

Preliminary Phytochemical Analysis of Red Sea Weed *Ceramium diaphanum*

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ABSTRACT

In the present study, preliminary phytochemical analysis of Chloroform extract of red seaweed *Ceramium diaphanum* was carried out by using simple chemical tests. The chloroform extract of red seaweed revealed a rich diversity of bioactive compounds, including steroids, triterpenoids, glycosides, carbohydrates, alkaloids, flavonoids, phenols, and tannins. These results may be helpful for use of this seaweed in the health care.

Key words: Phytochemical, Antimicrobial activity, *Ceramium diaphanum*.

Introduction

Currently, researchers are fully aware that valuable natural compounds can be obtained from seaweeds, namely phycocolloids, peptides, polysaccharides, polyunsaturated fatty acids, fibers, calcium carbonate, iodine, pigments, diterpenes, phlorotannin's, vitamins and phenols (Hentati *et al.*, 2020; Kalasariya and Pereira, 2022; Lopez-Santamarina *et al.*, 2020).

The extraction and characterization of these bioactive compounds from red seaweeds have become a focal point of research due to their potential health benefits and industrial applications. Traditional and modern extraction techniques have been employed to isolate these valuable Phytochemicals, with factors such as type of solvent, extraction method, and algal species significantly influencing the yield and bioactivity of the extracts (Nurlindah Hamrun, 2022; Sobuj *et al.*, 2021).

In this study, we aim to investigate the phytochemical composition of extracts obtained from red seaweed. By employing various extraction techniques. We seek to contribute to the growing body

of knowledge on the potential applications of red seaweed-derived compounds in various industries.

Materials and Methods

Red Sea weed extract preparation

The specimen of *Ceramium diaphanum*, a type of red seaweed, was obtained from the Kunkeshwar coastline in Maharashtra, India. The crushed plant material (10 g) was placed into a thimble and subsequently positioned inside a Soxhlet extractor. The thimble was filled with 250 ml of ethanol/chloroform as the organic solvent for extraction. The side arm of the extractor was insulated with glass wool. The solvent was heated using a heating mantle, initiating evaporation and movement through the apparatus to the condenser. The condensate dripped into the reservoir containing the thimble. Once the solvent level reached the siphon, it returned to the flask, restarting the cycle. This extraction process was carried out for a total of 8 hours. After completing 7 extraction cycles, the extracted plant samples were air-dried and collected in the extraction collector for further use.

Phytochemical analysis for chloroform extract (A2)

Test for Glycosides

Keller killani test

To test for cardiac glycosides, a few drops of glacial acetic acid were added to the test solution, followed by the addition of 2 ml of ferric chloride solution. Concentrated sulfuric acid was then carefully added along the sides of the test tube. This procedure resulted in the formation of two distinct layers: a reddish-brown lower layer and a bluish-green upper layer.

Raymond's test

When treated with dinitrobenzene in hot methanolic alkali, the test solution produces a violet color.

Legal's test

When the test solution is treated with 1ml of pyridine and 1ml of sodium nitroprusside, a pink to red color becomes visible.

Test for Alkaloids

Mayer's test

When added to the test solution, the potassium mercuric iodide, Mayer's reagent, produces a cream-colored precipitate.

Wagner's test

A brown precipitate is produced when Wagner's reagent (iodine in potassium iodide) is added to the acidic test solution.

Hager's reagent

A yellow residue forms when Hager's reagent (Saturated picric acid solution) is added to the acetic test solution.

Test for Flavonoids

Ferric chloride test

A few drops of ferric chloride solution added to the test solution produce a vivid green color.

Shinoda test

The test solution, consisting of small pieces of magnesium ribbon and strong hydrochloric acid, shows a color ranging from pink to magenta red.

Zinc-Hydrochloric acid-reduction test

The test solution, when combined with zinc dust

and a small amount of hydrochloric acid, produces a vivid magenta red color.

Alkaline reagent test

When the test solution is treated with sodium hydroxide solution, the intensity of the yellow color increases and eventually turns colorless when a few drops of diluted acid is added.

Lead acetate solution test

A yellow precipitate is produced when a small amount of lead acetate solution (10% w/v) is added to the test solution.

Test for Steroids

Chloroform Test

The crude plant extracts (1 mg) were dissolved in 1 ml of chloroform in a test tube. An equal volume of concentrated sulfuric acid was then carefully added to the test tube along the sides. The upper layer in the test tube turned red, while the sulfuric acid layer exhibited a yellow color with green fluorescence, indicating the presence of steroids.

Salkowski's test

The second portion of the solution was carefully combined with concentrated sulfuric acid, ensuring that the acid formed a distinct lower layer. The interface between the two layers was then examined for the presence of a reddish-brown coloration, which is indicative of a steroid ring.

Test for Phenols

Ferric Chloride Test

A small amount of the ethanolic extract was taken with 1 ml of water in a test tube and 1 to 2 drops of Iron III chloride (FeCl_3) was added resulting in blue, green, red or purple color which is considered to be positive.

Test for Terpenoids

Salkowski test

When a few drops of concentrated sulphuric acid are added to the test solution, shaken, and allowed to stand, the lower layer turns red, indicating the presence of sterols.

Liebermann Burchard test

The test solution was mixed thoroughly with a few

drops of acetic anhydride. Upon the addition of concentrated sulfuric acid along the sides of the test tube, a brown ring forms at the interface of the two layers, and the upper layer turns green.

