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Phytochemical characterization of brown seaweed *Sargassum wightii*

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ABSTRACT

Objective: To explore the phytochemical properties of *Sargassum wightii*. **Methods:** Phytochemical screening of the extracts was carried out according to the standard methods. To identify the functional constituents present in the crude extracts, the spectroscopic and chromatographic analysis were performed. **Results:** The different extracts of *S. wightii* showed the presence of steroids, alkaloids, phenolic compounds, saponins and flavonoids with varied degree. TLC profile of *S. wightii* demonstrated three distinct phenolic spots in the methanolic extract of *S. wightii* with different Rf values 0.172, 0.534 and 0.810. Steroids profile displayed only one distinct spot with the Rf value 0.068. HPLC fingerprint profile of chloroform extracts of *S. wightii* displayed one prominent peak at a retention time of 3.060 min out of nine compounds separated. Benzene extract of *S. wightii* displayed one prominent peak at a retention time of 2.637 min. The crude powder of *S. wightii* was passed into the FTIR and it confirmed the presence of functional groups such as amides, phosphorus compound, alcohols, phenols and halogen compounds. **Conclusions:** The results of the present study confirmed that *Sargassum wightii* may be rich sources of phytoconstituents which can be isolated and further screened for various biological activities.

1. Introduction

The marine environment representing approximately half of the global biodiversity, is an enormous resource for new compounds. Sea weeds or marine algae are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents [1]. Seaweeds are marine macro algae and primitive type of plants, growing abundantly in the shallow waters of sea, estuaries and backwaters. They flourish wherever rocky, coral or suitable substrata are available for their attachment. Seaweeds have been used since ancient times as food, fodder, fertilizer and as source of medicinal drugs. Today seaweeds are the raw material for industrial production of agar, algin and carrageenan but they continue to be widely consumed as food in Asian countries [2].

Recently, chemists worldwide have paid attention to the potential of marine organisms as alternative sources

for the isolation of novel metabolites with interesting biological and pharmaceutical properties [3]. Biological compounds extracted from some seaweed species namely Phaeophyceae, Rhodophyceae and Chlorophyceae were proven to have potential medicinal activities such as antibacterial, antiviral, antitumour, antifungal, antiprotozoal, antioxidant, and mosquito and larva control [4–7]. The environment in which seaweeds grow is harsh as they are exposed to a combination of light and high oxygen concentrations. These factors can lead to the formation of free radicals and other strong oxidizing agents but seaweeds seldom suffer any serious photodynamic damage during metabolism. This fact implies that seaweed cells have some protective mechanisms and compounds. Although thousands of bioactive compounds have been discovered, the need for novel therapeutic compounds is still urgent in view of the emergence of a number of new diseases and the resistant strains of microorganisms [7].

Sargassum, one of the marine macroalgal genera belonging to the class Phaeophyceae, is widely distributed in tropical and temperate oceans. It belongs to the family Sargassaceae and order Fucales. It is a large, economically important and ecologically dominant brown algae present in much of the tropics. It is found to be the most diverse

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genus among Phaeophyta in India and is represented by 38 species. *Sargassum wightii* is one of the important species belonging to the genus *Sargassum* and a wide range of bioactive properties have been reported from this species [8]. It is widely distributed on the southern coasts of Tamilnadu, India and many parts of Asia and it is reported to be used as animal feed, food ingredients and fertilizer. *S. wightii* shows a good amount of flavonoids in support of its antioxidant activity [9]; indicate that this genus is an ideal target for investigating presence of bio-molecules for various medical and industrial applications. Thus, the present study was aimed to explore the phytochemical constituents of different extracts of *S. wightii* using TLC, HPLC and FTIR.

2. Materials and Methods

Sargassum wightii was collected by handpicking from the coast of Rasthacaud, Kanyakumari District, Tamil Nadu, India (Lat N 08008'308'' E77032'80''). The collected Seaweeds were cleaned well with sea water to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The collected seaweeds were then thoroughly washed with tap water followed by distilled water. For drying, washed seaweeds were blotted on the blotting paper and spread out at room temperature in shade. The shade dried seaweeds were grounded to fine powder using tissue blender. The powdered samples were then stored in refrigerator for further use.

2.1 Preparation of extract

10 g of air dried powder of *S. wightii* was extracted with 60 mL of solvents viz., petroleum ether, benzene, chloroform, acetone, methanol and aqueous. The sample was kept in dark for 72 h with intermittent shaking. After incubation, the solution was filtered through filter paper and the filtrate was collected (crude extracts).

2.2 Fluorescence analysis

The powdered materials were treated with various reagents such as 50% nitric acid, acetone, ethanol, 50% sulphuric acid, 1N HCl, 1N NaOH. The crude extracts (solvents extracted and acids/ base treated extracts) were examined under visible and UV light and changes in colour were recorded [10].

2.3 Phytochemical screening

The different extracts were tested for steroids, alkaloids, phenolic compounds, saponins, flavonoids and anthroquinones. Phytochemical screening of the extracts was carried out according to the standard method described by Harborne [11].

