

RESEARCH ARTICLE

Study on phytochemical properties and antioxidant activities of marine macroalgae *Padina tetrastromatica* Hauck

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ABSTRACT

An abundance of beneficial plant life can be found in aquatic areas, particularly in marine ecosystems. These resources have, therefore, been in use for hundreds of years. Seaweeds have long been closely associated with human life as a source of food, fodder, fine chemicals, fertilizer and pharmaceutically significant drugs. Algae are an important group of organisms with the largest source of biogenic compounds. They are found to possess significant antioxidant and phytochemical activities. The aim of the present study was to investigate the phytochemical and antioxidant activity of active compounds present in the methanol extract of the brown algae, *Padina tetrastromatica* Hauck. The antioxidant activity of extracts was evaluated by DPPH scavenging activity and Hydroxyl radical-scavenging activity assays. The phytochemical analysis revealed the presence of alkaloids, flavonoids, steroids, saponins, carbohydrates, proteins and phenolic compounds etc.

Keywords: Phytochemical, Antioxidant activity, *Padina tetrastromatica*, Biogenic compounds, Brown algae

1. INTRODUCTION

Seaweeds are the vital constituents of marine ecosystems. They possess substantial ecological, economic and nutritional importance [1,3]. Serving as prolific oxygen producers and nutrient cyclers, they stabilize coastlines, foster biodiversity and play a crucial role in marine food webs [5,8,9]. Beyond their ecological contributions, seaweeds are utilized in diverse industries—providing food, pharmaceutical compounds and industrial products like alginates and carrageenans. Nutrient-rich and harboring bioactive compounds, they offer significant health benefits, while their potential in bioremediation and climate regulation underlines their critical role in the environment [2,6]. Overall, seaweeds stand as multifaceted resources pivotal for ecological balance, economic sustenance and human well-being [4].

Marine macroalgae, also known as seaweeds, boast an impressive array of phytochemical compounds and antioxidant properties that contribute to their significant biological value. These algae are rich sources of various bioactive compounds such as flavonoids, phenolic compounds, carotenoids, and phycobiliproteins. These

phytochemicals exhibit potent antioxidant activity, effectively scavenging free radicals and mitigating oxidative stress within the body [10]. The diverse range of antioxidants present in marine macroalgae not only aids in their defense against environmental stressors but also offers immense health benefits to humans. Regular consumption of these algae has been linked to potential anti-inflammatory, anti-aging and disease-preventive effects due to their ability to neutralize harmful free radicals and support overall health and well-being. As a result, the exploration of marine macroalgae for their antioxidant and phytochemical properties holds promise for both pharmaceutical and nutritional applications [7].

The present study is mainly based on the phytochemical and antioxidant activities of selected macroalgae, *Padina tetrastromatica* Hauck which is collected from Kannur coast, Kerala.

2. MATERIALS AND METHODS

2.1. Collection of Sample

The algae were collected in polythene bags containing seawater from the coastal line of Dharmadam (9°16'37.34"N; 79°7'30.78"E), Kannur

district, India and transported to the laboratory. Then the algal materials were thoroughly washed in running tap water and then distilled water to remove the attached epiphytes and other marine debris. The dried material was ground to a coarse powder and stored in airtight containers for further study.

2.2. Qualitative Phytochemical Screening

The aqueous, chloroform and methanol extracts of *P. tetrastromatica* were subjected to preliminary phytochemical screening using standard methods. The aqueous, chloroform and methanol extracts were screened for different classes of phytoconstituents such as alkaloids, flavonoids, phenols, tannins, saponins, steroids, terpenoids, carbohydrates and proteins using specific standard reagents.

2.2.1. Test for alkaloids

The crude extract was added to 2 mL of 1% HCl and gently heated. The reagents from Mayer and Wagner were then added to the mixture. The presence of alkaloids was determined by the turbidity of the resultant precipitate.

2.2.2. Test for Flavonoid

A few drops of sodium hydroxide solution were added to the crude extracts. It is indicated by the formation of a bright yellow color that fades to colorless when dilute acid is added.

2.2.3. Test for Phenol

Ferric chloride solution 3–4 drops were added to the crude extracts. The presence of phenol is indicated by the formation of a bluish-black color.

2.2.4. Test for Tannin

1% gelatin solution containing sodium chloride was added to the crude extract. The presence of tannin is shown by the formation of a white precipitate.

2.2.5. Test for Saponins

0.5 mL of crude extract was shaken with 2 mL of water. Saponins are present if the foam formed lasts longer than 10 minutes.

