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Chemical Profile, Antioxidant Activity and α-Glucosidase Inhibition of Sea Grape *Caulerpa lentillifera* Collected from Different Sites in Thailand

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Abstract

Caulerpa lentillifera (sea grapes) stands out for its nutritional profile and associated health advantages. Seaweeds, including sea grapes, exhibit diverse chemical compositions that are influenced by environmental conditions. This study aimed to compare the chemical composition, metabolite content, antioxidant activity and α-glucosidase inhibition of sea grapes collected at different sites (Phetchaburi and Trang) and at different times during the month of January, April and July 2022. Both collection times and sites have influenced all assessed parameters. The sea grapes collected from Trang had a significantly higher amount of metabolite, antioxidant activity and α-glucosidase inhibitory activity than those from Phetchaburi at $p < 0.001$. The highest amount of phenolic compound (93.76 ± 2.39 mg GAE/g extract) and reducing power (EC_{50} = 25.86 ± 0.20 mg/mL) occurred in sea grapes in April month, while those collected in January and April had high DPPH and ABTS radical scavenging (EC_{50} < 7.50 mg/mL). The highest inhibitory activity against α-glucosidase (EC_{50} = 19.27 ± 0.40 mg/mL) was found in the sea grapes collected in January. The LC-QTOF MS/MS result of sea grape extract indicated similar compounds in amino acids, fatty acids, lipids, nucleic acids, carboxylic acids, cyanogenic glucosides, steroids, terpenoids and vitamins. The disparity in phenolic profiles between Phetchaburi (9) and Trang (14) sea grapes was related to higher phenolic compound and flavonoid contents, coupled with antioxidant properties in Trang. These findings supported the possible use of sea grapes from Trang as a reservoir of bioactive compounds.

Keywords: LC-QTOF MS/MS analysis, Metabolite, Phenolic compound, Seaweed

Introduction

Nowadays, there is a quest for supplementing human food with antioxidants and nutraceuticals from natural resources or nonconventional food sources. Seaweed is classified as an important choice due to its high content of health-beneficial bioactive compounds. Therefore, it has the advantage of being a better alternative food with therapeutic potential [1].

Caulerpa is green seaweed and is the only genus of the family Caulerpaceae that has a circum-tropical to warm temperate geographic distribution [2,3]. Among the genus *Caulerpa*, *C. lentillifera* J. Agardh holds high economic value because it is widely consumed throughout the Pacific and Southeast Asia [4].

C. lentillifera, also known as sea grapes, is referred to as green caviar in Europe, umibudo in Japan, or rong nho in Vietnam. This edible seaweed is rich in protein, amino acids, polyunsaturated fatty acids, minerals (macro-mineral and trace minerals), dietary fibers, vitamins (B1, B2, B3, A, C and E), along with bioactive compounds are beneficial to human health [3-6]. Sea grapes are typically consumed in the form of fresh salad.

Previously, several biological activities of sea grapes extract or compounds derived from sea grapes have been reported, including antioxidant [7,8], anti-diabetes [9], anticancer [10], anti-tumor [11], anti-hypertensive [5], anti-coagulant [12], anti-inflammatory [13], anti-pyretic [14], immunostimulatory [15], anti-bacteria [13] and chelating agent [16]. Moreover, there is no evidence of cellular toxic effects [7].

These observed biological activities suggest that sea grapes hold significant potential for pharmaceutical and nutraceutical uses that are harmless for human health.

Due to the high demand for sea grapes for human consumption, market prices for domestic production are rising, leading to an increase in commercial aquaculture production. Previously, sea grape species were mostly collected from the wild and sold in markets. However, there is now a range of culture techniques for sea grape farming, including bottom planting, off-bottom culture, floating long lines or land-based raceways [3]. In Thailand, there are numerous sea grape farms, particularly in the southern region. Sea grape aquaculture is primarily located in near-shore farming operations. The Andaman Sea and the Gulf of Thailand coastline in the south are key areas for economic sea grape cultivation, even though these two coasts differ in terms of topography and climate.

In general, the nutritional values and bioactive substances of seaweed can vary widely depending on the taxon, life cycle, harvesting season and environmental conditions in which the seaweed grows [3]. Seaweeds produce bioactive compounds and secondary metabolites due to the demanding, competitive and aggressive environments they inhabit, compared to terrestrial environments [3]. Recent reports have shown that the nutrient and phytochemical profile of sea grapes differs from one region to another depending on various external factors [6].

To the best of the authors' knowledge, data on the metabolite composition and antioxidant activity of sea grapes harvested from different locations in Thailand are not yet available. Sea grape samples collected in January, April and July from two different sites—the Gulf of Thailand in the east and the Andaman Sea in the west—were compared for their metabolite content and profile as well as biological activity. The three different time collections were conducted to gain insight into the chemical change during seasonal variations. This study aimed to assess the change in metabolite profile, antioxidant activity and α-glucosidase inhibitory activity of sea grapes harvested from two aquaculture sites in the coastal regions of Thailand at different times. The study is highly useful to identify the location and harvesting period of sea grapes with significant metabolite profile and to exploit as potential sources of healthy supplement.

Materials and methods

Reagents

The analytical graded chemicals and research-supporting materials were used for the analysis. Folin Ciocalteu (FC) reagent, quercetin and *p*-nitrophenyl-α-D-glucopyranoside were purchased from Merck (Darmstadt, Germany). Gallic acid, acarbose, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and α-glucosidase were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Ascorbic acid, Trolox, $K_2S_2O_8$, trichloroacetic acid (TCA), Na_2CO_3, $NaNO_2$, NaOH, ferric chloride, dimethyl sulfoxide (DMSO) and absolute ethyl alcohol were purchased from Daejung Chemical & Metals Co., Ltd. (Korea). Potassium ferricyanide, 1,10-phenanthroline and meta-phosphoric acid were purchased from Ajax Finechem Pty Ltd. (Australia). Distilled water was used throughout the experiments.

Seaweed samples and study sites

The seaweed, sea grape, was collected from two different study sites. Firstly, sampling in Phetchaburi was conducted at Ban Laem, Phetchaburi Province (13°02'14.4" N 100°05'11.6" E), which is the coastline in Gulf of Thailand. The collection took place in January, April and July 2022. The Gulf of Thailand is a semi-enclosed basin, and its environment is influenced by hydrodynamic circulation patterns. This location commercially cultivates seaweed in open monoculture ponds using the land-based raceway technique (**Figure 1a**). Seawater was pumped from the sea into the pond, which had a depth of approximately 1–2 m. Secondly, sampling was carried out within the local natural culture in Sikao, Trang Province (7°37'36.5" N 99°16'24.3" E), which is located along the coastline of the Andaman Sea. The sample collection occurred in January, April and July 2022. The Andaman Coastal Sea has a narrow continental shelf that slopes towards greater depths offshore. The shelf area is slightly wider in the south covers 6 provinces (Ranong, Krabi, Phangnga, Phuket, Trang and Satun) in the southwestern part of Thailand. The culture system in Trang was locally maintained using hanging nets in the seawater or the off-bottom cages technique (**Figure 1b**). Seawater circulation was facilitated by the tidal cycle along the coast. In general, Thailand's weather tends to be defined by 3 seasons: The hot season (March to May), the rainy season (May to October) and the cold season (November to February). Therefore, January, April and July were chosen to represent each season.

