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Nutritional values of green seaweed Cladophoraceae in brackish water bodies in the Mekong delta, Vietnam

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Abstract
The proximate composition and amino acid profiles were evaluated in seven common green seaweeds (Cladophoraceae) from the Mekong delta of Vietnam, namely *Cladophora* sp., *Cl. socialis, Cl. crispula, Cl. patentiramae, Rhizoclonium* sp., *Rh. kochianum*, and *Chaetomorpha linum*. Results showed that nutritional composition of green seaweed varied among species. Seaweed proximate contents varied in the ranges of 11.49-19.29% for protein, 0.81-2.29% for lipid, 20.87-24.75% for ash, 39.87-54.19% for carbohydrate, 0.67-1.58% and 0.08-1.39% for calcium and phosphorus, respectively. The highest contents of essential amino acids were observed for isoleucine, followed by leucine, especially methionine was relatively high. For the non-essential amino acids such as aspartic acid, serine and lysine were more dominant than other individual amino acids. Overall, these investigations provide nutritional values of autochthonous green seaweeds Cladophoraceae in the Mekong delta. These seaweed can be used as feed ingredients in aquafeeds.

Keywords: Green seaweeds, cladophoraceae, proximate composition, amino acid profile

1. Introduction
The genera of green seaweeds (*Cladophora*, *Cheatomorpha* and *Rhizoclonium*) belong to Cladophoraceae family (Chlorophyta) are widely distributed from the tropics to cold temperate waters in fresh, brackish and marine environments [1]. In Vietnam, the members of the Cladophoraceae have been found to be abundant year-round in the brackish water bodies of the Mekong delta of Vietnam [2, 3]. Like other seaweeds, Cladophoraceae is a good source of protein, amino acid, fatty acids, minerals, pigment, and bioactive compounds [4, 5].
Green seaweed (*Cladophora* sp.) could be used as a suitable feed source for Tilapia fish [6], and partially replaced commercial feed for the giant gourami fish *Osphronemus goramy* that help reduce the feed cost and improve water quality [7]. Fermented *Cladophora* included in Tilapia diet containing enhanced growth rate and feed efficiency of fish [8]. *Cladophora* meal could replace up to 20% of fishmeal protein in the tiger shrimp *Penaeus monodon* postlarvae diet that improved growth rate, feed efficiency, and stress resistance of shrimp [9]. Additionally, green seaweed Cladophoraceae species play an important role in bio-filter as they have high capacity absorb nutrients (nitrogen and phosphorus), therefore they are the potential candidates in co-culture with the black tiger shrimp *P. monodon* in Southeast Asian shrimp farming [10], and integration of *Chaetomorpha* sp. (Cladophoraceae) and *P. monodon* shrimp helped reduce feed costs in shrimp culture [11].
Previous studies have reported that the nutritional composition of seaweeds varied depending on species, developmental stages, seasonal change and geographic regions [12, 15] and affected by environmental factors and trophic level in their habitat [16, 17]. The nutritional composition of green seaweed species from the brackish waters in the Mekong delta of Vietnam is poorly known and utilization of these seaweeds in this region is still limited. Therefore, the purpose of this study was to determine the proximate composition and amino acid profiles of common green seaweed species to provide useful information for further application in aquaculture.

2. Materials and Methods
2.1 Seaweed collection and isolation
Green seaweed species belong to Cladophoraceae family was collected in different brackish water bodies (improved extensive shrimp ponds, abandoned ponds, and discharged canals) in

Mekong delta of Vietnam. These seaweeds were collected during rainy season from June to September, 2016. This period is optimal harvesting, as several of green seaweeds have high biomass for isolating different species. Approximately 4 to 5 kg of fresh biomass was collected in each harvest then they were transported to the laboratory in an ice-cold box containing seawater (the same salinity at the sampling site).

Some important environmental factors were monitored during sampling period. Salinity was recorded with a hand refractometer (Atago, Japan), water temperature and pH was recorded using a thermo-pH meter (YSI 60 Model pH meter, HANNA instruments, Mauritius). The concentrations of nutrients (NO_3^-, NH_4^+ and PO_4^{3-}) in water were measured using test kits (Sera, Germany).

