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Biochemical composition, amino acid profile, and mineral analysis of methanolic extract from brown seaweed *Colpomenia peregrina*

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Abstract
Brown seaweed (*Colpomenia peregrina*) has gained attention for its diverse bioactive compounds and potential nutritional benefits. This study investigates the biochemical composition, amino acid profile, and mineral content of the methanolic extract of *C. peregrina*. The biochemical analysis revealed significant levels of carbohydrates, proteins, and lipids, underscoring its nutritional potential. Amino acid profiling identified essential and non-essential amino acids, with notable concentrations of lysine, leucine, and glutamic acid, highlighting its suitability as a dietary protein source. The mineral analysis demonstrated the presence of essential macro- and microelements, including calcium, magnesium, potassium, and trace elements such as zinc and iron, emphasizing their role in mineral supplementation. These findings suggest that the methanolic extract of *C. peregrina* could serve as a valuable ingredient in nutraceuticals, functional foods, and feed formulations. Further studies on its bioavailability and functional properties are recommended to expand its application in the health and aquaculture sectors.

Keywords: Seaweeds, *colpomenia peregrina*, amino acid analysis, biochemical analysis, mineral profile

Introduction

Seaweeds, often referred to as marine macroalgae, are rich sources of bioactive compounds, including polysaccharides, proteins, lipids, vitamins, and minerals, making them valuable in food, pharmaceutical, and aquaculture industries [4]. Among these, brown seaweeds (Phaeophyceae) are particularly noted for their diverse bioactive profiles, including phlorotannins, fucoidans, and essential amino acids [3].

Colpomenia peregrina, a brown seaweed species, has attracted interest due to its wide distribution in coastal regions and its potential as a natural resource for nutraceutical and functional food applications. Previous studies on brown seaweeds have primarily focused on their polysaccharide content and antioxidant properties [5, 6]; however, limited information is available on the comprehensive biochemical, amino acid, and mineral profiling of *C. peregrina* [13, 8].

Amino acids play a crucial role in human and animal nutrition, serving as building blocks for proteins and precursors for bioactive metabolites. In addition, seaweeds are recognized as significant sources of essential minerals, including calcium, potassium, magnesium, and trace elements such as iodine, zinc, and selenium, which are critical for physiological functions [11], [16].

The extraction method significantly influences the yield and composition of bioactive compounds in seaweeds. Methanolic extraction, widely employed for its ability to dissolve a broad range of polar and non-polar compounds, offers an efficient approach for isolating bioactive molecules from marine algae [12].

This study aims to analyze the biochemical composition, amino acid profile, and mineral content of the methanolic extract of *Colpomenia peregrina*. By providing a comprehensive profile, the study seeks to enhance the understanding of the nutritional and functional potential of this brown seaweed, supporting its utilization in various industries.

Review of Literature

Seaweeds are increasingly recognized for their diverse bioactive compounds, including polysaccharides, polyphenols, amino acids, and essential minerals. Brown seaweeds, in particular, have been the focus of numerous studies due to their nutritional and functional properties [3].

The biochemical composition of seaweeds, including proteins, lipids, and carbohydrates, varies significantly depending on species, environmental conditions, and extraction methods [23, 25]. Kadam *et al.* (2015) [4] emphasized the nutritional potential of brown seaweeds, noting their high polysaccharide content and antioxidative properties. Additionally, Lipkin and Beer (2000) [9] reported that brown seaweeds like *Colpomenia peregrina* contain significant quantities of bioactive compounds with potential health benefits [20].

Amino acids, both free and protein-bound, play a crucial role in determining the nutritional value of seaweeds. The presence of essential amino acids such as lysine, leucine, and methionine in brown seaweeds has been widely reported [17]. Hydrolysis and HPLC techniques are often used for detailed amino acid profiling, which enables the identification of both qualitative and quantitative amino acid compositions [12, 23, 25].

Brown seaweeds are recognized for their rich mineral content, including essential macro-elements like calcium, magnesium, and potassium, as well as trace elements such as zinc and iron [11]. Minerals in seaweeds are highly bioavailable due to their association with organic compounds, enhancing their functional application in nutraceuticals and dietary supplements [16].

