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PHYTOCHEMICAL, AMINO ACID, FATTY ACID AND VITAMIN INVESTIGATION OF MARINE SEAWEEDS COLPOMENIA SINUOSA AND HALYMENIA PORPHYROIDES COLLECTED ALONG SOUTHEAST COAST OF TAMILNADU, INDIA

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ABSTRACT

Seaweeds are marine macroscopic algae which are considered as the important living marine organisms with high nutritive value and rich bioactive compounds present in the ocean. In the current study the phytochemical, amino acid, fatty acid and vitamin profile analysis of the seaweeds were conducted which were collected along the south east coast of India. The marine brown macro alga *Colpomenia sinuosa* (Mertens ex Roth) Derbes and Solier as well as the marine red alga *Halymenia poryphyroides* Boergesen were used in this study. The relationship between the nutritive components and the variation between phytochemical, amino acid, fatty acid and vitamin profile were mainly analyzed in the current study. The present analysis revealed that phytochemical contents like alkaloids, triterpenoids, steroids and glycosides were present in higher amounts in marine red alga *Halymenia porphyroides* compared to the marine brown alga *Colpomenia sinuosa*. Similarly the amino acid analysis showed higher percentage in marine red alga *Halymenia porphyroides* when compared to the marine brown alga *Colpomenia sinuosa*. The fatty acid profile also revealed higher content in *Halymenia porphyroides* when compared to *Colpomenia sinuosa*. On the contrary the vitamin profile analysis revealed higher amounts of vitamins content in *Colpomenia sinuosa* rather than *Halymenia porphyroides*. The presence of high phytochemical, amino acid, fatty acid constituents in *Halymenia porphyroides* makes this seaweed more important when compared *Colpomenia sinuosa* which were rich in vitamin content and can be used as an important vitamin source for human and animal diet.

KEYWORDS: Phytochemical composition, amino acid and fatty acid analysis, vitamin analysis, *Colpomenia Sinuosa*, *Halymenia porphyroides.*

INTRODUCTION

The macroscopic algae has always been associated with human and animal life for their innumerable beneficial properties and has been used as a source of food, feed, fertilizer, medicine and the availability of economically cheap phytochemicals.[1,2] Seaweeds are also used as raw material for many industrial products like agar, alginates and carrageenan.[3,4,5] Fresh and dry seaweeds especially brown and red macroalgae are extensively consumed by people especially living in the coastal areas as a vegetable and in some cases the mucilage are extracted from the thallus for use as gelling and thickening.[6,7] Secondary metabolites known as phytochemicals present in seaweeds which include alkaloids, phenols, flavonoids, saponins, steroids, tannins, triterpenoids, anthraquinones, glycosides and related active metabolites have been extensively investigated as a source of medicinal agents.[8] These phytochemicals plays an important role in antimicrobial activity and are used as a treatment for many microbial infections.[9,10] Also, secondary metabolites, act as hypolipemic and hypoglycemic agents, reduce blood pressure and regulate cholesterol levels.[11,12] Seaweeds are also an excellent source of vitamins A, Bl, B12, C, D and E, riboflavin, niacin, pantothenic acid, folic acid[13,14] and amino acids.[15,16] The fatty acid is an important constituent of seaweeds. The abundant composition of individual fatty acids in algae are species-dependent and depend strongly on environmental factors such as growth conditions (light, temperature and the availability of nutrients), growth phase and age.[17,18,19,20] Different species have different fatty acid patterns, which may also be important for taxonomic purposes.[21,22] Macro algae have been extensively utilized as ingredients in human and animal food preparations owing to their high contents of polyunsaturated fatty acids (PUFAs).[23,24] In addition the compounds with cytostatic, antiviral, anthelminthic, antifungal and antibacterial activities have been detected in brown and red algae.[25,26] In the present study the phytochemical, amino acid, fatty acid and vitamin composition of two seaweeds namely *Colpomenia sinuosa* (Mertens ex Roth) Derbes and Solier and *Halymenia porphyroides* Boergesen collected from south east coat of India were studied and analyzed for their nutritive content.