Approximately 10 kg of fresh samples were randomly collected from each culture pond or cage. To study seaweed biology, *Caulerpa* is difficult due to its fragmentation and stolon growth. The age of samples vary in the adult stage, become mature at the time of sampling. Stolons and rhizoids are creeping on the ground while having upright fronds. Thalli shows a complete fronds structure with ramuli arranged in an orderly manner (**Figure 2**).

Figure 1 Study sites at a) Phetchaburi and b) Trang provinces.

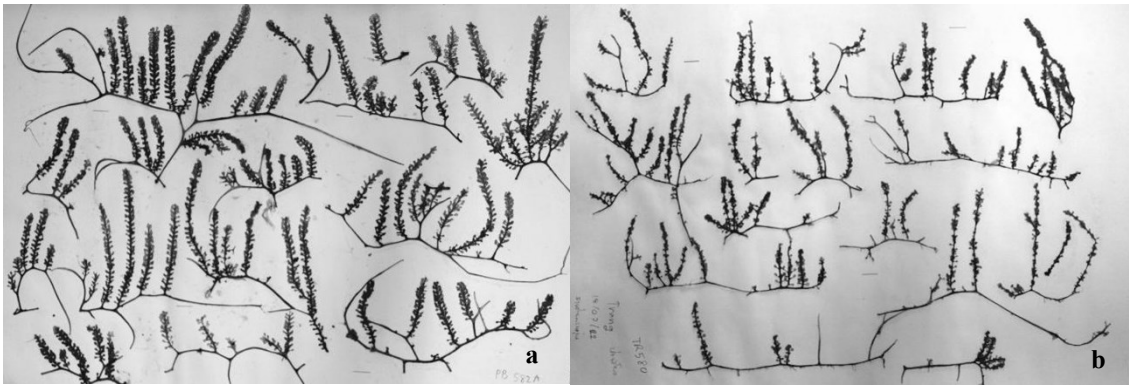

Figure 2 Sea grapes sample collected from a) Phetchaburi and b) Trang in July 2022.

Sea grapes crude extract preparation

The fresh thalli of sea grapes collected from the two study sites were underwent for cleaning process before being subjected to drying in a hot air oven (Memmert, Germany) at 70 °C for 2 days. Dried thalli were then milled using a milling machine (Polymix® PX-MFC90D, Kinematica, Switzerland) equipped with a 2 mm sieve to yield a powdered form. Each batch of dried seaweed powder, of 30 g, was separately subjected to extraction by soaking in 500 mL of absolute ethyl alcohol, with constant shaking at 100 rpm in dark conditions for 72 h. Following the extraction, the solution was filtered using Whatman no. 1 filter paper, and the resulting filtrates were subsequently evaporated to attain a state of dryness via a rotary evaporator. The yield of the crude extract was approximately 10 % (**Table 1**) in relation to the initial dry material. Each dried extract was then dissolved in the absolute ethyl alcohol to make a stock solution of 30 mg/mL. These solutions were preserved at a temperature of 4 °C for further analysis.

Chlorophyll content

Ethanolic crude extract of 5 mg/mL was prepared from the stock solution and the chlorophyll content was measured at 648.6 and 664.2 nm by spectrophotometer (UV 1800, Shimadzu, Japan). The total chlorophyll content was calculated using the equation:

$$\text{Total chlorophyll} = (5.24A_{664.2} + 22.24A_{648.6}) \times (\frac{V \times 1{,}000}{W})$$

where $A_{648.6}$ and $A_{664.2}$ are the absorbance values at 648.6 and 664.2 nm, V is the final volume of the extract, W is the weight of crude extract [17].

Table 1 Extraction yield of sea grapes crude extract collected from different sites and times.

Sites	Month	Extraction yield (%)*
Phetchaburi	January	10.00
	April	12.02
	July	13.06
Trang	January	10.43
	April	9.93
	July	11.01

* Extraction yield was calculated using this equation: Extraction yield (%) = (weight of crude extract (g)×100)/weight of dry sample (g) where weight of dry sample is 30 g.

Phenolic compound content

Phenolic content was determined by the Folin Ciocalteu method [18]. Fifty µL of ethanolic crude extract (30 mg/mL) was mixed with 250 µL of FC reagent. After appended at room temperature for 8 min, 750 µL of 20 % Na_2CO_3 and 950 µL of distilled water were added and incubated at room temperature for 30 min. The absorbance was taken at 765 nm by the spectrophotometer using gallic acid as a standard. The results were expressed as mg of gallic acid equivalent (GAE)/g extract.

Flavonoid content

Flavonoid content was determined by the aluminum chloride colorimetric method [19]. To 500 µL of ethanolic crude extract (30 mg/mL), 2 mL of distilled water and 15 µL of 5 % $NaNO_2$ were added, mixed and incubated at room temperature for 6 min. After that, 150 µL of 10 % $AlCl_3$, 2 mL of 2 M NaOH and 200 µL of distilled water were added to the solution. After incubation at room temperature for 30 min, the absorbance was measured spectroscopically at 415 nm and used quercetin as a standard. The results were expressed as mg of quercetin equivalent (QE)/g extract.

Ascorbic acid content

Ascorbic acid content was determined by ferric chloride in an acidic medium method [20]. One mL of ethanolic crude extract (30 mg/mL) was mixed with 1.0 mL of ethyl alcohol, 0.5 mL of 0.4 % phosphoric acid, 1.0 mL of 0.5 % 1,10-phenanthroline and 0.5 mL of 0.03 mg/mL ferric chloride. The absorbance was measured at 534 nm spectrometrically, and ascorbic acid was used as a standard.

Antioxidant activity

The antioxidant activity of sea grape extract was assessed through DPPH, ABTS and reducing power assays. DPPH and ABTS assays are methods employed to gauge the capacity of antioxidant compounds to scavenge free radicals, based on their ability to donate hydrogen atoms. Notably, ABTS is suitable for assessing both hydrophilic and lipophilic chemicals, while DPPH exhibits greater sensitivity towards hydrophobic molecules. On the other hand, the reducing power assay involves an electron transfer process where Fe^{3+} is reduced to Fe^{2+} by enhancing absorbance during the reduction reaction, the ability of the antioxidants in the sample to transfer electrons is enhanced by the ability to donate electrons [21].

