An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

PROXIMATE AND AMINO ACID COMPOSITIONS OF MARINE RED ALGA *LAURENCIA PAPILLOSA* (C.AGARDH) GREVILLE FROM SOUTH EAST COAST OF INDIA

Sathish Kumar, B and Murugesan, S*

Division of Algal Biotechnology and Bionano Technology,

PG and Research Department of Botany, Pachaiyappa's College, Chennai – 600 030.

*Corresponding author E-mail: **smurugesan5@gmail.com.**

ARTICLE INFO

Key Words

Proximate composition, Amino acid, Carbohydrate, Marine red alga, *Laurencia papillosa*.

ABSTRACT

Proximate and amino acid composition of marine red alga *Laurencia papillosa* were resolved to assess their potential nutritional value. In the present study, the proximate composition of *L. papillosa* demonstrated a higher contents of protein (341.25 ± 0.25 mg/g DW), carbohydrate (243.50 ± 0.23 mg/g DW) and low content of lipid (24.01 ± 0.19 mg/g DW). The free amino acids lysine (9.52 ± 0.31 mg/g DW) and leucine (8.83 ± 0.20 mg/g DW) were present in higher amount, whereas, tryptophan (1.84 ± 0.24 mg/g DW) and glutamic acid (2.00 ± 0.15 mg/g DW) were present in lower amounts. From this information, *L. papillosa* could be an incredible source of ingredients such as, dietary proteins, lipids and essential amino acids which enhances the nutritive value in human diets. *L. paillosa* specifically, to be secure for their versatile use and that they can add to a healthy balanced diet.

INTRODUCTION:

Marine macro algae are generally called as seaweeds which are macroscopic, multicellular and benthic algae. They are attached to solid substrates and rarely free. They are classified as three groups such as Chlorophyta, Phaeophyta and Rhodaphyta based on their major pigment content. Since the nutritious estimation of seaweed is great, the western countries also additionally impressively expending seaweed as food [**1**, **2**].

In India the seaweed consumption as raw food is known lesser compared to other coastal countries like Japan, South Korea, Philippines, etc. Aside from nourishment and industrial value, seaweeds also been used as manure, liquid fertilizer [**3**], colouring pigments [**4**], animal fodder [**5**], medicine and cosmetics products [**6**, **7**], water treatments [**8**], and bioethanol production [**9**]. They are known as natural sources of secondary metabolites, high level of proteins, minerals, fibers, essential amino acids, polyunsaturated essential fatty acids [**10, 11, 12**], antioxidants [**13]** and of hydrosoluble and liposoluble vitamins [**14]**. These properties make them as add-on in foods [**15]**. Over the most recent couple of decades the examination on metabolite and biological activities from seaweeds has been expanded fundamentally. Bioactive substances from seaweeds currently receive more consideration from the pharmaceutical and nutraceutical companies and the researchers as well [**16, 17, 18]**. Considering the consistently expanding population, it appears to be important to decide the chemical content of macro algae to assess their potential use as food sources directly or indirectly. *Laurencia papillosa* (C.Agardh) Greville belongs to class Rhodophyceae is an edible and economically important as a source of agar but its proximate composition and amino acid composition are inadequately known. Consequently, the goal of the present examination is to investigate the nutritive estimation of *L. papillosa* collected from South East Coast of Tamil Nadu, to decide the proximate and amino acid compositions.

MATERIALS AND METHODS

Sample collection

The red alga *Laurencia papillosa* (C.Agardh) Greville was collected from the coastal region of the Mandapam, Tamil Nadu, India. The experimental alga was distinguished by standard manual [**19**]. The algal samples were brought to the laboratory using sterile polythene bags for biochemical characterization. Upon collection the samples were washed repeatedly under running tap water followed by distilled water twice to eliminate the adhering impurities. The samples were then air-dried prior proximate composition evaluation.

Sample preparation: The experimental alga was shade dried, dehydrated and pulverized using electric blender. The pulverized powder was stored in a container in the dark. All experimental determinations were performed in triplicates from the pulverized alga.