MATERIALS AND METHODS

Collection and preparation of seaweeds

The marine brown alga *Colpomenia sinuosa* (Mertens ex Roth) Derbes and Solier was collected from the intertidal regions of Leepuram, Kanyakumari District, the South East Coast of Tamilnadu, India and the marine red alga *Halymenia porphyroides* Bøergesen was collected on summer season in the from 2.5 metre rapid intertidal regions of the Gulf of Mannar–Mandapam, Ramanathapuram District, South East Coast of Tamilnadu, South India. Collected seaweed was washed with sea water for eliminating impurities such as sand, rocks, epiphytes and epifauna. The washed samples were transported to the laboratory in a box containing slush ice. In the laboratory, the samples were washed thoroughly in running tap water to remove salt and were shade dried for 48 hours, pulverized to a fine powder and packed in airtight container and were stored at room temperature.

Estimation of phytochemicals

Shade dried, powdered material of the experimental algae (100 g) was extracted repeatedly by maceration for 6 hrs with 100 mL of 80% ethanol in the cold. The extract was then dried over anhydrous calcium chloride in a desiccator and the residue was redissolved in 100 mL of 80% ethanol. Tests for various phytochemicals were carried out using the residue extract.[27]

Estimation of Amino acids

Free and protein amino acids were estimated by the pthaldialdehyde method.[28] Extractions of free amino acids and soluble proteins from the algal tissues are described elsewhere. Concentrated 80% ethanolic extract was directly used for qualitative and quantitative estimation of free amino acids. For protein amino acids, protein in the extract was precipitated by adding an equal volume of 10% TCA and dried in vacuum. To the known quantities of dried protein (usually 75 mg), 2.0 mL of 6.0 N HCl was added and was hydrolyzed at 110ºC for 18 hours. After hydrolysis, the hydro lysates were allowed to evaporate to dryness and the dried material was used for HPLC analysis.

Estimation of fatty acids

Fatty acids in the sample were identified and quantified by the methyl esters in the NEON II gas chromatography instrument following the procedure outlined by Miller and Berger (1985).[29]

Estimation of vitamins

The estimation of vitamins was done according to Giorgi et *al*., 2012.[30] An AGILENT 1100 chromatographic system[31] was used for the analysis and quantification of vitamins in the algal samples. The chemstation software controlled the whole chromatographic system. To the dry powdered algal biomass, 100 mM perchloric acid and acetonitrile (92: 1 v/v) solution were added and left in a water bath at 50°C for 30 minutes. The resulting solution was centrifuged at 6000 rpm and the upper layer was used for the HPLC analysis. The HPLC system (Shimadzu) equipped with UV-detector was used for the estimation of vitamins B1, B2, B6, and B12.

RESULTS AND DISCUSSION

In the preliminary phytochemical analysis of the crude extracts of experimental algae *Colpomenia sinuosa* and *Halymenia porphyroides* showed the presence of phenols, alkaloids, triterpenoids, steroids, tannins, saponins, flavonoids, anthraquinones and glycosides. The extract of *Colpomenia sinuosa* had higher amounts of phenols (56.45 ± 0.01 mg/g dry wt) and lesser amounts of steroids (20.13 ± 0.01 mg/g dry wt), tannins (15.45 ± 0.08 mg/g dry wt), alkaloids (12.35 ± 0.01 mg/g dry wt., flavonoids (2.13 ± 0.01 mg/g dry wt) and glycosides (9.05 ± 0.01 mg/g-1 dry wt) than that in *Halymenia porphyroides* where the amount of steroids (30.47 ± 0.01 mg/g dry wt), glycosides (23.46 ± 9.08 mg/g dry wt), alkaloids (20.35 ± 0.01 mg/g dry wt), tannins (15.35 ± 0.01 mg/g dry wt), triterpenoids (9.33 ± 0.01 mg/g dry wt) were high while the amount of phenols (9.02 ± 3.32 mg/ g dry wt) was low (Table.1; Fig.1). *Colpomenia sinuosa* had low levels of triterpenoids (3.45 ± 0.01 mg/g dry wt), anthraquinones (3.35 ± 0.01 mg/g dry wt) and saponins (2.35 ± 0.01 mg/g dry wt). The extract residue of *Halymenia porphyroides* showed the least amount of flavonoids (4.43 ± 0.01mg/g dry wt), anthraquinones (4.15 ± 0.02 mg/g dry wt) and saponins (3.47 ± 0.01 mg/g dry wt) (Table.1; Fig.1). The total phenol content of edible Irish brown seaweed, *Himanthalia elongata* was found to be at a higher level.[32] These reports are in line with the current study. In the present study, alkaloid content of *Colpomenia sinuosa* was less (12.35 ± 0.01 mg/g dry wt.) as compared to *Halymenia porphyroides* (20.35 ± 0.01 mg/g dry wt). The presence of alkaloids in the experimental algae may be used in formulations of nutraceutical industry and could be used as antimalarial agent, central nervous system stimulant and as an antibacterial agent. The triterpenoids content of marine brown algae *Colpomenia sinuosa* were less as compared to that of *Halymenia porphyroides*. Triterpenoids are the most abundant secondary metabolite present in marine algae[33], and marine-derived fungi.[34] The

