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Fatty acid composition of *Sargassum wightii* and *Amphiroa anceps* collected from the Mandapam coast Tamil Nadu, India

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ABSTRACT

The fatty acid composition of two sea weeds viz, Sargassum wightii and Amphiroa anceps were evaluated using a Gas Chromatograph. Upon analysis it was found that Sargassum wightii (Phaeophytae) had the highest amount of Polyunsaturated fatty acid (PUFA) while Amphiroa anceps (Rhodophytae) showed comparatively greater amount of saturated fatty acid. Among the various other fatty acids, both the algal species showed significantly higher proportions of Myristic acid, Stearic acid, α-linoliec acid, Palmitaleic acid, Arachidonic acid and oleic acids. Sargassum wightii showed comparatively larger amount of omega3, 6 and 9 classes of fatty acids than Amphirova anceps. Both the species showed comparatively moderate amount of monounsaturated fatty acids.

Key Words: Sargassum, Gas Chromatography, Fatty acid, PUFA, Omega three acids.

INTRODUCTION

The quest for bio active molecules from natural products have begun since the evolution of mankind, but it was only until the last five decades that marine sources began to elicit interest in pharmaceutical companies and research institutions with regard to drug discovery and development[1]. Till now we have described well over 14,000 different natural products from marine organisms [2], hundreds of patents have been filed describing new bioactive marine natural products [3] and approximately 10 – 15 different marine natural products are currently in clinical trials mostly in the areas of cancer, pain or inflammatory diseases [4]. With improvement

in the marine sciences and greater knowledge of the marine flora and fauna, researchers are exploring newer aspects of marine resources apart from pharmaceuticals like neutraceuticals and dietary supplements.

Marine macroalgae, one of the largest and versatile groups of marine organisms, are very commonly used in the diet, particularly in the Asian subcontinent [5]. Macro algae have been of great interest to researchers and nutritionists due to their low calorie content and high vitamin, mineral and dietary fiber ingredients which make them a potential resource in the cosmetic and food industries [6-9]. Innumerous studies from across the world have demonstrated that marine algae also possess a number of biological activities beneficial for human health, including antimicrobial, cytotoxic, antimitotic, anticancer, and antimutagenic activities[10-15].

Fatty acids are important for a wide range of cell structure components and for many biochemical reactions occurring in the body including hormonal and energy activities. Moreover they play a significant role in establishing a lipid barrier in the skin to block irritants and infectious agents from entering the body and some of them are reported to have beneficial effects on human health such as cardio protective, cytotoxic, antimitotic, anticancer, and antimutagenic activities [16].

The present study was conducted to characterize the fatty acid composition of the of two marine alga species, viz *Sargassum wightii* and *Amphiroa anceps* collected from the Mandapam coast, Tamil Nadu, India.

EXPERIMENTAL SECTION

Collection and processing of sea weeds:

The two sea weeds were collected from the CSMCRI-MARS (Central Salts and Marine Chemicals Research Institute-Marine Algal Research Station) Mandapam coast Tamil Nadu. The collected sea weeds were washed in sea water to remove the sand and other debris and shade dried till all the moisture has evaporated. The samples were then stored in polythene covers in dark till further use. The samples were identified as *Amphirova anceps* (Rhodophyta) and *Sargassum wightii* (Phaeophyta) by Dr Eshwaren of CSMCRI- MARS Mandapam, Tamil Nadu India.

Preparation of extracts for fatty acid composition

Twenty grams of the two samples were powdered and weighed and extracted with 300 ml of cold Chloroform-methanol mixture (2:1) in 3 successive steps of 100 ml each. Each time the samples were homogenized for 15 minutes and collected in a round bottom flask after filtering under vacuum. The samples were then flash evaporated and the final volume was made up to 10 ml using cold Chloroform-methanol mixture.

Analysis of fatty acid composition (FAME)

Fatty acids were analyzed according to the method of AOAC (1980). Lipid content of the samples was estimated by the method of Folch *et al*. (1957). Methyl esters of fatty acids prepared from lipid extract were separated and detected by gas chromatography. The main reagents used

were boron triflouride (BF_3), methanolic sodium hydroxide solution, Petroleum ether, Sodium sulphate etc.

