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PRELIMINARY PHYTOCHEMICAL SCREENING, QUANTITATIVE ANALYSIS OF MARINE RED ALGAE *ACTINOTRICHIA FRAGILIS*

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ABSTRACT

The use of marine algae plays a vital role to maintain the human health. Due to the high demand for traditional medicines, there is a need for continuous research on marine algae for their therapeutic effects. Therefore, to investigate the preliminary phytochemical screening present in the of marine red algae *Actinotrichia fragilis*. Five different solvent extracts of the algae selected for the study were prepared using (Hexane, Ethyl acetate, Acetone, Methanol and Chloroform) of *Actinotrichia fragilis* were prepared by cold maceration method and the extracts were subjected to preliminary phytochemical screening and antibacterial activity against *Escherichia coli, Salmoella, Bacillus subtills, Streptococcus and Stapylococcus aureus*. The phytoconstituents such as carbohydrates and glycosides, proteins and free amino acids, phenolic compounds and tannins, flavonoids and terpenoids were found to be present in the extracts. The total tannin content, phenolic content and flavonoid content were determined by colorimetric method. The presence or absence of phytocostituents depends upon the solvent medium used for extraction and the physiological property of the seaweeds. From the results of the present study, it can be concluded that the seaweeds may be used abroad spectrum antimicrobial and bioactive agents after extensive investigation.

KEYWORDS

Actinotrichia fragilis, Total tannin, Total phenolic and Total flavonoids.

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INTRODUCTION

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities[1,2]. Antiviral, antibacterial[3], antifungal and anti tumoric activities that have been detected in green, brown and red algae giving an alternative approach to the use of the synthetic antimicrobial agents. However, the potent antimicrobial effect of seaweeds resides in

the efficiency of the extraction method, the algal species and the solvents being used[4-6].

Moreover, higher medicinal effect was obtained from dry seaweed samples than from fresh samples as indicated by many studies which reported that extracts prepared from fresh seaweeds showed negligible antimicrobial activity compared to that obtained from dried seaweeds[7-9]. In addition, revealed that algal sample preparation method greatly affects the bioactivity of testing algae (e.g. Lyophilization generally allows greater compound extraction and hence gives highest antimicrobial activity)[10].

Many investigations have demonstrated that a high dietary intake of natural phenols with the presence of several types of antioxidants such as flavonoids commonly found in plants and seaweeds is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, and various types of cancer[11-14]. Since seaweeds are known to contain a wide variety of bioactive compounds such as offering a rich source of new drugs with potentially lower toxicity.

Actinotrichia fragilis is red or yellow with cylindrical branches about 1 mm in diameter that are covered with closely spaced rings of short, dark, stiff hairs, Figure No.1. This lightly calcified seaweed is from 2 to 6 cm high and grows in tide pools, on reef flats, and in deeper subtidal habitats. *Actinotrichia fragilis* is a small (1±5–5 cm high) calcified, dichotomously divided multiaxial species, with an Indo-Pacific tropical distribution[15]. Tetrasporophytes and dioecious gametophytes are isomorphic. Therefore, the present investigation was attempting to study the antioxidant properties of five different solvent extracts of marine diatom red algae *Actinotrichia fragilis.*

MATERIAL AND METHODS

Chemicals

Methanol, ethyl acetate, hexane, acetone and chloroform were of HPLC grade (Lab-Scan, Dublin, Ireland). All the other reagents were of analytical grade and obtained from Merck (Darmstadt, Germany).

Algal materials

Actinotrichia fragilis red algae were collected from the Rameswaram area on January 5th, 2018. The freshly collected seaweeds were washed with clean seawater to remove salt, epiphytes and sand attached to the surfaces of the samples and transported to the laboratory. The samples were carefully rinsed with tap water, wiped with paper towel. For *Actinotrichia fragilis* the stipes and hapteres were removed and the new and old parts of the blades were separated. The samples were lyophilized for 72 h, pulverized into powder and stored at 80ºC prior to extraction.