steroid content of *Colpomenia sinuosa* was less (20.13 ± 0.01 mg/g dry wt.) as compared to *Halymenia porphyroides* 30.47 ± 0.01 mg/g dry wt.). The presence of steroids in experimental algae could be used in treating delayed puberty and as a supplement for building lean muscle mass. The tannins are found to have antiviral, antibacterial, ant parasitic effects, anti-inflammatory, antiulcer and antioxidant properties for possible therapeutic applications.[35] In the present study, considerable amounts (> 15.45 ± 0.08 mg/g dry wt.) of tannins were found in the experimental algae *Colpomenia sinuosa* and *Halymenia porphyroides*. The presence of tannins in the experimental algae could be used to treat diseases like ulcer, gonorrhea and leucorrhoea, after clinical screening. Seenivasan *et al.*, (2012)[36] reported the presence of saponins in the seaweeds *Codium adharens*, *Sargassum wightii* and *Acanthophora spicifera*. The saponin content was found in trace amounts (< 3± 0.01 mg/g dry wt.) in the experimental algae *Colpomenia sinuosa* and *Halymenia porphyroides*. Flavonoids are polyphenolic compounds that occur ubiquitously in plants, marine algae and are known to contain a broad spectrum of chemical, biological activities including antioxidant and free radical scavenging properties.[37] The flavonoids content was found in trace amounts (< 4 ± 0.01 mg/g dry wt) in *Colpomenia sinuosa* and *Halymenia poryphyroides*. The presence of flavonoids in the experimental algae may be useful as an antioxidant agent as well as an antimicrobial agent. Shalaby (2011)[38] reported the presence of anthraquinones from an ethyl acetate fraction of *Acanthophora spicifera*. In the present study, the anthraquinones content was found in trace amounts (<4.15 ± 0.02 mg/g dry wt.) in the experimental algae *Colpomenia sinuosa* and *Halymenia porphyroides*. The presence of anthraquinones in the experimental algae may find applications as an antioxidant. Yang *et al.*, (1992)[39] reported the presence of glycosides in the macro algae *Zostera* sp., *Zhongguo Haiyang Yaowu*. In the present investigation, *Halymenia porphyroides* (23.46 ± 9.08 mg/g dry wt.) showed higher amounts of glycoside content compared to that of *Colpomenia sinuosa* (9.05 ± 0.01 mg/g dry wt). The presence of glycosides in the experimental algae may find its applications in the antioxidant, anti-inflammatory properties and could be used as a supplement in the treatment of cancer.

