

Original Research Article

<https://doi.org/10.20546/ijcmas.2026.sp13.047>

Phytochemical Analysis and Identification of Bioactive Substances from Green and Brown Marine Algae

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ABSTRACT

Keywords

Marine algae, phytochemicals, bioactive, tannins and antimicrobial

Article Info

Received:

25 August 2026

Accepted:

30 September 2026

Available Online:

10 October 2026

Marine algae are a promising source of diverse bioactive compounds with potential pharmaceutical and therapeutic applications. The present study investigated the phytochemical composition of selected green and brown marine algae collected from the Kovalam coastal region. Algal samples were shade-dried, powdered, and extracted separately using aqueous and ethanol solvents through Soxhlet extraction. The extracts were qualitatively screened for major phytochemicals, including alkaloids, tannins, phenols, flavonoids, saponins, glycosides, sterols, carbohydrates, amino acids, coumarins, and quinones. The findings revealed considerable variation in phytochemical profiles between green and brown algae and between the two extraction solvents. Brown algae showed a greater diversity of bioactive compounds, particularly alkaloids, sterols, tannins, saponins, phenols, and terpenoids in ethanol extracts. The presence of tannins and phenolic compounds highlights the potential of these marine algae as sources of biologically active natural products. Overall, the study emphasizes the phytochemical richness and therapeutic potential of marine algae, supporting their further exploration for antimicrobial and pharmaceutical applications.

Introduction

The ocean is the mother of life and it is believed that the most primitive forms of life originated from this "primordial soup". It harbors a vast variety of marine organisms that are diverse in their physiology and adaptations. These marine inhabitants have served as a model for various studies ranging from the deep knowledge about nerve transmission (squid and its giant nerve axons) to the mesenteries of vision (eyes of horseshoe crabs, sharks and skates). The surf clam is

being used as an excellent model for the cell cycle and its regulation while the sea urchin is a model for understanding the molecular basis of cellular reproduction and development. As they thrive in a different kind of climate, these organisms develop certain adaptation mechanisms which may be useful for their defense and the result of these adaptations may be useful for human beings in many forms. Production of bioactive metabolites is one such adaptation mechanism, which helps in survival from predators. (Ira Bhatnaar *et al.*, 2010)

Algae consist of a large group of biologically important marine plants which have gained huge attraction for the commercial exploitation globally. The marine algae including micro- and macro-algae (seaweeds) have encountered new horizons in the field of marine pharmacology, bioremediation, and tissue culture technology. Algae as a photosynthetic plants form important marine living resources abundant in shallow, coastal and backwater substrates. In addition, algae have also been found to present on rocks, dead corals, stones, pebbles and solids substances as well as on other plants. Interestingly in intertidal and shallow water, algae provide a yield of 6000 tons of total agar yield. Brown algae are a large group of mostly marine multicellular algae possessing a wide range of bioactive secondary metabolites. They play an important role in marine environments, both as food and for the habitats they form. Various bioactivities of compounds from brown algae have been extensively reported (Fang *et al.*, 2015; Manilal *et al.*, 2016; Gupta *et al.*, 2011).

Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as “man- friendly medicines”.

Although a significant amount of research has been made on biosynthesis and desired chemical molecules which confer potential biological effects on therapeutic efficacy, there is still availability of the sources to be explored for more potent and biologically active compounds especially from marine resources. Marine organisms are a rich source of biological diversity with protection of structurally diverse, novel and biologically active marine metabolites. In the present study an attempt was made on phytochemical properties of some green and brown marine algae.

Materials and Methods

Collection of Marine Algae

Algae were collected from rocks in kovalam coastal area. The algal mats were shade dried until all the water molecules were evaporated and algae became well dried for grinding. After drying, the algal mats were ground well using mechanical blender into fine powder and transferred into airtight containers.

Preparation of algal extracts

Solvent extraction

Crude algal extract was prepared by Soxhlet extraction method. About 25g of powdered algal material was uniformly packed into a thimble and extracted with 150ml of different solvents separately. Solvents used were aqueous and ethanol. The process of extraction was continued for 24h till the solvent in siphon tube of an extractor become colourless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40°C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4°C for their future use in phytochemical analysis.

Phytochemical analysis of Crude extracts

Extracts were tested for the presence of active principles such as Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Amino acids, Carbohydrates, Sterols and phenols.

Test for Alkaloids (Mayer's test)

About 0.5 ml of extracts were separately treated with few drops of 1 ml 2N HCl and filtered. (Mayer's reagent: 1.36 g of mercuric chloride was dissolved in 60 ml distilled water and 5 g of potassium iodide was dissolved in 10 ml of distilled water. The two solutions were mixed and made up to 100 ml of distilled water). Few drops of reagent were added to 1.0 ml of acidic aqueous solution of samples. Formation of white or pale precipitate showed the presence of alkaloids.

Test for Glycosides: A small amount of alcoholic extract of sample was dissolved in 1.0 ml of water and then aqueous solution of sodium hydroxide was added. Formation of a yellow color indicated the presence of glycosides.

Test for Flavonoids: In a test tube containing 0.5 ml of extract of the samples, 5-10 drops of dilute hydrochloric acid and a small piece of Mg were added. The solution was boiled for few minutes. In the presence of flavonoids, the reddish pink or dirty brown colour was produced.

Test for Saponins: The test solution was vigorously shaken with distilled water. Appearance of stable foam indicated the presence of saponin.

Test for Tannins: Gelatin Test – Test solution when treated with gelatin solution would give white precipitate indicating the presence of tannins.

Test for Free Amino Acids: Ninhydrin Test – Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple colour suggesting the presence of free amino acids.