For isolation of seaweed species, each seaweed species was identified morphologically and isolated under stereomicroscope with the help of available taxonomic manuals [18, 19]. After isolation, the green seaweed samples were washed thoroughly in freshwater to remove foreign particles and shade to dry with a thin layer in air for three days. The dried seaweeds were then pulverized in the commercial grinder and the powdered seaweed samples were stored in a freezer (-15 ^oC) and used for further analysis.

2.2 Chemical analysis

Proximate analysis (moisture, crude protein, total lipid, fibre, ash, calcium and phosphorus) of seaweed samples were carried out according to the standard methods of AOAC (2000) [20]. Carbohydrate (CHO) was estimated on a dry weight basis by subtracting the percentages of crude protein, lipids, crude fiber and ash from 100%. The amino acid profile of experimental green seaweed samples were analyzed by the method of CASE.SK.0013. All the seaweed samples were analyzed by the Can Tho Technical Center of Standard Metrology and Quality, Can Tho city, Vietnam.

2.3 Statistical analysis

The percentage values were normalized through a square root arcsine transformation before statistical analysis. Data for all measured parameters were analyzed using SPSS for Windows, Version 16.0. Variations nutrient contents of different seaweed species were compared by one-way ANOVA. The Tukey HSD post hoc was used to identify significant differences at $P< 0.05$.

3. Results and discussion

3.1 Green seaweed species

In this investigation, seven common species of green seaweeds (Cladophoraceae) belong to three genera (*Cladophora*, *Chaetomorpha* and *Rhizoclonium*) were collected from the brackish water of the Vietnamese Mekong delta namely *Cladophora* sp., *Cl. socialis*, *Cl. crispula* and *Cl. patentiramea*, *Chaetomorpha linum*; *Rhizoclonium* sp. and *Rh. kochianum.* These seaweeds were analyzed the nutritional composition.

3.2 Environmental parameters in the study areas

Some important environmental factors during sampling green seaweeds in different brackish water bodies (the improved extensive shrimp ponds, abandoned ponds, and discharged canals) are presented in Table 1. Results showed that variation in salinity during collecting green seaweed species, this could be due to collecting different time in the rainy season (June - September 2016). *Cl. patentiramea* samples were collected at the lower salinities (between 6 and 7 ppt) than other because this time coincided with continuous heavy rainfall. Water temperature were not much different during sampling period, ranging from 26.4-32.5 ^oC. According to previous studies green seaweed species belonging to Cladophoraceae are euryhaline and eurythermal, they can adapt to extreme environmental conditions [1, 19]. Turbidity at the period collecting seaweed samples fluctuated from 28 to 51 cm, this range is suitable for seaweed growth [1].

Table 1: Environmental parameters during sampling period in each green seaweed species

Seaweed species	Salinity (ppt)	Temperature (^oC)	Turbidity (cm)	NH_4^+ (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)
Cladophora sp.	13–15	28.5–30.1	35–42	0.32–0.97	0.85–2.71	0.12–0.34
Cl. socialis	11–14	28.2– 1.1	32–47	0.25–0.54	0.68–3.15	0.18–0.62
Cl. crispula	10–12	27.5–30.8	30–51	0.48–0.47	1.82–4.07	0.26–0.75
Cl. patentiramea	6–8	28.4–31.4	33–45	0.51–0.85	0.84–2.74	0.24–0.65
Rhizoclonium sp.	12–16	27.6–30.8	28–50	0.57–0.96	0.94–1.63	0.15–0.28
Rh. kochianum	10–15	28.1–32.5	32–48	0.18–1.19	1.17–2.33	0.22–0.69
Chaetomorpha linum	15–19	26.4–29.7	30–46	0.55–1.82	1.41–3.52	0.41–1.25

For nutrient concentration**,** the levels of NH_4^+, NO_3^- and NO_3^- in the water bodies varied in the ranges of 0.32–1.82, 0.68–3.52 and 0.12–1.25 mg/L, respectively. These compounds showed large variation during sampling period. *Chaetomorpha linum* was collected in the discharge canals, which contained higher nutrient levels than in other water bodies (the improved extensive shrimp ponds and abandoned ponds). Early findings confirmed that the environmental conditions have strongly affected the nutrition composition of seaweeds where they live in [14-17].