The free amino acid profiles of the experimental algae are presented in Table.2 & 3; Fig.2. A dried sample of the experimental algae consists of 20 amino acids. The amino acid analysis of *Colpomenia sinuosa* showed marginally high content of essential amino acids (62.06%) as compared to *Halymenia porphyroides* (62%) (Table.2 & 3). Nevertheless, non-essential amino acids of *Colpomenia sinuosa* were slightly lesser in quantity (37.94%) as compared to

that in *Halymenia porphyroides* (38%) (Table.2). *Colpomenia sinuosa* showed a high level of lysine (8.99%), histidine (8.84 %), methionine (7.91%), tyrosine (7.41%) and cysteine (6.25%) and a high level of non-essential amino acid, arginine (6.92%), alanine (5.84%), glycine (5.77%) and glutamine (5.41%) (Table.2; Fig.2) whereas, *Halymenia porphyroides* showed a high level of histidine (9.93%), lysine (9.33%), tyrosine (8.93%), methionine (8.81%) and cysteine (7.69%) and a high level of non-essential amino acid, arginine (7.71%), alanine (6.75%) and glycine (6.20%) (Table.2 & 3; Fig.2). The above results reveal that the marine macro algae *Colpomenia sinuosa* and *Halymenia porphyroides* have higher amino acid content and may be used as a dietary supplement in animal and human feed. These results suggest that marine algae *Colpomenia sinuosa* and *Halymenia porphyroides*, with respect to their high protein level and their amino acid composition appear to be an interesting potential nutritional source of food proteins. Besides, as source of proteins, they can be used as excellent binders in formulating feeds.

The total fatty acid composition of the experimental algae is shown in Table.4 & 5. The experimental algae *Colpomenia sinuosa* and *Halymenia porphyroides* contain fatty acids such as palmitic acid, margaric acid, stearic acid, oleic acid, linolenic acid, alpha linolenic acid and morotic acid (Table.4 & 5; Fig.3). *Colpomenia sinuosa* contained high levels of polyunsaturated fatty acids (PUFA), which constituted 51.03% of total fatty acids. The saturated fatty acids (SFA) constituted about 42.66%, whereas; the levels of monounsaturated fatty acids (MUFA) were only 6.31%. The alga *Halymenia porphyroides* also had high levels of polyunsaturated fatty acids (PUFA) (51.47% of total fatty acid content) and saturated fatty acids (SFA) constituted 39.90%, whereas monounsaturated fatty acids (MUFA) were observed in 8.63% (Table.4 & 5). Higher levels of stearic acid (28.47%), alpha linolenic acid (27.12%) and linolenic acid (15.59%) were observed in *Colpomenia sinuosa* whereas, high level of alpha linolenic acid (31.21%), stearic acid (29.12%) and linolenic acid (16.14%) were observed in *Halymenia porphyroides* (Table.4 & 5; Fig.3). The results are in agreement with the earlier investigations reported by Bhaskar *et al*., (2004)[40]; Khotimchenko *et al*., (2002)[41] and Gressler *et al*., (2010).[42] The saturated fatty acids (SFA) of the experimental algae exhibited lower content as compared to that of polyunsaturated fatty acids (PUFA), which were similar to the findings of the earlier investigations reported by Venkatesalu *et al*., (2004)[43]; Venkatesalu *et al*., (2003b)[44] and Fayaz *et al*., (2005).[45] The major monounsaturated fatty acid (MUFA) found in the experimental algae was found to be oleic acid, which comprised of 6.31% of *Colpomenia sinuosa* and 8.63% of *Halymenia*

porphyroides. The presence of highly unsaturated fatty acids in brown seaweeds has been reported.[46,47]

The experimental algae *Colpomenia sinuosa* and *Halymenia porphyroides* showed the presence of water soluble vitamins such as vitamin B6, vitamin B12, vitamin-C and fat soluble vitamins such as vitamin A, vitamin-D, vitamin-E, and vitamin-K (Fig.4). *Colpomenia sinuosa* contained high amounts of vitamin A (23.45 ± 0.01 µg/g) as compared to other vitamins whereas, *Halymenia porphyroides* contained high amounts of vitamin A (34.5 ± 0.11 µg/g) and vitamin C (8.33 ± 0.01 µg/g) as compared to other vitamins. Vitamin C levels were low in *Colpomenia sinuosa* (0.0145 ± 0.09 µg/g) while it was vitamin B12 that registered low levels in the tissues of *Halymenia porphyroides* (0.0010 ± 0.01 µg/g) (Table.6; Fig.4). Seaweeds contain both water-soluble vitamins (Vitamin-B and Vitamin-C) and fat-soluble vitamins (Vitamins-A, D, E and K).[48] The presence of higher vitamin content in *Colpomenia sinuosa* compared to *Halymenia porphyroides* may be used as vitamin supplement in pharmaceutical industry for human and animal diets.

