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Screening of Bioactive Phytochemicals in Marine Macrophytes

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ABSTRACT

Bioflx crowns represent an innovative advancement in pediatric dentistry, addressing the growing demand for aesthetic and functional solutions in the restoration of primary molars. Combining the desirable features of traditional stainless steel crowns and zirconia crowns, bioflx crowns are designed to be highly flexible, visually appealing, and durable. This paper reviews the characteristics, benefits, and clinical applications of bioflx crowns in pediatric dental practice.

Introduction

The marine environment is abundant in biodiversity and holds numerous potential benefits, containing bioactive compounds with unique structural and physical characteristics that cannot be replicated by natural molecules from land sources (Mehendran et al., 2022). Seaweeds, also referred to as marine macroalgae, are tiny, multicellular, photosynthetic eukaryotic organisms. They can be divided into three groups based on their colors and taxonomic classification: Rhodophyta (red), Phaeophyceae (brown), and Chlorophyta (green). Approximately 9800 of the estimated 164,000 species of algae (micro and macro) in the world are seaweeds, of which only 0.17% have been domesticated for commercial

use (El-Beltagi et al., 2022). The marine ecosystem's extraordinarily sustainable resources include sea weeds, which have been utilized for food, feed, and medicinal purposes. Annually, around 20 million tones of seaweeds are harvested, with half designated for human consumption (Buschmann et al., 2017; Martelli et al., 2020). Moreover, the bioactive compounds and secondary metabolites found in seaweeds are highly sought after for various applications in the cosmetics and pharmaceutical sectors (Choudhary et al., 2021). Seaweeds contain high-quality proteins, dietary fibers, polysaccharides, macro and micronutrients, vitamins, minerals, fatty acids, and phytochemicals/bioactive compounds that exhibit a wide range of biological activities (Collins et al., 2016/ Amoriello et al., 2021).

Pyropia perforata (J. Agardh) S.C. Lindstrom)

It is a species of red algae (previously known as *Porphyra perforata*) belonging to the family Bangiaceae and it thrives in temperate intertidal zones and shallow waters. It is characterized by its discoid holdfast and short stripe and reddish brown in appearance. It possesses folded, membranous, monochromatic blades that range in color from red and brown to dark green. When unfolded, these blades resemble delicate fronds. While some species can reach up to one meter in length, most blades are approximately 20 centimeters in diameter. Recent research has explored the potential use of *P. perforata* as a consolidant for deteriorated linen fibers, highlighting its biotechnological and industrial relevance (Plate 1).

Sargassum muticum (Yendo) Fensholt.

S. muticum (Family: Sargassaceae), a brown seaweed that can grow up to 10 meters long, displays shades ranging from brown to yellowish. It exhibits a wide range of biological activities (Yende et al., 2014; Liu et al. 2012) (Plate 1).

Halimeda tuna (J. Ellis & Solander) J.V. Lamouroux.

It belongs to the family Halimedaceae and it is calcareous green seaweed anchored to the seafloor by a holdfast. Each thallus, or frond, consists of a single, tubular cell containing multiple nuclei. Within the cell wall, the cytoplasm remains mobile, allowing the nuclei, chloroplasts, and other cellular components to move freely. The thallus is composed of flattened, disc-like segments connected by flexible joints. These segments have

swollen patches called utricles on their surfaces, which together create a tabular "cellular pavement." (Nemcova et al., 2023) (Plate 1).