The sample (lipid of known weight) was added to a flask followed by 6 ml methanolic NaOH and boiling chip. A condenser was attached, and refluxed until fat globules disappear (usually 5-10 min). Then 6-7 ml of BF_3 solution was added from the bulb or automatic pipette through the condenser and continued boiling for 2 min. After removing the heat, the mixture was condensed and added 15 ml of saturated NaCl solution. The flask was stoppered and shaken vigorously for 15 seconds while the solution was tepid. The aqueous phase was transferred to a 250 ml separator. This was then extracted with two 30 ml portions of petroleum ether (b.p 60-80^{o}C).The combined extracts were washed with 20 ml portions of H_2O, dried over anhydrous Na_2SO_4, filtered and evaporated the solvent under stream of nitrogen on steam bath.

Gas Chromatograph analysis

Methyl esters of the fatty acid thus obtained were separated by gas liquid chromatography (Varian CP 3800. U.S.A) equipped with a capillary column (Elite 225, 30 m long and 0.25 mm diameter) and a flame ionization detector in the presence of hydrogen and air. The experimental conditions for the Gas Chromatograph were maintained as follows; the carrier gas was nitrogen and the flow rate was 0.5ml/min the chromatograph temperature started at 150^{o}C and was increased 4^{o}C/min until a temperature of 250^{o}C was obtained. Fatty acids separated were identified by the comparison of retention times with those obtained by the separation of a mixture of standard fatty acids. Measurement of peak areas and data processing were carried out by Star WS software package. Individual fatty acids were expressed as weight percentage of total fatty acids.

RESULTS AND DISCUSSION

From the fatty acid composition of the two algal species it was found that Myristic acid, Palmitaleic acid, Oleic acid, Linoleic acid, α-linolenic acid and Arachidonic acids are the predominant acids in the Sargassum species (Table 1). Of these oleic acid (27%) was found to be the highest followed by Arachidonic acid (17%), linoleic acid (11%) and myristic acid (9%). In the red alga, *Amphirova anceps* the predominant acids where Oleic acid (24%), Stearic acid (17%), myristic (17%) and Palmitaleic acid.

Sargassum species had comparatively much greater content of polyunsaturated fatty acids (70%) than the red algae species *Amphirova anceps* (35%) (Table 2). While *Amphirova anceps* exhibited 16% content of monounsaturated fatty acids, the Sargassum species showed 11%. The content of saturated fatty acids was also found to be high in Amphirova species (49%) than the Sargassum (20%). While *Sargassum wightii* showed trace amounts of Caproic acid, it was absent in the Amphiroa species. The red algae showed trace amounts of caprylic acid while it was completely absent in Sargassum.

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Table 1 Fatty acid profile of *Sargassum wightii* and *Amphiroa anceps*

CARBON NUMBER	FATTY ACID NAME	% of fatty acid in terms of total fatty acids	
		SARGASSUM WIGHTII	AMPHIROA ANCEPS
C6	Caproic acid	0.12	Nil
C8	Caprylic acid	Nil	0.19
C10	Capric acid	0.10	0.36
C12	Lauric acid	1.25	4.83
C13	Tridecylic	0.13	0.18
C14	Myristic acid	9.38	17.0
C15	Pentadecyclic acid	1.22	3.90
C16:1	Palmitaleic acid	5.67	8.48
C17	Margaric acid	0.48	1.73
C18	Stearic acid	2.91	17.2
C18:1n-9	Oleic acid	27.6	24.8
C18:2n-6	Linoleic acid	11.0	4.53
C18:3n-6	γ-Linolenic acid	0.56	Nil
C18:3n-3	α Linolenic acid	8.72	0.27
C20	Arachidic acid	0.58	0.85
C20:3n-6	dihomo-γ-linolenic acid	1.12	0.25
C20:4	Arachidonic acid	17.5	3.10
C21	Heneicosylic acid	0.13	Nil
C20:5n-3	Eicosapentaenoic acid	2.48	1.27
C22	Behenic acid	1.62	0.66
C22:6n-3	Docosahexaenoic acid	0.21	0.31
C24	LIgnoceric acid	1.20	1.47

