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Research Article

BIOCHEMICAL PROFILE OF *STOECHOSPERMUM MARGINATUM*

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ABSTRACT

The irrational exploitation of the available resources of land and the ever increasing human population have kept the human community in constant search for new resources to meet the growing demand for food, medicine and energy. Poorly exploited marine ecosystem with its rich resources appears to be a candidate as a natural resource for these needs. Thus a brown alga abundant in Indian shores was studied for its biochemical properties .Its a rich source of all essential nutrients which is promising as a source of pharmacognosical value.

Keywords: Algae, Phaeophyceae, Nutrients, Stoechospermum

INTRODUCTION

A perusal of literature indicates that the initial studies on Indian algae were confined to macrophytic seaweed (Iyengar,1922;Biswas1932) and extensive contributions of Boergesen1936;Desikachari,1968 are worth mentioning. In general the microscopic algae are studied for their potentiality or exploring their usage in the field of pharmaceuticals, phaeophyceae members or commonly called as brown algae constitute the major component of the seaweed population of the tropical countries of the world hence *Stoeochospermum marginatum* a monotypic genus belonging to the order Dictyotales of Phaeophyta was selected for the study.

The plant is brown to yellowish brown in colour,thallus is flat, foliaceous5-55cm long and1-3cm wide,irregularly branched into dichotomously strap shaped segments with entire margins.apical margins are involute and the growth of the thallus occurs by means of a marginal meristem.Hairs are scattered all over the surface of the thallus.Reproductive structures occur as marginal sori..

MATERIALS AND METHOD

Stoechospermum marginatum was collected from the rocky shores of Leepuram of Kanyakumari district,(lat.80ºN) Tamilnadu.The collections were made from the month of November to December.During this period the experimental algae were usually in the saprophytic phase.The collection contained juveniles and few gametophytic thalli. Freshly collected algae were shade dried for about a week then the dried samples were powdered using a coffee blender,packed in sterile polythene bags and stored at 4ºC until use.Herbaria were simultaneously prepared.

The carbohydrates as total sugars, were estimated following the procedure of Roe,1955. Known quantity of the alga was ground in a glass mortar and pestle with 80% ethanol and a little acid washed sand and filtered through Whatman No. 1 filter paper. The filtrate was centrifuged at 8000 x g for 20 minutes and the supernatant was made to 5.0 mL with 80% ethanol. The final volume of the extract was made to 10.0 mL by the addition of distilled water. To 1.0 mL of the above sample, 4.0 mL of anthrone reagent was added through the sides of the test tube. A glass marble was placed on top of the test tube and heated in a boiling water bath for 10 minutes. The tubes were then removed, cooled to room temperature and the absorbance was read at 620 nm using HITACHI UV 2001 Spectrophotometer. A reagent blank was run simultaneously. Glucose (Analar) was used as the standard.

Lowry *et.al.,*1951 procedure was followed to estimate the protein content. Freshly collected algae were used for the estimation of total soluble protein. Known quantities of the algae were ground with potassium phosphate buffer (pH 7.4) containing 20% polyvinyl pyrrolidone (PVP) and a little acid washed sand using a glass pestle and mortar and filtered through four layers of muslin cloth. An equal volume of 10% trichloroacetic acid (TCA) was added to precipitate protein in the filtrate. The precipitate was removed by centrifugation at 9000 x g for 15 minutes and dissolved in known volume of 1.0 N NaOH to give the protein solution.

The procedure outlined by Bligh and Dyer1959 was used with modifications to determine the total lipids in the sample. Known quantities of clean, dry and finely powdered thalli were extracted with known volume of chloroform : methanol (2:1 v/v) in a hot air oven at 45°C to defat the material. :The mixture was then centrifuged at 800 rpm for five minutes and the supernatant was saved. To the supernatant, 1/3 volume of distilled water was added and vortexed. To this, a little amount of sodium sulphate crystal was added and again vortexed to remove moisture. The resulting solution was kept in a separating funnel to initiate phase separation. The lower chloroform phase containing lipids recovered in pre-weighed crucibles were kept in a hot air oven at 40°C to evaporate the solvent. The dry residue in crucibles were treated with pure acetone to defat and dried again. The final residue thus obtained was weighed to get the lipid content of the sample.