Preparation of sample extract

10 grams of powdered samples were extracted with 50 ml of solvents, such as methanol, ethyl acetate, ethanol, acetone and chloroform. The samples were kept in the dark for 72 h with intermittent shaking. After incubation, the solution was filtered through filter paper, and the filtrate was collected (crude extracts) and stored in the refrigerator until further use.

Preliminary phytochemicals test

Phytochemical analysis was performed to determine the presence of different phytochemicals as described by Sadasivam and Manickam[16].

Estimation of flavonoid content

Total flavonoid content was determined according to the method[17]. A 1ml of each extract was mixed with 0.1 ml of 10% aluminium chloride and 0.1 ml of 1 M potassium acetate. Methanol (2.8 ml) was added and kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm. The flavonoid content was expressed in mg/g, and Rutin was used as a standard compound.

Estimation of tannin content

Total tannin content was determined according to the method[18]. Briefly, 50 l of seaweed extract was mixed with 1.5 ml of 40% vanillin (prepared with methanol), and then 750 l of HCl was added. The solution was shaken vigorously and left to stand at room temperature for 20 min in darkness. Absorbance against a blank was read at 500 nm. Catechin was used as standard

Estimation of phenol content

The total phenol content was measured using the Folin–Ciocalteu method[19]. Extract (100 ml) was mixed with 2 ml of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. Then, 100 ml of 50% Folin–Ciocalteu phenol reagent was added. After incubation for 30 min at room temperature in darkness, the absorbance was read at 720 nm. The total phenol content of the samples was expressed as mg Gallic acid per gram.

Determination of antibacterial activity

Antibacterial activities of the selected algal extracts were tested using pathogenic bacteria kindly supplied by the Sree Narayana Guru College, Microbiology department. The pathogens included *Escherichia coli, Salmoella, Bacillus subtills, Streptococcus and Stapylococcus*. The bacterial strains were grown in Nutrient agar medium at 37ºC. Stock cultures were maintained in nutrient medium at 4ºC and sub cultured at regular intervals[20]. The nutrient agar medium contained g/L: 5 g peptone, 3 g beef extract, 5 g NaCl, and 20 g agar agar in distilled water. Antimicrobial activity was evaluated using the agar diffusion technique in Petri dishes. Briefly, sterile filter paper discs, 6 mm in diameter (E-760), were loaded with 20 ml of the different antibacterial compound extracts and air dried. Discs containing standard concentrations of water used as a control. The discs were placed on Muller Hinton agar plates inoculated with each of the previously mentioned microorganisms. Plates were incubated for 24 h at 37 ºC and the inhibition zones that formed around the discs were measured (mm diameter). Each set was prepared in triplicate. The control discs were prepared with the solvents alone.

Statistical analysis

All the assays were carried out in triplicate. Experimental results are expressed as mean ± standard deviation. The results were analyzed using one-way analysis of variance and the group means were compared using Duncan's multiple range tests using SPSS version 16.

RESULTS AND DISCUSSION

The antioxidant activity of marine algae may arise from pigments such as chlorophylls, carotenoids, vitamins and vitamin precursors, including cophenol, carotene, niacin, thiamine, ascorbic acid and phenolic compounds, such as polyphenols, hydroquinones and flavonoids. Phospholipids, particularly phosphatidylcholine, terpenoids, peptides, and other antioxidative substances, directly or in directly contributed to the inhibition or suppression of oxidation processes[21,22]. The dried five different extracts of the *Actinotrichia fragilis* red algae species of seaweeds were found to have good antioxidant and antimicrobial activities. Similar results were previously obtained[23]. Although a variety of solvents have been employed in screening algae for antioxidant and antimicrobial activity, it is still unclear what type of solvent is the most effective and suitable for extraction of seaweeds[24,25].