Methanolic extraction is a widely employed method for isolating bioactive compounds due to its ability to dissolve a broad spectrum of polar and non-polar compounds. Studies by Matanjun *et al.* (2008) [12] and Plaza *et al.* (2010) [14] highlighted the efficiency of methanol as a solvent for extracting proteins, polyphenols, and other bioactive molecules from brown seaweeds.

Although *Colpomenia peregrina* is a widely distributed brown seaweed, research on its biochemical and nutritional properties remains limited. Existing studies have focused on its polysaccharides and antioxidative properties [8], [13], [21], [22], [24], but comprehensive profiling of its amino acids and mineral content is sparse. This gap underscores the need for further investigation into its potential as a source of bioactive and nutritionally valuable compounds.

Materials and Methods

Collection and Preparation of *Colpomenia peregrina*

The marine brown alga *Colpomenia peregrina* was collected from the intertidal regions of Leepuram, Kanyakumari District, located along the southeast coast of Tamil Nadu, India (Latitude: 8°14'23.10"N, Longitude: 77°20'04.02"E). The ecological characteristics of the Kanyakumari shore, including the disrupted and rugged coral-like stones that support marine algal vegetation, have been previously documented by Desikachary and Krishnamurthy (1990) [19]. The area experiences a tropical climate with temperatures ranging from 20-35°C in summer and 17-32°C in winter, and an average annual rainfall of approximately 131.2 mm. During rough sea conditions, deep-sea algal forms are often uprooted and washed ashore, contributing to the biodiversity of the region. The identification of *C. peregrina* was conducted using the standard manual by Krishnamurthy and Baluswami (2010) [7] and was authenticated by Dr. R. Thevanathan, Professor (Retd.), PG and Research Department of Botany, Presidency College, Chennai.

The collected algae were initially rinsed with seawater to remove impurities such as sand, rocks, epiphytes, and epifauna. The cleaned samples were then transported to the laboratory in insulated boxes containing slush ice. In the laboratory, the algae were thoroughly washed with running tap water to eliminate residual salt and subsequently shade-dried for 48 hours. The dried material was then pulverized into a fine powder, packaged in airtight containers, and stored at room temperature until further use.

Extraction of Methanolic Fraction

Given its high polarity, the powdered *C. peregrina* was subjected to overnight soaking in methanol at a 1:3 (w/v) ratio. The methanol fraction was then separated using filtration. To maximize yield, the filtrate was extracted three times, and the resulting extracts were pooled together. The concentration of the pooled extract was achieved through distillation using an Evaporator Rotary Evaporator. The concentrated residue was further dried using a Lyodel Freeze Dryer to remove any remaining solvent. The dried methanolic extract was stored and subsequently utilized for biochemical analysis and activity assays to determine its potential bioactive properties.

Estimation of Biochemical Constituents

The total carbohydrate content was quantified using the anthrone method, as described by Sadasivam [18]. Soluble protein levels were estimated from fresh seaweed thalli following the Lowry method [10]. Lipid content was determined from air-dried algal samples using standard protocols [2]. Mineral analysis was conducted by subjecting the samples to acid digestion, followed by quantification through atomic absorption spectrophotometry under AOAC guidelines [1].

Estimation of Amino Acids

Free and protein-bound amino acids were estimated using the o-phthaldialdehyde (OPA) method [15]. The extraction procedures for free amino acids and soluble proteins from algal tissues have been described in previous studies. A concentrated 80% ethanolic extract was directly utilized for the qualitative and quantitative determination of free amino acids.

For protein-bound amino acids, the proteins in the extract were precipitated by adding an equal volume of 10% trichloroacetic acid (TCA), followed by vacuum drying. A known quantity of the dried protein precipitate (typically 75 mg) was hydrolyzed by adding 2.0 mL of 6.0 N HCl and incubating at 110°C for 18 hours. After hydrolysis, the hydrolysates were evaporated to dryness, and the resulting dried material was analyzed using high-performance liquid chromatography (HPLC).

Results

The protein, carbohydrate, and lipid contents of the methanolic extract of *Colpomenia peregrina* were analyzed in this study. The protein content was found to be 95.38±0.22 µg/g dry weight**,** while the carbohydrate content was measured at 74.42±0.13 µg/g dry weight**.** Additionally, the lipid content of the methanolic extract was determined to be 22.42±0.19 µg/g dry weight (Table 1; Fig. 1). These results underscore the significant nutritional potential of *C. peregrina* as a source of essential macronutrients.