Pyropia perforata

Sargasum muticum

Halimeda tuna

Plate 1: Marine macrophytes

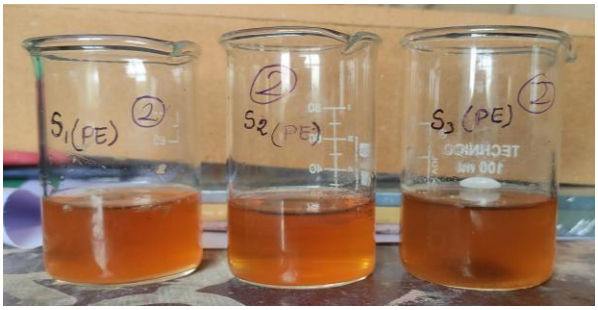
Materials and methods

Collection of seaweeds

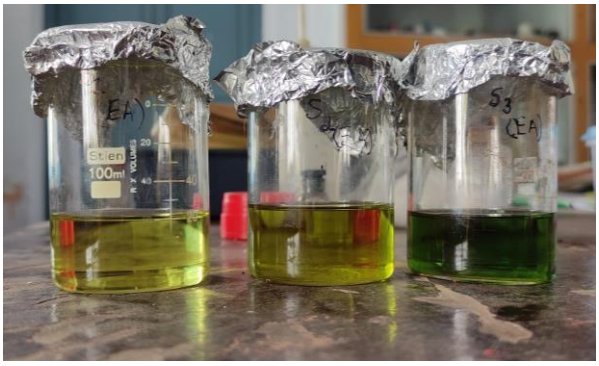
The seaweeds were collected from Manapadu, Thoothukudi District, Southern Coast of India. The specimens were authenticated by using standard floras. The collected seaweeds were thoroughly washed with seawater to remove all the extraneous impurities such as epithets, sand particles, pebbles, and shells and brought to the laboratory in plastic bags aseptically in ice boxes for further processing. The samples were again washed with tap water followed by distilled water to ensure removal of residual impurities. The washed seaweeds were blotted on the blotting paper, shade dried at ambient temperature and the samples were grounded into a fine powder using tissue blender. The powdered samples were then stored in the refrigerator until for further use.

Preparation of crude extracts

Ten grams of each powdered samples were extracted with 50 ml of different solvents, such as petroleum ether, ethylacetate and ethanol. The sample mixtures were kept in the orbital shaker for 48 hrs with intermittent shaking. After incubation, the mixtures were filtered through filter paper, and the filtrates were collected and stored in for subsequent analysis. The same procedure was used for the preparation of aqueous extracts (Plate 2).



Petroleum ether extracts



Ethyl acetate extracts

Ethanol extracts

Plate 2: Preparation of crude extracts

Screening of phytochemicals

Preliminary phytochemical screening of crude extracts of the selected seaweeds was carried out according to the methods described by Trease and Evans (1989 & 1983). Qualitative phytochemicals screening of the crude extracts (Petroleum ether, Ethyl acetate, Ethanol and Aqueous) are used for the detection of phytochemicals like as a saponins, tannin, phenols and terpenoid, flavonoid, coumarin, *etc.*

Test for alkaloids

One gram (1 g) of the each extract was dissolved in 5 ml of 10% ammonia solution and extracted with 15 ml of chloroform. The chloroform portion was evaporated to dryness and the resultant residue dissolved in 15 ml of dilute sulphuric acid. One quarter of the solution was used for the general alkaloid test while the remaining solution was used for specific tests

Test for Coumarins

0.5 g of the moistened crude plant extracts were taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

Test for Flavonoids (Shindo's test)

A few chop of 1% NH3 solution is added to the aqueous extract of each plant sample in a test tube. A yellow coloration is observed if flavonoids compound are present.

Test for Tannins

0.5g of powdered sample of each plant is boiled in 20ml of distilled water in a test tube and filtered 0.1% FeCl3 is added to the filtered samples and observed for brownish green or a blue black colouration which shows the presence of tannins.

Test for Steroids (Libermann-Burchard Test)

The amount of 0.5 g of the each extracts were dissolved in 10 ml anhydrous chloroform and filtered. The solution was divided into two equal portions for the following tests. The first portion of the solution above was mixed with one ml of acetic anhydride followed by the addition of 1 ml of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green colouration as indicative of steroids.

Test for terpenoids

5ml of aqueous extract of each plant sample is mixed with 2ml of CHCl3 in a test tube 3ml of concentrated H2SO4 is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

Results and Discussion

Phytochemical profiling of seaweeds serves as a fundamental need in drug design and development against various clinical complications. In modern era, the phytochemistry of aquatic resources especially in seaweeds has gained significant global attention due to their reservoir of secondary metabolites such as alkaloids, glycosides, flavonoids, saponins, tannins, steroids, and other related active metabolites with potential pharmaceutical applications. In the present study, the crude extracts of *P. perforata, S. muticum* and *H. tuna* were screened their bioactive compounds, revealing the different secondary metabolites including alkaloids, flavonoids, steroids, terpenoids and tannins (Tables 1 – 3).