Table 1: Phytochemical analysis of the experimental marine seaweeds.

S.No	Phytochemicals	*Colpomenia sinuosa*	*Halymenia porphyroides*
1	Phenols	56.45±0.01	9.02±3.32
2	Alkaloids	12.35±0.01	20.35±0.01
3	Triterpenoids	3.45±0.01	9.33±0.01
4	Steroids	20.13±0.01	30.47±0.01
5	Tannins	15.45±0.08	15.35±0.01
6	Saponins	2.35±0.01	3.47±0.01
7	Flavonoids	12.13±0.01	4.43±0.01
8	Anthraquinones	3.35±0.01	4.15±0.02
9	Glycosides	9.05±0.01	23.46±9.08

Values are expressed as Mean ± SEM, n=3 as ANOVA test p <0.05% level.

Table 2: Total amino acid analysis of the experimental marine seaweeds.

S.No	Total amino acids	*Colpomenia sinuosa*	*Halymenia porphyroides*
1	Aspartic Acid	304.66±0.14	334.66±0.14
2	Glutamic Acid	112.66±0.08	503.63±0.14
3	Asparagine	204.6±0.11	113.5±0.11
4	Serine	113.47±0.01	394.53±0.08
5	Glutamine	545.6±0.15	473.76±0.08
6	Glycine	221.7±0.11	893.5±0.11
7	Threonine	182.33±0.14	193.5±0.11
8	Arginine	436.7±0.11	771.5±0.11

9	Alanine	113.53±0.08	783.53±0.08
10	Cysteine	203.5±0.11	769.43±0.08
11	Tyrosine	304.7±0.11	893.46±0.08
12	Histidine	335.46±0.12	993.53±0.14
13	Valine	110.53±0.12	334.7±0.11
14	Methionine	654.5±0.11	880.7±0.11
15	Isoleucine	323.7±0.11	340.06±0.08
16	Phenylalanine	215.73±0.08	304.47±0.01
17	Leucine	113.6±0.15	353.5±0.11
18	Lysine	335.26±0.08	933.43±0.23
19	Proline	403.5±0.11	113.56±0.12
20	Tryptophan	115.56±0.01	203.66±0.14

Values are expressed as Mean ± SEM, n=3 as ANOVA test p <0.05% level.

Table 3: Total amino acid analysis (%) of the experimental marine seaweeds.

S.No	Amino acids	*Colpomenia sinuosa* (%)	*Halymenia porphyroides* (%)
Essential amino acids			
1	Threonine	1.82	1.94
2	Cysteine	6.25	7.69
3	Tyrosine	7.41	8.93
4	Histidine	8.84	9.93
5	Valine	3.01	3.35
6	Methionine	7.91	8.81
7	Isoleucine	4.91	3.4
8	Phenylalanine	5.11	3.04
9	Leucine	4.34	3.54
10	Lysine	8.99	9.33
11	Tryptophan	3.47	2.04
Total essential amino acids		**62.06**	**62**
Non-essential amino acids			
12	Aspartic acid	3.05	3.35
13	Glutamic acid	2.79	3.04
14	Arginine	6.92	7.71
15	Alanine	5.84	6.75
16	Asparagine	2.05	1.14
17	Serine	2.1	3.94
18	Glutamine	5.41	4.73
19	Glycine	5.77	6.2
20	Proline	4.01	1.14
Total non-essential amino acids		**37.94**	**38**
Total amino acids		**100**	**100**

Table 4: Fatty acid analysis of the experimental marine seaweeds.

