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Comparative Studies on Fatty Acid Composition of Three Marine Macroalgae Collected from Mandapam Region: South East Coast of India

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Abstract: Owing to the discrepancies in elucidating the fatty acid composition of seaweeds, the role of seaweeds in nutrition and pharmaceutics has been qualm. Hence, there is a need for comprehensive studies on the fatty acid composition of the seaweeds to set the benchmark for the incorporation of seaweeds in all the possible fields. Therefore, this study has been designed to investigate and compare the fatty acid profile of the three seaweeds, *Ulva lactuca, Sargassum wightii* and *Kappaphycus alvarezii* collected from the mandapam coast, Tamilnadu. The analysis of the fatty acids (as methyl esters) by Gas Chromatography-Mass Spectrometry (GC-MS) revealed the presence of 16 fatty acids in *U. lactuca*, 20 in *S. wightii* and 14 in *K. alvarezii.* Highest relative percentages of saturated and unsaturated fatty acids were observed in *K. alvarezii* and *S. wightii* respectively. Palmitic acid was the dominant fatty acid in all three seaweeds. The erucic acid, a potential component for biodiesel was observed in all the three seaweeds. Highest Omega-3 and Omega-6 fatty acid content was observed in *S. wightii* whereas *K. alvarezii* reported the high Omega-9 fatty acid content. Some rare fatty acids like eicosadienoic acid, eicosatrienoic acid and 7-hexadecenoic acid were also observed.

Key words: Erucic acid · *Kappaphycus alvarezii* · Palmitic acid · *Sargassum wightii* · *Ulva lactuca*

INTRODUCTION

Marine organisms are a rich source of structurally novel and biologically active metabolites which are of great interest in the pharmaceutical industry. Marine sources are the contents of functional ingredients such as polyunsaturated fatty acids, β-Carotene and their pigments carotenoids, sulphated polysaccharides and sterols. Such compounds with magnificent importance are fatty acids of marine algae containing low lipid value which makes them interested to the researchers for nutritional and potential medical applications [1-5]. Seaweeds have some of the valuable medicinal value components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Seaweeds are macroalgae living in sea or brackish water. They are often referred to as sea vegetables [6, 7]. They are the simple organisms with unique properties. In comparison to cultivated vegetables, edible seaweeds are potentially good sources of proteins, minerals, non-starch polysaccharides, fibre, vitamins and some trace elements [6, 8]. Marine algae are the excellent source of bioactive compounds such as carotenoids, dietary fibre, protein, essential fatty acids, vitamins and minerals [8, 9]. Seaweed has little fat, ranging from 1-5% of dry matter, although seaweed lipids have a higher proportion of Essential Fatly Acids (EFA) than land plants. The occurrence of polyunsaturated fatty acids (PUFAs) in general and η-3 fatty acids in particular is an unique feature of lipids of marine origin and the PUFAs have considerable health and economic significance [1, 10]. The fatty acids of seaweeds generally have linear chains and even number of carbon atoms with one or more double bonds [11].

Seaweeds have been studied for long for the production of wide range of chemicals, some of which stand the only natural resource, e.g. agar, carrageenan and alginates. However, they have not been looked upon as sources of lipids. There is a general agreement in human nutrition on the desirability of replacing saturated animal fat with unsaturated oils rich in PUFA. Among them, fish oil, with its high content in n-3 PUFA, was

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shown to exert important biological effects on several pathways predisposing to cardiovascular diseases. Hence there is an increasing need to search for the new PUFA sources to replace the conventional ones [7]. Seaweed lipids can be used as an alternative for the conventional PUFA sources. It has been reported that several seaweeds have balanced ratio of n3 and n6 fatty acids, which is a property of good neutraceutical [5]. However there is a need for further research on the nature of the seaweed lipids and seaweed fatty acids. So far, much work has been done on algal fatty acids by several researchers [8, 12-17]. However, there are many discrepancies in literature in elucidating the fatty acid composition of the same or related species. This may be attributed to the environmental, genetic as well as the taxonomic factors [9, 11, 18]. Hence, the present work was undertaken to study and compare the fatty acid composition of three marine algae *Ulva lactuca* Linnaeus, *Sargassum wightii* Greville and *Kappaphycus alvarezii* Doty of three distinct algal classes Cholorophyta, Phaeophyta and Rhodophyta.

