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Extraction and Profiling of Fatty Acids obtained from different Marine Seaweeds/Macro Algae

Research Article

Shubham S Gupta[1*], Tirupati M Rasala[1], Gulshan A Gurunani[1], Abhay M Ittadwar[1]

1. Gurunanak College of Pharmacy,
Rashtrasant Tukadoji Maharaj Nagpur University (RTMNU), Nagpur, Maharashtra, India.

Abstract

Seaweeds are acknowledged as potentially abundant sources of bioactive molecules with diverse applications in food, nutraceutical, and pharmaceutical industries. This study investigated the fatty acid (FA) composition of selected seaweed species collected from the Indian coastline, encompassing representatives from the Chlorophyta, Phaeophyta, and Rhodophyta phyla. Using gas chromatography, approximately 20 FAs were identified and quantified, including polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs). Among SFAs, palmitic acid (16:0) was the most prevalent across all species, followed by oleic acid (18:1) among MUFAs and linoleic acid (18:2) and eicosapentaenoic acid (20:5) among PUFAs. The variation in FA profiles among species suggests species-specific metabolic pathways influenced by environmental conditions. Additionally, the availability of essential FAs and notable PUFA/SFA ratios indicates the nutritional value of these seaweeds. These findings not only enhance our understanding of the biochemical diversity in Indian seaweeds but also support their potential utilization in health-promoting and therapeutic formulations.

Keywords: Seaweeds, Extraction, Physiochemical analysis, Fatty acids profiling, Nutraceuticals.

Introduction

Global food security has become a major problem in recent years because of factors including climate change, population growth and diversifying terrestrial food sources to meet energy demands (1). Furthermore, the geographical divisions between states and the boundaries between human races are becoming less distinct as a result of the globalisation of markets and the resulting increased globalisation of foods. Additionally, there has been an effort to improve and augment the nutritional value of human diets by investigating and using foods from unconventional sources, both terrestrial and marine. Subsequently, this relieves the increasing strain on traditional meals. Seaweeds, sometimes referred to as marine macroalgae, are one of the oceans' living, renewable resources that can be utilized for its potential food applications (2).

The pharmaceutical sector is very interested in the structurally unique and physiologically active metabolites found in marine species (3). As significant biological resources, seaweeds are an essential component of marine ecosystems. As primary producers, they contribute significantly to the diversity and productivity of marine populations. Moreover, they provide food and refuge to a variety of marine species at different stages of life (4). In an investigation conducted by Sohrabipour et al. (5), importance of seaweeds from 3 different species - red(Rhodophyta), green(Chlorophyta) and brown(Phaeophyta) - was assessed depending on their FA composition and medical applications in the treatment of specific human ailments. The study investigated the possibility that certain algae species may be useful resources for therapeutic uses.

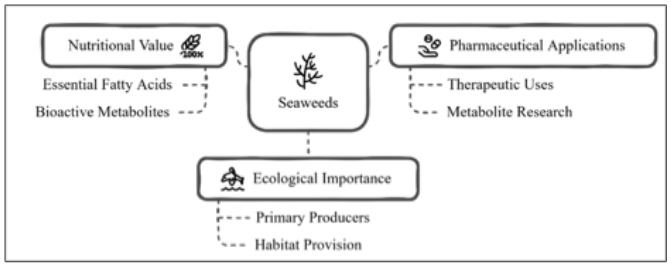
The FA content of seaweeds exhibits certain traits. Their fatty acids usually consist of one or more double bonds and linear chains of carbon atoms (even number) (6). Essential FAs, particularly PUFAs like ω-3 and ω-6, are prevalent in seaweeds and are crucial for both human and animal nutrition. The significant quantities of ω-3 and ω-6 FA in seaweeds maintains the ω-6/ω-3 ratio in accordance with WHO dietary guidelines (5).

