Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(10):4637-4641

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Biochemical investigation on red algae family of *Kappahycus Sp*

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ABSTRACT

This paper reports the details of experimental investigations carried out on the Kappaphycus sp., a family of red algae. Chemical constituents such as carbohydrates, lipids, proteins, fat, amino acids, fatty acids, phenols, sterols were estimated from Kappaphycus sp. Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of wet and living sample. Standard material methods have been followed for estimation of chemical constituents available in Kappaphycus sp. From the experimental studies, it is observed that the carbohydrate available in Kappaphycus alvarezii is 2.67 gm/100gm. The total lipid content is 1.09gm/100gm. The total protein content is very high about 18.78gms/100gm compared to all other substances. Fat content in the species is 1.09gm/100gm. Total phenol content is 4.565gm/100mg. Further, it can be noted that the phenolic content is very much less than proteins but more than carbohydrates, lipids and fats.

Key words: Red algae , *k.alvarezii*, Chemical constituents, Biological values

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INTRODUCTION

Marine organisms are a rich source of structurally novel and biologically active metabolites. Secondary or Primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. Microalgal metabolites have attracted attention for two main reasons, first, because they are the source of toxins in harmful algal blooms and secondly because they are a potentially rich source of new drug candidates. Commercially available varieties of marine macroalgae are commonly referred to as seaweeds. Seaweeds of the marine macrophytic algae are an assemblage of the members of Chlorophycease, Phaeophycease and Rhodophycease and, are the common inhabitants of the tidal and inter-tidal environments of the marine ecosystem. Seaweeds have been widely used for human consumption in many parts of the world. In general marine algae contains (i) protein with all the essential amino acids-unlike most plant foods (ii) a high carbohydrate content (iii) an extensive fatty acid profile, including Omega 3 and Omega 6 and (iv) an abundance of vitamins, minerals and trace elements in a naturally-occurring synergistic design. Macroalgae can be classified as red algae (*Rhodophyta*), brownalgae (*Phaeophyta*) or green algae (*Chlorophyta*) depending on their colour, nutrient and chemical composition. Red and brown algae are mainly used as human food sources. Seaweed species are rich in beneficial nutrients, in countries such as China, Japan and Korea, they have been commonly utilized in human alimentation.

Seaweeds have been consumed in Asia since ancient times. Further, marine algae have been utilized in Japan as raw materials in the manufacture of many seaweed food products, such as jam, cheese, wine, tea, soup and noodles and in the western countries, mainly as a source of polysaccharides for food and pharmaceutical uses [1-3]. In Europe, there is an increasing interest in marine seaweeds as a food, nevertheless, at present there are no European union specific regulations concerning their utilization for human consumption. Ke Li et al. [4] determined various chemical constituents of the red alga *Grateloupia turuturu*. Few seaweeds such as laminaria, focus are used as cattle feed and or believe to be good source of trace elements to animals. Protein content in these algae is high. However,

metabolizable carbohydrates are much less. Marine algae can serve as a source of minerals, vitamins, free amino acids and poly unsaturated fatty acids.

The determination of lipid composition in a given species is essential for further studies on lipid metabolism and on the effect of environmental factors. Lipid profiles assist the assignment of algal taxonomic position, and also provide signature lipid profiles for use in organic geochemistry and food web studies. Such biochemical analyses may also prove useful in studying the abundance and ecology of these species in marine environments. From chemotaxonomic point of view lipids e.g. fatty acids and sterols provide interesting information for taxonomic purposes. Whereas higher plants have received extensive attention regarding lipid content, benthic algae have received relatively little attention and our knowledge is still rather poor. Some papers, however, have been published on the Rhodophyta lipid area, but the classification was confused, as in some cases especially, large variations occurred in the fatty acid or in the sterol composition within the same genera or sometimes within the same species. The use of algal oils containing long chain polyunsaturated fatty acids as nutritional supplements has been recommended and algal sources are being identified for the presence of docosahexaenoic acid and eicosapentaenoic acid. These fatty acids are involved in the regulation of neurological, coronary and reproductive physiology in humans. They are conditionally essential nutrients during infancy and a number of nutritional and professional organizations including world health organistion have recommended the inclusion of these fatty acids in infant nutritional formula [5]. In general, from the critical review of literature, it is observed that the most of studies on the nutrient contents of seaweeds have concerned fresh plants. Little is known of the effects of processing by drying or canning. The present investigation aims at on the following parameters from *Kappaphycus sp*:

- Estimation of total carbohydrates
- Estimation of total lipids
- Estimation of total proteins
- Extraction and estimation of free phenols
- Estimation of total fat

EXPERIMENTAL SECTION

Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of dry and living material. Algae samples were cleaned and removed from epiphytes and necrotic parts. Samples were rinsed with sterile water to remove any associated debris. Sample was kept under sunshade for 7 days. After drying the sample, it was ground thoroughly to powder form. The powder was then used for the estimation of chemical constituents such as total carbohydrates, total lipids, free phenols, total fat, amino acids, fatty acids, sterols and minerals. This powder was stored in cold conditions in an airtight container and analysis was carried out within three months of processing.