3.3 Proximate composition of green seaweeds

Resulted showed that moisture contents (water contents of fresh green seaweeds) were not statistical differences among species ($P>0.05$), varying in the range of 86.32-90.21%. Protein levels of seaweed could be divided into two groups, the group (*Cl. Socialis*, *Cl. Patentiramea*, *Rhizoclonium* sp. and *Chaetomorpha linum*) with high values were between 17.34–19.29% and the other group (*Cladophora* sp., *Cl. crispula* and *Rh. Kochianum*) varied from 11.64% to 13.68%, there was significantly different between these two groups ($P<0.05$). The lipid contents of macroalgae were highly variable among species, fluctuated from 0.81–2.09%, of which *Rhizoclonium* sp. and *Ch. linum* had significantly higher values than that of other species.

Table 2: Proximate composition (% dry weight) of seven common green seaweed species collected in the Mekong delta, Vietnam

	Cladophora sp.	*Cl. socialis*	*Cl. crispula*	*Cl. patentiramea*	*Rhizoclonium* sp.	*Rh. kochianum*	*Chaetomorpha linum*
Moisture	87.13±0.99^{a}	87.0±11.6^{a}	86.32±0.92^{a}	89.41±0.94^{a}	87.88±0.88^{a}	86.65±0.76^{a}	90.21±0.76^{a}
Protein	13.68±0.56^{a}	17.73±0.58^{b}	11.94±0.71^{a}	17.34±0.78^{b}	18.31±0.51^{b}	12.58±0.43^{a}	19.29±0.51^{b}
Lipid	0.96±0.11^{a}	1.27±0.21^{ab}	0.81±0.12^{a}	1.43±0.22^{abc}	2.09±0.22^{c}	0.82±0.06^{a}	1.89±0.23^{bc}
Ash	23.07±0.85^{ab}	22.85±0.95^{ab}	24.75±0.88^{b}	20.87±0.83^{ab}	20.9±61.64^{ab}	19.29±0.68^{a}	23.1±40.73^{ab}
Fiber	13.9±0.51^{abc}	14.39±0.48^{abc}	14.01±0.49^{abc}	15.14±0.66^{bc}	12.89±0.52^{a}	13.12±0.58^{ab}	15.82±0.39^{c}
CHO	48.38±2.02^{bc}	43.76±1.8^{ab}	48.48±0.98^{bc}	45.21±2.06^{ab}	45.75±1.44^{ab}	54.19±0.89^{c}	39.87±1.4^{a}
Calcium	1.44±0.08^{b}	1.42±0.04^{b}	1.39±0.08^{b}	1.39±0.16^{b}	0.77±0.07^{a}	0.67±0.1^{a}	1.58±0.05^{b}
Phosphorus	0.08±0.04^{a}	0.79±0.09^{a}	0.91±0.04^{ab}	1.17±0.06^{bc}	1.06±0.03^{ab}	1.01±0.09^{ab}	1.39±0.09^{c}

Mean values in each row with different letter are significantly different from each other ($P < 0.05$).
Data are means ± SD of three replicates. CHO: carbohydrate