Seaweeds have a low lipid content but a high proportion of PUFAs with a distinctive FA pattern, however they are underutilized irrespective of their abundance. A few bioactive metabolites that may be beneficial to human health are also present in them, including amino acids, carotenoids, chlorophylls, phenolics, polysaccharides and sterols. Algal blooms and invading seaweeds are examples of low-cost biomass that shows great promise (7) (8). Seaweeds are preferred because of their high PUFA content, quick growing rate, and simpler structure. However, studies are currently in progress to use seaweeds for FA, most likely because of this resource's abundance (9). Hence

*** Corresponding Author:**
Shubham S Gupta
Gurunanak College of Pharmacy,
Rashtrasant Tukadoji Maharaj Nagpur University (RTMNU),
Nagpur, Maharashtra, India.
Email Id: shubhamgupta0918@gmail.com

this study's goal is to determine the qualitative and quantitative content of total FA as well as their profiles in a few seaweeds that were gathered from the Indian coastlines.

Figure 1: Nutritional and pharmaceutical potentials of seaweeds



Materials and methods

Seaweeds collection and preparation

The three different species of seaweeds viz, *Chaetomorpha linum*, *Padina pavonica* and *Gracilaria foliifera* were procured and authenticated from Annakkili Amma Research Institute (AARI), Medavakkam, Chennai. To get rid of epiphytes, dirt, and other foreign objects, the freshly harvested seaweeds were first cleaned with seawater. The seaweeds were thoroughly cleaned with filtered water to remove any remaining salt and sand particles. In a shady location, the samples were allowed to air dry at ambient temperature (25–30°C) for 3-5 days. After that, they were dried at 38±2°C until their weight remained constant. After the samples had dried fully, they were broken up into tiny pieces and crushed into a coarse powder. Impurity removal for further extraction and analysis was guaranteed by this preparation technique.

Extraction of total lipids

The modified Folch technique (10) was used to extract the lipid, but with minor changes. The sample of dried seaweed that had been roughly powdered was extracted using a solvent combination of chloroform and methanol (2:1, v/v). The combination was maintained at room temperature on a rotatory shaker set to 120 rpm for 48 hours. Whatman filter paper (No. 1) was used to filter the homogenate in order to extract the liquid phase. To create phase separation, the same amount of water and chloroform (1:1 v/v) combination was added. After being collected in a glass vial that had been previously weighted, the organic phase of the lipid extract was dried at 40–45°C until its weight remained constant. By this technique, the lipid extract was prepared for further examination.

Phytochemical evaluation

- Alkaloids: Small quantity of sample was combined with Mayer's reagent. Presence of alkaloids were indicated by a cream-colored precipitate.
- Steroid: Conc. sulfuric acid and acetic anhydride (2ml each) were mixed with the sample. Change in colour to bluish green from violet confirms the steroids test.
- Terpenoids: Conc. sulfuric acid and chloroform (1ml each) are incorporated into the sample. Terpenoids are indicated by a reddish-brown interface.
- Flavonoids: Magnesium was introduced into the solution of sample and then overlaid with strong hydrochloric acid; flavonoids are indicated by intense red coloration.
- Phenolic Compounds: Addition of ferric chloride reagent to the sample was done; blue-green or violet color indicates the phenolic compounds.
- Tannins: The lead acetate solution was combined with the sample. Presence of tannins was confirmed by an intense white solution.
- Cardiac Glycosides: Ferric chloride solution, glacial acetic acid, and conc. sulfuric acid was carefully added to the sample. Deoxy-sugar was indicated by a brown ring.

Physicochemical tests

Determination of density

A 25 ml empty density bottle's weight was determined with an electronic weighing scale. Then it was determined how much the bottle and sample weighed when it was completely filled to the brim with sample. The following formula was used to get the density.

$$\text{Density} = \frac{\text{Mass}}{\text{Volume}}$$

Saponification value:

A measured portion (2g) of sample was taken in 250 mL round-bottom flask, to which 0.5 M ethanolic KOH (25ml) was added. After that, a reflux condenser was utilized, and the mixture was subjected to heat in a water bath for half an hour while being constantly swirled until it began to gently boil. While still hot, the remaining unreacted KOH was back titrated with 0.5 M HCl after adding phenolphthalein which acts as indicator. Under the same circumstances, a blank titration was also carried out using distilled water. The saponification value was subsequently calculated using the obtained titration data.

$$\text{Saponification value} = \frac{28.05 * (\text{Blank titration reading} - \text{Sample titration reading})}{\text{Weight of oil sample taken}}$$