MATERIAL AND METHODS

Seaweed Collection and Preparation: The seaweeds were collected from Mandapam region, southeast coast of India. Immediately after collection, they were washed in fresh seawater to remove the epiphytes, sand and other extraneous matter. Further, the samples were washed thoroughly using tap water to remove the surface salt. The water was drained off and the seaweeds were spread on blotting paper to remove excess water. The samples were air dried for a period of 3-4 days under shade. Having completed the shade drying process they were cut into pieces and dried in an oven at 45°C. Completely dried material was weighed and ground finely in a mechanical grinder.

Fatty Acid Analysis: The samples were homogenized with chloroform: methanol (2:1 v/v) mixture and they were extracted using the method of Bligh and Dyer [19]. The AOAC method [20] was followed to esterify the lipid extract, fatty acid methyl esters (FAME) was prepared from the lipids extracted from the seaweed sample by heating with the methanolic NAOH first and then with BF_3 methanol for esterification, 5ml n-heptane was added to recover the methyl esters in organic phase. Saturated NaCl solution was added to the mixture and the aqueous and organic were separated using a separating funnel. The upper n-heptane phase was pipetted out and stored in 10ml glass vials in refrigerator until further analysis.

Fatty acids were separated by using a Schimadzu QP2010 quadrupole Gas Chromatography Mass Spectrometer (GC-MS) instrument equipped with a Carbowax (30m x 0.25 mm ID; 0.25-μm film thickness) capillary column (Cromlab S.A.). Helium was used as the carrier gas. Injector and detector temperature were set at 250°C. Injection was performed in split mode (1:15). The column temperature was programmed initially at 50°C for 2 min and then increased at a rate of 10°C per min to a final temperature of 230°C. FAME esters were separated at a constant pressure (23.1 kPa). Individual fatty acid methyl esters (FAME) were identified by comparing the relative retention times (RRT).

RESULTS AND DISCUSSION

The chromatogram of fatty acid profile in the seaweeds *Ulva lactuca*, *Sargassum wightii* and *Kappaphycus alvarezii* is shown in the Figure 1. The fatty acid composition of the three seaweeds and the relative percentages of the fatty acids are presented in the Table 1. Comparison of total saturated fatty acid and unsaturated fatty acid content of the three seaweeds is presented in Figure 2.

The fatty acids like lauric acid, myristic acid, pentadecanoic acid, palmitic acid, heptadecanoic acid, stearic acid, arachidic acid, palmitoleic acid, oleic acid, erucic acid and Linoleic acid were present in all three seaweeds. Palmitic acid is the major fatty acid in all the three seaweeds. Among the seaweeds *Ulva lactuca* contained the high amount of palmitic acid (57.81%) and *Sargassum wightii* contained the least (44.97%). The saturated fatty acid content of all three seaweeds was higher than the unsaturated fatty acid content which is in conformity with the previous reports [5, 12-14, 16, 21-26]. Maximum saturated fatty acid content was observed in *K. alvarezii* (75.8%) followed by *U. lactuca* (72.09%) and *S. wightii* (58.13%). High saturated fatty acid content in *K. alvarezii* from the present study deviates from the reports of Fayaz *et al.* [8] on *K. alvarezii* where high unsaturated fatty acid than the saturated fatty acid content has been observed. In the case of mono unsaturated fatty acid, highest amount was observed in *S. wightii* (22.78%) followed by *K. alvarezii* (19.1%) and *U. lactuca* (18.8%). Highest Poly unsaturated fatty acid content was observed in *S. wightii* (19.09%) followed by *U. lactuca* (9.11%) and *K. alvarezii* (5.1%). Presence of high concentration of unsaturated fatty acids in brown seaweeds has been reported by other researchers

Table 1: Fatty acid content of seaweeds (value expressed in % with reference to the total fatty acid)