DPPH radical scavenging assay

Ethanolic crude extract of sea grapes in concentrations of 2.5, 5, 10 and 20 mg/mL was prepared from the stock solution. The DPPH radical scavenging of the crude extract was carried out by the method of Brand-Williams *et al.* [22] with some modifications. Each concentration of the crude extract (1.9 mL) 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 with the spectrophotometer. Calculating the percentage of DPPH radical scavenging using the equation: $[(A_0 – (A_1 – A_2))/A_0]×100$, where A_0 is the absorbance of the ethyl alcohol + DPPH, A_1 is the absorbance of the extract solution + DPPH, A_2 is the absorbance of the extract solution + ethyl alcohol. The observed result was reported as the effective concentration of sample required to scavenge DPPH radical by 50 % (EC_{50}). Trolox was used as a standard.

ABTS radical scavenging assay

The ABTS radical cation scavenging activity was determined according to Re *et al.* [23] with some modifications. ABTS radical was generated by reacting 5 mL of 7 mM ABTS aqueous solution with 88 µL of 140 mM $K_2S_2O_8$ (2.45 mM of final concentration) and kept in the dark for 16 h. Then, the solution was diluted in ethanol to an absorbance of 0.70 ± 0.20 at 734 nm before use. Appropriate concentration (2.5, 5, 10 and 20 mg/mL) of the extracts (0.2 mL) was mixed with 2.0 mL of ABTS radical solution and incubated for 15 min. The solution was measured at 734 nm with the spectrophotometer. The percentage of ABTS radical scavenging was calculated using the equation: $[(A_0 - A_1)/A_0]\times100$, where A_0 is the absorbance of the control (ethanol), A_1 is the absorbance of the extract solution. The observed result was reported as EC_{50}. Trolox was used as a standard.

Reducing power assay

Reducing power assay of sea grape crude extract was performed according to Su *et al.* [24]. Each concentration (2.5, 5, 10 and 20 mg/mL) of the crude extract (0.2 mL) was added to 0.5 mL of 0.2 M potassium phosphate (KP) buffer (pH 6.8). The solution was then reacted with 1 % potassium ferricyanide and incubated at 50 °C for 20 min. After that the mixture was mixed with 0.5 mL of 10 % TCA, 1.5 mL of distilled water and 0.1 mL of 0.1 % (w/v) ferric chloride and incubated for 10 min at room temperature, subsequently the absorbance was measured spectrometrically at 700 nm. The result was reported as EC_{50} value (the effective concentration of the extract at which the absorbance was 0.5). Trolox was used as a standard.

α-Glucosidase inhibition assay

The sea grape crude extract with a concentration of 5, 10, 20 and 40 mg/mL was prepared in 10 % DMSO and used for the determination of enzyme α-glucosidase inhibition [25]. Each 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. Future 50 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside was added, the absorbance at 405 nm was immediately monitored using the spectrophotometer at 0 and 6 min. The percentage inhibition of α-glucosidase activity was calculated using the equation: $[(\Delta A_{control} - \Delta A_{sample})/\Delta A_{control}]\times100$, where $\Delta A_{control}$ is the change of absorbance at 405 nm of control (10 % DMSO) and ΔA_{sample} is the change of absorbance at 405 nm of the extract. EC_{50} value was also reported. Acarbose was used as a standard.

LC-QTOF MS/MS analysis

LC-QTOF MS/MS analysis was conducted using ExionLC 2.0 system (SCIEX, MA, USA) coupled with X500R QTOF mass spectrometer (SCIEX, MA, U.S.A.). Chromatographic separation was achieved using Kinetex® 100 A° C18 (150×30 mm^2, 2.6 µm). The two LC-MS/MS parameters (Method I and Method II) were used for analysis.

Method I: The column temperature was maintained at 30 °C. The autosampler had an injection volume of 10 mL with a flow rate of 0.5 mL/min. The mobile phases were 0.02 % acetic acid in DI water (A) and 0.02 % acetic acid in LC-MS grade acetonitrile (B). Gradient elution was started at 5 % mobile phase B and increased to 60 % B until 11 min. At 13 min, the gradient increased to 95 % B until 17 min and began re-equilibration at 19.00 min at 5 % B until the end of the run at 22 min. The QTOF-MS/MS detection was performed in positive electrospray mode (ESI), with the detection range being set from 100 to 1,000 m/z. The collision energy was set at 10 and 35 eV. Air zero and dried nitrogen were used as a carrier and the gas temperature was set at 120 °C. Other parameters were: Curtain gas 25 psi, ion spray voltage 5,500 V, ion source gas 1 is 20 psi, and ion source gas 2 is 35 psi.

Method II: The sample solution (10 mL) was injected into and separated using, a Kinetex® 100 A° C18 (150×30 mm^2, 2.6 µm) column, gradient elution with 0.5 % acetic acid in deionized water (A) and LC-MS grade methanol (B). Gradient elution was started at 10 % mobile phase B until 2 min and increased to 70 % B until 7 min and maintained until 10 min. At 10.50 min, the gradient began re-equilibration at 10 % B until the end of the run at 15 min. The flow rate was 0.5 mL/min, and the column temperature was set a 40 °C. The QTOF MS/MS was operated in positive ESI mode. The detection range was set from 100 to 1,000 m/z. The collision energy was set at 10 and 35 eV. Air zero and dried nitrogen were used as a carrier and the gas temperature was set at 350 °C. Optimized source parameters were: Curtain gas 25 psi, ion spray voltage 5,500 V, ion source gas 1 is 20 psi, and ion source gas 2 is 40 psi.

Statistical analysis

Data were expressed as mean ± standard error (SE). Statistical analysis was conducted using the R program package [26]. A two-way analysis of variance (ANOVA) was applied to assess the combined

impact of site and season on both metabolite content and bioactivity. Duncan's multiple range test was employed for post hoc multiple comparison testing, with a significance level of $p < 0.05$ indicating statistical significance. The correlation between the metabolite content and bioactivity was evaluated using Pearson's correlation coefficient.

Results and discussion

Metabolite content

The metabolite quantity like chlorophyll, phenolic compounds, flavonoids and ascorbic acid were significantly influenced by the collection site and the interaction between these factors. However, flavonoids remained unaffected by the collection time (**Table 2**). Sea grapes collected from Trang exhibited significantly higher metabolite content compared to those from Phetchaburi. Notably, the sea grapes from Trang in July displayed the highest chlorophyll content (4.52 ± 0.01 mg/g extract) (**Figure 3a**). Chlorophyll, being the most abundant pigment in the *Caulerpa* genus, possesses antioxidant properties [27]. Supporting these findings, a previous study using ethanol to extract sea grape lipids reported chlorophyll a and chlorophyll b contents of 1.77 ± 0.25 and 0.91 ± 0.09 mg/g extract, respectively [28].

In terms of phenolic compounds, the content of sea grapes from Phetchaburi and Trang ranged between 39.59 ± 2.67 to 51.18 ± 1.25 and 45.21 ± 0.69 to 93.76 ± 2.39 mg GAE/g extract, respectively. The highest phenolic compound content was observed in sea grapes from Trang during April (**Figure 3b**). Flavonoid content revealed that sea grapes from Trang (ranging from 22.21 ± 0.95 to 23.05 ± 0.85 mg QE/g extract) exhibited higher values than those from Phetchaburi (ranging from 11.56 ± 2.36 to 19.15 ± 2.78 mg QE/g extract) (**Figure 3c**). Similar study in a sea grape extract by Srinorasing *et al.* [28], reported less phenolic compound and flavonoid as 2.07 ± 0.34 mg GAE/g sample and 5.40 ± 0.76 mg QE/g sample, respectively. The increased polyphenol production in these samples may be attributed to environmental factors.