Total free amino acid content of freshly collected frozen tissues of algae was estimated by ninhydrin method (Moore and Stein, 1948). To suitable aliquots of the algal extract, water was added to make the total volume to 4.0 mL. To this, 1.0 mL of ninhydrin reagent was added, mixed and kept in a boiling water bath for 15 minutes. The tubes were then removed, cooled and 1.0 mL of 50% ethanol was added. The pink colour developed was measured at 550 nm in a Spectronic 21 photocolorimeter. A composite mixture of alanine, aspartic acid, tryptophan, proline and lysine (in equal weights) was used as the standard.

Vitamin content of dry, powdered sample of algae was estimated by HPLC (AOAC, 1990). To the dry powdered algal tissues, 100 mM perchloric acid : acetonitrile (2 : 1 v/v) solution was added and left in a water bath at 50°C for 30 minutes. The resulting solution was centrifuged at 6000 x g and the upper layer was used for HPLC analysis. HPLC system (SCHIMADZU) equipped with UV detector was used under the following analytical conditions for the estimation of nicotinic acid, vitamin B_1, vitamin B_6 and vitamin B_2 .For vitamin A the algal powder was saponified with ethanolic KOH for 30 minutes and transferred to a separating funnel and repeatedly extracted with n-hexane. The final pooled extract was evaporated to dryness under reduced pressure in a rotaevaporator and vitamin A level was determined by HPLC.

Fatty acids were determined and quantified byNEON II gas chromatography analysis outlined by Niller and Bligh,1985.The total free amino acid content of the algae was estimated by Ninhydrin method(Moore and Stein,1948)

Mineral analysis or elemental analysis of the shade dried sample was carried out by procedure outlined by Perkin Elmer atomic absorption spectrophotometry,1981.Standards for the above elements were prepared according to PERKIN – ELMER'S manual.

Extraction of fucan was done following the method outlined by Preeprame *et al*. (2001) with some modifications. Fucoidan was

extracted from the algae with water at room temperature and purified through ethanol precipitation. Ten gram of the powdered seaweed was extracted with water under mechanical stirring, for 12 hours at 25°C which was later centrifuged. The supernatant was concentrated and the residue was discarded. The supernatant was concentrated, dialyzed against water and lyophilized to yield colourless residue. This was then weighed and the yield was estimated.

Alginic and alginate from the algal samples were extracted following the methods outlined by Umamaheswara Rao (1969) with some modification.Twenty grams of powered dry algal sample was soaked in 1.0 N HCI for 2 hrs. The soaked algal material was washed and treated with 6 mL of 3% sodium carbonate for 24 hrs. The mixture was then pressure filtered through fine muslin cloth. The residue was mixed with 20 ml of distilled water and pressure filtered once again. The two filtrates were pooled and made up to 200 ml with distilled water. To this, 100 ml of 10% calcium chloride solution was added and mixed by vigorous shaking for 15 minutes. During the process, calcium alginate precipitated. The precipitate was washed in cold water, dried and weighed calcium alginate thus obtained was treated with 1.0 N HCI to obtain alginic acid, which was centrifuged at 10,000 rpm for 20 minutes to pellet the alginic acid in the solution. The pellet of alginic acid was then washed twice with water, centrifuged and the resulting dried in oven and weighed. Dry alginic acid was moistened with water and powdered sodium carbonate was added until the alginic acid dissolved to form a homogeneous solution. This solution containing sodium alginate coagulated with the addition of alcohol. The coagulated material was dried and weighed to determine the yield of sodium alginate.