Table 2 Percentage composition of saturated, Monounsaturated and Polyunsaturated fatty acid distribution in the two sea weeds

FATTY ACID	SARGASSUM WIGHTII	AMPHIROA ANCEPS
SATURATED FATTY ACID	19.14	48.53
MONO UNSATURATED FATTY ACID	10.75	16.63
POLY UNSATURATED FATTY ACID	70.05	34.81

Heneicosylic acid and γ-Linolenic acid was noted in trace amounts in the Sargassum species while the Amphirova species completely lacked both. Eicosapentaenoic acid (EPA) was found in trace amounts in both the species studied, the higher being in the Sargassum species. Both the species of the sea weeds show significantly high levels of both the classes of the essential fatty acids (EFA), viz the n-6 or omega 6 and the n-3 or the omega 3 fatty acids. *Sargassum wightii* was found to be rich in omega-3 or the n-3 class of fatty acids (11%) while the red algae contained only 2% of omega-3 acids (Table 3). Omega -6 as well as omega-9 family of fatty acids also showed significantly higher levels in *Sargassum wightii* (13 and 27% respectively) than *Amphiroa anceps.*

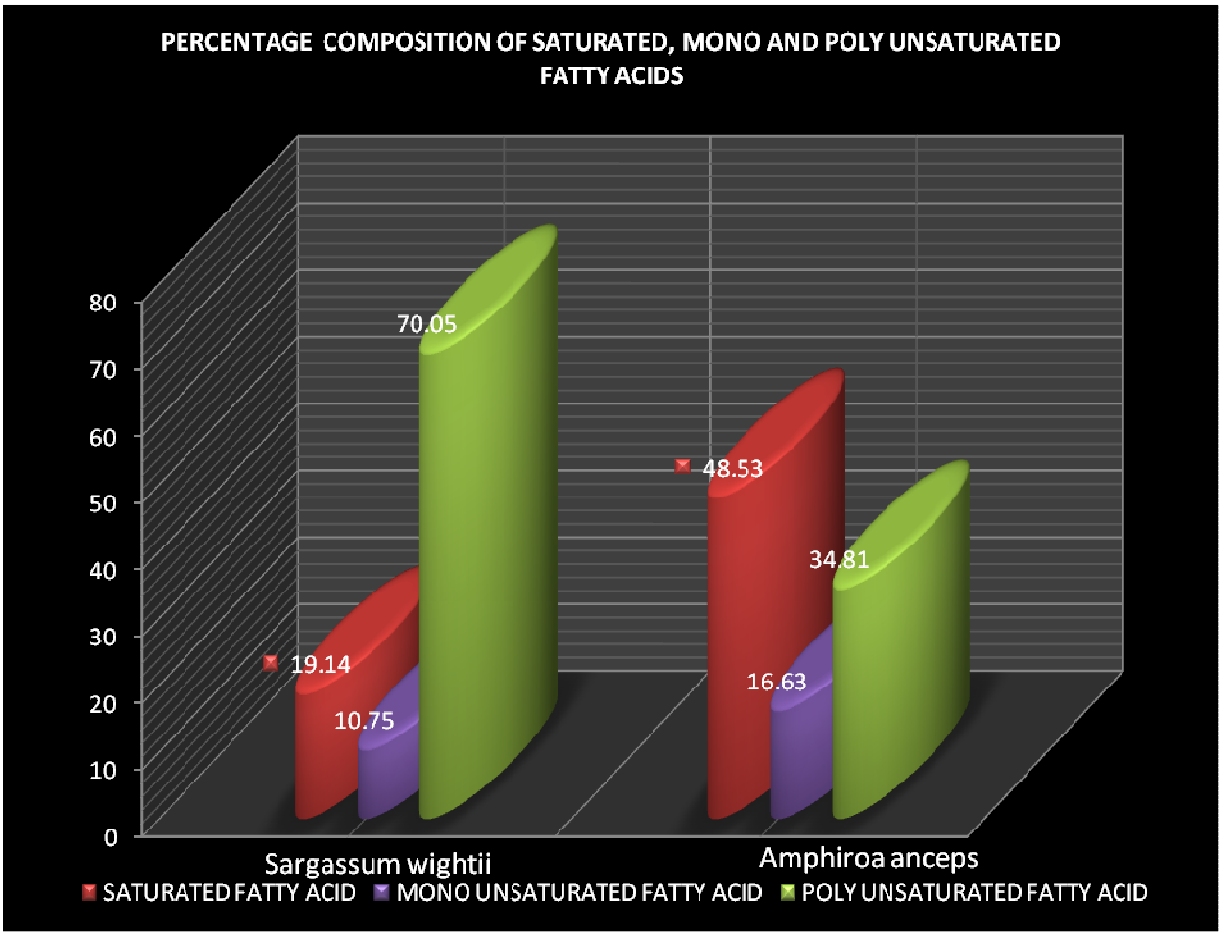

Fig 1 Percentage composition of the three classes of fatty acids in *Sargassum wightii* and *Amohiroa anceps*

Arachidonic acid was found abundantly in the Sargassum species (17%) while only trace amounts were determined in the Amphirova species (3%). Docosahexaenoic acid (DHA) was found in trace amounts in both the species of the algae. Arachidonic acid is significant in the sense that it is a major constituent of cell membranes and it plays an important role in the production of prostaglandins in the body. It acts as the major precursor of series -2 prostaglandins and thromboxanes and series-4 leukotrienes, while eicosapentanoic acid is the precursor of series-3 prostaglandins and thromboxanes and series-5 leukotrienes. Eicosanoids from Arachidonic acid have the property of raising platelet activity and immune response[17].

Table 3 Composition of essential fatty acids

ESSENTAL FATTY ACID	SARGASSUM WIGHTII	AMPHIROA ANCEPS
OMEGA -3	11.42	1.86
OMEGA-6	12.76	4.78
OMEGA-9	27.61	24.86