Regarding ascorbic acid, a previous study highlighted that ascorbic acid in fresh sea grapes was the predominant and major contributor to antioxidant activity [6]. However, in this study, ascorbic acid was found in the ethanolic extract of sea grapes and highest content occurred from Trang during April (**Figure 3d**).

Table 2 Analysis of variance and means comparison for phytochemicals content and antioxidant activity.

	Chlorophyll (mg/g)	Phenolic (mg GAE/g)	Flavonoid (mg QE/g)	Ascorbic acid (mg/g)	EC_{50} (mg/mL)			
					DPPH	ABTS	Reducing power	α-Glucosidase
Site (S)								
Phetchaburi	2.44 ± 0.10b	44.69 ± 1.91b	14.31 ± 1.66b	1.04 ± 0.09b	14.56 ± 0.32a	7.23 ± 0.51a	41.50 ± 2.41a	50.68 ± 1.75a
Trang	4.18 ± 0.12a	65.18 ± 7.40a	22.49 ± 1.13a	1.66 ± 0.12a	8.62 ± 0.75b	5.33 ± 0.38b	29.49 ± 1.07b	34.99 ± 4.19b
Month (M)								
January	3.49 ± 0.36a	49.93 ± 3.19b	21.10 ± 1.57a	1.20 ± 0.18c	10.70 ± 1.45b	6.49 ± 0.99b	31.77 ± 1.07c	37.43 ± 8.14b
April	3.16 ± 0.26c	66.68 ± 12.21a	17.21 ± 2.37a	1.50 ± 0.26a	13.57 ± 0.92a	7.15 ± 0.25a	32.92 ± 3.17b	44.84 ± 2.99a
July	3.28 ± 0.56b	48.19 ± 1.48b	16.88 ± 3.08a	1.35 ± 0.02b	10.50 ± 1.66b	5.20 ± 0.26c	41.79 ± 3.88a	46.24 ± 1.49a
Significance								
S	***	***	***	***	***	***	***	***
M	***	***	Ns	***	***	***	***	***
S×M	***	***	*	***	***	***	***	***
CV. (%)	0.10	6.01	21.28	6.93	4.21	7.56	2.23	6.73

Mean with different letters in the same column are significantly different ($p < 0.05$). Ns, *, *** mean not significant, significant at $p < 0.05$ and $p < 0.001$, respectively.

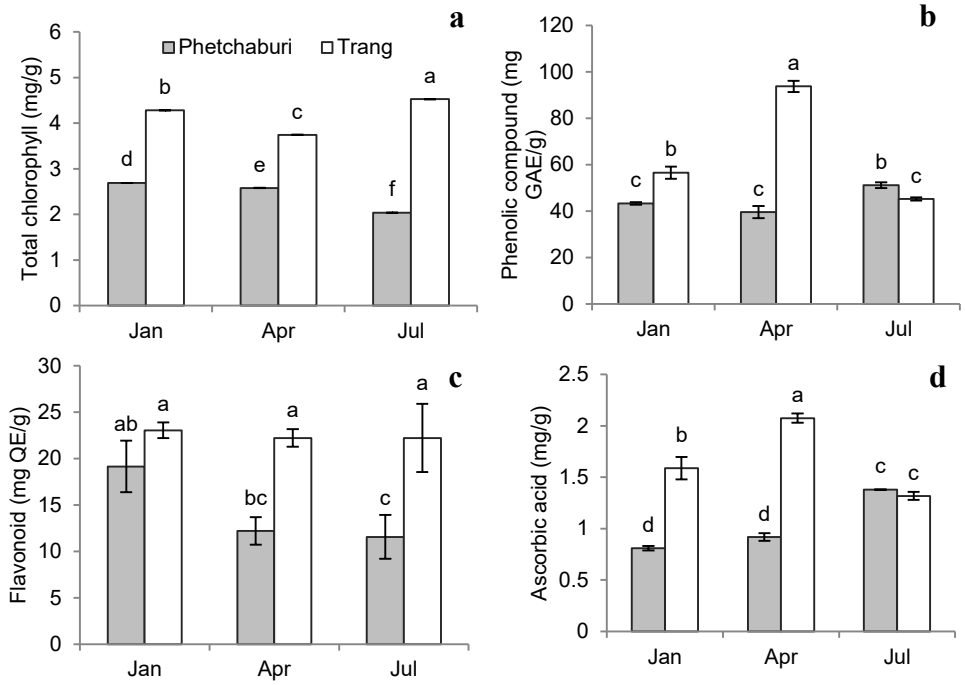

Figure 3 The content of secondary metabolites, a) total chlorophyll, b) phenolic compound, c) flavonoid and d) ascorbic acid, of sea grapes crude extract collected from different sites and times. The data represent the mean ± SE (n = 3). Different letters indicate statistical differences ($p < 0.05$).

Antioxidant activity

The antioxidant activity of sea grape extract was assessed through DPPH, ABTS and reducing power assays, and the results were expressed as EC_{50} value relative to the reference compound, trolox. The antioxidant activity was significantly influenced by the collection site, collection time and their interaction ($p < 0.001$) (**Table 2**). Sea grapes collected from Trang exhibited significantly higher antioxidant activity compared to those from Phetchaburi. The sea grapes from Trang collected in January (EC_{50} value: 7.47 ± 0.10 and 4.28 ± 0.18 mg/mL by DPPH and ABTS methods, respectively) and July (EC_{50} value: 6.80 ± 0.07 and 5.03 ± 0.35 mg/mL by DPPH and ABTS methods, respectively) showed the highest free radical scavenging activity. Additionally, sea grapes from Trang collected in April demonstrated the highest reducing power, with an EC_{50} value of 25.86 ± 0.20 mg/mL (**Figures 4a–4c**).

The study revealed that sea grape extract exhibited antioxidant activity in both radical scavenging and reducing power capacities, consistent with a previous study [7]. The antioxidant activity of sea grapes is likely attributed to their content of chlorophyll, phenolic compounds, flavonoids and ascorbic acid. Pearson's correlation test was performed to establish relationships between metabolite content and biological activity. The correlations between chlorophyll and antioxidant activity as measured by DPPH ($r = -0.929$, $p < 0.001$), ABTS ($r = -0.521$, $p < 0.05$) and reducing power ($r = -0.774$, $p < 0.001$); phenolic compound and reducing power ($r = -0.535$, $p < 0.05$); flavonoid and DPPH ($r = -0.681$, $p < 0.01$) and reducing power ($r = -0.765$, $p < 0.001$); as well as ascorbic acid and ABTS ($r = -0.509$, $p < 0.05$) were observed (**Table 3**). Sea grapes exhibit a wide range of antioxidant molecules, likely as an adaptation to varying environmental conditions. Given their habitat's susceptibility to stress, seaweeds generally produce oxidizing agents, leading to the generation of free radicals within cells [29].

