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An Investigation on the evaluation of heavy metals in *Kappaphycus alvarezii*

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ABSTRACT

This paper presents the details of estimation of heavy metals available in Kappaphycus sp. Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of dry and living sample. It is observed that total amount of the heavy metals is 30.4ppm. Iodine is the major constituent (56.77ppm) then boron (6.66ppm) and lead (3.45ppm) and nickel (2.43ppm). The composition of cobalt is 1.56ppm, mercury is 1.44ppm and cadmium is 1.02ppm. Very less amount of metals are arsenic (0.897ppm) and tin (0.89 ppm).

Keywords: Seaweeds, red algae, *Kappaphycus*, heavy metals

INTRODUCTION

Marine algae are an important source of dissolved organic carbon in coastal waters. The organic carbon is represented by carbohydrates, polysaccharides, nitrogenous and polyphenolic materials. Marine algae are the excellent source of bioactive compounds such as carotenoids, dietary fibres, proteins, essential fatty acids, vitamins and minerals. Marine algae are exploited mainly for the industrial production of phycocolloids such as agar-agar, alginate and carrageenan, not for health aspects. Different types of antioxidants are available in various kinds of plants. 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 [4] determined various chemical constituents of the red alga *Grateloupia turuturu*.

Antioxidants are effective in protecting the body against damage by reactive oxygen species. There is an increasing interest in natural antioxidants because of the safety and toxicity problems of synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) that are commonly used in lipid containing food [5]. Many natural antioxidants have already been isolated from different kinds of plant, such as oilseeds, cereal crop, vegetables, leaves, roots, species and herbs [6]. Among natural antioxidants, phenolic antioxidants are in the fore front as they are widely distributed in the plant kingdom. Plants contain diverse group of phenolic compounds, including simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid datives and flavonoids. Reactive oxygen species (ROS) is generated in living organisms during metabolism [7]. Excess amounts of ROS may be harmful because they can initiate biomolecular oxidants which lead to cell injury and death and create oxidative stress which results in numerous diseases and disorders such as cancer, stroke, mycocardial infraction, diabetes,

septic and haemorrhagic shock alzhemer's and parkinsons diseases. The negative effects of oxidative stress may be mitigated by antioxidants. Marine algae extracts have been demonstrated to have strong antioxidant properties [8, 9]. Some of the seaweeds are considered to be a rich source of antioxidants)[10]. New technologies involving the removal of metals ions from waste waters have directed attention to biosorption based on metal binding capacities of various biological materials. Biosorption is an innovative technology that employs inactive and dead biomass for the recovery of heavy metals from aqueous solution. Research in the field of biosorption has mostly concerned itself with brown algae [11, 12] and to a less extent with red algae [13]. Literature survey found that the marine red algae belonging to this family are rich sources of phenolic compounds, especially bromophenols [14]. Phenolic compounds play an important role in one antioxidative properties of many plant derived antioxidants. Phenolic substances were also reported to possess a wide range of biological effects, including antioxidant, antimicrobial, anti-inflammatory and vasodilatory actions.

Ascorbic acid (vitamin c) is an essential nutrient required to maintain normal physiological functions in animal cells. It is generally believed that the ability to synthesize ascorbic acid is absent in some animals including invertebrates and fishes due to the lack of L-gulonolactone oxidase that catalyzes the terminal step in the conversion of glucose to ascorbic acid [15]. A dietary requirement for vitamin C has been reported for a no. of species of crustaceans [16]. Abalone are large algivorous marine molluscs of the genus, *Haliotis*. They are the most commercially important gastropods in aquaculture. It has been reported that an increased level of vitamin C was incorporated into abalone feeds. A vitamin mixture including vitamin C, usually a commercial product or experimental formulation similar to that for other aquatic animals, is generally supplemented to test diets for abalone at a level of 1.5-2.5% [17, 18].

In general, from the critical review of literature, it has been observed that the most 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 dtermination of individual heavy metals and total heavy metals in *Kappaphycus sp.*

EXPERIMENTAL SECTION

Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of dry and living sample. Algae samples were cleaned at epiphytes and necrotic parts were removed. 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 heavy metals. This powder was stored in cold conditions in an airtight container and analysis was carried out within three months of processing. Material methods for the estimation of heavy metals is described below.

Limit Test for Heavy Metals in Kappaphycus sp.

