercase superscripts in same row are significantly ($p < 0.05$) different based on by t test.

† EC_{50} DPPH, reducing power and ABTS of Trolox were 9.58 µg/mL, 89.93 µg/mL and 25.75 µg/mL, respectively.

‡ EC_{50} tyrosinase of arbutin was 81.44 µg/mL.

§ EC_{50} α-amylase and α-glucosidase of acarbose were 46.20 µg/mL and 0.15 µg/mL, respectively.

found that crude lipid and purified lipid extracts from *C. lentillifera* waste contained total phenolic compounds of 2.07 mg GAE/g sample and 17.46 mg GAE/g sample, respectively. The flavonoid content of the *C. lentillifera* waste in the current study was 1.91 mg QE/g DW (equivalent to 19.15 mg QE/g extract) which was higher than the crude lipid extract but lower than the purified lipid extract from *C. lentillifera* waste (5.40 mg QE/g sample and 55.48 mg QE/g sample, respectively) reported by Srinorasing et al. (2021). This indicated that the ethanolic crude extract of *C. lentillifera* waste had a higher phenolic compound and flavonoid contents compared to the polysaccharide and lipid extracts from *C. lentillifera* waste, which may be due to the polysaccharide and crude lipid extraction process causing the loss of some phenolic compounds.

Generally, the antioxidant effects (DPPH and ABTS radical scavenging and reducing power) of *C. lentillifera* crude extract in both the food and waste grades increased with increasing concentrations of the samples (Figs. 3A–3C). This was in agreement with Matanjun et al. (2008), Nguyen et al. (2011) and Osotprasit et al. (2021), who reported that *C. lentillifera* crude extract exhibited antioxidant activity. The EC_{50} values of DPPH and ABTS of *C. lentillifera* waste crude extract in this study (Table 3) were lower than those of the polysaccharide extract from *C. lentillifera* waste (74.03 mg/mL and 10.31 mg/mL for DPPH and ABTS, respectively), according to Chaiklahan et al. (2020), but higher than for lipid extraction (Srinorasing et al., 2021). This revealed that the ethanolic crude extract of *C. lentillifera* waste had greater antioxidant activity than the polysaccharide extract but lower than the purified lipid extract from *C. lentillifera* waste.

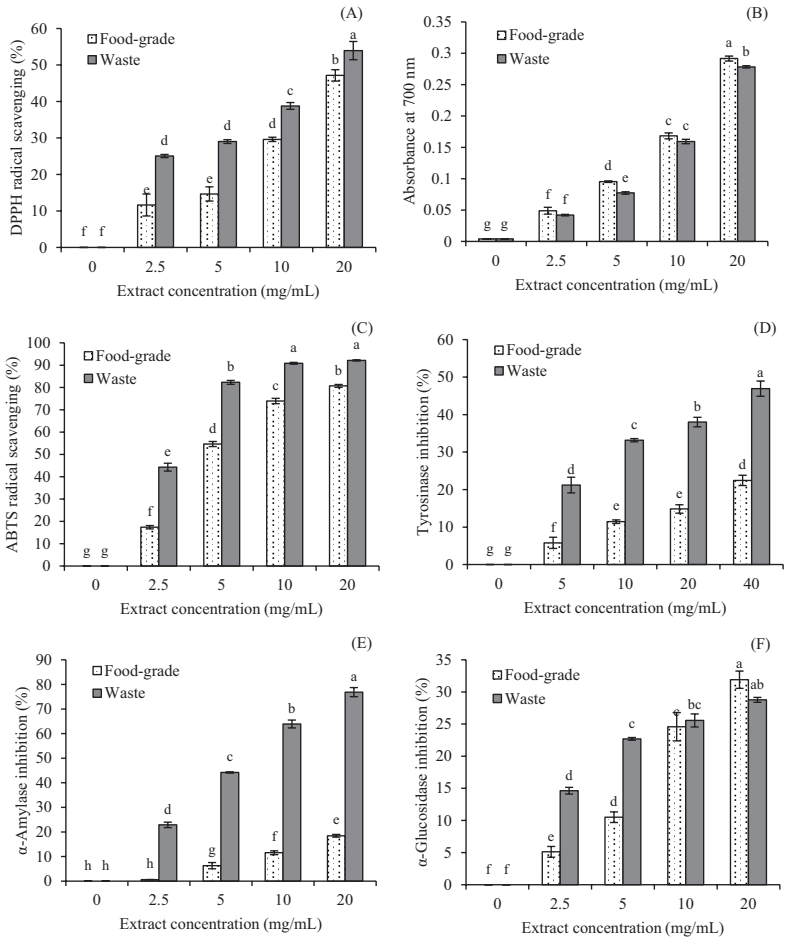

Fig. 3 Biological activities of *Caulerpa lentillifera* ethanolic crude extract: (A) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging; (B) reducing power; (C) 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging; (D) tyrosinase inhibition; (E) α-amylase inhibition; (F) α-glucosidase inhibition, where bars and error bars represent means and ± SD, respectively. Different lowercase letters above bars denote significant ($p < 0.05$) difference among means.

The crude extract of *C. lentillifera* waste exhibited tyrosinase inhibitory activity in a concentration-dependent manner, with EC_{50} values of 91.30 mg/mL and 37.59 mg/mL for the food and waste grade samples, respectively (Fig. 3D and Table 3). It appeared that the current study was the first to report on the evaluation of the anti-tyrosinase activity of *C. lentillifera*, with the results suggesting that the natural substances derived from *C. lentillifera* waste could contribute as raw materials for the development of safe cosmetics.

The crude extract of *C. lentillifera* had carbohydrate-hydrolyzing enzyme inhibitory activity in a concentration-dependent manner (Figs. 3E and 3F), with the crude extract from *C. lentillifera* waste exhibiting α-amylase and α-glucosidase inhibitory activity levels that were higher than for the food grade, with lower EC_{50} values (Table 3). The EC_{50} value of α-glucosidase of waste crude extract (38.46 mg/mL) was higher than that of polysaccharide (13.59 mg/mL) and crude lipid extract (8.97 mg/mL) from *C. lentillifera* waste (Chaiklahan et al., 2020; Srinorasing et al., 2021). The potential of *C. lentillifera* ethanolic extract for antidiabetic activity was consistent with Sharma and Rhyu (2014), who reported that the ethanolic extract of *C. lentillifera* from the Philippines minimized dipeptidyl peptidase-IV and α-glucosidase enzyme activity. In addition, the *C. lentillifera* ethanolic extract collected from Okinama, Japan, had an anti-diabetic property by regulating the glucose metabolism in mice (Sharma et al., 2015). Therefore, the ethanolic extract of *C. lentillifera* could be a potential candidate for the prevention of diabetes.

The waste from *C. lentillifera* contained higher amounts of bioactive compounds and demonstrated greater antioxidant and anti-enzyme activity levels compared to the food grade. These bioactive compounds contributing to the antioxidant activity of *C. lentillifera* include chlorophyll, carotenoids, phenolic compounds, flavonoids and ascorbic acid, all of which could play an important role in its bioactivity. Furthermore, one studied showed that the ethanolic extract of *C. lentillifera* was non-toxic and had a high antioxidant capacity (Osotprasit et al., 2021). The presence of bioactive compounds and the biological activities in *C. lentillifera* waste suggest substantial commercial potential in various fields such as medicine, food production and the cosmetics industry. Utilizing *C. lentillifera* waste presents an alternative approach with substantial benefits.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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