Carbon no.	Fatty Acid	*Ulva lactuca*	*Sargassum wightii*	*Kappaphycus alvarezii*
Saturated Fatty Acid (SFA)				
12:0	Lauric acid	1.06	0.51	0.54
14:0	Myristic acid	3.47	8.45	4.98
15:0	Pentadecanoic acid	1.77	0.80	0.76
16:0	Palmitic acid	57.8	44.97	49.72
17:0	Heptadecanoic acid	0.7	0.30	9.53
18:0	Stearic acid	3.35	2.16	6.89
20:0	Arachidic acid	1.1	0.58	1.14
22:0	Behenic acid/ Docosanoic acid	2.84	-----	1.72
24:0	Lignoceric acid	-----	0.36	0.52
Mono Unsaturated Fatty Acid (MUFA)				
16:1,n-9	7-Hexadecenoic acid	3.11	----	1.0
16:1,n-7	Palmitoleic acid	1.84	6.60	3.46
18:1,n-9	Oleic acid	5.15	13.10	13.08
18:1,n-7	Vaccenic (asclepic) acid	8.15	0.83	-----
20:1,n-9	Gondoic acid	------	1.30	-----
22:1,n-9	Erucic acid	0.55	0.95	1.56
Poly Unsaturated Fatty Acid (PUFA)				
16:2,n-4	Hexadecadienoic acid	-----	0.17	-----
18:2,n-6	Linoleic acid	5.48	5.53	5.1
18:2,n-3	Cis-12,trans-15-Octadecadienoic acid	0.48	-----	-----
18:3,n-3	α- Linolenic acid	3.15	4.25	-----
20:2,n-7	Eicosadienoic acid	-----	0.2	-----
20:3,n-7	Eicosatrienoic acid	-----	1.11	-----
20:4,n-6	Arachadonic acid	-----	7.4	-----
20:4,n-3	Eicosatetranoic acid	-----	0.43	-----
TOTAL Saturated Fatty Acid (SFA) content		72.09±1.05[b]	58.13±0.81[a]	75.8±0.96[c]
TOTAL Unsaturated Fatty Acid (UFA) content		27.91±0.67[b]	41.87±0.93[c]	24.2±0.84[a]

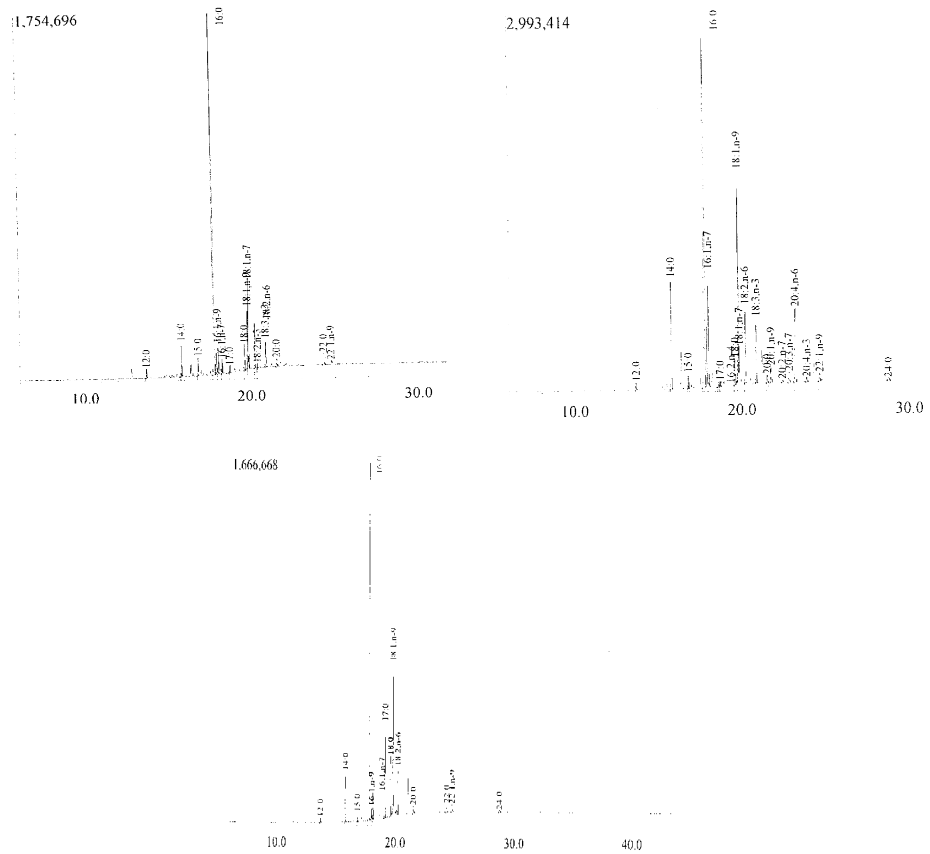

Fig. 1: Chromatogram of fatty acid profile in A) *Ulva lactuca* B) *Sargassum wightii* and C)*Kappaphycus alvarezii*

