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Phytoconstituents screening and *in-vitro* evaluation of total antioxidant activity of marine red algae *Gracilaria fergusonii* J. Agardh

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ABSTRACT

Evaluation of various organic solvent extracts of marine red algae Gracilaria fergusonii used for priliminary screening of phytoconstituents and in-vitro total antioxidant activity. The phytochemical screening was carried out by cooling percolation extraction using different solvents like acetone, ethanol, chloroform, petroleum ether, benzene, methanol and water. The standard regression of alpha tocopherol gives linear regression [y=0.0004x + 0.0807 (R^2= 0.9814)] obtained and used to calculate the standard and the total antioxidant activity are expressed as equivalents of vitamin E (µg g^{-1}). The present study reavealed that most of the phytochemicals like alkaloids, coumarin, flavonoids, phenol, quinons, steroids, saponin, tannins, glycosides, amino acid, sugar, protein and fatty acid were present in the dried algal sample. The total antioxidant activity was done by phosphomolybdate method using α- tocopherol as standard.

Keywords: *Gracilaria fergusonii*, phytoconstituents, antioxidant, α- tocopherol
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INTRODUCTION

The phytochemical from marine algae are extensively used in various industries such as food, confectionary and textile, pharmaceutical, dairy and paper mostly as gelling, stabilizing and thickening agents. Seaweeds are reservoirs of carotenoids, pigments, polyphenols, enzymes and diverse functional polysaccharides. Seaweeds are excellent source of vitamin A, B_1, B_{12}, C, D and E. Seaweeds have been one of the richest and most promising sources of bioactive primary and secondary metabolites [1]. Phytochemical constituents of a drug are helpful for identification purpose, when the drug is mixed together in compound drug formulation. Marine algae are produced a wide variety of chemically active metabolites in their surroundings, these active metabolites also known as biogenic compounds such as halogenated compounds, alcohols, aldehydes, terpenoids are produced by several species of marine algae and have antioxidant, antibacterial, antialgal and antifouling properties.

Antioxidants play an important role as health protecting factor. Antioxidants are molecules, capable of inhibiting the oxidation of molecules and protect the environment. Free radicals are produced mainly by oxidation reactions and, thereby these radicals can establish chain reaction. Because of these reactions in a cell, it gets damaged and finally leads to death. Antioxidants cause these sequences of reactions by eradicating free radical intermediates and reduce further oxidation reactions. Antioxidants do the above said by being oxidized themselves. Food antioxidants such as α-tocopherol, ascorbic acid, carotenoids, amino acids, peptides, proteins, flavonoids and other phenolic compounds might also play a significant role as physiological and dietary antioxidants, thereby accumulating the body's natural resistance to oxidative damage [2]. In recent years, marine algal sulfated polysaccharides have been demonstrated to have antioxidant activity. Sulfated polysaccharides comprise a complex group of macromolecules with a wide range of important biological properties. In the present study revealed that the presence of phytoconstituents and antioxidant activity of marine red algae *G. fergusonii.*

MATERIALS AND METHODS

2.1 Collection and preparation of seaweeds

Red alga *Gracilaria fergusonii* was collected from Manapad coast of Tamil Nadu, India (8.3775°N; 78.0522°E) at low tide. Specimen was washed thoroughly in seawater to remove extraneous matter such as epiphytes and sand. After collection, fresh samples were taken into plastic jar and brought back to the laboratory immediately. Samples were washed by tape water for several times, then gently brushed and rinsed with distilled water and then dried. The samples were dried under room temperature and powdered using blender.

2.2 Extraction and phytochemical screening

The active compounds in the powdered sample were extracted using organic solvents like acetone, ethanol, chloroform, petroleum ether, benzene, methanol, and water by percolation method. The powder was soaked in respective solvents for 48 hours and this procedure was repeated when the sample decolorized for thrice. Qualitative analysis of various extracts were subjected to identifying bioactive constituents like alkaloids, anthraquinones, catachin, coumarin, flavonoids, phenol, quinons, steroids, saponin, tannins, glycosides, amino acid, sugar, protein, fixed oil, xanthoprotein and fatty acid using standard procedures [3-5].

2.3 Evaluation of total antioxidant activities

2.3.1 Algal extracts preparation

10g of powdered samples were subjected to extraction with methanol and in a Soxhlet extractor for six hours and the extraction was repeated twice. Similar extracts were also prepared using chloroform and distilled water. The extracts were then concentrated to dryness under reduced pressure and controlled temperature (40-50ºC). The resultant residues were stored in a refrigerator till further use.