The ash contents of seaweeds varied from 19.29 to 24.75% with the highest value was observed for *C. crispula* but significant difference was not detected among seaweed species (P>0.05). The fiber contents among species were in the range of 12.89 –15.82%, of which *Ch. linum* had a highest value and statistical differences (P<0.05) compared to *Rh. kochianum* and *Rhizoclonium* sp. The carbohydrate (CHO) were most dominant compared to other components with the lowest and highest concentrations were observed for *Ch. linum* (39.87%) and *Rh. kochianum* (54.19%), respectively, other green seaweed species, CHO levels varied between 43.76 and 48.38%. Statistical indicated that the CHO level in *Rh. kochianum* was significantly higher (p<0.05) than those in *Cl. socialis*, *Rhizoclonium* sp. and *Ch. linum*. The contents of calcium among green seaweed species fluctuated 0.67–1.58%, of which *Rhizoclonium* sp. and *Rh. kochianum* had significantly lower values (p<0.05) compared to other species. The phosphorus contents of these green seaweeds ranged from 0.79 to 1.39% where *Ch. linum* and *Cl. patentiramae* contained highest levels and statistical differences (P<0.05) from other green seaweed species.

Early finding found that [2] that biochemical composition of family Cladophoraceae had protein contents of 10.71-17.69%, lipid 2.04-2.56% and carbohydrate 52.54-60.98%. Additionally, *Cladophora* sp. contained 0.78-1.87% lipid, 14.45-26.55% protein and 14.69-39.25% ash (in dry weight) [5]. *Cl. glomerata* contained 20.38% protein, 1.1% lipid and 44.83% carbohydrate [21]. The proximate compositions of green seaweed species in the present study are relatively comparable to that of these early studies.

It was noted that proximate composition of green seaweeds in this study showed relatively variation among species, *i.e.* some species contained high level of protein while other species had higher lipid or ash contents. The differences in proximate composition could be due to green seaweed samples were collected in different water bodies and different time that mean the environmental conditions in their habitats change with the season, especially salinity and nutrient status (as mentioned in Table 1). According to several researchers, nutrition composition of seaweeds was greatly affected by environmental conditions such as temperature, salinity, light and nutrient status in the habitat where they live [14-17]. Similarly, the seaweeds showed high fluctuation in the nutrient concentrations, which are related to several environmental factors that are water temperature, salinity, light and nutrient [22, 23]. Most of the environmental parameters fluctuate according to season and the changes in ecological conditions that can stimulate or inhibit the biosynthesis of several nutrients [22, 24]. Additionally, the seaweeds show great difference in proximate composition within and between the species [17, 24, 25]. Also, protein content showed remarkable variation among seaweed groups with higher protein content in species of Rhodophyta, moderate in Chlorophyta and the lowest in Phaeophyta [24].

In the present study, the significant variation in the protein level of these seaweed species, which could be attributed to the difference in nutrient level of the water bodies where they inhabited. According to previous studies [2, 16, 17, 22], protein level of seaweed tissue was greatly influenced by trophic conditions where they live. *i.e.* Seaweeds collected in oligotrophic marine waters with low availability of nitrogen had lower protein concentrations than those collected in eutrophic waters. The current investigation support to previous studies mentioned above.

Furthermore, Banerjee, *et al.* [26] evaluated proximate composition of three macroalgae (*Enteromorpha intestinalis*, *Ulva lactuca* and *Catenella repens*) collected from Gangetic Delta at the Apex of Bay of Bengal, India. Authors found that protein content of these macroalgae were significantly positive correlated with nitrate level in their habitat. Their lipid values showed significant negative correlation with water temperature while carbohydrate contents of seaweeds had positive relationships with temperature. Besides, two green seaweeds (*Ulva pertusa* and *U. intestinalis*) collected in the rainy season (low temperature and salinity, and high nutrient availability) had higher contents of protein, lipid, ash and mineral (K Na, Cu, and Zn) than those harvested in summer [25].

3.4 Amino acid profiles of green seaweeds

The amino acid profiles of seven common species of green seaweeds are shown in the Table 3. It was found that all seaweed species contained nine essential amino acids but no have tryptophan, and seven non-essential amino acids (absence of proline).

Non-essential amino acids: the contents of aspartic acid and serine varied in the ranges of 2.07-6.75 and 1.07-6.48 mg/g, of which *Ch. linum* had the lowest value (p<0.05) compared to other seaweed species. Glycine was most dominant (2.14-6.09 mg/g), followed by alanine (2.09-3.41), glutamine (0.45-1.92 mg/g), tyrosine (0.21-0.52 mg/g), and the least proportion was observed for cystine (0.21–0.54 mg/g). Statistical analysis indicated that significant differences were found among some green seaweed species.