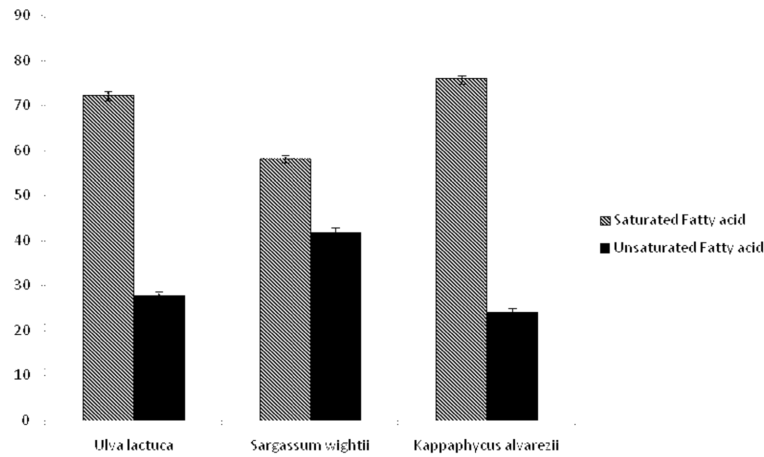

Fig. 2: Comparison of saturated and unsaturated fatty acid content of three seaweeds

[6, 27]. Essential fatty acids play an important role in the life and death of cardiac cells, the high percentage of unsaturated fatty acids might protect against Parkinson disease [28].

The results showed that green seaweed, *Ulva lactuca* contains 16 different fatty acids. Among those major fatty acid was palmitic acid followed by vaccenic (asclepic) acid, linoleic acid, oleic acid and myristic acid. Cis-12, trans-15-Octadecadienoic acid of linoleic fatty acid series was observed in least amounts. Palmitic acid was reported as the major fatty acid commom to *Ergrezia menziesii, Chondracanthus canaliculatus* and U*lva lobata* [29]. The highest relative percentages of lauric acid and pentadecanoic acid were observed which has been supported by Shameel [21] and Khotimchenko *et al*. [24]. Significant amount of behenic acid and oleic acid were observed in *U. lactuca* which is supported by Nelson *et al*. [29]. Considerable amount of stearic acid, α-linolenic acid, 7-hexa decenoic acid, palmitoleic acid and arachidic acid were observed. Erucic acid, a monounsaturated omega-9 fatty acid, which has a potential to be a component of bio-diesel was observed in minor quantities in *U. lactuca*.

Twenty different fatty acids were identified in brown seaweed *Sargassum wightii.* Among those major fatty acid was palmitic acid followed by oleic acid, myristic acid, arachadonic acid, palmatoleic acid, linoleic acid and hexadecadienoic acid which was observed at lowest levels. Presence of high percentage of palmitic acid was supported by Bhaskar *et al*. [5]. C18 and C20 unsaturated fatty acids were the major components in *S. wightii*. Arachadonic acid and oleic fatty acids predominated among polyenoic acids. Small amounts of C20:2 nonmethyl interrupted fatty acid was found. The above results for *S. wightii* were supported by Khotimchenko [30]. Oleic acid, myristic acid, palmitoleic acid, linoleic acid and α-linolenic acid were significantly more when compared to other two seaweeds. Similarly a high content of linoleic acid was found in *Codium fragile* [23, 25]. Fatty acids like stearic acid, pentadecanoic acid, lauric acid, arachidonic acid and heptadecanoic acid were observed in less relative percentage. Mono unsaturated ω-9 fatty acid like gondoic acid and erucic acid and saturated fatty acid like lignoceric acid were noticed in small amounts. Some of the rare fatty acids like eicosadienoic acid, eicosatrienoic acid and 7-hexadecenoic acid were also observed in *S. wightii.*

Kappaphycus alvarezii fatty acid profile showed 14 different fatty acids. However, presence of only eight fatty acids in *K. alvarezii* collected from Rameswaram coast was reported by Rajasulochana *et al*. [17, 31]. Palmitic acid was the prominent fatty acid and the lignoceric acid was present in minor quantities. Significant quantities of oleic acid, linoleic acid and myristic acid were also observed. The higher relative percentage of heptadecanoic acid was recorded in *K. alvarezii*. Stefanov *et al*. [32] reported the presence of heptadecanoic acid in *Phyllophora nervosa*. The relative higher percentage of stearic and arachidic acid were observed in *K. alvarezii.* Highest relative percentage of erucic acid was found in *K. alvarezii* than in the other seaweeds. The results obtained in this study were different from the reports of Rajasulochana *et al*. [17, 31].