Moisture content: The moisture content was determined by following the technique of Kumar *et al.*, [**20]**. The fresh experimental alga was dried using oven at 60°C for constant weight and after that by subtracting the dry weight from the wet weight.

Total ash content: The ash content was determined gravimetrically based on Kumar *et al*., [**20]** after heating at 550°C for 18 h in a muffle furnace.

Carbon and Nitrogen content: The total carbon and nitrogen content and its ratio were determined using the CHN Elemental Analyzer by combusting the experimental alga.

Estimation of total protein: The total protein was evaluated using the Folin-Ciocalteu Phenol method of Lowry *et al*., [**21**].

Estimation of total carbohydrates: The total carbohydrate was evaluated following the Phenol-sulphuric acid method of Dubois *et al*., [**22**].

Estimation of total lipids: The extraction of lipid was determined by the chloroform-methanol mixture by following Folch *et al*., method [**23**].

Energy content: The energy content of the experimental alga was determined by multiplying 17, 17 and 37 kJ/g with the values of protein, carbohydrate and lipid, respectively [**24]**.

Amino acid analysis: Amino acids were determined by high-performance liquid chromatography (HPLC) according to the method described by Rajendra [**25**]. Hydrolysis tube containing a seaweed protein of 75 mg was added with 2 ml of 6.0 N HCL. The solution was incubated in an oven at 110°C for 18 h and dried *in vacuo* using rotavapor. Equal voulume (20 µL) of the OPA reagent and amino acid sample was added in a vial together for derivatization (2 min). After this, 50 µL of Borate buffer (1 M, pH 9.0) was added and mixed well. Filtered and derivatized amino acid sample (20 µL) was injected into a HPLC containing a C18 reverse phase, ion exchange chromatography (Shimatzu-High Performance Liquid Chromatography LC 6A) and were analyzed using sodium acetate buffer with tetrahydrofuran (THF), triethylamine (TEA) and sodium acetate with methanol, acetonitrite as mobile phase A and B respectively. A variety of amino acid standards were injected simultaneously. By comparing the sample retention time (Rt) with that of the standard amino acids run at identical conditions, the amino acids present in the sample were identified and quantified.

Statistical analysis: All data are expressed as mean standard deviation (n = 3). Statistical comparisons of the results were performed using SPSS ver.19 by one-way analysis of variance (ANOVA) using Microsoft Office Excel 2007 (Microsoft, USA). A significant difference was considered at the level of $p < 0.05$ and <0.01.