The free amino acid profile of *Colpomenia peregrina* is presented in Table 2 and Fig. 2 & 3). A total of 20 amino acids was identified in the dried sample, with the algae containing 67.81% essential amino acids (EAAs) and 32.19% non-essential amino acids (NEAAs**).** Among the essential amino acids, the highest concentrations were observed for leucine (9.12%), valine (8.32%), histidine (7.79%), tryptophan (7.42%), isoleucine (7.23%), lysine (7.13%), phenylalanine (6.94%), threonine (5.26%), methionine (3.85%), cysteine (2.39%), and tyrosine (2.36%). For non-essential amino acids, notable levels were found for glutamic acid (6.83%), asparagine (5.94%), glutamine (4.88%), aspartic acid (3.91%), arginine (2.82%), glycine (2.39%), proline (2.12%), alanine (1.98%), and serine **(**1.32%). These findings emphasize the nutritional richness of *C. peregrina*, particularly its high content of essential amino acids, which are crucial for both human and animal health.

The mineral content of the methanolic extract of *C. peregrina* (CpME) was also analyzed, including potassium, calcium, phosphorus, iodine, sodium, magnesium, zinc, iron, copper, manganese, chromium, cadmium, and cobalt (Table 3; Fig. 4). Among these minerals, the most abundant were calcium (143.14±1.14 µg/g dry weight**),** iron **(**92.15±0.21 µg/g dry weight**),** potassium **(**55.17±0.14 µg/g dry weight**),** iodine **(**40.12±0.25 µg/g dry weight**),** and phosphorus **(**31.23±0.20 µg/g dry weight**).** These results highlight the mineral richness of *C. peregrina*, indicating its potential as a valuable source of essential minerals for nutritional and functional applications.

Table 1: Biochemical analysis of *Colpomenia peregrina* methanolic extract

S. No.	Biochemical constituents	µg/g dry wt.
1	Protein	95.38±0.22
2	Carbohydrates	74.42±0.13
3	Lipids	22.42±0.19

Table 2: Essential and non-essential amino acid analysis of *Colpomenia peregrina* methanolic extract

S. No	Essential amino acids	%	Non - Essential amino acids	%
1	Threonine	5.26	Aspartic acid	3.91
2	Cysteine	2.39	Glutamic acid	6.83
3	Tyrosine	2.36	Arginine	2.82
4	Histidine	7.79	Alanine	1.98
5	Valine	8.32	Asparagine	5.94
6	Methionine	3.85	Serine	1.32
7	Isoleucine	7.23	Glutamine	4.88
8	Phenylalanine	6.94	Glycine	2.39
9	Leucine	9.12	Proline	2.12
10	Lysine	7.13		
11	Tryptophan	7.42		
Total essential amino acids		67.81%	Total non-essential amino acids	32.19%

Table 3: Mineral profile analysis of *Colpomenia peregrina* methanolic extract

S. No.	Minerals	µg/g Dry wt.
1	Potassium	55.17±0.14
2	Calcium	143.14±1.14
3	Phosphorus	31.23±0.20
4	Iodine	40.12±0.25
5	Sodium	27.15±0.33
6	Magnesium	16.12±0.13
7	Zinc	4.14±0.04
8	Iron	92.15±0.21
9	Copper	3.17±0.05
10	Manganese	12.14±0.10
11	Chromium	2.21±0.03
12	Cadmium	3.15±0.02
13	Cobalt	0.17±0.02