2.3.2 Preparation of the extracts and standard

Weighed quantities of methanol, chloroform and water residues were dissolved in the respective solvents. The stock solutions were serially diluted with respective solvents to get lower concentrations (1000, 750, 500, 250, 100 µg ml^{-1}). Each concentration was prepared in triplicate. These were subjected to the in vitro assays total antioxidant activity. Vitamin E standard (alpha tocopherol) was dissolved in methanol and diluted quantitatively with methanol to obtain a concentration of 100µg ml^{-1}.

The total antioxidant activity was evaluated by the method of phosphomolybdate using α- tocopherol as the standard [6]. An aliquot of the extract (100-1000µg ml^{-1}, 0.1ml) was combined in an eppendorf tube with 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). In case of blank, 0.1ml of methanol or distilled water was used in place of sample. The tubes were capped and incubated in a boiling water bath at 95ºC for 90min. After the samples had cooled at room temperature, the absorbance was measured at 695 nm against the blank using an UV spectrophotometer. The blank solution contained 1.0 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as rest of the sample. The total antioxidant capacity was expressed as µg g^{-1} equivalents of α-tocopherol by using the standard α-tocopherol graph.

RESULTS AND DISCUSSION

3.1 Phytochemical analysis

Phytochemical analysis of *G. fergusonii* is presented in Table – 1. Most of the bioactive compounds like alkaloids, anthraquinones, catachin, coumarin, flavonoids, phenol, quinons, steroids, saponin, tannins, glycosides, amino acid, sugar, protein, fixed oil, xanthoprotein and fatty acid were tested. Whereas, compounds like anthraquinon, catachin and xanthoprotein are not found. Alkaloids are the naturally occurring secondary metabolites comprising basic nitrogen synthesized from building blocks of amino acids with various radicals replacing hydrogen in the peptide ring and most containing oxygen. Here alkaloids are positive results shown in all extracts except in petroleum ether extract. Phenols or polyphenols are compound containing a hydroxyl group attached an aromatic ring. It is readily soluble in ethanol and other organic solvents used as anesthetic and ointments. Aromatic organic compound like quinons are cyclic unsaturated diketeones are biologically important constituent present most of the extracts are considerable feature of this study.

Coumarin is a phytochemical with a vanilla like flavor and oxygen heterocycle. Coumarin can occur either free or combined with the sugar glucose (coumarin glycoside). Coumarin has blood-thinning, anti-fungicidal and anti-tumor activities. In present study coumarin and flavonoid are found in ethanol, chloroform, methanol and aqueous extract of *G. fergusonii.* Flavonoids being the largest group of phenolic compounds are known to contain a broad spectrum of chemical and biological activities including antioxidant and free radical scavenging properties [7]. Steroids are widely distributed in marine alage [8]. Sterol was predominant in red seaweeds, because presence of fatty acid and

sterol of algae were used for the chemotaxonomy [9]. The qualitative phytochemical screening showed the presence of fixed oil in aqueous extracts of *G. fergusonii.* Steroid and tannin were present in acetone, ethanol, chloroform and methanol extract of *G. fergusonii*. Organic acid and phenolic compounds of marine algae especially polyphenols and tannins have also been shown to have antimicrobial activities [10].

The presence of saponin was found in chloroform, petroleum ether, benzene, methanol extract of *G. fergusonii*. Saponin is useful medicine and pharmaceutical industry due to its foaming ability that produces frothy effect. Saponin is used also in the manufacture of shampoos, insecticides and various drug preparation and synthesis of steroid hormones [11]. Sugar bound molecules like glycosides play numerous roles in living organisms. In the phytochemical screening, glycosides are examined by two methods are anthrone and Borntrager's test. Among this, glycosides positively observed in anthrone test of most of the extracts of *G. fergusonii*.

3.2 Evaluation of Total Antioxidant Activities

The extractives of *G. fergusonii* were assay for *in-vitro* total antioxidant activity of methanol, chloroform and aqueous extracts equivalent with Vitamin E (α- tocopherol) and results are presented in Table-3. The standard Vitamin E values were tabulated in Table-2. From the standard regression (fig.1) of Vitamin E (α- tocopherol) as constructed using concentration between 100-1000 µg/ml the linear regression equation [$y=0.0004x + 0.0807$ ($R^2= 0.9814$)] obtained by MS office excel. Using this equation calculated to estimate the total antioxidant activity of methanol, chloroform and aqueous extracts. Antioxidants are basically reducing agents. Molybdate ion reduction assay is a quick, reliable method to determine the total antioxidant capacity of seaweed. Significant amount of total antioxidant activity was obtained from methanol extract 142.5 to 412.5 µg/*g* equivalent of vitamin E followed by chloroform and water (155.0 to 602.5 µg/*g* and 175.0 to 352.5 µg/*g*) respectively.