Results and discussion

The red alga *L. papillosa* from South East coast of Tamil Nadu demonstrated a significant proximate composition (Table.1) and amino acid composition (Table.2 and 3) which are all expressed in mg/g dry weight (DW). The moisture content of *L. papillosa* was observed to be 680.84 ± 0.08% (mg/g) dry weight (DW). Possibly it can accumulate water and contain considerable values of water content, thus suggesting a new insight that these marine organisms have developed this ability to respond to different marine habitat, season and environment variation [**26**, **27**]. The determination of the water content of sea foods is enormously significant because it affects their sensorial quality, microbiological stability, physical characteristics and shelf life. Experimental alga exhibited 193.43 ± 0.37 mg/g DW of ash content (Table.1) which is relatively higher than the terrestrial plants with 5–10% DW [**20**]. The high ash content always indicates the presence of significant amounts of different mineral elements [**14**]. The ash content in the present study was compared to *Laurencia* species that reported by [**28]**. Neda Mehdipour [**29**] reported the ash content of *L. caspica* (26.82 ± 0.31 % DW) which is higher than *L. papillosa*. Gressler *et al.,* [**30**] revealed ash contents of *G. domingensis, G. birdiae, L. filiformis* and *L. intricata*, ranged from 22.5 ± 0.3 to 38.4 ± 0.1% dry. In our case, the ash content of *L. papillosa* was about one fold lower than those *L. intricata*. The distinction in ash contents of the seaweeds depends on their species, physiological factors, environmental changes, methods of mineralization and type of processing adopted [**31]**. In the present study proteins were the significant component in the proximate composition. Algae are regularly named as superfood because of their high protein content [**32]**. The protein content of *L. papillosa* was generally high 341.25 ± 0.25 mg/g DW (Table.1) and was in accordance with those recorded for green and red seaweeds (10-47% of the dry weight) [**14]**. Seaweed appeared to be a fascinating potential source of food proteins. According to Mabeau and Fleurence [**15**], the protein content of some marine algae was in addition to those found in terrestrial high protein plants, such as soybeans (35% of the dry mass). The protein content of this species contains all essential amino acids compared to those of the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) requirement. In this way, this seaweed can be a good source of proteins; however an examination on the digestibility *in vivo* of these algal proteins will be useful to determine the degradation process by human and animal gastrointestinal tract enzymes [**34]**. The carbohydrate content was observed to be 243.50 ± 0.23 mg/g DW in the red alga analyzed (Table.1). The carbohydrate content of the present study was higher to that of *L. papillosa* (155.23 ± 1.79 mg/g DW) estimated by Funda Turan *et al.,* [**28**]. Mohammadi *et al.,* [**35]** revealed that carbohydrate content for the same species *L. papillosa* (25% DW) collected from the Caspian Sea, which is more or less equivalent to our findings. The outcomes were comparatively higher than those outcomes by Hind *et al*., [**36**] for *Ulva lactuca* (17.5%), and Garcia *et al*., [**37**] reported in *Ulva lactuca* (11.5%). Carbohydrate is the most vital component of metabolism as it supplies the energy needed for respiration and other metabolic processes [**38]**. The carbohydrate content of the present study is in a concurrence with the converse relationships between carbohydrates and proteins corresponding to a pattern observed in several species of macroalgae [**39**]. The amount of lipid extracted was recorded in Table.1. The results of the present study revealed that a difference in the lipid contents between other studied *Laurencia* species. Our value (24.01 ± 0.19 mg/g DW or 2.42%) was higher than the amount reported for *L. intricate* (1.1 ± 0.0% DW) [**30]**, *L. cruciate* (1.53 ± 0.05 % DW) and *L .papillosa* (1.73 ± 0.11% DW) [**40]** and *Ulva lactuca* (0.8-1.2% DW) [**41]**. Deyab [**42**] detailed the lipid content of *L. obtusa* which ranged from 2.3 ± 03 to 13.7 ± 07 mg/g DW which is collected at various locations and in different seasons. This information obviously reflects the connection between the habitat, season and environment conditions in deciding the biochemical content of species [**2**]. In light of the outcome found in this investigation, the *L. papillosa* can be viewed as an elective source of a healthy food for human which has high protein with low fat content. The carbon content is predominantly associated with the combination of storage/structural polysaccharides, which gives energy and supports growth while the nitrogen is the principle component involved in generation of amino acids, peptides, DNA and ATP in the cell, supports reproduction and furthermore helps in keeping up neutral pH conditions essential for cell, growth. In this manner carbon/nitrogen (C/N) ratio is an important index of the nutritional quality of food. The total carbon and total nitrogen content of the experimental alga was resolved as 405.23 ± 0.20 and 54.40 ± 0.15 mg/g DW, respectively. In the present study, the C/N proportion was observed to be 7.44 ± 0.17 which is very low compared to *Caulerpa* species extended from 16.33 ± 1.89 to 18.45 ± 1.28% DW [**20]**. Carbohydrates, fats and proteins comprise ninety percent of the dry weight of food which gives the majority of the energy for living organisms [**43]**. Some food components such as water, minerals, fibers and vitamins gives little or no energy may still require for health benefits and survival for other purposes. Animals, including humans need a minimum intake of food energy to carry out metabolism and to drive their muscles. The energy content of the investigated alga was 11.50 kJ/g DW which ranges higher than the green algae *Caulerpa* ranged from 8.70 ± 0.56 to 10.91 ± 1.74 kJ/g DW [**20]**.