The fatty acids observed in all the three seaweeds and their possible applications are summarized in the Table 2. The possible applications of the fatty acids present in the seaweeds decide their importance in different fields.

Table 2: Fatty acids and their applications

Fatty acid	Applications
Lauric acid	Possess antibacterial, antioxidant and antiviral properties. Induces the expression of cyclooxygenase-2.
Myristic acid	Acts as lipid anchor in biomembranes and also used in topical medicinal preparations. It is the most hypercholesterolemic of all the saturated fatty acids. It is a marker of excessive ethanol consumption that can be isolated from the hair of an individual.
Pentadecanoic acid	Used as a marker for butterfat consumption.
Palmitic acid	Most abundant saturated fatty acid in plants and animals. It induces the expression of cyclooxygenase-2 and after protein acylation, are used to confer lipid anchoring to a variety of signaling molecules.
Heptadecanoic/ Margaric acid	Generally observed in ruminants. The content of heptadecanoic acid in the subcutaneous adipose tissue of humans appears to be a good biological marker of long-term milk fat intake.
Stearic acid	Stearic acid can be used as a hardening agent for vegetable oils and due to its negligible effect on cholesterol metabolism it may be used as an alternative to modification of fatty acids by partial hydrogenation.
Arachidic acid	Because of its surfactant-like properties, arachidic acid is used in the manufacture of pharmaceuticals, soaps, cosmetics and food packaging.
Behenic/ Docosanoic acid	Commercially, behenic acid is often used to give hair conditioners and moisturizers their smoothing properties. It is a cholesterol-raising saturated fatty acid in humans.
Lignoceric acid	In mammals, it is synthesized during brain development and is found in cerebrosides.[1]The deficient peroxisomal oxidation of very-long-chain fatty acids, including lignoceric acid, contributes to certain syndromes, including Zellweger cerebro-hepato-renal syndrome and X chromosome-linked adrenoleukodystrophy.
7-Hexadecenoic acid	*cis*-7-Hexadecenoic acid has been isolated from autotrophic bacterial cultures associated with the accumulation of sulfate in biofilters. It is used for identifying specific genera of bacterial populations in natural environments.
Palmitoleic acid	Palmitoleic acid is an ω-7 monounsaturated fatty acid. Palmitoleic acid is assumed to be a signaling molecule which can help to fight weight gain.
Oleic acid	Oleic acid is a monounsaturated fatty acid and is one of the major components of membrane phospholipids. It is used as an excipient in pharmaceuticals and also as emulsifying or solubilizing agent in aerosol products.
Vaccenic (asclepic) acid	It is a naturally occurring trans fat found in the fat of ruminants and in dairy products such as milk and yogurt. Studies showed that feeding of vaccenic acid in rats resulted in lowered total cholesterol, lowered LDL cholesterol and lower triglyceride levels.
Gondoic acid	It is an monounsaturated omega-9 fatty acid found in a variety of plant oils. It is also found in the red cell membrane with increased concentrations in children with regressive autism.
Erucic acid	It can be a potential component for the bio-diesel due to its unique properties. It is a component in Lorenzo's oil which is used to treat adrenoleukodystrophy. The C-1 amide of erucic acid has been identified as one of the anandamide-related neurotransmitters associated with sleep.
Hexadecadienoic acid	It is a naturally occurring fatty acid. It possesses antimicrobial properties. It is found to inhibit the human topoisomerase I enzyme.
Linoleic acid	It is an unsaturated omega-6 fatty acid and one of the two essential fatty acids. It possesses anti-inflammatory, acne reductive and moisture retentive properties. Deficiencies in linoleic acid are linked to defective wound healing, growth retardation and dermatitis.
α- Linolenic acid	It is an essential fatty acid. As part of a low saturated fat diet, it helps to prevent cardiovascular disease. α- Linolenic acid decreases blood pressure, serum cholesterol levels and platelet aggregation.
Eicosadienoic acid	Eicosadienoic acid is an uncommon, naturally occurring PUFA. It has a strong regulatory role of lipids, can promote the decomposition of cholesterol in the liver of bile acids excreted, there is a stronger cholesterol-lowering effect. Also enhance protein synthesis, inhibit catabolism, increase appetite, enhance physical fitness, promote the growth and development and the promotion of tissue regeneration role.
Eicosatrienoic acid	Eicosatrienoic Acid (20:3ω-3) is a rare polyunsaturated fatty acid of the ω-3 series. one of the most active essential fatty acids when assayed for the inhibition of fatty acid elongation/desaturation reactions which convert dietary C-18 fatty acids to C-20 eicosanoid precursors.
Arachadonic acid	It is a polyunsaturated omega-6 fatty acid. Arachidonic acid is an essential fatty acid and a precursor for all prostaglandins, thromboxanes and leukotrienes. It is necessary for the repair and growth of skeletal muscle tissue and also used as an anabolic bodybuilding supplement.
Eicosatetranoic acid	It is a nonspecific inhibitor of cyclooxygenases and lipoxygenases.