The petroleum ether extract of P. perforata showed the presence of three bioactive compounds, namely alkaloids, coumarins and tannins. However, steroids, terpenoids and flavonoids were not detected in this extract. In contrast, the ethyl acetate extract of P. perforata revealed the presence of two bioactive compounds – tannins and terpenoids while steroids, coumarins, flavonoids and alkaloids were absent. The ethanol and aqueous extracts of P. perforata exhibited comparatively fewer bioactive constituents. The ethanol extract contained steroids and terpenoids, while the aqueous extract revealed the presence of only tannins (Table 1; Plate 3).

Table 1. Qualitative phytochemical screening of *Pyropia perforata*

Bioactive compounds	Crude extracts			
	Petroleum ether	Ethyl acetate	Ethanol	Aqueous
Alkaloids	+	-	-	-
Coumarins	+	-	-	-
Flavonoids	-	-	-	-
Tannins	+	+	-	+
Steroids	-	-	+	-
Terpenoids	-	+	+	-

'+' Present **'-' Absent**

The petroleum ether extract of *S. muticum* showed the presence of four bioactive compounds, namely alkaloids, coumarins, flavonoids and terpenoids. However, steroids and tannins were not detected in this extract. In contrast, the ethyl acetate extract of *S. muticum* revealed the presence of two bioactive compounds – tannins and steroids while terpenoids, coumarins, flavonoids and alkaloids were absent. The ethanol extract demonstrated four bioactive compounds namely, coumarins, tannins, steroids and terpenoids and aqueous extract of *S. muticum* exhibited comparatively fewer bioactive constituents. The aqueous extract revealed the presence of only tannins (Table 2; Plate 3).

Table 2. Qualitative phytochemical screening of *Sargassum muticum*

Bioactive compounds	Crude extracts			
	Petroleum ether	Ethyl acetate	Ethanol	Aqueous
Alkaloids	+	-	-	-
Coumarins	+	-	+	-
Flavonoids	+	-	-	-
Tannins	-	+	+	+
Steroids	-	+	+	-
Terpenoids	+	-	+	-

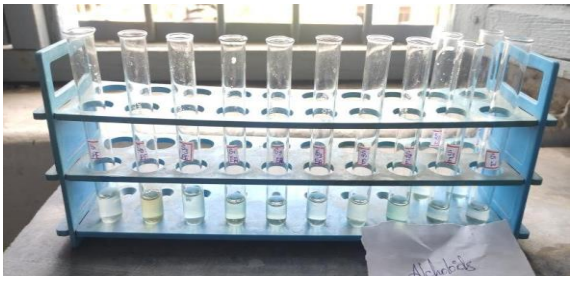
'+' Present **'-' Absent**

The petroleum ether extract of *H. tuna* showed the presence of only one bioactive compound, namely terpenoids. However, alkaloids, coumarins, flavonoids, tannins and steroids were not detected in this extract. In contrast, the ethanol extract of *H. tuna* revealed the presence of two bioactive compounds – alkaloids and steroids while terpenoids, coumarins, flavonoids and tannins were absent. The aqueous extract demonstrated two bioactive compounds namely, tannins and steroids (Table 3; Plate 3). These findings suggest that the solvent polarity plays a critical role in the extraction efficiency of different phytochemical constituents from the selected seaweeds.

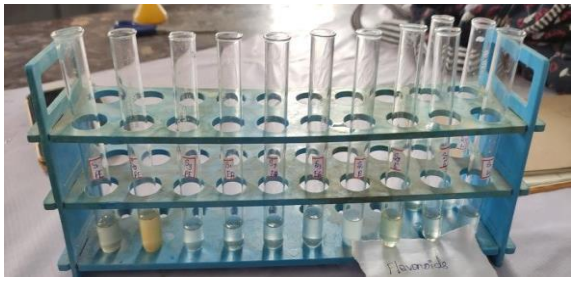
Table 3. Qualitative phytochemical screening of *Halimeda tuna*