The amino acid contents of *L. papillosa* are magnificent because of their high protein value. Totally 20 amino acids have been identified in the protein hydrolysate of seaweeds of which 11 Essential Amino Acids (EAA) and 9 Non Essential Amino Acids (N-EAA) were present (Table 2 and 3). In general, EAA constitute 63.20% and N-EAA represented for 36.80% of total amino acids (Table 3). In the total amino acids, lysine (9.52 ± 0.31 mg/g DW) was highest in content and tryptophan was lower in content (1.84 ± 0.24 mg/g DW) both are EAA. Among the N-EAA, arginine (8.18 ± 0.17 mg/g DW) was higher in content and glutamic acid (2.00 ± 0.15 mg/g DW) was lower in content. The ratio of EAA to total amino acids was 0.63 and also demonstrated a good ratio of EAA to non-EAA (1.17) in seaweed (Table.2). From the findings of the present study, it is clear that all or more amino acids, both EAA and N-EAA were distributed comparatively. With good content EAA it is clear that the *L. papillosa,* can be used as alternative nutrient sources of amino acid and protein for human and animal consumption [**44, 45]**. All the above conflicting values may be as a result of seaweed species, seasonal variation of the year and/or environmental growth parameters, methods and type of processing [**30]**.

Table 1 Proximate compositions of *Laurencia papillosa**

Proximate compositions	(mg/g DW)
Carbon	405.23 ± 0.20^{h}
Nitrogen	54.40 ± 0.15^{d}
C/N ratio	7.44 ± 0.17^{a}
Moisture	680.84 ± 0.08^{i}
Ash	193.43 ± 0.37^{e}
Protein	341.25 ± 0.25^{g}
Carbohydrate	243.50 ± 0.23^{f}
Lipid	24.01 ± 0.19^{c}
Energy (kJ/g DW)	11.50 ± 0.01^{b}
P-Value	0.000
F-Value	124.60

***Values are expressed as Mean ± SEM, n=3; Means in each column with different superscripts letters are significantly different at p<0.05, while others at p<0.01.

Table 2 Amino acid profile of *Laurencia papillosa**

S.No	Name of amino acids	(mg/g DW)
1	Aspartic acid	3.11± 0.15 ^{c}
2	Glutamic acid	2.00 ± 0.15 ^{b}
3	Asparagine	5.04 ± 0.18 ^{f}
4	Serine	2.33 ± 0.29 ^{b}
5	Glutamine	5.84 ± 0.23 ^{g}
6	Glycine	3.19 ± 0.16 ^{c}
7	Threonine	7.07 ± 0.12 ^{h}
8	Arginine	8.18 ± 0.17 ^{i}
9	Alanine	2.33 ± 0.19 ^{b}
10	Cysteine	4.27 ± 0.23 ^{e}
11	Tyrosine	6.77 ± 0.21 ^{h}
12	Histidine	7.33 ± 0.12 ^{h}
13	Valine	3.39 ± 0.24 ^{cd}
14	Methionine	5.76 ± 0.29 ^{g}
15	Isoleucine	2.03 ± 0.18 ^{b}
16	Phenylalanine	3.82 ± 0.17 ^{de}
17	Leucine	8.83 ± 0.20 ^{j}
18	Lysine	9.52 ± 0.31 ^{k}
19	Proline	3.30 ± 0.26 ^{cd}
20	Tryptophan	1.84 ± 0.24 ^{b}
21	Total Amino acid	95.95 ± 0.0 ^{n}
22	EAA	60.63 ± 0.0 ^{m}
23	Non-EAA	35.32 ± 0.0 ^{l}
24	EAA/ Non-EAA	1.17 ± 0.0 ^{a}
25	EAA/ Total Amino acid	0.63 ± 0.0 ^{a}
P-Value		0.000
F-Value		126.80

***Values are expressed as Mean ± SEM, n=3; Means in each column with different superscripts letters are significantly different at p<0.05, while others at p<0.01.