Essential amino acids: These green seaweeds were rich in isoleucine (9.37-18.82 mg/g), except for *Cl. socialis*, which contained lowest quantity (0.40 mg/g). Other EAA contents such as leucine was in the range of 2.18-6.23 mg/g, methionine (1.50-2.88mg/g), threonine (0.73-2.44mg/g), valine (0.5-1.17 mg/g), arginine (0.85-2.44 mg/g), phenylalanin (0.52-1.2 mg/g), histidine (0.24-0.53 mg/g), and lowest value was found for valine (0.14-0.37mg/g).

Generally, *Ch. linum* was poorer in amino acid profile than that of other green seaweed species.

Total non-essential amino acid ((∑non-EAA), total essential amino acid (∑EAA) and total amino acid (∑AA) varied in the ranges of 8.9-25.17, 9.12-33.32 and 26.59-58.4 mg/g, respectively. Overall, *Ch. linum* and *Cladophora* sp. contained lowest quantities AA while *Cl. patentiramea* and *Rh. kochianum* had highest amounts of ∑AA, and were significant differences with other seaweed species (P<0.05). These results indicated that green seaweed species in the present investigation showed relatively high nutrition values that could be a suitable food source for herbivorous aquatic organisms.

Table 3: Amino acid profiles (mg/g of seaweed) of seven common green seaweed species collected in the Mekong delta, Vietnam

	Cl. socialis	*Cl. crispula*	*Cl. patentiramea*	*Cladophora* sp.	*Rh. kochianum*	*Rhizoclonium* sp.	*Ch. linum*
Non-essential amino acid (Non-EAA)							
Alanine	2.34±0.09^a	2.09±0.08^a	2.43±0.08^a	3.05±0.08^b	3.41±0.08^c	2.82±0.08^b	2.14±0.09^a
Aspartic acid	4.87±0.19^b	4.43±0.16^b	4.85±0.16^b	6.51±0.17^c	6.75±0.17^c	5.01±0.15^b	2.70±0.13^a
Cystine	0.52±0.02^c	0.28±0.02^{ab}	0.37±0.02^b	0.42±0.04^{bc}	0.54±0.06^c	0.40±0.03^{bc}	0.21±0.06^a
Glutamine	1.61±0.06^c	1.60±0.06^c	1.25±0.04^b	1.39±0.04^b	1.92±0.05^d	1.83±0.05^d	0.45±0.04^a
Glycine	3.51±0.14^b	3.66±0.13^b	4.30±0.14^c	5.16±0.13^d	6.09±0.15^e	4.80±0.14^{cd}	2.14±0.11^a
Serine	4.30±0.17^b	3.84±0.14^b	4.21±0.14^b	6.48±0.17^c	5.99±0.15^c	4.12±0.12^b	1.07±0.05 ^{a}
Tyrosine	0.33±0.02^b	0.36±0.02^{bc}	0.37±0.03^{bc}	0.45±0.03^{cd}	0.47±0.03^d	0.35±0.02^b	0.18±0.02^a
Essential amino acid (EAA)							
Arginine	0.86±0.03^a	0.85±0.03^a	0.73±0.02^a	1.73±0.04^d	1.50±0.04^c	1.15±0.03^b	1.11±0.05^b
Histidine	0.24±0.01^a	0.30±0.02^a	0.25±0.02^a	0.53±0.06^b	0.66±0.02^c	0.48±0.04^b	0.27±0.04^a
Isoleucine	0.40±0.0^a	12.18±1.01^{bc}	13.32±1.68^{bc}	16.10±0.98^{cd}	18.82±0.87^d	17.9±0.96^d	9.37±0.82^b
Leucine	2.45±0.1^a	2.18±0.08^a	2.67±0.09^a	7.69±0.37^d	4.95±0.12^b	6.23±0.19^c	4.23±0.21^b
Lysine	0.43±0.02^a	0.45±0.02^{ab}	0.44±0.02^{ab}	0.37±0.04^a	0.65±0.02^c	0.55±0.03^{bc}	0.63±0.03^c
Methionine	1.84±0.09^b	2.39±0.07^c	2.83±0.08^d	2.29±0.07^c	2.88±0.07^d	2.39±0.07^c	1.50±0.07^a
Phenylalanine	0.89±003^{bc}	0.93±0.03^{bc}	1.20±0.03^d	0.87±0.03^b	1.20±0.03^d	1.00±0.03^c	0.52±0.03^a
Threonine	1.63±0.06^b	1.99±0.06^c	2.29±0.06^{de}	1.45±0.06^b	2.44±0.06^e	2.12±0.06^{cd}	0.73±0.04 ^{a}
Valine	0.16±0.01^a	0.20±0.02^a	0.39±0.02^b	0.18±0.02^a	0.14±0.02^a	0.37±0.02^b	0.21±0.02^a
∑ Non-EAA	16.27±0.61^b	17.77±0.6^{bc}	23.45±0.65^d	17.47±0.7^{bc}	25.17±0.68^d	19.32±0.61^c	8.9±0.5^a
∑ EAA	20.44±1.33^{bc}	22.9±2.03^c	33.21±1.67^d	9.12±0.38^a	33.23±1.24^d	32.2±1.44^d	18.58±1.3^b
∑AA	36.71±1.94^b	40.67±2.63^b	56.65±2.32^{cd}	26.59±1.08^a	58.40±1.92^d	51.52±2.05^c	27.45±1.8^a

