Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(1):33-37

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Potential Application of *Kappaphycus alvarezii* in Agricultural and Pharmaceutical Industry

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ABSTRACT

Marine organisms are a rich source of structurally novel and biologically active metabolites. Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of dry and living sample. After drying the sample, it was ground thoroughly to powder form. The powder was then used for the estimation of parameters. From estimation of biochemical composition, it is observed that the protein is as high as 18.78 gm compared to all other substances like, phenols, lipids, carbohydrates, fat, sterols. Hence the species can serve as functional food with vital nutritional and biologicalvalue. .Antioxidant potential of the red algae (Kappaphycus alvarezii) was determined by estimation of vitamin C, vitamin E and heavy metals such as Selenium and Magnesium. The primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. Results of this study suggested that the utility of Kappaphycus alvarezii proved to be a promising area of pharmaceutical study From the study, it was observed that kappaphycus alvarezii maximum activity against pseudomonas flourescence, staphylococcus aureus and less inhibition on vibriochloera and proteus mirabilis. India are potential sources of bioactive compounds..The XRD spectrum of thin film of the biomass that has synthesized gold nano particles was confirmed the presence of gold nanoparticles and its good crystalline nature.

Key words: Red algae, *Kappaphycus alvarezii*, proteins, antibacterial, pharma-industry biological aspects.

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, 2, 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*.

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.

The present investigation explores the possibility of utilizing the marine red algae *Kappaphycus alvarezii* and their active principles in agriculture and medicine.

EXPERIMENTAL SECTION

Carbohydrates were extracted and estimated as the procedure of Roe (1955) and Indian Pharmacopeia (2007). The procedure outlined by Bligh and Dyer (1959) and Indian Pharmacopeia (2007) was used to determine the total lipids in the samples .

Protein in the solution was estimated by following procedure of Lowry et al. (1951). Procedure is outlined below:

Total free phenols were estimated as per British Pharmacopeia (2007) and by the method of Maxon and Rooney (1972).

Free and protein amino acids were estimated by O-pthaldialdehyde method described by Rajendra (1987).

Fatty acids in the samples were identified and quantified as methyl esters in NEON 11 gas chromatography instrument following the procedure outlined by Niller and Berger (1985).

The antibacterial activity of the extract was assayed using the disc diffusion method (Bauer et al., 1996). For inoculum preparation and assay of antibacterial activity, Muller-Hinton agar was used. The bacteria were sub cultured and routinely maintained on both nutrient agar and Muller-Hinton agar. Antimicrobial activity was evaluated using the agar diffusion technique in petridishes. Each extract was loaded on sterile filter paper discs and air dried. Indicator microbes were spread on Muller-Hinton agar plates with sterile effusion the discs were placed on plates. After incubation for 24 hours at 30°c, a clear zone around a disc was evidence of antimicrobial activity. Discs loaded with the extracting agents were tested as controls (Inci Tunes et al 2006).

In this study, five test pathogens were considered, namely, *Pseudomonas fluorescence*, *Staphylococcus aureus*, *Vibrio cholera*, *Proteus mirabilis* towards study of inhibition of microbial growth by methanol extract. The plates were incubated at 37^{o}C. The zone of inhibition of assay was scored (+), if it is ‹2mm, double positive (++), if the zone is 2mm.

Material used for the synthesis of gold nanoparticles is chloroauric acid ($HAuCl_4^-$). Formation of Au (0) was carried out by taking 100 mg of seaweed powder in a 500 mL Erlenmeyer flask with 100 mL of 10^{-3} M aqueous $HAuCl_4^-$ solution. The 95% of the bioreduction of $HAuCl_4^-$ ions occurred within 2 hr. under stirring condition.

The methods and equipment were used to characterize gold nanoparticles using standard protocols UV–Visible spectra,,Fourier transform infrared (FT-IR) spectrum- a Shimadzu, FTIR spectrophotometer TEM-JEOL TEMSCAN2000EX, X- Ray diffraction (XRD) analysis-D8 Rich Seifert P3000 instrument operating at a voltage of 40 kV and a current of 30 mA with Cu Kα1 radiations.

RESULTS AND DISCUSSION

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.

Total 18 amino acids were found in the dried powder of *K. alvarezii*. Among all the amino acids, lysine is the major constituent and followed by asparagines, histidine, isoleucine, phenylalamine, tryptophan. In the case of fatty acids, eight components were identified including two components, namely, palmitic acid and cervonic acid in traces. Alpha linolenic acid (n-3) and linoleic acid are the major components. Macrominerals were identified by using flame atomic absorption spectrophotometry and it was found that red algae contained various amounts of macrominerals such as Sodium (23.4 mg), Potasium (12.44 mg), Magnesium (23.56 mg), Phosphorous (19.5 mg) per 100mg and rich in calcium (3.565 gm/100 gm). The studies showed that red seaweeds could be used as a food supplement to meet the recommended daily intake of some essential minerals. From the overall study, it can be concluded that the *K. alvarezii* can serve as functional food with vital nutritional and biological values.