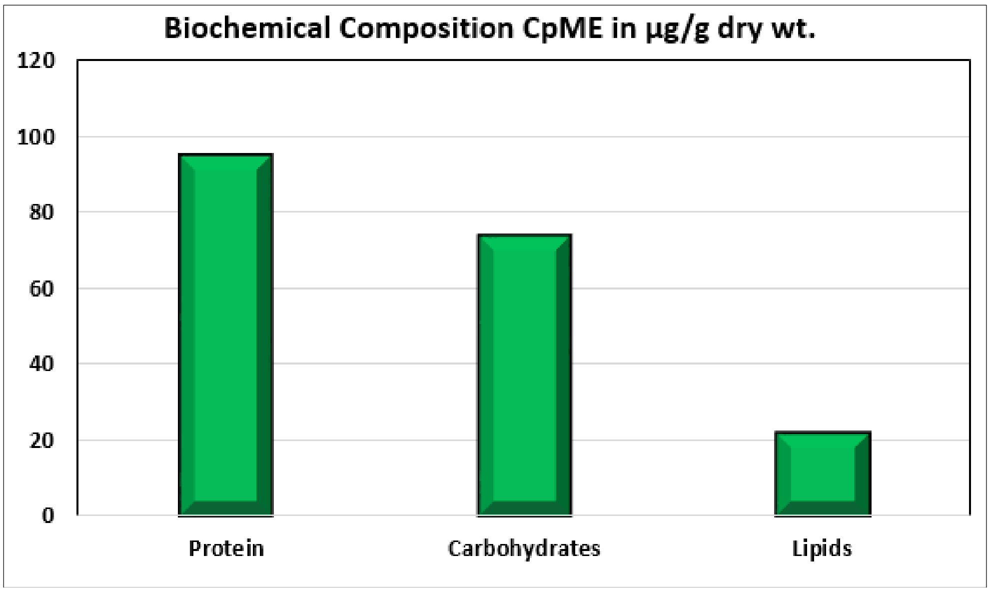

Fig 1: Biochemical Composition CpME in µg/g dry wt.