Shade dried powdered material of the experimental algae were used in the investigations unless otherwise mentioned. Wet weight and dry weight estimations were made from the samples of alga *vide* Materials and Methods.

2.1 Estimation of total carbohydrates

Carbohydrates were extracted and estimated as the procedure given by Roe [6] and Indian Pharmacopeia [7]. Freshly harvested, frozen thalli were used within two days of collection. 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's 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 then made up to 10.0 ml by adding distilled water. To 1.0 ml of the above sample, 4.0 ml of anthrone reagent was added by the sides of the test tube. A glass marble was then 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 absorbance was read at 620 nm using Hitachi UV 2001 spectrophotometer. A reagent blank was run simultaneously. Glucose (ANALAR) was used as the standard.

Anthrone reagent

Anthrone (50.0mg) and thiourea (1.0 g) were added to 100.0 ml of 72% cold sulphuric acid and stored in a dark bottle. Freshly prepared reagent was used for every estimation.

2.2 Estimation of total lipids

The procedure outlined by Bligh and Dyer [8] and Indian Pharmacopeia [7] was used to determine the total lipids in the samples. 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^{o}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 was recovered in pre-weighed crucibles and kept in a hot air oven at 40^{o}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 weighted to get the lipid content of the sample.

2.3 Estimation of total proteins

Freshly collected, frozen algae were used for total soluble protein estimation. 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, mortar and filtered through the four layers of muslin cloth. An equal volume of 10% trichloro acetic acid (TCA) was added to precipitate potein 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. Protein in the solution was estimated by following procedure of Lowry et al. (1951). Procedure is outlined below:

Reagents

- Alkaline copper sulphate solution was prepared freshly by mixing 1.0 mL of 0.5% $CuSO_4.5H_2O$ in 1% Potassium sodium tartarate with 50.0 mL of 2% sodium carbonate in 0.1 N NaOH.
- Diluted Folin-phenol reagent was prepared by diluting Folin-ciocalteu reagent obtained with an equal volume of glass distilled water.

Procedure

To one milliliter of protein solution, 5.0 ml of alkaline copper sulphate solution was added and allowed to stand at room temperature for 10 minutes. Then 0.5 ml of diluted Folin-phenol reagent was added and mixed well. The solution was allowed to stand for 30 minutes and absorbance was read at 500 nm using a Hitachi UV 2001 spectrophotometer. Bovine serum albumin (BSA) (Sigma fraction V) was used as standard.

2.4 Extraction and estimation of total free phenols

Total free phenols were estimated as per British Pharmacopeia [9] and by the method of Maxon and Rooney [10].

Reagent

Diluted Folin–phenol reagent was prepared by diluting Folin –Ciocalteu reagent obtained commercially with an equal amount of distilled water.

Procedure

One gram of air-dried red algae flour was placed in a 100-ml flask, with 50 ml of 1 % (v/v) HCl in methanol. The samples were shaken on a reciprocating shaker for 24 h at room temperature. The contents were centrifuged at 10,000 X g for 5 min and the supernatant was used for further analysis. The extracted free phenol was estimated. One-ml aliquots of the above extract were pipetted into different test tubes to which 1-ml of Folin-phenol reagent and 2 ml of 20 % (w/v) Na_2CO_3 solution were added. The tubes were shaken and placed in a boiling water bath for exactly 1 min. and then were cooled under running tap water. The resulting blue solution was diluted to 25 ml with distilled water and the absorbance was measured at 650 nm with a Spectronic 20D spectrophotometer. If precipitation occurred, it was removed by centrifugation at 5000 X g for 10 min. before measuring the absorbance. The amount of phenol present in the sample was determined from a standard curve prepared with catechol. A blank containing all the reagents minus plant extract was used to adjust the absorbance to zero. Average values of triplicate estimations were expressed as 100 g^{-1} of the sample on a dry weight basis.

2.5 Estimation of total fat

3.0 g of sample was dissolved in 100 ml of water, transferred to a separating funnel, acidified with 1M sulphuric acid and extracted with successive quantities of 50, 40 and 30 ml of ether. Mixed ether solutions were taken in a separating funnel and washed with water until the washings were free from mineral acid .The ether solution was transferred to a tared flask, removed the ether and dried the residue of fatty acids to constant weight at 80°C.

A. Base wash: 10.8 g of NaOH dissolved in 900 ml of distilled water.

B. Hundred milligrams of each sample was taken in Teflon-lined screw cap tube and 1.0 mL of reagent A was added. The tube was closed and boiled for 30 minutes with intermittent shaking. Two milliliters of reagent B was then added and boiled

again in a water bath at 80^{o}C for 20 minutes. Following for 30 minutes with intermittent shaking, two milliliters of reagent B was then added and boiled again in a water bath at 80^{o}C for 20 minutes.