S.No	Fatty acids	*Colpomenia sinuosa*	*Halymenia porphyroides*
1	Palmic Acid	113.5±0.11	119.36±0.14
2	Margaric Acid	112.46±0.20	3.53±0.08
3	Stearic Acid	203.56±0.17	303.5±0.11
4	Oleic Acid	334.23±0.14	863.5±0.11
5	Linoleic Acid	193.7±0.11	781.73±0.08
6	Alpha Linoleic Acid	203.56±0.12	831.73±0.08
7	Morotic Acid	119.5±0.11	335.7±0.11

Values are expressed as Mean ± SEM, n=3 as ANOVA test p <0.05% level.

Table 5: Fatty acid analysis (%) of the experimental marine seaweeds.

Fatty acids	*Colpomenia sinuosa* (%)	*Halymenia porphyroides* (%)
Saturated fatty acids		
Palmitic acid	3.13	2.19
Stearic acid	28.47	29.12
Margaric acid	11.06	8.59
Total	**42.66**	**39.9**
Monounsaturated fatty acids		
Oleic acid	**6.31**	**8.63**
Polyunsaturated fatty acids		
Linolenic acid	15.59	16.14
Alpha linolenic acid	27.12	31.21
Morotic acid	8.32	4.12
Total PUFA	**51.03**	**51.47**
Total	**100**	**100**

Table 6: Vitamin analysis of the experimental marine seaweeds.

S.No	Vitamins	*Colpomenia sinuosa*	*Halymenia porphyroides*
1	Vitamin B6	2.7±0.11	0.33±0.01
2	Vitamin B12	0.057±0.01	0.10±0.01
3	Vitamin K	1.79±0.05	1.7±0.02
4	Vitamin D	2.33±0.14	1.83±0.02
5	Vitamin A	23.47±0.01	34.7±0.11
6	Vitamin E	33.66±0.14	2.24±0.02
7	Vitamin C	1.45±0.09	8.35±0.01

Values are expressed as Mean ± SEM, n=3 as ANOVA test p <0.05% level.

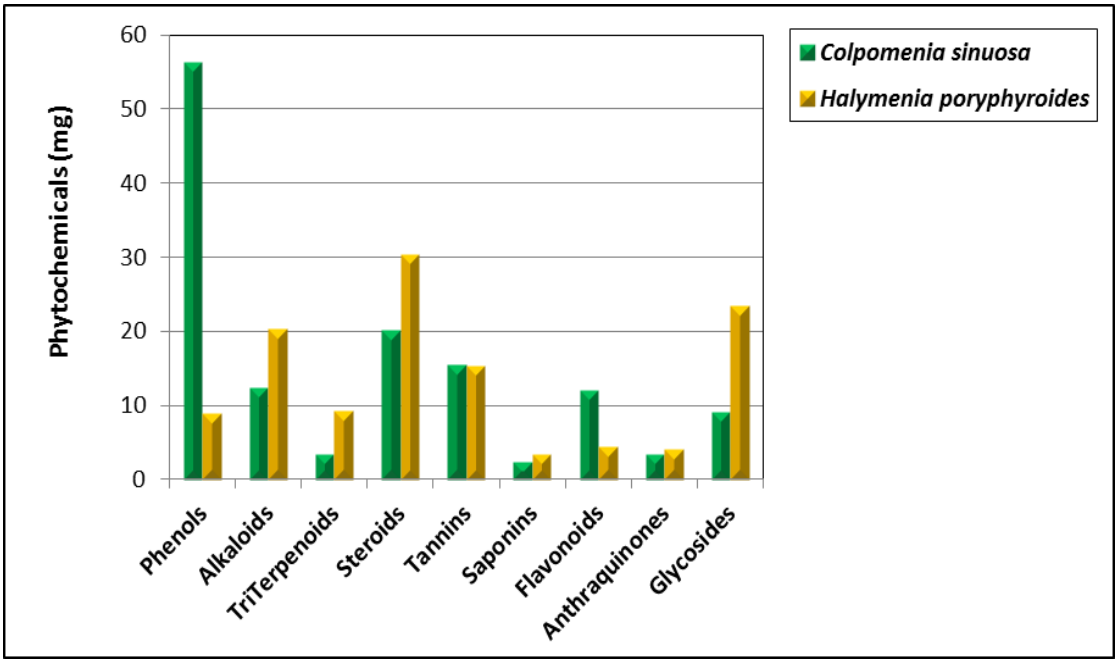

Figure 1: Phytochemical analysis of the experimental marine seaweeds.