Reduction of the aqueous chloroaurate ions during exposure to the biomass of alga *Kappaphycus alvarezii* may be easily followed by UV-Vis spectroscopy. The significant reduction in the reaction time with the *Kappaphycus alvarezii* is an important result and will enable nanoparticle biosynthesis methods to compete favorably with chemical methods for the formation of gold nanoparticles that are currently much more rapid and reproducible. It is well known that gold nanoparticles exhibit lovely pink-ruby red colors, these colors arises due to excitation of surface plasmon vibrations in the gold nanoparticles[18] as shown in Figure 1.

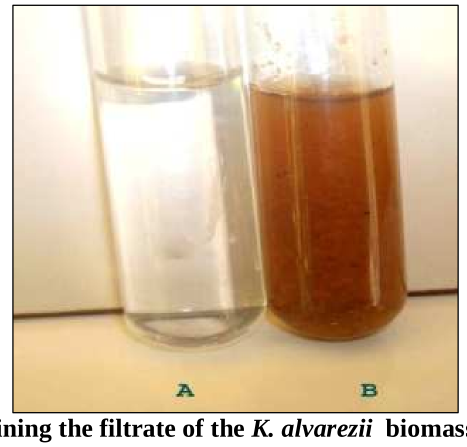

Fig. 1. Picture of the test tube containing the filtrate of the *K. alvarezii* biomass in aqueous solution of 1 x10^{-3} M at the beginning of reaction (A) and after 2 hr. of reaction (B)

The result obtained from the TEM study gives a clear indication regarding the shape and size of the nanoparticles. On careful observations of various magnifications of TEM images of gold nanoparticles (figure 2), it is noted that the particles are of different size ca. ranges from 10 to 40 nm and were predominantly polydisperse in size. From the size distribution of gold nanoparticles it is observed that maximum number of gold nanoparticles having size around 25 nm. The particle size histogram derived from the particles shown in this image and other similar images is shown in figure 3.

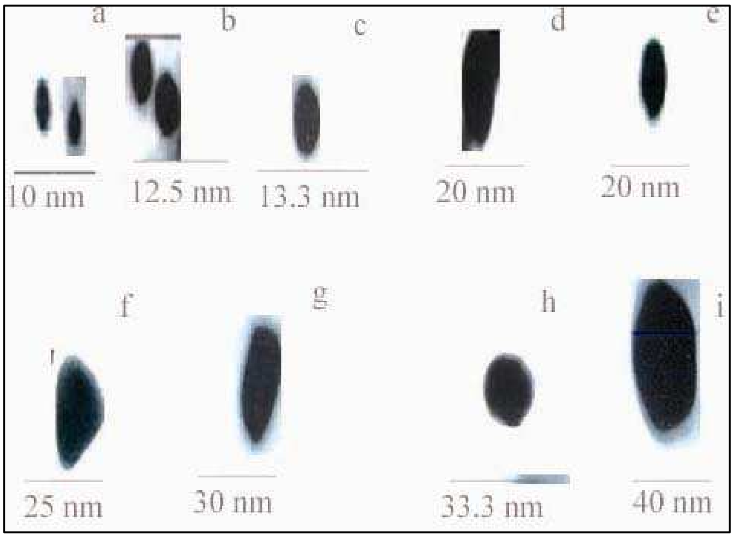

Fig. 2 TEM images of gold nanoparticles synthesized using *K. alvarezii*

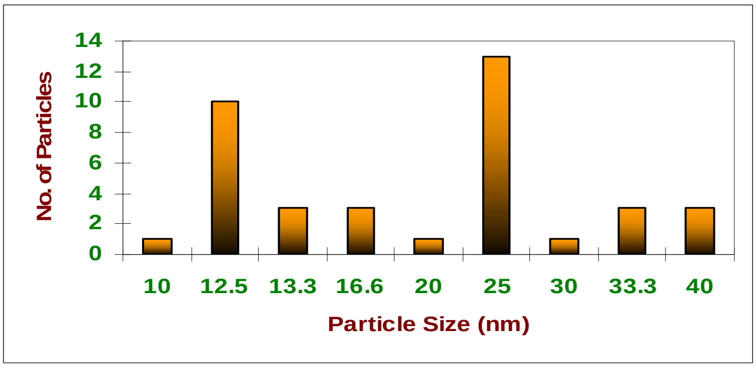

Fig.3. A particle size distribution histogram of as synthesized gold nanoparticles determined from TEM images

The present investigation shows the importance of surface characteristics and the type of functional groups of supporting polymer micro spheres to achieve a uniform anchorage of gold nanoparticles.

In the present study, in an eco-friendly method using *Kappaphycus alvarezii* biomass deposited gold nanoparticles at room temperature and neutral pH. Furthermore, the extracellular synthesis would make the process simpler and easier for downstream processing. Our results suggest that this methodology for gold nanoparticles synthesis using the algal biomass extracts is an attractive green process which is cost effective affordable and environmentally benign, and is useful for achieving a high yield of gold nanoparticles.

CONCLUSION

In the study, it is observed that *Kappaphycus alvarezii* maximum activity against *Pseudomonas flouresences, Staphylococcus aureus* and less inhibition on *Vibriochloera* and *Proteus mirabilis.* Our results suggest that this methodology for gold nanoparticles synthesis using the algal biomass extracts is an attractive green process which is cost effective affordable and environmentally benign, and is useful for achieving a high yield of gold nanoparticles.

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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