Test for phenols: DilHNO₃Test: Test solution when treated with dilute HNO₃ solution, reddish colour appeared suggesting the presence of phenols

Test for Carbohydrate: Benedict's test – Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

Test for sterols: Test solution was mixed with 2ml of chloroform and 1ml of sulphuric acid (H₂SO₄) Formation of reddish brown ring at interface indicates the presence of sterols.

Test for Coumarins: Test solution was mixed with 1ml of 10% NaOH. Formation of yellow colour indicates the presence of coumarins.

Test for Quinones: Test solution was mixed with 1ml of concentrated sulphuric acid (H₂SO₄). Formation of red colour indicates the presence of quinones.

Results and Discussion

Infectious diseases are the world's leading cause of premature deaths. In recent years, drug resistant to human pathogenic bacteria has been commonly reported from all over the world (Piddock and Wise 1989). Even though pharmaceutical companies produce a number of new antibacterial drugs, drug resistant bacteria increased and become global concern; therefore the importance of identifying new effective antimicrobial agents cannot be over emphasized, the use of algal extracts or phytochemicals with known antimicrobial properties can be great significance in therapeutic treatments. The marine algae selected for this study consist of various bioactive substances like tannin, phenols, alkaloids, carbohydrates and terpenoids and also this study suggested that green and brown marine algae are rich in tannin. (Table 1a and 1b) Tannic acid in the algae

represents the major constituents. Some studies reported that tannic acid is bacteriostatic or bactericidal to some Gram positive and Gram negative pathogens. Phenol compounds suggested to be the major chemical components of algal cells could have an activating or inhibiting effect on microbial growth depending on their constitution and concentration.

Marine algae are eukaryotic organism that live in salty water in the ocean and is recognized as a potential source of bioactive natural products (Michael TM *et al.*, 2005). 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 (Smit AJ., 2004). Tannins play a major role as antihemorrhagic agent and showed to have immense significance as antihypercholesterol, hypotensive, and cardiac depressant properties. (Cherian S *et al.*, 1995). Phenolic compounds may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration. (Shan B *et al.*, 2007).

The main phytochemical present in the aqueous extract of green algae are alkaloids, tannins. In ethanol extract of green algae consist of major bioactive compounds they are alkaloids, tannins, sterols and carbohydrates. Brown algae of aqueous extract contain alkaloids and phenols. In ethanol extract of brown algae consist of alkaloids, sterols, tannins, saponins, phenols and terpenoids. Brown algae exhibits more bioactive compound rather than green algae.

The result further revealed that antibacterial potency of the bioactive compounds was not affected when extracted in boiling water indicating that the algal material contains thermostable bioactive compounds. It is also important to note that susceptibility of the pathogen was varied to ethanol extract and aqueous extract.

In conclusion, the present study demonstrates that marine algae are promising natural sources of bioactive compounds with potential antibacterial properties. Phytochemical screening revealed the presence of several biologically active constituents, including alkaloids, tannins, phenolic compounds, sterols, saponins, carbohydrates, and terpenoids in the selected green and brown marine algae.



Green Algae – Embedded in rocks Brown algae – Embedded in rocks

Preparation of algal extracts - Soxhlet apparatus



Phytochemical analysis of the Green and Brown Marine algae using Aqueous and Ethanol extract



Alkaloid



Aminoacid



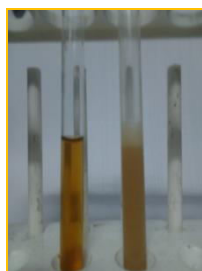
Carbohydrate



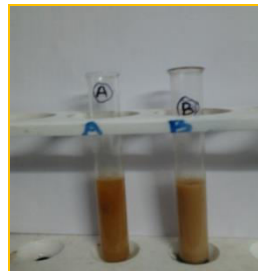
Phenols



Glycosides



Saponins



Tannins

Table.1a Phytochemical analysis of Marine Green algae

S. NO	Test	Aqueous Extract	Ethanol Extract
1	Alkaloids	+	+
2	Flavonoids	-	-
3	Saponins	-	-
4	Carbohydrates	-	+
5	Tannins	+	+
6	Sterols	-	+
7	Glycosides	-	-
8	Phenolic compound	-	-
9	Protein	-	-

Table.1b Phytochemical analysis of Marine Brown algae

S. NO	Test	Aqueous extract	Ethanol extract
1	Alkaloids	+	+
2	Flavonoids	-	-
3	Saponins	-	+
4	Tannins	-	+
5	Sterols	-	+
6	Phenol	+	+
7	Terpenoids	-	+
8	Coumarins	-	-
9	Quinones	-	-

(+) Positive (-) Negative

Among the investigated algae, brown algae exhibited a greater diversity of phytochemicals compared with green algae, particularly in the ethanol extracts.

Overall, the findings support the potential of marine algae, particularly brown algae, as valuable sources of naturally occurring antimicrobial compounds. Further studies involving isolation and characterization of the individual bioactive compounds, determination of minimum inhibitory and bactericidal concentrations, toxicity evaluation, and investigation of their mechanisms of antibacterial action are required. Such studies may contribute to the development of novel algal-derived antimicrobial agents to complement existing approaches for addressing the increasing problem of bacterial drug resistance.

Author Contributions

Dr. P. Sumithira: Conceived the original idea and designed the model and wrote the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Sumithira P. 2026. Phytochemical Analysis and Identification of Bioactive Substances from Green and Brown Marine Algae. *Int.J.Curr.Microbiol.App.Sci*. Special Issue-13: 461-466.

doi: <https://doi.org/10.20546/ijcmas.2026.sp13.047>