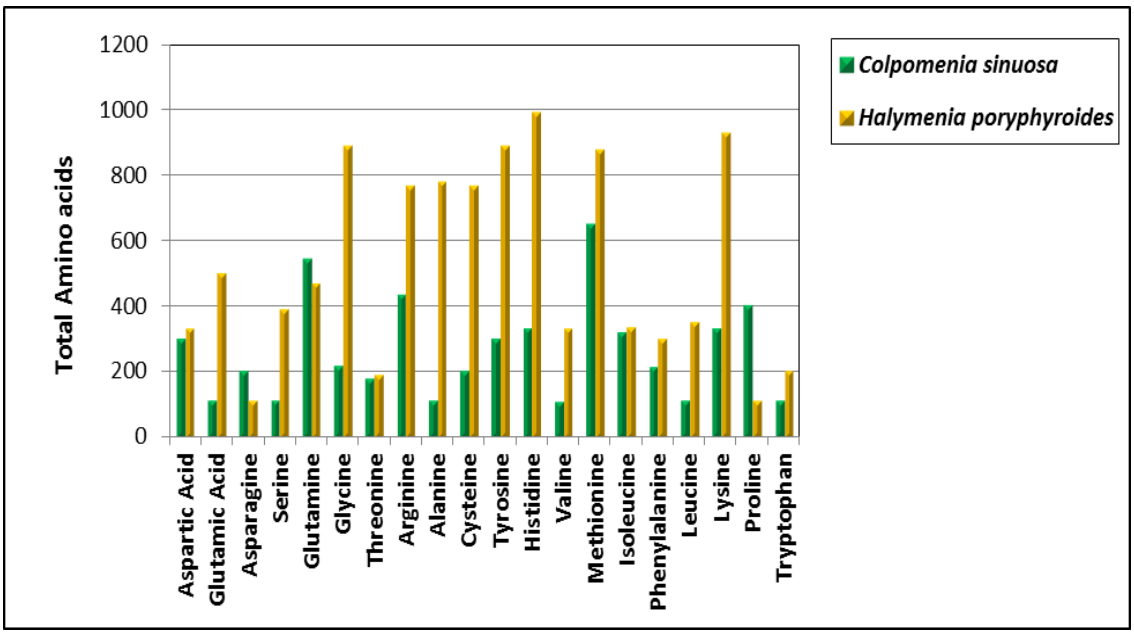

Figure 2: Total amino acid analysis of the experimental marine seaweeds.

