Qualitative and Quantitative Analysis Of Phytochemical Variation in G. corticata And K. alvarezii

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Abstract--- Seaweeds are one of the most important renewable wealth of marine source which have been used as food in diet for centuries. They are source of wide range of secondary metabolites such as carotenoids, dietary fibres, proteins, essential fatty acids, vitamins and minerals. For these study seaweeds samples of Gracilaria corticata and Kappaphycus alvarezii were collected from Rameshwaram. These samples were subjected to preliminary phytochemical screening and then quantitative estimation of photochemical. The samples were found positive for alkaloids, anthraquinones, flavinoids, lignin, proteins and saponins. In our observation phenolic compound and flavonoids were founf in these species which have been reported to be potent free radical scavengers. In our study Gracilaria corticata was found to contain more phenolic content and flavonoids compared to Kappaphycus alvarezii.

Keywords: G. corticata, K. alvarezii, Phytochemicals, Secondary Metabolites

I. INTRODUCTION

Diet plays the most important role in sustaining human health which challenges the food industry to provide healthy and nutritious food [1]. Seaweeds are considered as healthy food with fiber, mineral, protein, vitamins, and trace elements of wide range of secondary metabolites [2]. Seaweeds are known to contain secondary metabolites such as carotenoids, phycobilins, fatty acids, polysaccharides, vitamins, sterols, tocopherol and phycocyanins [3]. Seaweeds also contain many minerals and trace elements, due to their capacity to retain inorganic marine substances [4, 5]. These mineral accumulate in seaweeds at much higher levels and can accounts for up to 36% of dry matter of seaweed in some species [6]. Seaweed secondary metabolite is known to provide innumerable health benefits such as reduction of CHD, anticarinogenic and anti-inflammatory activities [2].

Seaweed are used commercially as a gelling, emulsifying, thickening and stabilizing agent in both pharmaceutical and nutraceutical products [7]. Considering the great diversity, investigations related to the search of new bioactive compounds from the seaweeds is unrestrained area of research [8, 9]. Seaweeds derived compounds can act as allelopathic, antimicrobial, antifouling, and herbivore deterrents, or as ultraviolet-screening agents [10]. The chemical and nutritional composition of seaweeds depends on many factors, including species, geographical origin or area of cultivation, seasonal, environmental, and physiological variations, time of harvest, water temperature, and processing methods [11-12]. In this study two species of seaweed were selected, Gracilaria which has more than 150 species reported across the world and consists of many commercially important agarophytes and Kappaphycus which is one of the main seaweed cultivated around the world [13, 14]. In this study our aim is to screen seaweeds from different environments to determine their phytochemical constituent by qualitative as well as quantitative method.

II. MATERIALS AND METHODS

A. *Sample collection*

Seaweeds samples of Gracilaria corticata and Kappaphycus alvarezii were collected from the Rameshwaram.

B. *Preliminary Phytochemical Analysis*

Phytochemical analysis was performed according to the standard protocol described [15]. All the prepared seaweed extracts were subjected to preliminary phytochemical screening for the presence of anthraquinones, flavinoids, glycosides, lignin, proteins, saponins, tannins and terpenoids.

C. *Estimation of phenolic content*

The total phenolic content was determined spectrophotometrically by using Folin- Ciocalteau reagent according to the modified method. 50µl of the methanolic extract was dissolved in gallic acid in methanol (1mg/ml) and Added 1.5ml of Folin-Ciocalteau reagent (Dilution 1:2) and 1.2 ml of 20% sodium carbonate. Incubated in dark for 30 minutes and the absorbance was recorded at 765nm and expressed as µg of gallic acid per gm of sample.

D. *Estimation of Flavonoid*

The methanolic extract of sample was filtered through whatman filter paper No 42 (125mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

E. *Quantitative estimation of Alkaloids*

To 5 g of the sample 200 ml of 10% acetic acid in ethanol was added and kept for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue (alkaloid) was dried and weighed.

F. *Determination of Total Carbohydrate*

100 mg of the sample was added to 5 mL of 2.5 N HCl kept in boiling water bath for 3 hours. It was neutralized with sodium carbonate and centrifuged. The sup supernatant was used for analysis. Standards were prepared and 4 mL of

anthrone reagent was added. Heated for 8 minutes in a boiling water bath then cooled rapidly. The absorbance was recorded at 630 nm. Standard graph was plotted using concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph the amount of carbohydrate calculated.

III. RESULTS AND DISCUSSION

The extracts of seaweed samples were prepared and used for preliminary phytochemical screening for the presence of phenolic content, cardiac glycosides, anthraquinones, alkaloids, terpenoids, proteins, flavinoids, saponins, tannins and lignin as chemical constituents. The detailed results of all tests for phytochemical screening of each species are summarized in table 1.

S.No	Test	Gracillaria	Kappaphy cus
1	Reducing sugars	+	-
2	Cardiac glycosides	-	-
3	Anthraquinones	+	+
4	Terpenoids	-	-
5	Proteins	+	+
6	Flavonoids	+	+
7	Saponins	+	+
8	Tannins	-	-
9	Lignins	+	+
10	Phenol	+	+
11	Alkaloids	+	+

Table 1: Qualitative analysis of phytochemicals

The seaweeds samples were found positive for all phytochemical except cardiac glycosides, anthraquinones and terpenoids. Phytochemicals are compounds from food and medicine to protect and maintain human health. These have antioxidant or hormone-like effect which helps to fight against diseases like cancer, heart disease, diabetes, high blood pressure and preventing the formation of carcinogens on their target tissues. It is reported earlier that seaweeds are also rich in polysaccharides such as alginates, fucans, and laminarans which possess medicinal values [11]. Seaweed content of proteins, carbohydrates, lipids, fibre, metabolites, etc. can be influenced by their growing parameters. For this reason seaweeds can be considered as natural bioreactors, able to provide different types of compounds at different quantities. The quantitative estimation of phytochemicals was showed in table 2

S.No	Test	Gracillaria	Kappaphycus
	Phenolic content	7.412 (mg/g)	Very less(<5 mg/g)
2	Flavonoids	6.5 %	3 %
3	Alkaloids	2.9 %	1.5%
4	Carbohydrate	5.1 %	9.6 %
5	Proteins	6.5 (mg/g)	2.3 (mg/g)

Table 2: The estimated quantity of phytochemicals

Seaweed is also known to possess antioxidant potential due to these secondary metabolites which enhance their property as healthy food. The presence of flavonoids correlates with the reported antimicrobial, antiviral and spasmolytic activity of these two species. The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. In our observation both of them were present in these species. Antioxidative properties of polyphenols are due to high reactivity as hydrogen or electron donors to stabilize and delocalize the unpaired electron and their potential to chelate metal ions [16]. The observations of present study directly coincided with the previous observations.

IV. CONCLUSION

The present study analyzed the bioactive content of seaweeds from different environment and demonstrates the quantitative variation due to environmental changes. In our study Gracilaria corticata was found to contain more phenolic content and flavonoids compared to Kappaphycus alvarezii.

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