Table 3 Percentage of amino acids of *Laurencia papillosa*

Essential amino acids (%)	
Threonine	7.37
Cysteine	4.45
Tyrosine	7.06
Histidine	7.64
Valine	3.53
Methionine	6.01
Isoleucine	2.11
Phenylalanine	3.99
Leucine	9.2
Lysine	9.92
Tryptophan	1.92
Non-essential amino acids (%)	
Aspartic acid	3.24
Glutamic acid	2.08
Arginine	8.52
Alanine	2.43
Asparagine	5.25
Serine	2.43
Glutamine	6.09
Glycine	3.32
Proline	3.44
Total essential amino acids	63.20
Total non-essential amino acids	36.80
EAA/ Non-EAA	1.22
EAA/ Total Amino acid	0.66

CONCLUSION

The seaweeds are a potential and healthy food supplement for human diets and may be of use to the food industry as a source of ingredients with high nutritional value, and its commercial value can be enhanced by improving the quality and extending the scope of seaweed-based products. The proximate and amino acid composition were high in content and conflicted with other studied algae. It is apparent that the *L. papillosa* could be a potential source of food ingredients for human and animal diets. The nutritional value of this species mainly derives from their high proteins and carbohydrates together with the low content of fat and Energy. The inclusion of *L. papillosa* in the diet could be beneficial for health. As such, nutrition or health researchers should collaborate early on with food technologists, food industry in order to design and develop suitable appealing products with these ingredients.

REFERENCES

1. Kilinc, B., Cirik, S., Turan, G., Tekogul, H. and Koru, E. 2013. Seaweeds for food and industrial applications, food industry. In: Innocenzo Muzzalupo (ed) InTech, pp 735–748.
2. Zawawi, M.H., Idris, M.H., Kamal, A.H.M. and Wong, S.K. 2014. Seaweed composition from Bintulu coast of Sarawak, Malaysia. *Pakistan Journal of Biological Sciences*. 17(8): 1007-1014.
3. Yasmin Akhtar., Farha Aziz and Amina Ajmal. 2015. Studies on the effect of soil amendment seaweed fertilizer (ssf) of *Botryocladia leptopodia* (J.Agardh) Kylin (Red Algae) on growth and biochemical

constituents of *Cicer arietinum* (Chick pea). *International Journal of Recent Scientific Research Research*. 6 (9): 6246-6250.