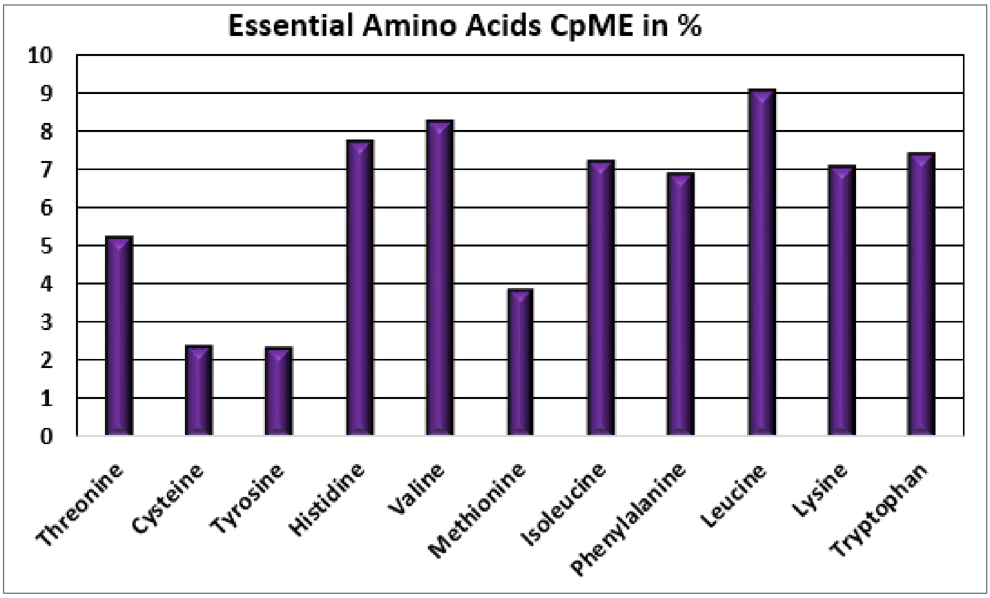

Fig 2: Essential Amino Acids CpME in %

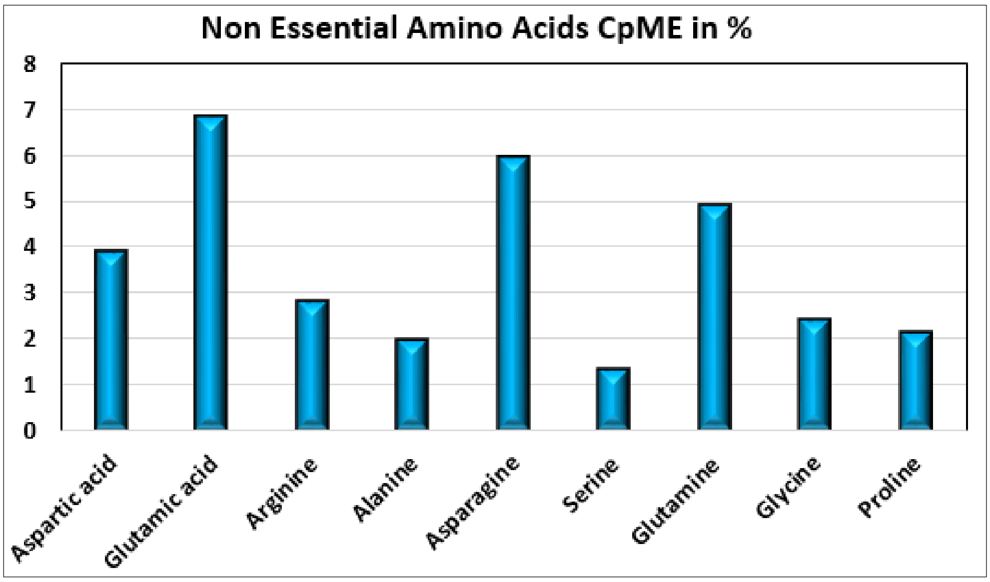

Fig 3: Non-Essential Amino Acids CpME in %

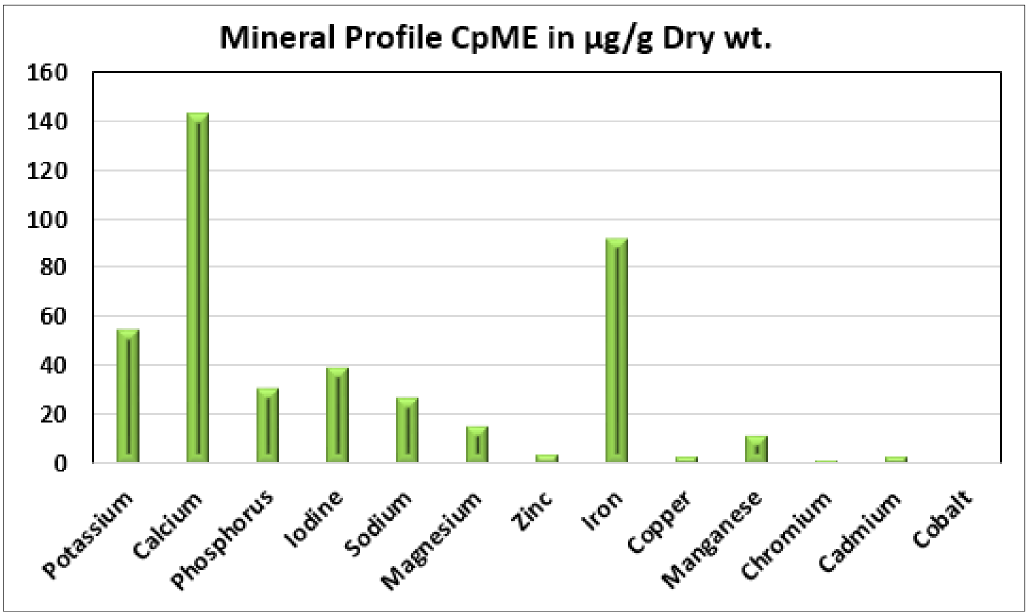

Fig 4: Mineral profile analysis CpME in µg/g Dry wt.

Discussion

The present study focused on the biochemical composition of *Colpomenia peregrina* (C. peregrina), particularly its protein, carbohydrate, lipid, amino acid, and mineral contents. The findings highlight the substantial nutritional potential of *C. peregrina* as a rich source of macronutrients and essential minerals, suggesting its possible use as a dietary supplement or functional food. The protein content of *C. peregrina* was found to be 95.38±0.22 µg/g dry weight, which is notably high. This indicates that *C. peregrina* could serve as an excellent source of plant-based protein. Compared to other marine algae, this protein concentration is consistent with findings in species like *Gracilaria* and *Ulva*, which also have high protein content, but *C. peregrina* stands out for its notable protein yield. Proteins from marine algae are of particular interest due to their essential amino acid profile, which supports tissue repair, immune function, and other critical biological functions.