Acid value

A combination of equal parts ethanol and ether was taken in a 250 ml round-bottom flask along with the sample (10g). With regular shaking, the flask's content were gradually warmed by a reflux condenser until the oil sample was fully dissolved. After that, 0.1 N KOH was used to titrate the content, with phenolphthalein serving as indicator to obtain the faint pink colour after shaking for 30 sec.

$$\text{Acid value} = \frac{5.61 * \text{Vol. of potassium hydroxide required}}{\text{Weight of oil sample taken}}$$

Iodine Value

The sample (1g) was placed in a 250ml iodine flask and stored in the drawer for exactly 30 minutes. To wash away any iodine that could have been on the

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stopper, a 15% W/V potassium iodine solution (10ml) were introduced to the flask. This was titrated until the sodium became bright yellow against 0.14 M Na_2SO_3. Following this the starch indicator was added and the titration continued until the blue color simply disappeared. For a blank determination, the same conditions were applied using distilled water. The titre reading was noted and utilised to determine the iodine value as shown below.

$$\text{Iodine value} = \frac{1.269 * (\text{Blank titration reading} - \text{Sample titration reading})}{\text{Weight of oil sample taken}}$$

Hexabromide Test

Measure the sample (1g) into a wide-mouthed boiling tube with a 50 ml capacity. Add chloroform (5ml) gradually, then continue to add bromine (1ml), until a deep red coloration develops. Cool the tube by submerging it in cold water. While gently shaking the mixture, slowly introduce rectified spirit dropwise (1.5ml) until any initial precipitate dissolves completely. Then, add diethyl ether (10ml) and mix thoroughly. Put the tube back in the cold water and let it there for 20 mins. At this point, the development of precipitates suggests that PUFAs are present.

Fatty Acid Methyl Ester (FAME) analysis

Preparation of FAME

The transmethylation of lipids obtained from the seaweeds (*Chaetomorpha linum*, *Padina pavonica* and *Gracilaria foliifera*) and extraction of FAME was done (11). 200µL of sample was mixed with 1 mL Hexane, shaken for 10 seconds. 200µL of 2N Methanolic NaOH was added to the mix and vortexed. 200µL 2N Methanolic HCl was added and vortexed. The top layer was collected, passed through a nylon 13 mm 0.2µm syringe filter, and then injected into the GCMS.

GC-MS analysis of FAME

FAMEs were analyzed using Agilent 7890A GC paired with a 5975C MS instrument, equipped with DB-WAX capillary column (30 m length; 0.25 mm internal diameter; 0.25 µm film thickness). 2 µL sample was inserted using split ratio of 300:1. High-purity helium (99.9995%) was incorporated as carrier gas, flowing at a steady rate of 0.6 mL/min. The instrument operated in electron impact (EI) mode with an ionization energy of 70 eV. The injector temperature was kept constant at 250°C. The temperature schedule for the column oven as follows:

Oven	Rate (°C/min)	Value (°C/min)	Hold Time (min)
Beginning		80	5
Level 1	5	150	5
Level 2	2	175	1
Level 3	10	250	3

The chemical compounds were identified by matching the spectrum configurations with those available on mass spectral database (NIST -08 spectrum DATA).

Results

Phytochemical evaluation

Table 1: Phytochemical evaluation parameters

Test	*Chaetomorpha linum*	*Padina pavonica*	*Gracillaria foliifera*
Alkaloids	×	✓	✓
Steroids	×	✓	✓
Terpenoids	✓	×	✓
Flavonoids	✓	×	×
Phenolic compounds	✓	✓	×
Tannins	×	✓	✓
Cardiac Glycosides	×	×	×

Physicochemical tests

Table 2: Physicochemical test parameters

Test	*Chaetomorpha linum* (Chlorophyta)	*Padina pavonica* (Phaeophyta)	*Gracillaria foliifera* (Rhodophyta)
Density (g/ml)	0.930	0.864	0.892
Saponification Value (mg KOH/g)	185.2	286.2	221.6
Acid Value (mg KOH/g)	2.13	4.66	3.42
Iodine Value (mg I/g)	107.6	86.8	81.9
Hexabromide Test	+	+	+

