# Fatty acid profiles of marine red alga *Gracilaria* spp (Rhodophyta, Gigartinales)

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Species of *Gracilaria* from Gulf of Mannar were analyzed for their fatty acid composition. The major fatty acids such as myristic acid, myristoleic acid, palmatic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid were analyzed by gas liquid chromatography. The qualitative and the quantitative distribution of above fatty acids exhibited wide variation among the species of different habitat. The fatty acid content of the species collected from the same locality also showed quantitative variation depending on their distribution and the availability of light intensity to the particular species.

Red algae comprises the largest proportion of macroscopic seaweeds and the only source of phytochemicals such as agar-agar and carrageenan. Most of the seaweeds contain very limited quantity of lipids compared to the other major biochemical constituents[1] . These lipids are the common assemblage of number of fatty acids such as lauric acid (LAA, 12:0), myristic acid (MAA, 14:0), myristoleic acid (MOA, 14:1), palmatic acid (PAA, 16:0), palmitoleic acid (POA, 16:1), stearic acid (SAA, 18:0), oleic acid (OA, 18:1) and linoleic acid (LOA, 18:2) . Besides, it is characterized by high content of poly-unsaturated fatty acids (PUFA) mainly arachidonic acid (20:4 w6) and eicosapentanoic acid (20:5 w3)[2]. The use of fatty acid analysis to solve the taxonomic problems is particularly common in the bacteriological field. By comparing the whole cell fatty acids profiles, closely related organisms can be differentiated[3]. In the phycological area, some evidence suggests that fatty acids may be useful for taxonomic purposes but only up to division level[4-6]. The present work is aimed to find out the fatty acid composition of different species of *Gracilaria* from the Gulf of Mannar.

Considering the location specificity of seaweeds in their distribution, three centres namely Thonithurai, Mandapam and Pudumodom were selected for the collection of the samples in the Gulf of Mannar . All the three areas are within 30 km distance but exhibit different sea conditions. Pudumodom is about 20 km from Mandapam (9°17' N and 79° E) having a rocky coast and sandy bottom. Species like *Gracilaria corticata* var *corticata*, *G. corticata* var. *pudumodomsis* and *G. corticata* (green mutant) are found in this area. *Gracilaria. corticata* var *corticata* is found in plenty, attached to the rocks, exposed to direct sunlight and affected by strong wave action. *Gracilaria corticata* (green mutant) is rare in this locality and found some time along with *G. corticata* var *corticata* exhibiting a similar morphology but widely different in their color. *Gracilaria corticata* var *cylindrica* was collected from Mandapam (9°17' N and 79°10' E) within a depth of 10-20 cm . The plants are not very much exposed to direct sunlight or strong wave action as observed in *G. corticata* var *corticata*. Thonithurai is situated 8 km from Mandapam (9°17' N and 79°11' E). The coast is sparsely rocky and the sea bottom is muddy covered by seagrasses. Sea off Thonithurai is relatively calm due to the presence of chains of island protecting the strong wave action. *Gracilaria edulis* and *G. crassa* grow well in this area at a depth of 10-30 cm attached to either dead gastropod shells or small pebbles.

The samples were collected from the respective sites during low tide and transported to the laboratory in plastic bags containing seawater. The plants were brushed off epiphytes and washed 2-3 times in ordinary seawater followed by sterilized seawater and transported to the laboratory of Madurai Kamaraj University. Extraction of fatty acid methyl ester was carried out by the modified method of Levy *et al*[2]. The apical portion of the fresh seaweeds samples were cut and weighed, ground with 2 ml of 1.2 *N* sodium hydroxide in 50% aqueous methanol. Samples were boiled in sealed tubes for 30 min to saponify. Further, they were acidified with 0.6 ml of 10 *N* hydrochloric acid with the addition of 1 ml of 12% barium trichloride prepared with methanol. The sample heated for 10 min at 85°C. Extraction of fatty acid methyl esters was done by adding 1 ml of hexane/diethyl ether (1:1) to the

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sample. The organic phase is removed and mixed with 3 ml of 0.3 *N* sodium hydroxide solution, shaken properly and allowed to stand till both the liquid phase and organic phase got separated properly. The organic phase was removed carefully with the help of a micro-pipette and dried. Five hundred microlitre of hexane were added to solubulize the sample before injecting to the gas chromatograph. Two microlitres of the subsample were injected to GC. The methyl ester fatty acids were analyzed using gas chromatograph (Hewlett Packard, Model 5890 A) with flame ionization detector. The column (6 ft) was packed with 10% diethylene glycol succinate on chromosorb A. The flow rate of N2 and H2 gases were 30 ml/min. The chromatography conditions were maintained as follows:

| | |
|---|---|
| Oven temperature - 100°C | Final time - 30 min |
| Initial temperature - 100°C | Rate - 10 °C/min |
| Final temperature - 160°C | Injection temp. - 180°C |
| Oven maximum temp. - 200°C | Detector temp. - 180°C |
| Initial time - 10 min | Equilibrium - 3 |