RESULTS

The results are shown in the form of tables with statistical significance. Any macrobiotic diet should contain carbohydrates, lipids and protein which the algae possesss in the following proportion as given in Table 1. The algae is rich in amino acids a total of eighteen amino acids were reported (Table 2) . In addition to lysine, phenylalanine and aspartic acid, histidine too occurred as a major constituent. Table3 gives the vitamin content of the experimental algae .nicotinic acid, B1 and B2 were detected in the shade dried powdered samples of the experimental algae.B6 was present in huge amounts. Gas chromatographic analysis of the shade dried samples showed the presence of fifteen acids where palmitic acid formed the bulk of the total fatty acid content (Table4). Among the minerals present Calcium and iron formed the major ones as depicted in Table 5. The most important biochemical compound being the phycocolloids. Brown seaweeds are known to produce different polysaccharides namely alginate, laminarin and fucoidans. (Table 6)

Table 1: Total carbohydrates lipids and protein in the tissues of the experimental algae

Total Carbohydrate mg g-1 dry wt.(mean±S.E)	Total Lipid mg g-1 dry wt.(mean±S.E)	Total Protein mg g-1 dry wt. (mean±S.E)
50.9±0.52	184.0±1.155	12.6±0.162

Table 2: Relative levels of amino acid in *Stoechospermum marginatum*

Amino Acid	µg g-1 dry wt .(mean±S.E)
Aspartic acid	27.083±0.254
Glutamic acid	42.212±0.058
Asparagine	59.200±0.058
Serine	26.000±0.017
Glutamine	14.698±0.041
Glycine	33.013±0.369
Threonine	17.931±0.001
Arginine	24.562±0.030
Alanine	25.150±0.012
Cystine	26.995±0.012
Tyrosine	18.671±0.115
Histidine	44.898±0.018
Valine	45.332±0.018
Methionine	29.826±0.015
Isoleucine	74.393±0.005
Phenylalanine	50.595±0.049
Leucine	49.535±0.003
Lysine	5.022±0.013

Table 3: Vitamin content of the experimental algae

Vitamin	µg g-1 dry wt .(mean±S.E)
Nicotinic acid	68±1.732
Vitamin B6	115±0.577
VitaminB2	75±1.732
VitaminB1	46±2.309

Table 4: Relative levels of Fatty acid in *Stoechospermum marginatum*

Fatty Acid	µg g-1 dry wt . (mean±S.E)
Caprylic acid	22.0±0.866
Lauric acid	67.0±0.577
Tridecanoic acid	27.0±0.289
Myristic acid	11.067±0.346
Pentadecanoic acid	11.6±0.176
Palmitaloic acid	34.1±0.577
Heptadecanoic acid	27.3±0.173
Stearic acid	29.4±0.866
Oleic acid	24.0±0.808
Linoleic acid	47.7±0.981
α Linoleic acid	10.0±0.173
γ Linoleic acid	8.6±0.346
Palmitic acid	5001±15.011

Table 5: Mineral content of *Stoechospermum marginatum*

Mineral	µg g-1 dry wt .(mean±S.E)
Copper	8.641±0.173
Zinc	19.92±0.115
Manganese	8.75±0.144
Chromium	16.6±0.115
Lead	0.4±0.006
Nickel	25.2±0.115
Cadmium	5.9±0.058
Magnesium	9.6±0.116
Sodiom	39.11±0.058
Potassium	29.65±0.265
Cobalt	3.47±0.058
Calcium	2053.4±5.774
Nickel	858.5±0.25

Table 6: Extractive phycocolloids present in experimental algae

Fucoidan mg g-1 dry wt.(mean±S.E)	Alginic Acid mg g-1 dry wt.(mean±S.E)	Calcium Alginate mg g-1 dry wt. (mean±S.E)
119.0±0.01	14.9±0.021	26.2±0.324

DISCUSSION

The investigation revealed the richness of the algae in the carbohydrate and protein content, lipid content being far ahead. All the essential amino acids were present but isoleucine and asparagine occurred in huge amounts.Vitamin B6 formed the major part in addition to Vitamin B2 and Vitamin B1.The algae is a rich source of palmitic acid and calcium and iron formed the major bulks in the mineral content. The bulk of the algae contained a phycocolloid fucoidan which is a sulphated polysaccharide and trace amounts of alginate a carboxylated polysaccharide.These phycocolloids are promising in the field of pharmaceuticals. Thus the nutraceutical value of the alga shows its diversification in the field of medicine, nutrition and industry.

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