2.2.6. Test for Steroids

Crude extracts were combined with chloroform and a few drops of concentrated H₂SO₄ and shaken thoroughly and set aside for a while. The presence of steroids is indicated by the formation of a red color in the lower layer.

2.2.7. Test for Terpenoids

Crude extracts were mixed with a few drops of acetic anhydride, boiled and cooled, and then concentrated H₂SO₄ was added from the sides of the test tube for a brown ring created at the intersection of layers. Whereas, the presence of triterpenoids was indicated by the creation of a deep red color.

2.2.8. Test for Carbohydrates

When crude extracts were heated with 2 mL of Benedict's reagent, a reddish-brown precipitate appeared, indicating the presence of carbohydrates.

2.2.9. Test for Proteins

While crude extracts were combined with 2 mL of Millon's reagent, a white precipitate is formed, which turned red after gentle heating, indicating the presence of protein.

2.3. Antioxidant Analysis

2.3.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) Scavenging Activity

DPPH radical scavenging activity was measured using the Blois (1958) methodology. To make extract solutions, 0.05g of dry extract was dissolved in 50 mL of methanol. An aliquot of 2 mL is 0.004 percent DPPH solution and 1 mL plant extract at various concentrations (50–250 µg/mL) were mixed and incubated at 25°C for 30 minutes, with absorbance measured at 517nm using a spectrophotometer against a DPPH control containing only 1 mL of methanol in place of the extract. A DPPH solution was prepared on a regular basis prior to the absorbance measurements. Purple is the color of the stable free radical DPPH. Yellow Diphenyl Picryl Hydrazine is produced when diphenyl picryl hydrazine is reduced. All of the trials were carried out three times, with the results averaging. BHT and Rutin were used as points of comparison. Calculation done by using the following formula:

$$\% \text{ RSA} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100$$

Where, Abs control and Abs sample denote the absorption of a blank sample and a tested extract solution, respectively.

2.3.2. Hydroxyl Radical Scavenging Activity

A reaction mixture containing 3.75 mM 2-deoxyribose, 100 mM EDTA, 100 mM FeCl₃ (Merck), and 1 mM H₂O₂ was produced and added to plant

extracts at various concentrations (50–250 g/mL). At 37 °C, the reaction mixture was incubated for 1 hour. Following incubation, 1 mL of 2% Trichloroacetic acid (TCA) (SRL, India) and 1 mL of 1% Thiobarbituric acid (TBA) (SD Fine Chemicals, India) were added and incubated in a water bath at 90 °C for 20 minutes. After incubation, the solution was cooled and absorbance at 417 nm was measured using a UV spectrophotometer. Methanol was used as a blank. BHT and Rutin are used as standards.

$$\% \text{ RSA} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100$$

Abs control denoted the absorbance of the control, while Abs sample denoted the existence of absorbance in the samples.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Analysis

In the present study, a preliminary phytochemical screening was carried out on selected edible algal sample, namely *P. tetrastromatica*. The

presence of active constituents such as alkaloids, flavonoids, phenols, tannins, steroids, saponins, glycosides, terpenoids, carbohydrates and proteins were tested by using methanolic extract. In *P. tetrastromatica*. The phytochemical compounds namely carbohydrates, glycosides, flavonoids and proteins were present in all three solvents. Steroids were present only in chloroform extracts. Alkaloids, saponins and phenolic groups were present in chloroform and methanolic extracts of *P. tetrastromatica* (Table 1).

Preliminary phytochemical screening of methanolic extract from *P. pavonica* contains unsaturated sterols and/or triterpenoides, flavonoids, carbohydrates or glycosides, proteins, amino acids, tannins and coumarin (Al-Enazi et al., 2018). Acetone extract of *P. pavonica* contain phenolic, flavonoid and tannin (Bernardini et al., 2018). Phytochemical analysis of ethanol extract from *P. pavonica* revealed the presence of alkaloids, phenols, flavonoids, carbohydrates, glycosides, steroids and terpenoids (Ismail et al., 2019).

Table: 1 Phytochemical screening of *Padina tetrastromatica* Hauck

S. No	Compounds	<i>P. tetrastromatica</i>		
		Chloroform	Methanol	Aqueous
1	Alkaloids	+	+++	-
2	Flavonoids	+++	+++	+
3	Phenolic groups	+	+++	-
4	Tannins	-	-	-
5	Steroids	+	-	-
6	Saponins	++	+++	-
7	Glycosides	+	++	+
8	Terpenoids	-	+	-
9	Carbohydrates	+	++	+
10	Proteins	++	+++	++

Heavily present: +++; slightly present: ++; present: +; absent: -.