Table 1 explains the quantitative estimation of the major fatty acids expressed in percentage with reference to the total fatty acid content. MAA, MOA and PAA are found in all the species of *Gracilaria* exhibited wide variation in their quantity. The total quantity of these major fatty acids was found to be maximum in *G. crassa* (77 %) followed by *G. edulis* (74.7%) grown at Thonithurai. Both the species are grown in calmer area protected by islands. The fatty acids content of *G. corticata* var *cylindrica* was found to be 55.2% comparatively higher than the other sub species of *G. corticata* which ranged between 31.6 to 43.4 % of the total fatty acids. While comparing the fatty acids content of all these species, it was observed that the qualitative and quantitative status of the essential fatty acid showed similarity in the species grown in particular similar habitat.

The fatty acid composition in vegetative and reproductive plant of *G. corticata* collected from the same locality varied quantitatively . The vegetative thallus of the particular species contained higher quantity of fatty acid compared to the reproductive counterpart (Table 1). This may be explained here that the fatty acids might have been utilized during formation of the reproductive stage.

In the present study , the quantity of MOA is always higher than MAA in all the species of *Gracilaria* except in *G. corticata* var *pudumodamsis*. In this experiment palmitoleic acid has not been traced out in *G. edulis* and *G. corticata* var *pudumodamsis* but substituted by high quantity of palmitic acid. *Gracilaria crassa* contained higher quantity of PAA and POA compared to MAA and MOA. LAA and OAA were found in negligible quantities in some subspecies of *G.corticata* whereas stearic acid and linoleic acid contribute less percentage in *G. corticata.*

The morphological structure of the thallus may also contribute for the variations in fatty acid composition. *Gracilaria crassa*, being fleshy and bulbous in nature, available in the shallow depth got maximum fatty acid content compared to the cylindrical and flattened type of thallus. *Gracilaria corticata* var *cylindrica* though slightly flattened has a more fleshy structure of thallus compared to *G. corticata* var *corticata* . Its fatty acid content was found to be higher (55.2%) than that of *G .corticata* var *corticata.* Similarly, the fatty acid contents of *G. crassa* and *G. edulis* was found to be much higher due to the cylindrical and bulbous structure of the thallus. The fatty acid composition of the species growing in similar habitats also exhibit certain difference as seen in *G. corticata* var *corticata* and *G. corticata* var *pudumodamsis*. *Gracilaria corticata* var

Table 1—Quantitative estimation of major fatty acid in different species of *Gracilaria* (value expressed in percentage with reference to the total fatty acid)

| Location | Species | LAA 12:0 | MAA 14:0 | MOA 14:1 | PAA 16:0 | POA 16:1 | SAA 18:0 | OAA 18:1 | LOA 18:2 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Pudumodam | *G. corticata* (vegetative) | 0.431 | 1.419 | 7.341 | 6.994 | 11.038 | 5.364 | 0.349 | 3.904 | 36.840 |
| Pudumodam | *G. corticata* (reproductive) | 0.149 | 2.065 | 11.488 | 5.541 | 10.022 | 3.217 | --- | 5.125 | 32.482 |
| Pudumodam | *G. corticata* (green mutant) | 0.993 | 2.250 | 6.630 | 8.850 | 7.240 | 5.660 | --- | --- | 31.623 |
| Mandapam | *G. corticata* var. *cylindrica* | 0.360 | 8.840 | 22.260 | 16.000 | 6.770 | 0.690 | 0.240 | --- | 55.160 |
| Pudumodam | *G. corticata* var. *pudumodamsis* | --- | 17.130 | 11.300 | 14.920 | --- | --- | --- | --- | 43.350 |
| Thonithurai | *G. edulis* (vegetative) | --- | 21.210 | 34.210 | 19.260 | --- | --- | --- | --- | 74.680 |
| Thonithurai | *G. crassa* (vegetative) | 0.670 | 5.130 | 11.110 | 44.840 | 11.360 | --- | --- | 4.560 | 77.670 |

LAA -Lauric acid, MAA -Myristic acid, MOA -Myristoleic acid, PAA -Palmitic acid, POA -Palmitoleic acid, SAA -Stearic acid, OAA -Oleic acid and LOA -Linoleic acid

*corticata* grows luxuriantly on the rock surface exposed to direct sunlight and strong wave action. During lowest low tide the plants are exposed whereas *G. corticata* var *pudumodamsis* prefers to grow in the rock crevices away from direct sunlight. The wave action was also found to be comparatively less. It may be explained here that light intensity appears to play a major role in determining the fatty acid composition of different species of *Gracilaria.* Since the availability of light varies from place to place in the same locality the distribution pattern of these species depend on the photon flux density and contribute to differences in their fatty acid compositions. Present results are in agreement with Levy *et al*[2]. that fatty acid composition exhibit significant influence of PFD than temperature. It was also explained that the compositional characteristics of fatty acids in *Gracilaria tenuistipitata* was assumed to be affected by environmental factors such as seawater temperature and intense illumination[7].

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