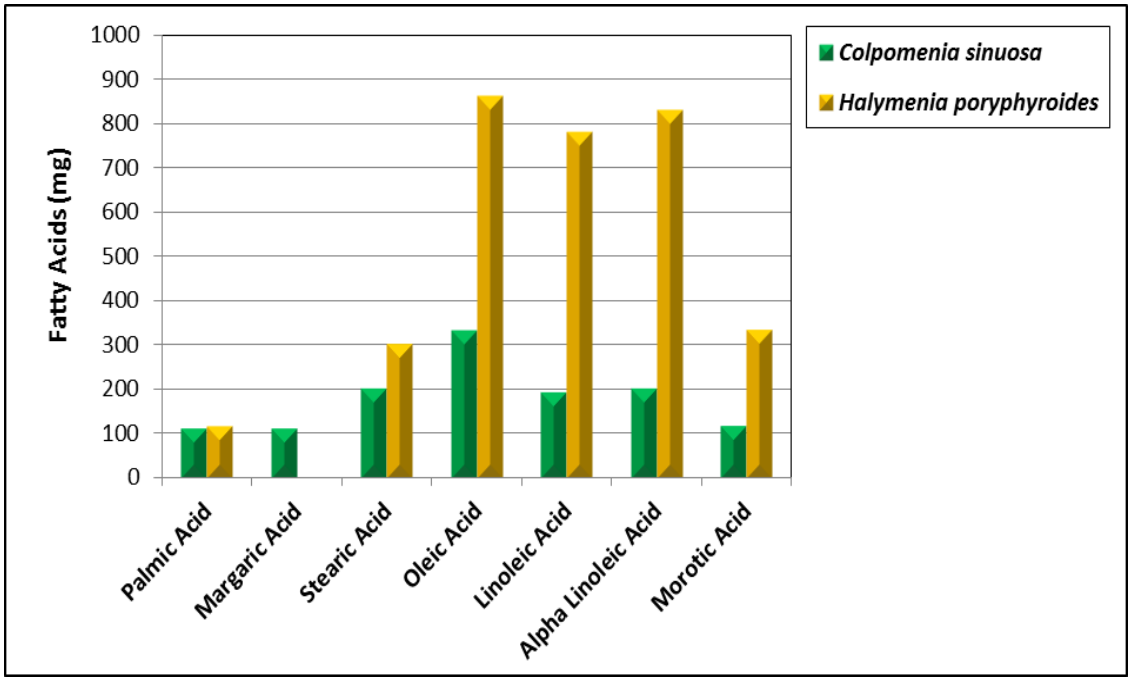

Figure 3: Fatty acid analysis of the experimental marine seaweeds.

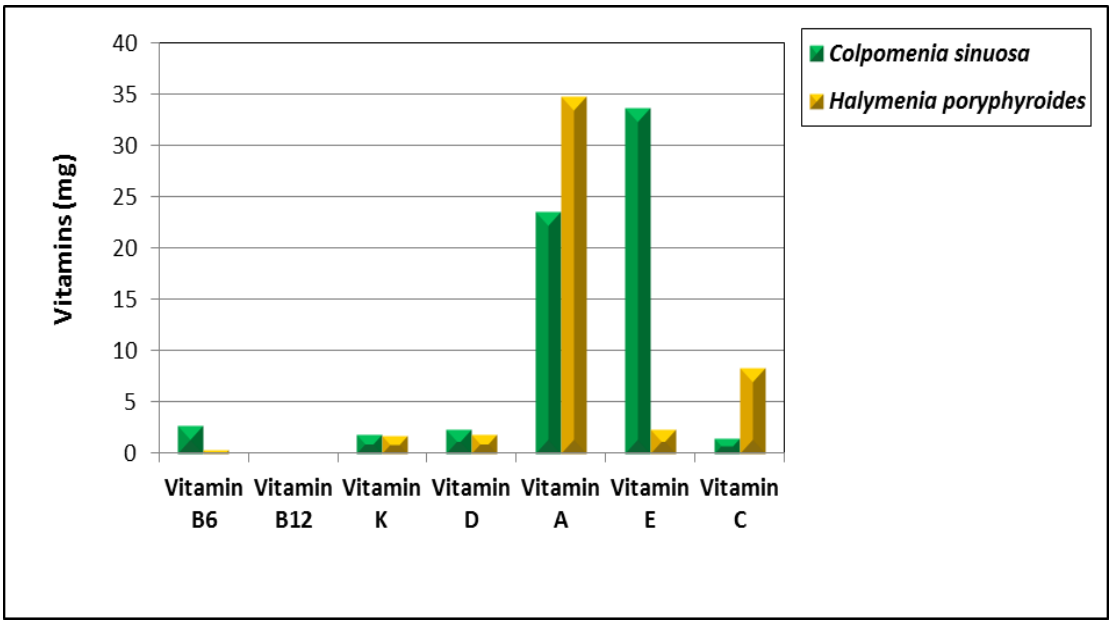

Figure 4: Vitamin analysis of the experimental marine seaweeds.

CONCLUSION

The present investigation on the phytochemical, amino acid, fatty acid and vitamin constituents from marine brown and red seaweeds concludes these seaweeds especially the red seaweed are rich source of phytochemicals as well as the amino acid and fatty acid contents. The brown seaweed indicated to possess high amount of vitamin content when compared to the red seaweed. The presence of these contents in high amounts may indicate a

possible pathway that these seaweeds can be used as the nutritive source for animal and human diet. The seaweeds can be used as raw material in food processing and pharmaceutical industries to make innovative nutritive products. More research is indeed required to exploit the full potential of these two seaweeds.

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