RESULTS AND DISCUSSION

Results obtained for various chemical constituents such as total carbohydrates, total lipids, free phenols, total fat, amino acids, fatty acids, sterols and minerals available in *Kappaphycus sp.* are shown in Table 1. Statistical distribution was carried out and standard deviation (SD) is estimated against each constituent.

Table 1 Various Chemical constituents of *Kappaphycus sp.*

S. No.	Chemical constituent	Composition	SD
1	Total Carbohydrates	2.675 gm/100gm	2.675±0.01
2	Total lipids	1.09 gm/100gm	1.09±0.01
3	Total Proteins	18.78 gm/100gm	18.78±0.01
4	Total phenols	4.565 gm/100gm	4.565±0.02
5	Total fat	1.67 gm/100gm	1.67±0.02

3.1 Total carbohydrates

From the Table 1, it is observed that the carbohydrate available in *Kappaphycus sp.* is 2.67 gm/100gm. Impellizzeri et al. [11] reported the occurrence of amino acids and low-MW carbohydrates in 18 macroscopic marine algae belonging to the division *Rhodophyta*, class *Florideophyceae*. Fayaz et al. [12] analysed the chemical composition, iron bioavailability and antioxidant activity of *Kappaphycus alvarezii* (Doty) and reported that *K. alvarezii* is rich in protein (16.24% w/w) and contains a high amount of fiber (29.40% w/w) and carbohydrates (27.4% w/w). Both biotic and abiotic factors form the micro and macro environment of the organism and the strong or less perceivable variations in the environmental parameters influence such distinct differential amount of carbohydrates.

3.2 Total lipids

In the present study, the total lipid content is observed to be 1.09gms/100gms. Fayaz et al. [12] determined the chemical composition of *Eucheuma (K. alvarezii*), the bioavailability of iron and the evaluation of the antioxidant activity. It was observed that *K. alvarezii* contains total lipid as 0.738%. Dawczynski et al. [13] mentioned that the marine macroalgae varieties tested demonstrated low lipid contents with 2.3±1.6 g/100g semi-dry sample weight.. The determination of lipid composition in a given species is essential for further studies on lipid metabolism and on the effect of environmental factor (Tasende, 2000). Dhamotharan [14] investigated the level of the total lipid content in brown algae and found that the levels of lipid content is twice in *stoechospernum marginatum (*181±1.155mg*)* as compared to *padina (*94±2.309mg*)*. The variations in the amount of lipid levels in the present study in comparison with previous studies may due to variations in the species and geographical factors.

3.3 Estimation of total proteins

In the present study, the total protein content is as high as 18.78 gm compared to all other substances like, phenols, lipids, carbohydrates, fat and sterols. Hence the species can serve as functional food with vital nutritional and biological values. Galland-Irmouli et al. [15] determined the nutritional value of P. palmate by analysis of seasonal variations in the protein and amino acid contents and evaluation of protein digestibility. Based on the nitrogen level, the protein amount varied from 9.7%to25.5% of the dry mass, with a yearly average of 18.3 ± 5.9%. Significant variations in protein content were observed according to the season. The highest protein content (21.9 ± 3.5%) was found in the winter-spring period and the lowest (11.9 ± 2.0%) on the summer- early autumn period. Similar observation was made in the present studies. Fayaz et al. [12] observed that K. alvarezii is rich in protein (16.24% w/w). Dhamotharan [14] investigated the level of protein contents in brown algae and found that the levels of total protein content is high in *stoechospernum marginatum (10.6 ± 0.162)* as compared to *padina (*9.95 ± 0.058). The variations in the amount of proteins are attributed due to seasonal and environmental variations which are transitory.

3.4 Estimation of total phenols

In the present investigation, total phenol content is observed to be 4.565gm/100mg. Further, it can be observed from the Table 2 that the phenolic content is very much less than proteins and it is more than carbohydrates, lipids and fats. Dhamotharan [14] estimated the total phenol content in brown algae and found that the levels of total phenol content in *stoechospernum marginatum (*920 µg/g dry wt.*)* is three folds that of padina (280 µg/g dry wt).

3.5 Estimation of total fat

Total fat content in the *kappaphycus sp.* is found to be 1.67gm.

CONCLUSION

Chemical constituents such as carbohydrates, lipids, proteins, fat, amino acids, fatty acids, phenols, sterols were estimated from *Kappaphycus sp*. Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in

the form of wet and living sample. Material methods for typical chemical constituents have been followed. From the studies, it was observed that the carbohydrate available in *Kappaphycus alvarezii* is 2.67 gm/100gm. The total lipid content was 1.09gm/100gm. The total protein content was very high about 18.78gms/100gm compared to all other substances. Fat content in the species is 1.09gm/100gm. Total phenol content was 4.565gm/100mg. Further, it was observed that the phenolic content was very much less than proteins and it was more than carbohydrates, lipids and fats. From the overall study, it can be concluded that the *K. alvarezii* can serve as functional food with vital nutritional and biological values.

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