α-Glucosidase inhibition

It is widely recognized that effective management of the metabolic disorder associated with type 2 diabetes can be achieved through the inhibition of α-glucosidase enzyme activity [30]. Extract from sea grapes originating in both Phetchaburi and Trang exhibited inhibitory effects on the α-glucosidase enzyme, albeit with lower potency than the reference compound, acarbose. The EC_{50} values ranged between 45.29 ± 2.89 and 55.59 ± 1.27 mg/mL for sea grapes from Phetchaburi, and between 19.27 ± 0.40 and 47.19 ± 1.39 mg/mL for sea grapes from Trang (**Figure 4d**). This study demonstrated that all sea grape samples exhibited moderate inhibition of α-glucosidase activity, comparable to the reported activity (EC_{50} = 8.97 mg/mL) of sea grape lipid extract [28].

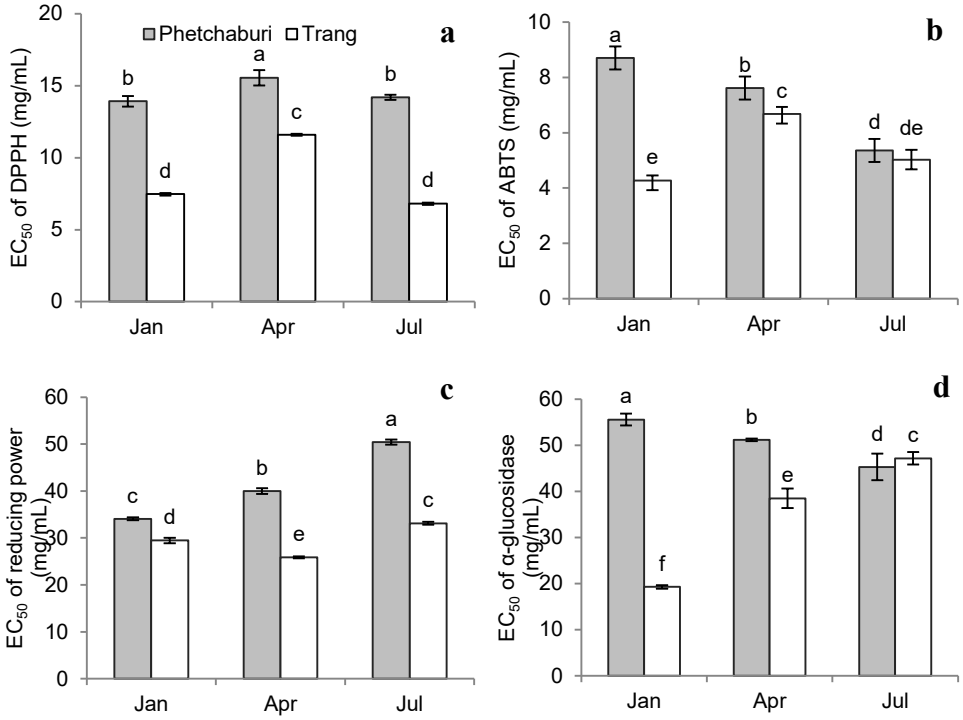

Figure 4 The EC_{50} values for antioxidant activities were determined using a) DPPH, b) ABTS, and c) reducing power assays, d) along with α-glucosidase inhibition of sea grapes crude extract collected from different sites and times. The data represent the mean ± SE (n = 3). Different letters indicate statistical differences ($p < 0.05$). The EC_{50} values of the standard compound, trolox, for DPPH, ABTS and reducing power assays were found to be 9.58 ± 0.52, 25.75 ± 0.41 and 89.93 ± 0.55 µg/mL, respectively. The EC_{50} value of the standard compound, acarbose, for α-glucosidase inhibition was determined as 15.00 ± 0.00 µg/mL.

Table 3 Correlation coefficients between responses studied.

	Chlorophyll	**Phenolic compound**	**Flavonoid**	**Ascorbic acid**	**DPPH**	**ABTS**	**Reducing power**	**α-Glucosidase inhibition**
Chlorophyll	1							
Phenolic compound	0.291	1						
Flavonoid	0.740***	0.372	1					
Ascorbic acid	0.470*	0.881***	0.409	1				
DPPH	−0.929***	−0.159	−0.681**	−0.434	1			
ABTS	−0.521*	−0.113	−0.228	−0.509*	0.695**	1		
Reducing power	−0.774***	−0.535*	−0.765***	−0.450	0.554*	0.004	1	
α-Glucosidase inhibition	−0.548*	−0.404	−0.347	−0.625**	0.597**	0.685**	0.391	1

*, **, *** = correlation significant at 0.05, 0.01 and 0.001 level, respectively.

An interesting correlation emerged between the α-glucosidase inhibitory activity and variables such as chlorophyll (r = −0.548, $p < 0.05$), ascorbic acid content (r = −0.625, $p < 0.01$) and the scavenging activities of DPPH and ABTS radical (r = 0.597 and 0.685, respectively, $p < 0.01$) (**Table 3**). This observation suggested that the α-glucosidase inhibitory activity of sea grapes might be linked to the accumulation of secondary metabolites like chlorophyll and ascorbic acid, in conjunction with their antioxidant capacity.

Effects of site and time on metabolite content and biological activity of sea grape

The sea grape samples collected from Phetchaburi and Trang during January, April and July were compared in terms of their metabolite content, antioxidant activity and α-glucosidase inhibition. The results indicated that both the sampling site and time exerted an influence on metabolite content and biological activities. Notably, sea grapes collected from Trang exhibited significantly higher metabolite content, antioxidant activity and α-glucosidase inhibitory activity than those collected from Phetchaburi. The collected data exhibited variations across different time periods or seasons. The practice of sub-harvesting sea grapes during cultivation, followed by replanting from young algae obtained through cutting, allows for samples to be accessible year-round, contingent on their growth. It's important to acknowledge that sea salinity and climate conditions can also affect seaweed yield.

Climate conditions can induce variations in the chemical composition of sea grapes. Pires-Cavalcante *et al.* [31] highlighted variability in the distribution of α-tocopherol in *Caulerpa* genus in response to seasonal changes. The data from the Thai Meteorological Department for the year 2022 illustrates consistent fluctuations in monthly rainfall, maximum and minimum temperatures, mean temperature and relative humidity in Phetchaburi and Trang throughout the year (**Table 4**). For example, January, as the collection month, exhibited the lowest rainfall and minimum temperature, whereas July (Phetchaburi) and April (Trang) recorded the highest maximum temperatures. Generally, various macroalgae in aquaculture are sensitive to extreme temperature events [32], which can induce stress and consequently lead to the accumulation of total chlorophyll, flavonoids and antioxidant molecules.