Apparatus The apparatus typically consists of the following: as digestion flasks, polytetrafluoroethylene flasks with a volume of about 120 ml, fitted with an airtight closure, a valve to adjust the pressure inside the container and a polytetrafluoroethylene tube to allow release of gas, a system to make flasks airtight, using the same torsional force for each of them, a microwave oven, with a magnetron frequency of 2450MHz, with a selectable output from 0 to 630 ± 70 W in 1 per cent increments, a programmable digital computer, a polytetrafluoroethylene-coated microwave cavity with a variable speed exhaust fan, a rotating turntable drive system and exhaust tubing to vent fumes, an atomic absorption spectrometer, equipped with hollow-cathode lamps as source of radiation and a deuterium lamp as background corrector; the system is fitted with: (a) a graphite furnace as atomisation device for cadmium, copper, iron, lead, nickel and zinc. (b) an automated continuous-flow hydride vapour generation system for arsenic and mercury.

Method In case alternative apparatus is used, an adjustment of the instrument parameters may be necessary. Clean all the glassware and laboratory equipment with a 10 g/l solution of nitric acid R before use.

Test solution In a digestion flask place the prescribed quantity of the substance to be examined (about 0.50 g of powdered (1400) or 0.50 g of fatty oil). Add 6 ml of heavy metal-free nitric acid R and 4 ml of heavy metal-free hydrochloric acid R. Make the flask airtight. Place the digestion flasks in the microwave oven. Carry out the digestion in 3 steps according to the following programme, used for 7 flasks each containing the test solution: 80 per cent power for 15 min, 100 per cent power for 5 min, 80 per cent power for 20 min. At the end of the cycle allow the flasks to cool in air and to each add 4 ml of heavy metal-free sulphuric acid R. Repeat the digestion programme. After cooling in air, open each digestion flask and introduce the clear, colourless solution obtained into a 50 ml

volumetric flask. Rinse each digestion flask with 2 quantities, each of 15 ml, of water R and collect the rinsings in the volumetric flask. Add 1.0 ml of a 10 g/l solution of magnesium nitrate R and 1.0 ml of a 100 g/l solution of ammonium dihydrogen phosphate R and dilute to 50.0 ml with water R.

Blank solution Mix 6 ml of heavy metal-free nitric acid R and 4 ml of heavy metal-free hydrochloric acid R in a digestion flask. Carry out the digestion in the same manner as for the test solution.

***Cadmium, copper, iron, lead, nickel and zinc*:** Measure the content of cadmium, copper, iron, lead, nickel and zinc by the standard additions method, using reference solutions of each heavy metal and the instrumental parameters described. The absorbance value of the blank solution is automatically subtracted from the value obtained with the test solution.

Arsenic and mercury: Measure the content of arsenic and mercury in comparison with the reference solutions of arsenic or mercury at a known concentration by direct calibration using an automated continuous-flow hydride vapour generation system. The absorbance value of the blank solution is automatically subtracted from the value obtained with the test solution.

Arsenic* sample solution:** To 19.0 ml of the test solution or of the blank solution as prescribed above, add 1 ml of a 200 g/l solution of potassium iodide R. Allow the test solution to stand at room temperature for about 50 min or at 70°C for about 4 min. Acid reagent heavy metal is free hydrochloric acid R. ***Reducing reagent A 6 g/l solution of sodium tetrahydroborate R in a 5 g/l solution of sodium hydroxide R.

***Mercury* sample solution:** Test solution or blank solution, as prescribed above. Acid reagent A 515 g/l solution of heavy metal is free hydrochloric acid R.

Reducing reagent: A 10 g/l solution of stannous chloride R in dilute heavy metal-free hydrochloric acid R.
Limit test for Nickel: Determine the nickel by atomic absorption spectrometry.

Test solution: Dissolve 20.0 g of the substance to be examined in a mixture of equal volumes of dilute acetic acid R and water R and dilute to 100.0 ml with the same mixture of solvents. Add 2.0 ml of a saturated solution of ammonium pyrrolidinedithiocarbamate R (about 10 g/l) and 10.0 ml of methyl isobutyl ketone R and then shake for 30 s protected from bright light. Allow the layers to separate and use the methyl isobutyl ketone layer.