As discussed, methanol extracts of red algae had higher total phenolic content and total antioxidant capacity as compared to five *Actinotrichia fragilis* extracts. The reducing power indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process, so they can act as primary and secondary antioxidants[26,27]. Overall, five different *Actinotrichia fragilis* extract were the most effective solvent for extraction of antioxidant properties from seaweeds, which may be due to *Actinotrichia fragilis* having a higher dielectric constant than five extracts. We found that for the red seaweeds, extracts of *Actinotrichia fragilis* Ethyl acetate more reactive than the other extracts.

Total flavonoids in the seaweeds ranged that flavonoids are probably the most important natural phenol due to their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties[28]. Flavonoids have been reported as antioxidants of a wide range of reactive oxygen species and inhibitors of lipid peroxidation and as potential therapeutic agents against a wide variety of diseases. Table No.1 and 2 shows the presence of phyto-constituents such as

flavonoids, tannins and polyphenols prevent a number of diseases through their free radical scavenging activity[29], and these phenolic compounds, which include phenol, tannin and flavonoids, have been found in appreciable amounts in the seaweeds of *Actinotrichia fragilis*.

The Ethyl acetate volatile oil extracts of the red alga Jania rubens were tested in vitro for their antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria. The Ethyl acetate extracts showed more potent antimicrobial activity than the hexane extracts of J. rubens[30]. Among the different extract species of algae collected from the sea water of the Red sea along the coastal region rameswaran of India. *Actinotrichia fragilis* were evaluated for their potential for bioactivity. Table No.3 and Figure No.2 shows revealed that the five different solvent algae extracts prepared with methanol, ethyl acetate, ethanol, acetone and chloroform had active principles that could inhibit growth of the pathogenic bacteria control in water and tested for methanol, ethyl acetate, hexane, acetone and chloroform extract only inhibited *Escherichia coli, Salmoella, Bacillus subtills, Streptococcus and Stapylococcus aureus*.

Table No.1: Qualitative phytochemical analysis different extracts of *Actinotrichia fragilis*

S.No	Phytochemicals	*Actinotrichia fragilis*				
		Hexane	**Ethyl acetate**	**Acetone**	**Methanol**	**Chloroform**
1	Alkaloids	--	++	+	+	+
2	Terpenoids	+	++	+	--	--
3	Tannins	+	++	--	+	+
4	Saponins	--	--	--	--	--
5	Flavonoids	+	++	++	++	+
6	Phenols	+	++	--	++	++
7	Amino acid	++	++	+	++	+
8	Aromatic acid	--	--	--	--	
9	Glycosides	--	+	--	+	--
10	Steroids	--	--	--	--	--
11	Carbohydrates	+	+	+	++	++
12	Essential oil and Resins	+	++	--	+	--
13	Pholabatanins	+	--	++	--	+
14	Xantho protein	--	++	--	+	--
15	Anthroquinones	--	--	--	--	++
16	Phytosterols	--	+	--	--	+
17	++: Intensely present, +: Present, -- : Absent					

Table No.2: Quantitative phytochemical analysis different extracts of *Actinotrichia fragilis*

S.No	Solvents	Total Phenolics (mg GAE/g dry wt)	Total Flavonoids (mg RUE/g dry wt)	Total Tannins (mg CAE/g dry wt)
1	Hexane	1.83 ± 0.06	0.86 ± 0.02	1.62 ± 0.21
2	Ethyl acetate	2.08 ±0.17	1.36 ± 0.09	2.43 ± 0.33
3	Acetone	2.16 ± 0.04	1.43± 0.07	1.37 ± 0.09
4	Methanol	2.31 ±0.15	1.65 ± 0.12	1.85 ±0.04
5	Chloroform	2.12 ± 0.08	1.48 ± 0.37	1.65 ±0.05

Values are means of three analyses of the extract ± Standard deviation (n=3)
(GAE: Gallic acid equivalent, RUE: Rutin equivalent, CAE: Catechin equivalent)