Test for Saponins

Foam test

When mixed with water and subjected to agitation, saponins produce stable foam that persists for a minimum of 15 minutes.

Raymond's test

When the test solution is treated with dinitrobenzene in hot methanolic alkali, it gives a violet color.

Test for Carbohydrates

Molisch's test

When a small amount of Molisch's reagent and two millilitres of concentrated sulphuric acid are added slowly to the test tube, a purple ring forms where the two liquids meet.

Benedict's test

When treated with Benedict's reagent and heated in a water bath, the test solution produces a reddish-brown precipitate.

Test for Proteins

Millon's test

The protein gets stained yellow when a test solution is heated in a water bath and treated with Millon's reagent.

Xanthoproteic test

When concentrated nitric acid is added to a test solution, it heats and produces a yellow precipitate.

Ninhydrin test

When the test solution is treated with ninhydrin reagent, it produces blue color.

Test for Starch

Starch Reagent Test

10 ml of NaCl solution was mixed with 1ml of extract. When the starch reagent is introduced after heating, a blue-purplish color indicates that starch is present.

Test for Tannins

Gelatin Test

Plant extract is dissolved in 10% NaCl, 1% gelatin solution, and 5 ml of distilled water. White precipitate results from the reaction.

NaOH Test

When 4 ml of 10% NaOH is mixed with 0.4 ml of extract and the mixture is shaken well, an emulsion is formed, indicating the presence of tannins.

Test for anthocyanin

HCl Test

When a small amount of ammonia is added to a mixture containing two millilitres of plant extract and two ml of 2N HCl, the pink-red solution turns blue-violet.

Results and Discussion

Phytochemicals analysis of red algae chloroform extract

The presence of various Phytochemicals in the chloroform extract of red seaweed suggests its potential for various biological and medicinal applications. Steroids and triterpenoids, both of which were found to be present, are known for their anti-inflammatory and anticancer properties (Kumar *et al.*, 2015). The detection of glycosides indicates potential cardiac benefits, as these compounds are often associated with heart health. The absence of saponins was confirmed, which means that the extract does not exhibit the typical foaming properties associated with these compounds. This can be advantageous or disadvantageous depending on the intended application of the extract.

Carbohydrates were present, which can serve as an energy source and may contribute to the overall nutritional value of the extract. Alkaloids, known for their wide range of pharmacological activities including antimicrobial, analgesic, and anticancer effects, were also present (El-Beltagi *et al.*, 2022). The significant presence of flavonoids highlights the potential antioxidant properties of the extract, as these compounds are known for their ability to scavenge free radicals (Mohy El-Din and El-Ahwany, 2016). Phenols, which also possess strong antioxidant properties, were detected, further supporting the potential health benefits of the extract (El-Beltagi *et*

Table 1. Different Phytochemicals analysis of red algae chloroforms extract.

Sr. No	Chemical Constituents	Tests	Result	Observation
1	Steroids	Chloroform test	+	Present
		Salkowski test		
2	Triterpenoids	Salkowski test	+	Present
		Liebermann Burchard test		
3	Glycosides	Raymond's test	-	Absent
		Keller killiani test	+	Present
		Legal's test		
4	Saponins	Foam test	-	Absent
		Raymond's test		
5	Carbohydrates	Molisch's test	+	Present
		Benedict's test	-	Absent
6	Alkaloids	Wagner's test	+	Present
		Hager's test	-	Absent
		Mayer's test	+	Present
7	Flavonoids	Lead acetate solution test	+	Present
		Shinoda test		
		Ferric chloride test		
		Zinc- Hydrochloric acid- reduction test		
		Alkaline reagent test		
8	Phenol	FeCl ₃ test	+	Present
9	Proteins	Xanthoproteic Test	-	Absent
		Miilion's Test		
		Ninhydrin Test		
10	Tannins	Gelatin test	+	Present
		NaOH Test		
11	Starch	Starch Reagent	-	Absent
13	Anthocyanin	HCl Test	-	Absent

al., 2022; Kumar *et al.*, 2015).

Proteins were notably absent, which suggests that the extract may not be suitable as a protein supplement but may still offer other health benefits due to the presence of other bioactive compounds. The presence of tannins indicates potential astringent properties, which can be beneficial for wound healing and other therapeutic applications (Das *et al.*, 2023). Starch and anthocyanins were absent, which limits certain uses of the extract but does not detract from its potential in other areas such as medicine and nutrition.

In summary, the chloroform extract of red seaweed was found to contain a variety of phytochemicals, including steroids, triterpenoids, glycosides, carbohydrates, alkaloids, flavonoids, phenols, and tannins. The absence of saponins, proteins, starch, and anthocyanins were confirmed. These findings suggest that the red seaweed extract possesses a range of bioactive compounds with potential health benefits, particularly in the areas of anti-inflammatory, anticancer, antioxidant, and as-

tringent activities (El-Beltagi *et al.*, 2022)

Conclusion

Based on the comprehensive analysis presented in this research paper, several key conclusions have been revealed regarding the phytochemical composition of red sea weed extracts. The chloroform extract of red sea weed revealed a rich diversity of bioactive compounds, including steroids, triterpenoids, glycosides, carbohydrates, alkaloids, flavonoids, phenols, and tannins. This diverse phytochemical profile suggested potential applications, particularly in medicine and nutrition.

Conflict of Interest - None

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