The choice of culture technique for sea grapes could also contribute to differences in metabolite contents. The off-bottom cages technique was employed for sea grape aquaculture in Trang, whereas the land-based raceway technique was used in Phetchaburi. In Trang, sea grapes are directly exposed to seawater due to the prevailing sea conditions, have significant effect. In contrast, in Phetchaburi, sea grape farming takes place in open ponds where seawater is pumped from the sea, thereby minimizing direct exposure to sea conditions. The land-based raceway technique offers a means to mitigate environmental variations, making it increasingly favored for sensitive *Caulerpa* species that are affected by factors like salinity, light, currents and sediment [3]. Seaweeds are typically subjected to stress due to their habitat, leading to the generation of free radicals in cells. The synthesis of secondary metabolites and free radical scavenging compounds represents an effective adaptive strategy and mitigation measure against the risk of extreme stress events.

Table 4 Monthly climate data of Phetchaburi and Trang province in 2022.

Climate	Province	Month												Mean
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
Rainfall (mm)	Phet[1/]	50.7	158.9	24.3	56.8	116.6	18.6	91.4	108	99.8	339	67.9	0.5	94.4
	Trang	61.1	160.6	167.9	142.9	237	369.8	309.1	229.8	285.6	485.9	193.5	238.5	240.1
Maximum temperature (°C)	Phet	30.2	30.2	31.7	32.6	31.8	33.3	33.2	32.8	32.3	31.6	31.8	30.3	31.8
	Trang	32.9	32.9	34.2	33.7	32.8	32.9	33.0	32.5	32.6	31.7	31.8	30.4	32.6
Minimum temperature (°C)	Phet	21.7	23.5	25.1	24.9	24.9	24.9	24.4	23.8	23.1	23.9	24.1	22.0	23.8
	Trang	23.1	24.0	24.2	24.3	24.8	24.4	24.7	24.3	24.1	24.1	24.0	23.6	24.1
Mean temperature (°C)	Phet	26.3	27.2	29.7	27.6	29.0	29.8	29.8	28.9	28.4	27	27.2	25.6	28.0
	Trang	28.5	28.9	29.5	29.6	29.4	29.4	29.2	29.2	29.1	28.7	28.5	26.9	28.9
Relative humidity (%)	Phet	78	78	81	75	81	79	76	77	80	80	78	72	78
	Trang	73	76	79	82	85	84	83	83	84	86	85	80	82

[1/] Phet = Phetchaburi
Source: Thai Meteorological Department (2023)

Chemical profile

LC-MS coupled with available database identification remains a widely used approach for non-targeted metabolic profiling within complex chemical compound mixtures [29]. This insight into the class

of active compounds holds significance for future research. The qualitative and identification analysis of chemical compounds in the 6 distinct sea grape samples was performed using LC-QTOF MS/MS in positive ionization modes, employing two methods as detailed in **Figure 5** and **Table 5**. The selection of a positive mode stems from the investigation's focus on sugar-free natural products. In total, 63 compounds were identified in the sea grape ethanolic extract, classified into amino acids (4), fatty acids (2), lipids (2), nucleic acids (5), carboxylic acids (4), alkaloids (3), cyanogenic glucosides (1), phenolic compounds (15), steroids (7), terpenoids (17) and vitamins (3). Notably, sea grapes collected from both Phetchaburi and Trang contained similar compounds in the classes such as amino acids, fatty acids, lipids, nucleic acids, carboxylic acids, cyanogenic glucosides, steroids, terpenoids and vitamins. However, variations arose in the classes of alkaloid and phenolic compounds.

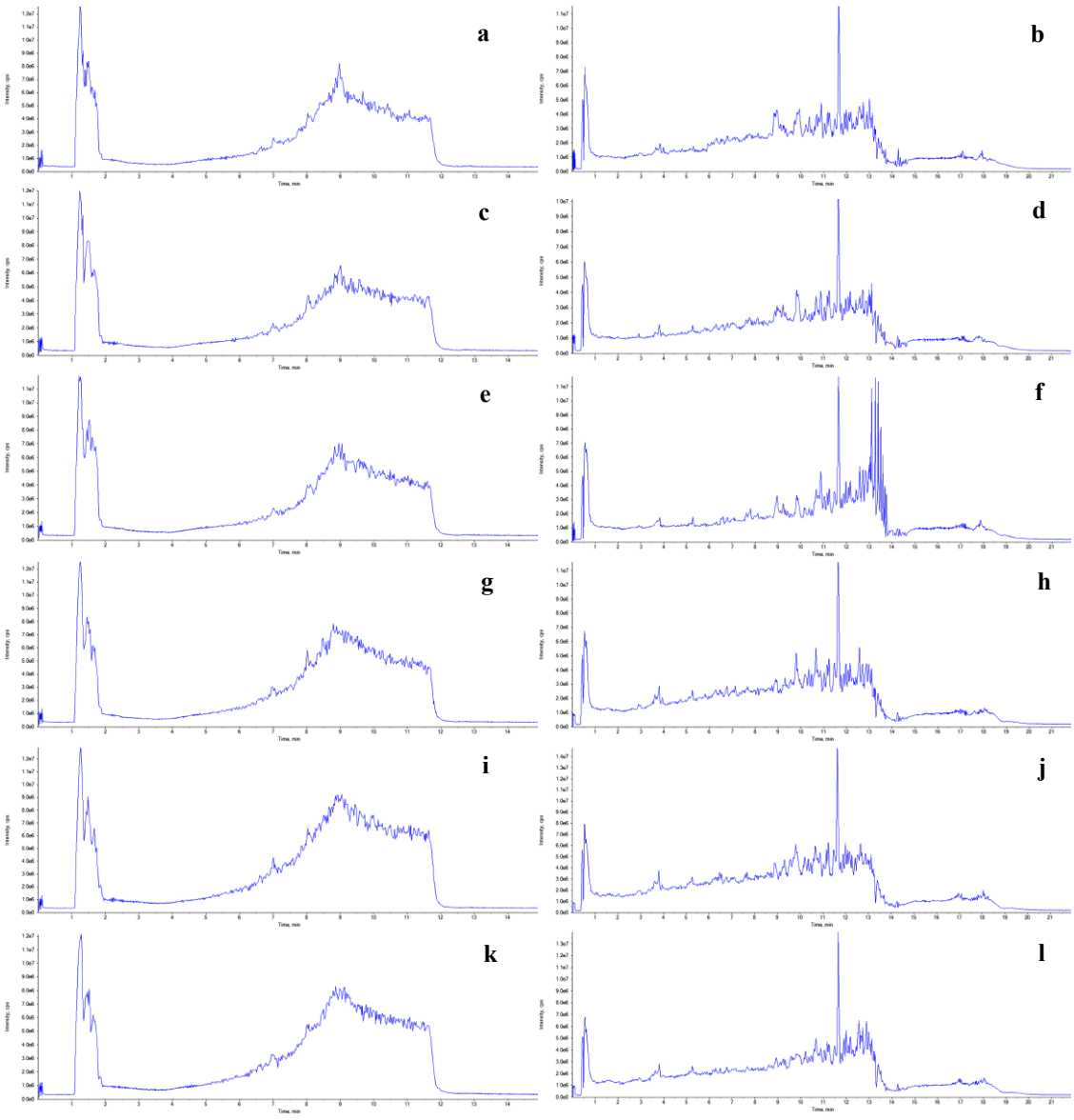

Figure 5 LC-QTOF-MS/MS chromatogram of sea grapes crude extract collected from different sites and times, Phetchaburi, January, a) method I and b) method II; Phetchaburi, April, c) method I and d) method II; Phetchaburi, July, e) method I and f) method II; Trang, January, g) method I and h) method II; Trang, April, i) method I and j) method II; Trang, July, k) method I and l) method II.