Mean values in each row with different letter are significantly different from each other (P < 0.05).
Data are means ± SD of three replicates.

In the current study, amino acid profiles of green seaweeds showed similar pattern as observed for proximate composition, different species contained different levels of individual amino acid. This is in accordance with the research of Rameshkumar *et al*. [24], who revealed that the distribution pattern of the amino acids showed some distinct differences among the species of Rhodophyceae, Phaeophyceae and Chlorophyceae. Glutamic acid and aspartic was the most abundant amino acid in all species while methionine quantity was observed to be low in all studied seaweed species. Similarly, several seaweed species had high concentration of arginine, aspartic acid and glutamic acid [27, 28]. Other findings [29, and 30] stated that seaweeds contained high levels of aspartic and glutamic acids that made the special flavour and taste of the seaweeds. Furthermore, the seaweeds were generally rich in glycine and alanine but poor in histidine [30].

Benjama and Masniyom [25] reported that the contents of some amino acids (glutamic acid, arginine, threonine and alanine) of two green seaweeds (*Ulva pertusa* and *U. intestinalis*) were higher in the rainy season than in the summer. Recent investigation [13] revealed that the distribution pattern of some amino acids showed pronounced differences among the five species of green macrolagae Chlorophyceae (*Caulerpa racemosa*, *Ulva lactuca*, *Ulva reticulate*, *Codium tomentosum* and Caulerpa *sertularioides*). For example, the highest concentration of glutamic acid and aspartic acid was found in *C. sertularioides* (16.3% and 15.6%, respectively), and lowest value of methionine was recorded in all species except *Ulva reticulata* (3.0%). These findings have supported the results of present study. Overall, nutritional composition of wild seaweeds such as mineral, proximate and amino acid, fatty acid profiles have changed with season and environmental conditions and species, geography, developmental stage of seaweeds and trophic levels in their habitats [13, 15, 17, 29, and 30]. Therefore, harvesting seaweeds should be considered some the important factors that can obtain the optimal seaweed products.

4. Conclusion

Proximate and amino acid compositions of seven common green seaweeds belonging to Cladophoraceae family, which contained relatively high contents of protein, ash fiber and carbohydrate as well as high essential amino acids. The present investigation could provide useful information for collecting the seaweeds from brackish water bodies of the Mekong delta, Vietnam for using in fish and shrimp feeds.

5. Conflict of interests

The author states that there is no conflict of interests regarding the publication of this paper.

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