3.2. Antioxidant Analysis

3.2.1. DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of the methanolic extract of *P. tetrastromatica* was assessed to determine the antioxidant activity. Different concentrations ranging from 100 to 250 µg/L were taken for the study and BHT and Rutin were used as standards. In general, the effect of radical scavenging appears to increase as concentrations rise. As a result, the extract's ability to scavenge DPPH radicals increased dose-dependently.

3.2.2. Hydroxyl Radical Scavenging Activity

The efficacy of a methanolic extract of *P. tetrastromatica* to quench hydroxyl radicals seems to be appreciable compared to the standard

antioxidants BHT and Rutin. The ability of the extract to scavenge hydroxyl has increased as the concentration of the extract has increased. This suggested that the extract was significantly higher than the synthetic antioxidant BHT and the natural antioxidant Rutin.

Aruoma OI, 2003 reported that the DPPH radical scavenging activity of the seaweeds in our study is considerable; they could be used as substitutes to replace harmful synthetic antioxidants commonly used in processed food products, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which have been reported to be carcinogenic and tumorigenic at high doses. Halliwell B *et al*, 1987 reported that when extracts are added to the reaction mixture, they remove the hydroxyl radicals from deoxyribose, thus directing the damage towards them and preventing the reaction.

Table 2. Anti-oxidant activities of *Padina tetrastromatica* Hauck.

S. No.	Extracts	DPPH (IC ₅₀) (µg/mL)	Hydroxyl radical scavenging activity (IC ₅₀)
1	Methanolic	51.44	43.8
2	BHT (std)	45.63	37.06
3	Rutin (std)	32.32	30.18

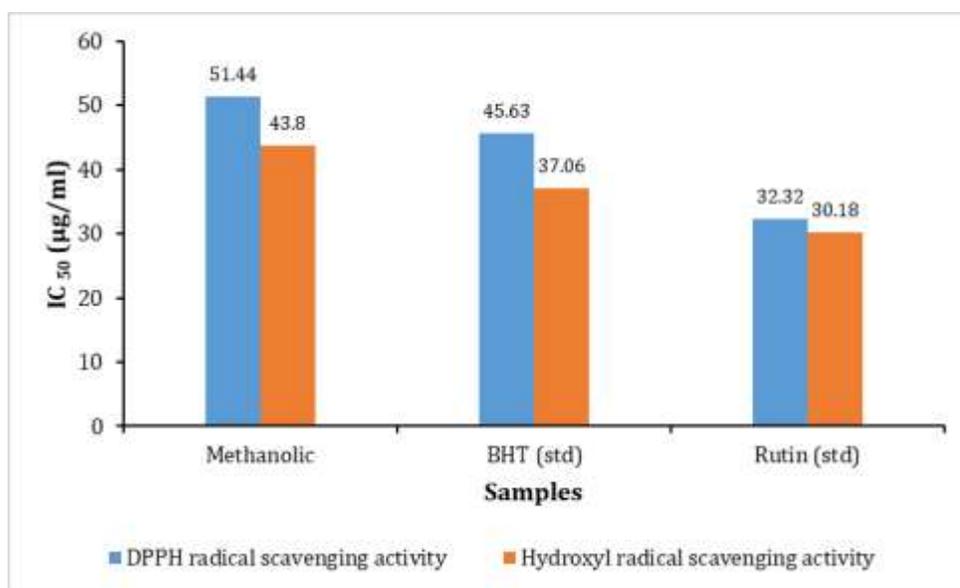


Figure 1. Anti-oxidant activities of *Padina tetrastromatica* Hauck.

4. CONCLUSION

Sea weeds are primitive, non-flowering plants with an immense source of secondary metabolites. The pharmacological activity of seaweeds is believed to be due to the presence of these compounds. There are several compounds in seaweeds that have the property to treat deadly diseases like cancer, arthritis and diabetics etc. Seaweeds are used in a wide variety of commercial applications and they are the source of agar-agar, carrageenan and algin, which are extensively used in various industries. From the present study, the identified phytochemical constituents are bioactive constituents that have great medicinal value, and are extensively used in the drug and pharmaceutical industries. Hence, these phytochemicals can be further screened for different kinds of biological activities depending on their therapeutic uses. This study concluded that methanolic extract of *P. tetrastromatica* exhibited the highest antioxidant potential and also good source of pharmaceutically important secondary metabolites which will be useful for pharmacological as well as functional food applications.

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