Fatty acid analysis

GC Spectra and results

Figure 2: GC profile of *Chaetomorpha linum*

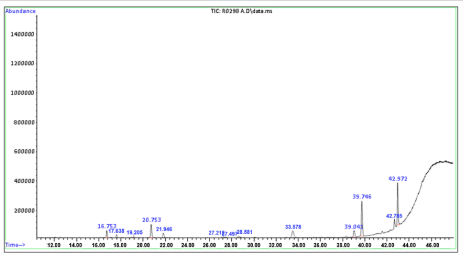

Figure 3: GC profile of *Padina pavonica*

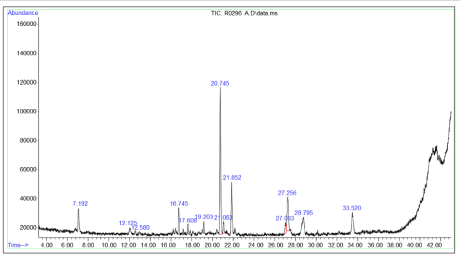

Figure 4: GC profile of *Gracilaria foliifera*

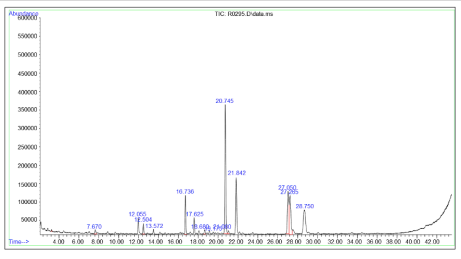

Table 3: Fatty acid profile of *Chaetomorpha linum*, *Padina pavonica* and *Gracilaria foliifera*

FAME	C:D	*Chaetomorpha linum*	*Padina pavonica*	*Gracilaria foliifera*
Capric acid	10:0	-	6.71%	1.35%
Undecylic acid	11:0	-	2.24%	4.05%
Lauric acid	12:0	-	1.58%	2.70%
Tridecylic acid	13:0	-	-	1.35%
Myristic acid	14:0	4.82%	6.84%	9.46%
Myristoleic acid	14:1	2.07%	3.16%	4.05%
Pentadecylic acid	15:0	-	-	1.22%
Pentadecyloleic acid	15:1	1.38%	3.68%	1.08%
Palmitic acid	16:0	8.28%	34.47%	31.08%
Palmitoleic acid	16:1	-	3.68%	0.95%
Margaric acid	17:0	3.45%	12.63%	13.78%
Stearic acid	18:0	1.38%	3.55%	10.27%
Oleic acid	18:1	0.69%	9.21%	9.46%
Linoleic acid	18:2	1.04%	4.60%	5.95%
Arachidic acid	20:0	4.14%	5.79%	-
Hexadecatetraenoic acid	16:4	4.48%	-	-
Ecosapentaenoic acid	20:5	22.07%	-	-
Docosapentaenoic acid	22:5	11.38%	-	-
Docosahexaenoic acid	22:6	32.76%	-	-

Discussion

The three different species of seaweeds viz., *Chaetomorpha linum*, *Padina pavonica* and *Gracilaria foliifera* were procured and authenticated. Total lipid was extracted from these seaweeds by modified Folch method employing a chloroform/methanol (2:1 v/v) solvent mixture as the extraction solvent. The portion of the extract that was subjected to phytochemical evaluation, according to the research findings, included terpenoids, flavonoids and phenolic compounds in *Chaetomorpha linum*; alkaloids, steroids, saponins, phenolic compounds and tannins in *Padina pavonica*; alkaloids, steroids, terpenoids and tannins in *Gracilaria foliifera*.

During the physicochemical evaluation, the physical attribute i.e. density was obtained in the range of 0.85-0.95 g/ml. The Lorentz–Lorenz relation may provide a good description of the RI-density relationship, according to a combination of theoretical and empirical investigation. Since more electric dipoles are produced by the applied electric field, a denser material usually has a higher refraction index (12).

The extracts' saponification values (SV) ranged from 180 to 290 mg KOH/g, indicating that the triglycerides present in the extract had a low molecular weight of FA (both saturated and unsaturated). The outcome showed a favorable comparison with soybean oil [13]. Thus the length of the FA chains generated from triacylglycerols determines the SV. Triacylglycerols with shorter FA chains are indicated by a high SV; conversely, a lower SV shows long chain FA on the glycerol backbone (13).