Table No.3: Antibacterial activity of different extracts of *Actinotrichia fragilis*

S.No		Concentration of *Actinotrichia fragilis*		
		Control	10µL	20µL
1	Escherichia coli (Hexane)	10.56 ± 1.16	16.42 ±1.46	22.26 ± 2.02
2	Salmoella (Ethyl acetate)	14.40 ± 2.09	16.18 ± 2.27	18.39 ± 1.98
3	Bacillus.subtills (Acetone)	12.76 ±1.28	17.08 ±1.42	19.17 ± 1.71
4	Streptococcus (Methanol)	9.48 ±0.37	13.42 ±1.24	15.26 ±1.39
5	Stapylococcus (Chloroform)	8.43 ± 0.42	14.51 ±1.07	19.86 ± 1.46

Values are means of three analyses of the extract ± Standard deviation (n=3)

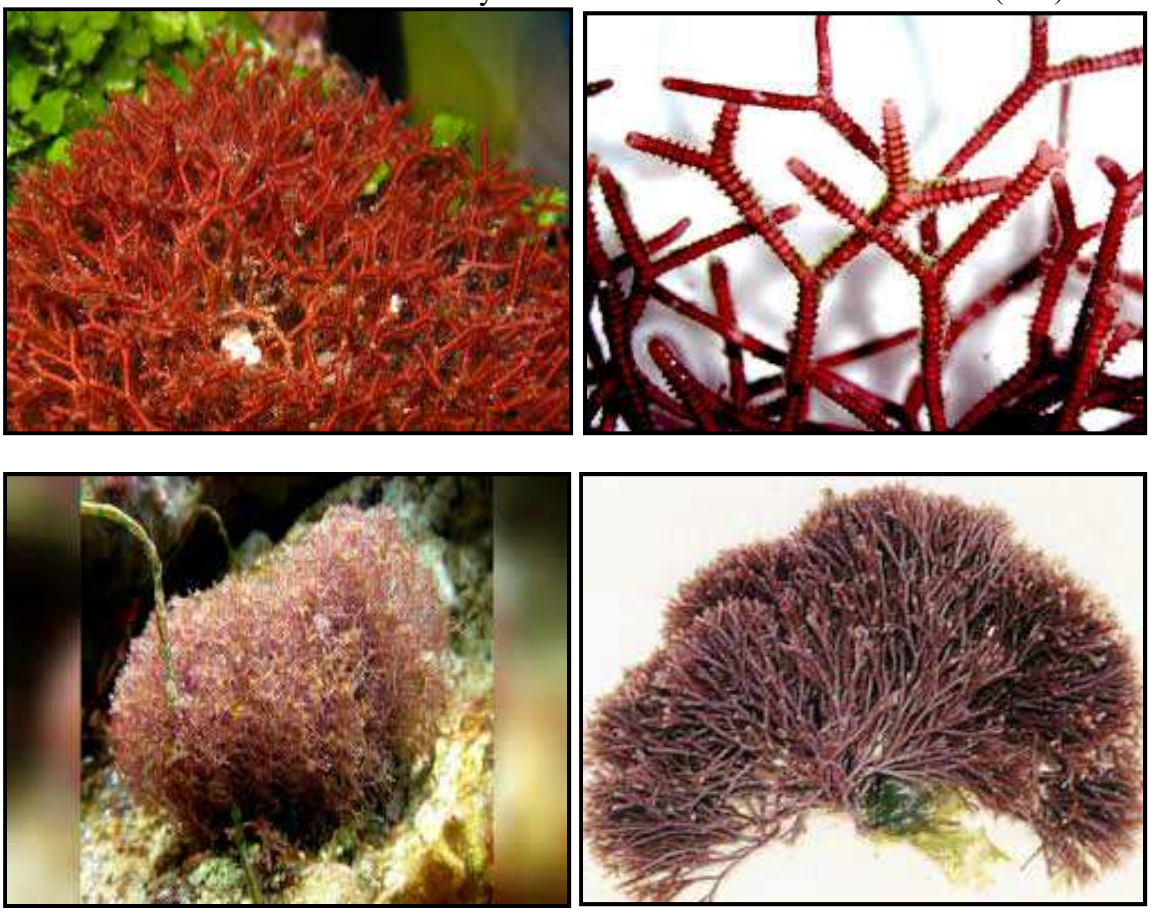

Figure No.1: Genus: *Actinotrichia* Species: *fragilis*

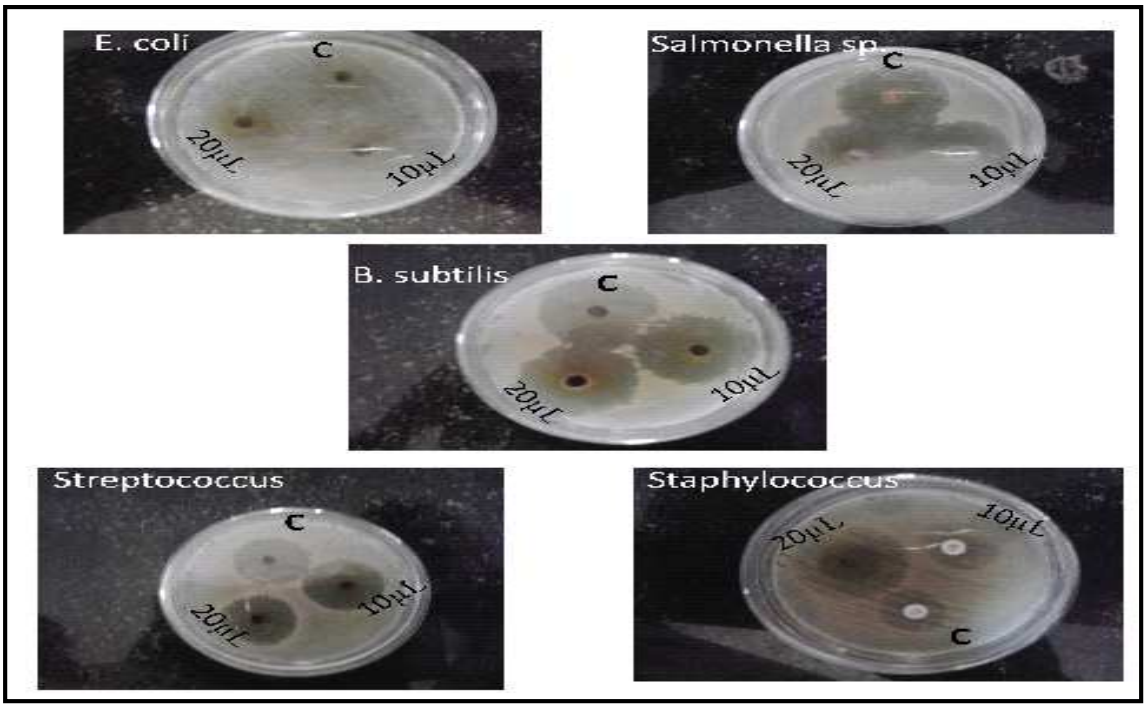

Figure No.2: Antibacterial activity on different extract of *Actinotrichia fragil*

CONCLUSION

It can be concluded that marine *Actinotrichia fragilis* red algae are a rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these red algae may be potential bioactive compounds of interest in the pharmaceutical industry and medicinal compounds. The present investigation adequate data on the phytochemical constituents of biochemical composition and antibacterial potential of the seaweed five different extracts for the synthesis of novel antibiotics. Bioactive compounds found in seaweeds await a major breakthrough in their potential application as natural antioxidants in different food and pharmaceutical products.

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CONFLICT OF INTEREST

The authors declare that they don't have any conflict of interest.

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