4. Jayasinghe., Pahalawattaarachchi, V. and Ranaweera, K.K.D. S. 2016. Seaweed extract as a natural food coloring agent in jelly desserts on chemical, microbial and sensory quality. *Academy of Agriculture Journal*. 1: 65-69.
5. Ktita, Chermiti, and Mahouachi. M. 2010. The use of seaweeds (*Ruppia maritima* and *Chaetomorpha linum*) for lamb fattening during drought periods. *Small Ruminant Research*. 91(1): 116 – 119.
6. Pooja, S. 2014. Algae used as Medicine and Food-A Short Review .*J. Pharm. Sci. & Res*. 6(1): 33 – 35.
7. Mouritsen, 2013. *Seaweeds Edible, Available & Sustainable*. The University of Chicago Press, Chicago & London, 287 pp.
8. Kim, Kraemer, Yarish, 2014. Field scale evaluation of seaweed aquaculture as a nutrient bioextraction strategy in Long Island Sound and the Bronx River Estuary. *Aquaculture*. 433: 148-156.
9. Adams,Gallagher, J. and Donnison, I. 2009. Fermentation study on *Saccharina lattisima* for bioethanol production considering variable pre-treatments. *Journal of Applied Phycology*. 21: 569-574.
10. Rasyid, A. 2017. Evaluation of nutritional composition of the dried seaweed *Ulva lactuca* from Pameungpeuk waters, Indonesia. *Tropical Life Sciences Research* 28(2):119–125.
11. Roohinejad, S., Koubaa, M., Barba, F.J., Saljoughian, S., Amid, M. and Greiner, R. 2017. Application of seaweeds to develop new food products with enhanced shelf-life, quality and health-related beneficial properties. *Food Res Int.* 99(Pt 3):1066-1083.
12. Thennarasan, S. and Murugesan, S. 2015. Biochecmial composition of marine brown alga *Lobophora variegata* from Mandapam in the South East Coast of Tamil Nadu. *International Journal of Phytopharmacy*. 5 (3) 25-29.
13. Mohanapriya, P., Murugesan, S. and Sivamurugan, V. 2017. *In vitro* Free Radical Scavenging Activity of *Tolypiocladia glomerulata* (C.Agardh) .Schmitz. *Saudi J. Med. Pharm. Si.* 3(4): 245-249.
14. Mohamed, S., Hashim, S.N. and Rahman, H.A. 2012. Seaweeds: A sustainable functional food for complementary and alternative therapy. *Trends in Food Science and Technology*. 23(2):83-96.
15. Mabeau, S. and Fleurence, J. 1993. Seaweed in food products: Biochemical and nutritional aspects. *Trends Food Sci Technol*. 4: 103-107.
16. Kausalya, M. and Narasimha rao, G.M. 2015. Antimicrobial activity of *Gelidium pusillum* and *Centroceros clavatum* from Visakhapatnam Coast, India. *J. Algal Biomass Utln*. 2015, 6 (2): 40- 48.
17. Vikash Kumar, Suvra Roy and Debtanu Barman. 2016. Bioactive Compounds From Algae And Bacteria In Marine Environment. *Aquafind*.
18. Murugesan, S., Bhuvaneswari, S., Mahadeva Rao, and Sivamurugan, V. 2017. Screening of Phytochemicals and Antibacterial Activity of Marine Red Alga *Portieria hornemannii* (Lyngbye) P. C. Silva. *Research Journal of Pharmacology and Pharmacodynamics.* 9(3):131-136.
19. Desikachary, T.V., Krishnamurthy, V. and Balakrishnan, M. S. 1998.

Rhodophyta Vol. II-Part-IIB. Madras Science Foundation, Chennai. pp: 359.

20. Kumar, Kumari, Reddy, and Jha, 2011. Assessment of nutrient composition and antioxidante potential of Caulerpaceae seaweeds. *Journal of Food Composition and Analysis*, 24(2):270-278.
21. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. *J. Biol Chem*. 193: 265-273.
22. Dubois, Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. 1956. Colorimetric method for determination of sugar and related substances. *Annal Chem*. 28: 350.
23. Folch, J., Lees, M. and Sloane Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol Chem*, 226: 497-509.
24. FAO (Food and Agriculture Organization). 2003. Food energy-methods of analysis and conversion factors. Report of a Technical Workshop. FAO Food and Nutrition Paper 77. Rome.
25. Rajendra, 1987. High performance liquid chromatographic determination of amino acids in biological samples by precolumn derivatization with O-phthaldehyde. *J Liq. Chromat*. 10: 941– 955.
26. Andrade, L.R., Leal, R.N., Noseda, M., Duarte, M.E.R., Pereira, M.S., Mourão, P.A.S., Farina, M., Filho, G.M.A. 2005. Brown algae overproduce cell wall polysaccharides as a protection mechanism against the heavy metal toxicity. *Marine Pollution Bulletin*. 60(9):1482-1488.
27. Rodrigues, Quinderé, A.L.G., De Queiroz, I.N.L., Coura, C.O., Benevides, N.M.B. 2012b. Comparative study of sulfated polysaccharides from *Caulerpa* spp. (Chlorophyceae). Biotechnological tool for species identification? *Acta Scientiarum. Biological Sciences*. 34(4):381-389.
28. Turan, F., Ozgun, S., Sayın, S. and Ozyılmaz, G. 2015. Biochemical composition of some red and green seaweeds from Iskenderun Bay, the northeastern Mediterranean coast of Turkey. *J. Black Sea/Mediterranean Environment*. 21(3): 239-249.
29. Mehdipour, N. 2015. Biochemical composition of the Caspian Sea red macroalga, *Laurencia caspica*. *J Marine Sci Res Dev*. 5:2.
30. Gressler, V., Yokoya, N.S., Fujii, M.T., Colepicolo, P., Filho, J. M., Torres, R.P. and Pinto, E. 2010. Lipid fatty acid, protein, amino acid and ash contents in four Brazilian red algae species. *Food Chemistry*. 120(2): 585-590, 2010.
31. Norziah, M.H. and Ching, C.Y. 2000. Nutritional composition of edible seaweed *Gracilaria changgi*. *Food Chemistry*. 68:69-76.
32. Chakraborty, S and Bhattacharya, T. 2012. Nutrient composition of marine benthic algae found in the Gulf of Kutch coastline, Gujarat, India. *J. Algal Biomass Utln.* 3 (1), 32-38.
33. Mabeau, S. and Fleurence, J. 1993. Seaweed in food products: Biochemical and nutritional aspects. *Trends Food Sci Technol.* 4: 103-107.
34. Charles, A.L., Chang, C.K., Wu, M.L. and Huang, T.C. 2007. Studies on the expression of liver detoxifying enzymes in rats fed seaweed (*Monostroma nitidum*). *Food and Chemical Toxicology*. 45(12): 2390-2396.