Bioactive compounds	Crude extracts			
	Petroleum ether	Ethyl acetate	Ethanol	Aqueous
Alkaloids	-	-	+	-
Coumarins	-	-	-	-
Flavonoids	-	-	-	-
Tannins	-	+	-	+
Steroids	-	+	+	+
Terpenoids	+	-	-	-

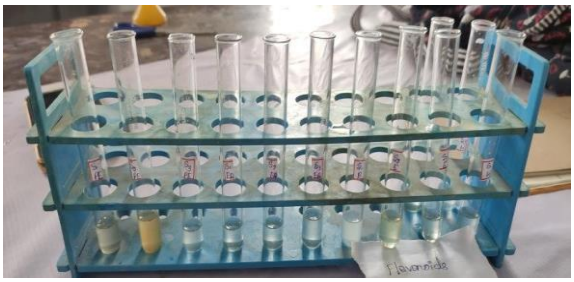
'+' Present **'-' Absent**



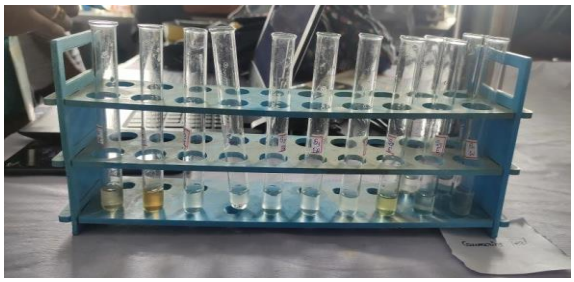
Alkaloids



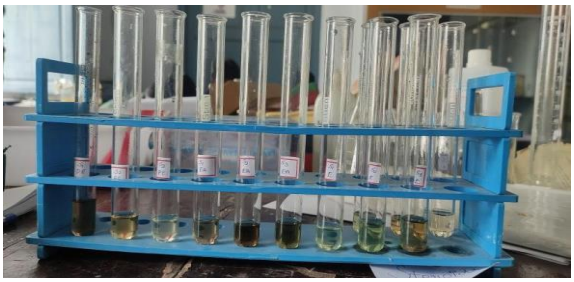
Flavonoids



Terpenoids



Coumarins



Steroids

Tannins

Plate 3. Phytochemical screening of crude extracts

Alkaloids have been extensively investigated for numerous pharmacological properties such as antiprotozoal, cytotoxic, antidiabetic and anti-inflammatory activities but until recently their antimicrobial properties were relatively under-reported. Contemporary reviews now

indicate that natural alkaloids exhibit significant antibacterial and antifungal activities, including efficacy against drug-resistant pathogens. For example, alkaloids have been shown to disrupt bacterial membranes, inhibit DNA topoisomerases, suppress functional proteases, and interfere with bacterial respiration and efflux pump mechanisms (Yan *et al*., 2021).

Phenolic compounds particularly flavonoids and tannins function as primary antioxidants or free-radical scavengers. Flavonoids are often described as "nature's biological response modifiers" because of their inherent ability to modulate the body's response to allergies, inflammation, microbial infection and cancer. In animal and in-vitro studies, flavonoids have demonstrated potent antihyperglycemic activity, improved blood circulation, reduced blood pressure and inhibition of enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase, ATPase, lipoxygenase and cyclooxygenase. (Shamsudin *et al*., 2022). They also influence the regulation of hormone systems (estrogens, androgens, thyroid) and modulate intracellular signalling pathways that underlie inflammation, oxidative stress and cellular proliferation.

Tannins are high-molecular-weight polyphenols that can chelate metal ions, bind to proteins/enzymes and thereby reduce oxidative stress, inhibit carbohydrate-digesting enzymes (e.g., amylase, lipase) and block microbial adhesion or enzyme activity. Tannins have been reported to exhibit antidiabetic, anti-inflammatory, antibacterial and antitumor activities. They also have shown specific antiviral potential, for example inhibiting HIV replication and other viral targets (Cosme *et al*., 2025). Plant tannins have traditionally been recognized for how they make foliage less palatable to herbivorous insects, reflecting their defensive role in nature. The present study showed that the selected macroalgae (*Pyropia perforata*, *Sargassum muticum* and *Halimeda tuna*) contains no of bioactive compounds indicating their potential utilization in various pharmacological and functional food applications.

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