Table 5 Characterization of chemical compounds in sea grapes collected from different sites and times by using LC-QTOF MS/MS analysis.

No	Proposed compounds	Molecular formula	RT (min)	Method	Molecular Weight	Theoretical (*m/z*)	Observed (*m/z*)	Samples					
								Phetchaburi			Trang		
								Jan	Apr	Jul	Jan	Apr	Jul
Amino acid													
1	2-Phenylglycine	$C_8H_9NO_2$	1.35	I	151.163	152.069	152.0697	√*	√	√	–	–	√
2	Betaine	$C_5H_{11}NO_2$	1.39	I	117.150	118.085	118.0852	–	√*	√	√	√	√
3	L-Phenylalanine	$C_9H_{11}NO_2$	2.76	I, II**	165.189	166.085	166.0851	–	√*	√	–	√	√
4	Nicotinamide	$C_6H_6N_2O$	0.91	II	122.120	123.054	123.0544	–	–	–	√*	√	–
Fatty acid													
5	Alpha-Linolenic acid	$C_{18}H_{30}O_2$	9.07	II	278.430	279.193	279.1931	√*	√	√	√	√	√
6	Methyl linoleate	$C_{19}H_{34}O_2$	6.31	II	294.500	295.224	295.2243	√*	√	√	√	√	√
Lipid													
7	Sphinganine	$C_{18}H_{39}NO_2$	8.49	II	301.508	302.303	302.3033	–	√*	√	√	√	–
8	Gingerglycolipid B	$C_{33}H_{58}O_{14}$	8.05	I	678.805	701.488[/1]	701.4883	√*	√	√	√	√	√
Nucleic acid													
9	6-hydroxypurine	$C_5H_4N_4O$	0.78	II	136.111	137.044	137.0447	√*	√	√	√	√	√
10	Adenine	$C_5H_5N_5$	0.76	II	135.130	136.061	136.0609	√*	√	√	√	√	√
11	Adenosine	$C_{10}H_{13}N_5O_4$	2.37	I	267.240	268.102	268.1028	√	√*	√	√	√	√
12	Flavin mononucleotide	$C_{17}H_{21}N_4O_9P$	16.23	II	456.344	457.347	457.3475	–	–	√*	√	√	√
13	Thymine	$C_5H_6N_2O_2$	1.85	I, II**	126.113	127.049	127.0492	√*	√	√	–	√	–
Carboxylic acid and derivative													
14	3-Phenylbutyric acid	$C_{10}H_{12}O_2$	6.41	I	164.201	165.090	165.0902	–	–	√*	√	–	–
15	Benzamide	C_7H_7NO	8.86	I, II**	121.137	122.096	122.0956	–	√*	√	–	√	√
16	Methylglutaric acid	$C_6H_{10}O_4$	1.71	I	146.141	147.049	147.0493	–	–	√*	√	√	–
17	Picolinic acid	$C_6H_5NO_2$	18.36	II	123.109	124.023	124.0232	–	√	–	–	–	–
Alkaloid													
18	Indole-3-carboxylic acid	$C_9H_7NO_2$	4.09	II	161.157	162.053	162.0536	√*	√	√	–	–	–
19	Nicotinic acid	$C_6H_5NO_2$	1.86	I	123.109	124.038	124.0384	√*	–	√	–	–	–
20	5-Methylmyosmine	$C_{10}H_{12}N_2$	7.01	I, II**	160.22	161.095	161.0955	√*	–	–	√	√	√
Cyanogenic glucoside													
21	Amygdalin	$C_{20}H_{27}NO_{11}$	7.34	I	457.428	475.324[/2]	475.3232	√*	√	√	√	√	√
Phenolic compound													
Hydroxycinnamic acid													
22	Cinnamic acid	$C_9H_8O_2$	7.79	I, II**	148.159	149.022	149.0222	√*	√	√	√	–	√
23	Cinnamaldehyde	C_9H_8O	3.83	II	132.159	133.100	133.1001	√*	√	√	√	–	√
Coumarin													
24	6-Methylcoumarin	$C_{10}H_8O_2$	7.01	I, II**	160.170	161.095	161.0951	√	√	√*	√	√	√
25	7-Hydroxycoumarin	$C_9H_6O_3$	5.66	II	162.140	163.111	163.1108	–	–	–	–	–	√
26	Coumarin	$C_9H_6O_2$	2.97	II	146.143	147.079	147.0794	–	–	–	–	√*	√
27	Esculetin	$C_9H_6O_4$	3.57	II	178.141	211.132[/3]	211.1317	–	–	–	–	√*	√
28	Hymecromone	$C_{10}H_8O_3$	6.60	I, II**	176.169	177.090	177.0901	√*	√	√	√	√	√
Stilbene													
29	Pterostilbene	$C_{16}H_{16}O_3$	9.13	I, II**	256.296	257.127	257.1273	–	–	√*	–	√	–
Flavonoid													
Flavanol													
30	(-)-Epigallocatechin	$C_{15}H_{14}O_7$	11.51	II	306.267	307.261	307.2612	–	–	–	√	–	–
Flavonol													
31	Fisetin	$C_{15}H_{10}O_6$	11.10	II	286.236	287.235	287.2352	–	–	–	√*	–	√
32	Galangin	$C_{15}H_{10}O_5$	12.16	II	270.237	271.204	271.2043	–	–	√	√*	–	√