The acid value of seaweeds *Chaetomorpha linum*, *Padina pavonica* and *Gracilaria foliifera* are 2.13 mg KOH/g, 4.66 mg KOH/g and 3.42 mg KOH/g respectively. Given that solvent extraction reduced the acid content, this low acid value highlights the advantages of solvent extraction versus mechanical extraction (14). This acid value indicates how well lipase action can break down the constituent glycerides.

The iodine value of the extracts was 80-110 mg I/g which is similar to those of sesame seed oil [115 mg I/g] and sunflower oil [124 mg I/g] (15). Usually, the high unsaturation is depicted by the higher iodine value and the more likely it is to experience oxidative rancidity (16).

A hexabromide test is used to measure the amount of unsaturation present in the sample. The presence of unsaturated FA is indicated by the production of a hexabromide precipitate when FA, such as extract containing linolenic acid, are treated with bromine in chloroform and subsequently with alcohol and ether (17).

Overall, there were notable differences in quantities of various kinds of FA across all species that were studied. FA composition of algal lipids changes greatly depending on the species, salinity, pollution, light, habitat and environmental factors (18). The FA composition was determined by capillary GC method of three different seaweeds and it indicates the presence of around 20 compounds. The FA content in the examined seaweeds was roughly the same but *Chaetomorpha linum* contains significant quantity of unsaturated FAs, subsequently *Padina pavonica*, and finally *Gracilaria foliifera*.

The current findings indicate distinct FA profiles across the studied seaweed species, characterized by notable amounts of SFAs, including stearic acid (18:0), margaric acid (17:0), palmitic acid (16:0) and myristic acid (14:0); as well as MUFAs such as oleic acid (18:1), pentadecyloleic acid (15:1) and myristoleic acid (14:1). These FAs together made up over half of the total FA composition in the examined samples. Among them, palmitic and oleic acids were particularly abundant in red and brown seaweeds. This trend is consistent with

earlier reports that identified palmitic acid as the dominant FA in various seaweed species (19).

Seaweed samples contain notable quantities of oleic acid, palmitoleic acid and palmitic acid, which could be valuable for use in nutritional products or as dietary supplements. These fatty acids have demonstrated significant antimicrobial effects against key oral pathogens, including *Fusobacterium nucleatum*, *Streptococcus mutans*, *Candida albicans* and *Porphyromonas gingivalis*. In addition, MUFAs derived from C16 and C18 chains are believed to play a protective role in various health conditions, especially those that have an effect on the cardiovascular system (20) (21).

In the case of Chaetomorpha linum, analysis of its FA profile revealed a predominance of PUFAs. The largest concentration among these was docosahexaenoic acid (DHA; 22:6), which was followed by docosapentaenoic acid (DPA; 22:5), eicosapentaenoic acid (EPA; 20:5), and hexadecatetraenoic acid (HDA; 16:4). Research by Moustafa and Batran (22) highlights the importance of PUFAs in the diets of humans and other vertebrates, as these organisms are unable to synthesize them on their own. Additionally, Erkkilä et al. (23) noted that an increased ratio of PUFAs to SFAs in the diet is linked with a reduced risk of cardiac diseases, supporting the recommendation to substitute SFAs with PUFAs for better heart health.

Conclusion

This study offers a thorough examination of the FA profiles of several seaweed species that were collected along the Indian coastline, highlighting their potential nutritional and pharmaceutical value. The predominance of saturated FA, particularly palmitic acid, along with significant levels of MUFAs and PUFAs such as linoleic acid, oleic acid and eicosapentaenoic acid, underscores the rich lipid diversity of these marine macroalgae. These bioactive compounds are not only essential for human health but also exhibit promising antimicrobial properties, suggesting their possible application as natural food supplements and therapeutic agents. Furthermore, the variation in fatty acid composition among different species and phyla emphasizes the need for species-specific investigations to fully exploit their functional properties. In broad terms, this research adds to the expanding corpus of information demonstrating the valorization of seaweeds as sustainable and valuable resources for the food, nutraceutical, and pharmaceutical industries.

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