Reference solutions Prepare 3 reference solutions in the same manner as the test solution but adding 0.5ml, 1.0ml and 1.5ml respectively of nickel standard solution (10 ppm Ni) R in addition to the 20.0 g of the substance to be examined. Set the zero of the instrument using methyl isobutyl ketone R treated as described for preparation of the test solution omitting the substance to be examined. Measure the absorbance at 232.0 nm using a nickel hollow-cathode lamp as source of radiation and an air-acetylene flame. The substance to be examined contains not more than 1 ppm of nickel, unless otherwise prescribed. that in the standard.

Table 1 Various heavy metals available in *Kappaphycus sp.*

Chemical constituent	Composition	SD
Iodine	56.77 ppm	56.77±0.012
Boron	6.66 ppm	6.66±0.01
Lead	3.45 ppm	3.45±0.02
Nickel	2.43 ppm	2.43±0.01
Cobalt	1.56 ppm	1.56±0.03
Mercury	1.44 ppm	1.44±0.03
Cadmium	1.02 ppm	1.02±0.02
Arsenic	0.897 ppm	0.897±0.02
Tin	0.89 ppm	0.89±0.01
Total heavy metals	30.4 ppm	30.4±0.01

RESULTS AND DISCUSSION

Results obtained for various heavy metals available in *Kappaphycus sp*. are shown in Table 1. Statistical distribution was carried out and standard deviation (SD) is estimated and is shown in Table 1 against each constituent.

Fig. 1 shows composition of various heavy metals available in *Kappaphycus sp.* From the studies, it is observed that (Table 1, Fig. 1), total account of the heavy metals are 30.4ppm. Iodine is the major constitutent (56.77ppm) then boron (6.66ppm) and lead (3.45ppm) and nickel (2.43ppm). The composition of cobalt is 1.56ppm, mercury is 1.44ppm and cadmium is 1.02ppm. Very less amount of metals are arsenic (0..897ppm) and tin (0.89 ppm). Romera et al [19] studies biosorption capacity of six different algae and evaluated in the recovery of cadmium, nickel, zinc, copper and lead from aqueous solutions. It was found that the optimum pH was 6 for the recovery of Cd, Ni and Zn and less than 5 for Cu and Pb. The best results were obtained with the lowest biomass concentration used (0.5g/l) and the sequence of sorption values was Pb>Cd>Cu>Zn>Ni. The brown algae achieved the lowest metal concentration levels in solution. Fayaz et al [20] reported that *Kappaphycus alvarezii* contain calcium, 159.5, iron 33.8 and zinc, 1.58 mg/100g of the sample (w/w). The presence of significant amounts of calcium and iron in *K.alvarezii* may be due to its metabolic system in which it is capable of directly absorbing elements from the seawater.

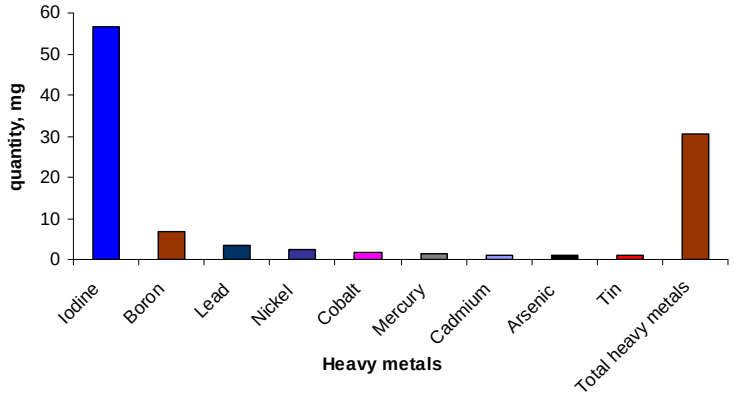

Fig. 2. Plot of heavy metals

CONCLUSION

Heavy metals were estimated on the *Kappaphycus sp*. Sample, which was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of dry and living sample. Various heavy metals such as Cadmium, copper, iron, lead, nickel and zinc were estimated by using the standard additions method and reference solution. . It is observed that total amount of the heavy metals is 30.4ppm. Iodine is the major constituent (56.77ppm) then boron (6.66ppm) and lead (3.45ppm) and nickel (2.43ppm). The composition of cobalt is 1.56ppm, mercury is 1.44ppm and cadmium is 1.02ppm. Very less amount of metals are arsenic (0.897ppm) and tin (0.89 ppm).

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