No	Proposed compounds	Molecular formula	RT (min)	Method	Molecular Weight	Theoretical (*m/z*)	Observed (*m/z*)	Samples					
								Phetchaburi			Trang		
								Jan	Apr	Jul	Jan	Apr	Jul
33	Kaempferol	$C_{15}H_{10}O_6$	11.11	II	286.236	287.235	287.2350	–	–	–	√	√*	√
34	Quercetagetin	$C_{15}H_{10}O_8$	10.96	I	318.235	319.187	319.1870	√*	√	√	√	√	√
Polyphenol													
35	Curculigoside	$C_{22}H_{26}O_{11}$	12.86	II	466.435	489.325[/4]	489.3250	–	√	–	√	√	√
36	Icarrin	$C_{33}H_{40}O_{15}$	6.70	II	676.662	677.177	677.1768	√*	√	–	–	–	–
Steroid													
37	16-Dehydroprogesterone	$C_{21}H_{28}O_2$	8.92	II	312.446	313.191	313.1910	√*	√	√	√	√	√
38	Androstanedione	$C_{19}H_{28}O_2$	8.59	I	288.424	289.179	289.1785	√*	√	√	√	√	√
39	Androsterone	$C_{19}H_{30}O_2$	6.78	II	290.440	291.194	291.1938	√*	√	√	√	√	√
40	Canrenone	$C_{22}H_{28}O_3$	15.88	II	340.456	341.264	341.2637	√*	–	–	√	–	–
41	Cholestenone	$C_{27}H_{44}O$	14.46	II	384.638	385.290	385.2898	–	√*	–	√	–	√
42	Pregnenolone	$C_{21}H_{32}O_2$	7.23	I, II**	316.478	317.209	317.2093	√*	–	√	√	√	√
43	Resibufogenin	$C_{24}H_{32}O_4$	15.89	II	384.509	385.290	385.2896	√*	√	√	√	√	√
Terpenoid													
Monoterpenoid and derivative													
44	Camphor	$C_{10}H_{16}O$	2.90	II	152.233	153.090	153.0900	–	√*	√	√	–	√
45	Cantharidin	$C_{10}H_{12}O_4$	3.84	II	196.200	197.115	197.1144	√*	√	√	√	√	√
46	Loganin	$C_{17}H_{26}O_{10}$	13.01	II	390.382	391.280	391.2795	√*	√	√	√	√	√
47	Pulegone	$C_{10}H_{16}O$	2.93	II	152.233	153.090	153.0899	√*	√	√	√	√	√
Sesquiterpenoid													
48	Alpha-cyperone	$C_{15}H_{22}O$	10.69	II	218.33	219.209	219.2093	√*	√	√	√	√	√
49	Atractylenolide I	$C_{15}H_{18}O_2$	9.53	II	230.302	231.173	231.1729	–	√*	√	–	√	–
50	Costunolide	$C_{15}H_{20}O_2$	7.75	I	232.318	233.076	233.0756	√*	√	√	√	√	√
51	Curcumol	$C_{15}H_{24}O_2$	9.13	II	236.350	237.220	237.2199	√*	–	√	–	√	√
52	Curdione	$C_{15}H_{24}O_2$	5.83	II	236.350	237.147	237.1464	√*	√	√	√	√	√
53	Dehydrocostus lactone	$C_{15}H_{18}O_2$	6.66	II	230.302	231.137	231.1366	√	√	√	√*	√	√
54	Furanodiene	$C_{15}H_{20}O$	8.90	II	216.319	217.194	217.1938	√	√	√	√*	√	√
55	Germacrone	$C_{15}H_{22}O$	7.86	I	218.335	219.173	219.1734	√*	√	√	√	√	√
56	Linderane	$C_{15}H_{16}O_4$	8.81	I	260.285	261.109	261.1088	√*	√	–	√	√	√
57	Nardosinone	$C_{15}H_{22}O_3$	7.27	II	250.333	251.199	251.1988	√	√*	√	√	√	√
58	Parthenolide	$C_{15}H_{20}O_3$	8.04	I	248.317	249.109	249.1090	√*	√	√	√	√	√
Diterpenoid and derivative													
59	Ambroxane	$C_{16}H_{28}O$	11.48	II	236.39	237.220	237.2194	√*	√	–	√	√	√
60	Kaurenoic acid	$C_{20}H_{30}O_2$	11.46	II	302.451	303.288	303.2876	√*	–	–	–	√	√
Vitamin													
61	Vitamin A	$C_{20}H_{30}O$	10.14	I, II**	286.452	269.171	269.1713	√*	–	–	√	√	√
62	Vitamin B_2	$C_{17}H_{20}N_4O_6$	6.34	I	376.364	377.145	377.1444	√*	√	√	√	–	–
63	Vitamin D_2	$C_{28}H_{44}O$	13.31	II	396.648	397.380	397.3803	–	–	√*	√	√	√

*Compound was detected in more than 1 samples, data presented in the table are form single asterisk sample. **Compounds were detected in both 2 methods while only method I data was presented.
[/1] Gingerglycolipid B +Na, [/2] Amygdalin + NH_3, [/3] Esculetin + CH_3OH + H^+, [/4] Curculigoside + Na.
RT = stands for retention time. √ = Detect, – = Not detect.
Molecular formular and molecular weight were confirmed from ChemSpider Compound Database: http://www.chemspider.com/ and PubChem Compound Database: https://pubchem.ncbi.nlm.nih.gov/

Concerning alkaloids, sea grapes from Phetchaburi primarily contained indole-3-carboxylic acid and nicotinic acid, while those from Trang contained 5-methylmyosmine. In the term of phenolic compound, sea grapes from Trang exhibited the presence of coumarin—7-hydroxycoumarin, coumarin, and esculetin—, flavanol—epigallocatechin—, and flavonol—fisetin and kaempferol—, which were absent in

Phetchaburi. Nonetheless, compounds such as cinnamic acid, cinnamaldehyde, 6-methylcoumarin, hypercromone, pterostilbene, galangin, quercetagetin and curculigoside were found in both Phetchaburi and Trang. Notably, 15 phenolic compounds were identified in the sea grape extract, with disparities noted between Phetchaburi and Trang sea grapes, underscoring the relevance of chemical profiling for biological activity assessment. Phenolic compounds including hydroxycinnamic acid, coumarin and flavonoid exhibited potent antioxidant activity and function as free radical scavengers [33]. The greater number of phenolic compounds in Trang sea grapes (14) compared to Phetchaburi (9) correlates with their heightened total phenolic compound, total flavonoid content and antioxidant properties.

Terpenoids constituted a significant portion of the sea grape extract and were consistently present in both Phetchaburi and Trang samples. Apart from phenolic compounds, terpenoid, including monoterpenoids, sesquiterpenoids, diterpenoids and tetraterpenoid, have been reported for their antioxidant properties [34]. Many compounds detected in sea grape extract have been documented as pharmaceutical attributes. For example, cinnamaldehyde prevented UVB-induced collagen degradation [35], while esculetin exhibited anti-tumor effects on endometrial cancer [36], kaempferol displayed anti-Alzheimer's effect [37] and curculigoside stimulated glucose uptake [38].

Conclusions

The synergistic effects of the chemical compounds within the sea grape extract were responsible for its antioxidant capabilities. These capabilities indicate biological activities, such as the inhibition of α-glucosidase. This study investigated variations in the bioactive constituents present in sea grapes, which are contingent upon the sampling site and time. These variations hold significance in pinpointing the ideal harvest site and time to maximize the yield of bioactive compounds. This information could prove valuable in advancing their potential application as a source of bioactive compounds for both nutraceutical and pharmaceutical purposes. Additionally, this study utilized LC-QTOF MS/MS to elucidate the chemical profile of the ethanolic sea grape extract. Nonetheless, further investigations are necessary to quantify the main compounds and assess their corresponding bioactivity.

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