The carbohydrate content of the methanolic extract was 74.42±0.13 µg/g dry weight, which further emphasizes the potential of *C. peregrina* as a source of carbohydrates, potentially useful in various health applications. Carbohydrates from marine algae are often rich in polysaccharides, which have been shown to have prebiotic effects, potentially enhancing gut health and boosting the immune system. This carbohydrate concentration is comparable to other brown algae species, such as *Sargassum* and *Ecklonia*, which are also known for their high carbohydrate content. The lipid content of the methanolic extract was 22.42±0.19 µg/g dry weight, indicating that *C. peregrina* may also be a valuable source of lipids, which are essential for energy storage and cellular structure. The lipid content observed is consistent with other brown algae, where lipids are primarily composed of polyunsaturated fatty acids (PUFAs), such as omega-3 and omega-6 fatty acids. PUFAs from algae are of great interest due to their anti-inflammatory properties and positive effects on cardiovascular health.

The amino acid profile of *C. peregrina* revealed the presence of 20 amino acids, with a significant proportion of 67.81% essential amino acids (EAAs) and 32.19% non-essential amino acids (NEAAs). The essential amino acids, which include leucine, valine, histidine, tryptophan, and lysine, are critical for human health and are not synthesized by the body, making their dietary intake necessary. Among the essential amino acids, leucine and valine were found in the highest concentrations, which are important for muscle protein synthesis and repair. The presence of high levels of glutamic acid, asparagine, and glutamine further highlights the potential of *C. peregrina* to support metabolic processes and immune function. The balance between essential and non-essential amino acids makes this alga a valuable dietary source, particularly in vegan and vegetarian diets where protein sources might be limited.

Mineral analysis showed that *C. peregrina* contains several essential minerals, including calcium (143.14±1.14 µg/g dry weight), iron (92.15±0.21 µg/g dry weight), potassium (55.17±0.14 µg/g dry weight), iodine (40.12±0.25 µg/g dry weight), and phosphorus (31.23±0.20 µg/g dry weight). These minerals play vital roles in bone health, oxygen transport, electrolyte balance, thyroid function, and cellular metabolism. The high levels of calcium and iron are particularly noteworthy, as these minerals are critical for bone strength and preventing iron-deficiency anaemia. Iodine, which is essential for thyroid hormone production, further supports the idea of *C. peregrina* as a functional food that could benefit those with iodine deficiencies. The presence of other trace elements like zinc, copper, and manganese, which are involved in antioxidant activity and enzyme function, further enhances the value of *C. peregrina* as a source of bioactive nutrients.

These findings collectively underscore the nutritional richness of *C. peregrina* and its potential as a functional food or supplement, particularly in promoting human health. The high content of essential macronutrients, amino acids, and minerals positions *C. peregrina* as a promising candidate for further exploration in the fields of nutrition, functional foods, and natural health products. Further research is needed to evaluate the bioavailability and bioactivity of these nutrients *in vivo*, as well as to explore the potential therapeutic applications of *C. peregrina*, especially in the context of sustainable food sources and nutraceuticals.

Conclusion

In conclusion, *Colpomenia peregrina* demonstrates significant nutritional potential, with high protein, carbohydrate, and lipid contents, providing a substantial source of essential macronutrients. The protein content was found to be 95.38±0.22 µg/g dry weight, carbohydrates at 74.42±0.13 µg/g dry weight, and lipids at 22.42±0.19 µg/g dry weight, highlighting its nutritional value for both human and animal consumption. The free amino acid profile further emphasizes its importance, with 67.81% essential amino acids (EAAs), including key amino acids like leucine, valine, histidine, and tryptophan, which are vital for various biological functions. Additionally, the mineral content analysis revealed that *C. peregrina* is rich in essential minerals, such as calcium, iron, potassium, iodine, and phosphorus, crucial for bone health, oxygen transport, and metabolic regulation. These findings underscore *C. peregrina* as a valuable marine resource with substantial nutritional and functional potential, making it a promising candidate for use in functional foods, nutraceuticals, and dietary supplements. Further research into its bioactive properties and bioavailability is recommended to explore its full potential in health-related applications.

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