Docosahexaenoic acid (DHA) has the property of reducing blood pressure, and other n-3 acids especially from marine sources have been reported to reduce the plasma cholesterol and triacyleglycerol levels thereby decreasing the chances of thrombosis and related cardiac

complications. Many studies have justified the use of n-3 acids for the prevention and treatment of acute coronary syndromes. n-3 acids are also reported to have potential for the effective treatment and prevention/curing tumor development. Extensive research is going on, to evaluate the effects of n-3 acids and EPA on neuropsychiatric disorders such as depression and schizophrenia [17]. All this data support the traditional usage of marine plants in the diet in some Asian countries like China and Japan and emphasize the need to include marine resources in the daily diet due to the multitude of essential minerals and health promoting components present in them.

CONCLUSION

All these data suggests that the use of these species of sea weeds in the diet may prove to be a very effective way of supplementation of essential nutrients and minerals for the body. The sea weeds because of their relative abundance can be potential alternative sources of polyunsaturated fatty acids that have an important role in the normal metabolic functioning of the body. They are also found to be rich in many saturated as well as unsaturated fatty acids that have proved to be very much necessary for the normal and pathologic state functioning of the body. Many chronic disease conditions and nutrient deficiency syndromes that plague the 21st century mainly stems from the incomplete nutritional diet and the deficiencies of the basic components that is indispensible for the proper functioning of the body that arises thereby. Marine sources may prove to be an efficient solution of an alternative source of fatty acids especially stearic, oleic and myristic acids. The two species of sea weeds under study may be promising alternative sources of Oleic acid, Arachidonic acid and myristic acid. Furthur research into the isolaton and purification of the essential acids from these sea weeds may yeild very promising results for large scale manufacture of these acids with emphasis on the pharmaceutical and nutraceutical industry.

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