Source:

1) Beare-Rogers *et al.* [33],

2) http://en.wikipedia.org/fattyacids

3) http://www.hmdb.ca/metabolites

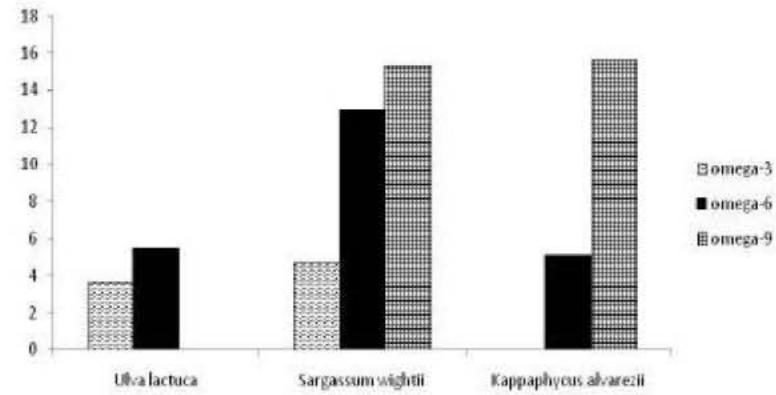

Fig. 3: Essential fatty acid composition of three seaweeds

Omega (ω)-3 fatly acids have recognized anti.infiammatoiy, antioxidant actions, that may contribute to their beneficial cardiac effects [34-36]. ω-3 PUFAs from both seafood and plant sources may reduce Coronary Heart Disease (CHD) risk [35, 36]. ω-6 fatty acids have been shown to be beneficial in the reduction of cholesterol levels when they are substituted for saturated fats in a person's diet. ω-9 is necessary yet non-essential because the body can manufacture a modest amount on its own, provided EFAs are present. ω-9 is mainly used when there is an insufficiency of ω-3, ω-6 or both. ω-3, ω-6 and ω-9 fatty acid composition of the three seaweeds was given in Figure 3. Present study showed that *Sargassum wightii* consists of high amounts of ω-3 and ω-6 fatty acids. High ω-9 fatty acid content was observed in *Kappaphycus alvarezii*. ω-9 fatty acids were absent in *Ulva lactuca* and ω-3 fatty acids were absent in *K. alvarezii*. However, *S. wightii* contained all three types of essential fatty acids.

Fatty acid content of the seaweeds is influenced by many factors. Some researchers have opined that the differences in the fatty acid composition of the algae are connected with environmental factors [18]. Some scientists have also found that distribution of fatty acids in marine plants get closely linked with taxonomic position [11]. Sanchez-Machado *et al.* [9] stated that the variations in the fatty acid content are due to the both environmental as well as genetic factors. However, in the present study only three different seaweeds have been compared from the different classes of algae. There is an urgent need for the large scale comparison of algae of the different classes to set a benchmark for the incorporation of the seaweeds in all the possible fields to exploit the vast seaweed resources including the biomedical advances.

CONCLUSION

The present study revealed that palmitic acid is the prominent fatty acid in all the three seaweeds. Erucic acid, which is a potential component for bio-diesel, is present in minor quantities in all the seaweeds. Some of the rare fatty acids like eicosadienoic acid, eicosatrienoic acid and 7-hexadecenoic acid were also observed. High amounts of omega-3 and omega-6 fatty acids were reported in *Sargassum wightii* and high omega-9 fatty acid content was observed in *Kappaphycus alvarezii*. By studying the fatty acid content and their application, it can be concluded that brown seaweed, *S. wightii* has the vast potential for incorporation in nutritional and biomedical fields when compared to other two seaweeds. Overall, it can be concluded that all the three Seaweeds fatty acid composition was unique and different from one another indicating their importance in divergent fields.

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