Table 1: Phytochemical screening of different solvent extracts of *Gracilaria fergusonii*

Sl. No	Tests	Solvents						
		Acetone	Ethanol	Chloroform	Petroleum Ether	Benzene	Methanol	Water
1.	Alkaloids I. Mayer's test	+	+	-	-	-	+	+
	II.Wagner's test	-	+	+	-	+	+	+
2.	Anthraquinones (Borntrager's test)	-	-	-	-	-	-	-
3.	Catachin	-	-	-	-	-	-	-
4.	Coumarin	-	+	+	-	-	+	+
5.	Flavonoid	-	+	+	-	-	+	+
6.	Phenol	+	+	+	-	-	+	+
7.	Quinons	+	+	+	-	-	+	+
8.	Saponin (Foam test)	-	-	+	+	+	+	-
9.	Steroids	+	+	+	-	-	+	-
10.	Tannins	+	+	+	-	-	+	-
11.	Sugar I. Benedict's test	-	-	+	+	-	-	-
	II. Fehling's test	+	-	+	+	-	+	-
12.	Glycosides I.Anthrone test	+	-	+	-	+	-	-
	II. Borntrager's test	-	-	-	-	+	-	-
13.	Amino acids (Ninhydrin test)	+	+	+	+	-	-	+
14.	Xanthoprotein	-	-	-	-	-	-	-
15.	Fixed oil	-	-	-	-	-	-	+
16.	Protein	+	+	+	+	+	+	+
17.	Fatty acid	+	+	+	+	+	+	+
	Saturated fatty acid	+	+	+	+	+	+	+

'+' / '-' indicates the presence or absence of compounds

Table 2: Total Antioxidant Activity of vitamin E (α- tocopherol)

Standard	Concentration (µg/ml)	Absorbance* (λ 695 nm)
Vitamin E standard (α- tocopherol)	100	0.105±0.001
	250	0.177±0.006
	500	0.259±0.010
	750	0.378±0.015
	1000	0.421±0.011

Values are expressed as mean ± SEM (Standard Error Mean) n=3

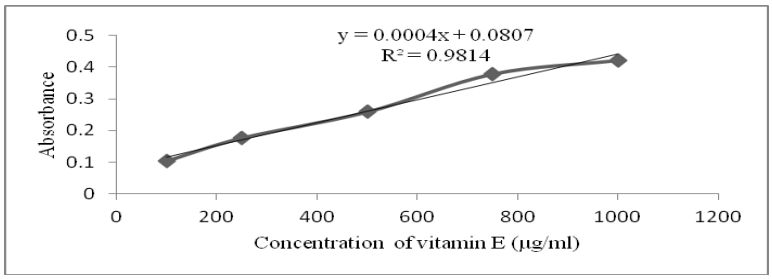

Fig 1: Standard curve for total antioxidant activity assay using α-tocopherol (100-1000 µg/ml)

Table 3: *In-vitro* total antioxidant activities of various extracts of *G. fergusonii* expressed as ascorbic acid equivalents µg/g

Sample	Concentration (µg/ml)	Absorbance (λ 695 nm)*	µg/g equivalent of vitamin E (a-Tocopherol)
Methanolic extract	100	0.057±0.001	142.5
	250	0.058±0.002	145.0
	500	0.078±0.003	195.0
	750	0.158±0.002	395.0
	1000	0.165±0.009	412.5
Chloroform extract	100	0.062±0.002	155.0
	250	0.065±0.005	162.5
	500	0.098±0.002	245.0
	750	0.172±0.004	430.0
	1000	0.241±0.026	602.5
Water Extract	100	0.070±0.003	175.0
	250	0.071±0.002	177.5
	500	0.099±0.001	247.5
	750	0.115±0.002	287.5
	1000	0.141±0.003	352.5

Values are expressed as mean ± SEM (Standard Error Mean) n=3

CONCLUSION

Primary and secondary products like alkaloids, coumarin, flavonoids, phenol, quinons, steroids, saponin, tannins, glycosides, amino acid, sugar, protein and fatty acids are maximum present in all the organic solvents whereas anthraquinones, xanthoprotein and fixed oil are observed in least or absent of above extracts. The methanolic extract has maximum scavenging activity compared with other two. The presence of phenol and flavonoid are the notable characters of this study. These compounds are the major source of antioxidants and these have more bioactivity. On the basis of this result, isolation and characterization of secondary metabolites are under process using advanced techniques.

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