35. Mohammadi, Tajik, and Hajeb, P. 2013. Nutritional composition of seaweeds from the Northern Persian Gulf. *Iranian Journal of Fisheries Sciences*. 12(1) 232- 240
36. Hind, Z., Rabah, A., Christelle, B., Hacène, B. and Yves, B. 2014. Chemical and biological evaluation of the nutritive value of Algerian green seaweed *Ulva lactuca* using in vitro gas production technique for ruminant animals. *International Journal of Advanced Research*. 2:916- 925.
37. Garcia, Palacios, V and Roldán, A. 2016. Nutritional Potential of Four Seaweed Species Collected in the Barbate Estuary (Gulf of Cadiz, Spain). *Journal of Nutrition & Food Sciences*.6.3
38. Khairy and El-Shafay S.M. 2013. Seasonal variations in the biochemical composition of some common seaweed species from the coast of Abu Qir Bay, Alexandria, Egypt. *Oceanologia*. 55(2): 435-452.
39. Mourandi-Givernaud, Givernaud, T., Morvan, H. and Cosson, J., 1993. Annual variations of the biochemical composition of *Gelidium latifolium* (greville) Thuret et Bornet. *Hydrobiologia*. 260/261, 607–612.
40. Mehdipour, N., Sheijooni Fumani, N. and Rahnama, R. 2014. Proximate and Fatty acid Composition of the Southern Caspian Sea Macroalgae. *Journal of the Persian Gulf. Marine Science*. 5(18) 10:63-72.
41. Abirami, and Kowsalya, S. 2011. Nutrient and nutraceutical potentials of seaweed biomass *Ulva lactuca* and *Kappaphycus alvarezii*. *Journal of Agricultural Science and Technology* 5(1): 1939- 1250.
42. Deyab, M.A.I 2011. Biochemical constituents and bioactivity of *Laurencia obtuse* (ceramiales, Rhodophyta) in relation to seasonal and ecological factors of Red Sea Coast- Egypt. Egypt. *Egyptian J. of Phycol*. 12. 33-46.
43. The Merck Manual. 1899. Carbohydrates, Proteins and fats.
44. Ommee, and Payap. 2012. Biochemical composition and physicochemical properties of two red seaweeds (*Gracilaria fisheri* and *G. tenuistipitata*) from the Pattani Bay in Southern Thailand. *Songklanakarin Journal of Science and Technology*. 34(2): 223-230.
45. Xiren and Aminah. 2017. Proximate composition and total amino acid composition of *Kappaphycus alvarezii* found in the waters of Langkawi and Sabah, Malaysia. *International Food Research Journal*. 24(3): 1255-1260.