2.4 TLC analysis

TLC was carried out on 10 × 20 cm silica gel plates (Merck, Germany). The phenolic and steroids compounds present in *S. wightii* were qualitatively detected by TLC. The chloroform and methanol (9:1 ratio) was served as mobile phase for phenolic compound. Folin ciocalteau reagent was served as spraying agents to detect the phenolic compound present in the *S. wightii*. The occurrence of blue colour spot in the TLC chromatogram indicated the presence of phenolic compounds in the *S. wightii* extracts. For steroids, benzene and methanol (9:1 ratio) was used as mobile phase. 5% alcoholic sulphuric acid was exercised as the spraying agent to detect steroid presence in the *S. wightii*. The appearance of bluish green colour spot in the TLC chromatogram indicated the presence of steroid compounds in the *S. wightii*.

2.5 HPLC analysis

The different extracts of *S. wightii* were centrifuged at 3000 rpm for 10 min and then filtered through Whatmann No.1 filter paper using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvents. HPLC method was performed on a Shimadzu LC-10AT VP HPLC system, equipped with a model LC-10AT pump, UV-Vis detector SPD-10AT, Rheodyne injector fitted with a 20 µL loop and auto injector SIL-10AT. A Hypersil BDS C-18 column (4.6 × 250 mm, 5 µm size) with a C-18 guard column was used. The elution was carried out with gradient solvent systems with a flow rate of 1 mL min⁻¹ at ambient temperature (25–28°C). The mobile phase was consisted of 0.1% v/v methanol (solvent A) and water (solvent B). The mobile phase was prepared daily, filtered through a 0.45 µm and sonicated before use. Total running time was 15 min. The sample injection volume was 20 µL while the wavelength of the UV-Vis detector was set at 254 nm [12, 13].

2.6 FTIR analysis

FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum [14].

3. Results

Preliminary phytochemical screening of six different chemical compounds (steroids, alkaloids, phenolic groups, saponins, flavonoids and anthroquinones) were tested in six different extracts of *S. wightii*. Thus out of (1 × 6 × 6 = 36) tests for the presence or absence of the above compounds,

12 tests gave positive results and the remaining 24 gave negative results (Table 1). The 12 positive results show the presence of alkaloids, steroids, phenolic groups, saponins and flavonoids with varied degree. Anthroquinone did not show any positive result for their presence in any of the tested six extracts of *S. wightii*. Among the six different extracts tested, aqueous extract failed to show the presence of any of the compounds.

S. wightii showed the maximum presence of steroids in four different extracts except petroleum ether and aqueous, followed by flavonoids in three extracts, saponins and phenolic compounds in two extracts. Alkaloids are present only in benzene extract of *S. wightii*. Among the six different extracts tested, benzene extracts showed the presence of maximum number (5/6) of compounds. Methanolic and chloroform extracts showed the presence of 3 compounds each. Next to that acetone extracts showed the presence of only one compound and petroleum ether extracts failed to show the presence of any of the secondary metabolites.

Table 1

Preliminary phytochemical analysis of crude extracts of *S. wightii*

Name of the extract	Alkaloids	Phenolics	Steroids	Saponins	Flavonoids
Aqueous	–	–	–	–	–
Methanol	–	+	+	–	+
Acetone	–	–	+	–	–
Benzene	+	+	+	+	+
Chloroform	–	–	+	+	+
Pet. Ether	–	–	–	–	–

The characteristic fluorescent properties or colours emitted by the powdered thallus of *S. wightii* before and after treating with various reagents were recorded. The powdered thallus as such appeared very pale brown under daylight and ultraviolet radiation. After treating with various reagents, under daylight, it showed different shades of brown. However, under ultraviolet radiation, alkaline solutions like 1N aqueous sodium hydroxide and acids like 50% sulphuric acid, 1 N HCl and 50% Nitric acid, with green, blackish brown and yellowish brown colours respectively. The characteristic fluorescent properties or colours recorded through this study could be used as a standard in the identification and authentication of the thallus of *S. wightii* in its crude form.

Phenolics and steroids present in methanolic extracts of *S. wightii* were separated using TLC. Three distinct phenolic spots were detected in the methanolic extract of *S. wightii* with different Rf values 0.172, 0.534 and 0.810. Steroids profile displayed only one distinct spot with the Rf value 0.068.

The qualitative HPLC fingerprint profile of chloroform extracts of *S. wightii* were selected at a wavelength of 254 nm due to sharpness of the peaks and proper baseline. Chloroform extract prepared by cold extraction was subjected to HPLC for the isolation and identification of constituents present in the *S. wightii*. Nine compounds were

separated at different retention time viz., 1.830, 2.152, 2.293, 2.437, 2.590, 3.060, 3.543, 11.383 and 13.473 respectively. The profile displayed one prominent peak at a retention time of 3.060 min and some moderate peaks were also observed at a retention time 2.590 min, 1.830 min, 2.293 min, 2.437 min and 3.543 min respectively (Fig. 1). Benzene extracts of *S. wightii* illustrated with four compounds with the retention time 1.950, 2.157, 2.303 and 2.637 min respectively. The profile displayed one prominent peak at a retention time of 2.637 min and some moderate peaks were also observed at a retention time of 1.950, 2.157 and 2.303 min respectively (Fig. 2)

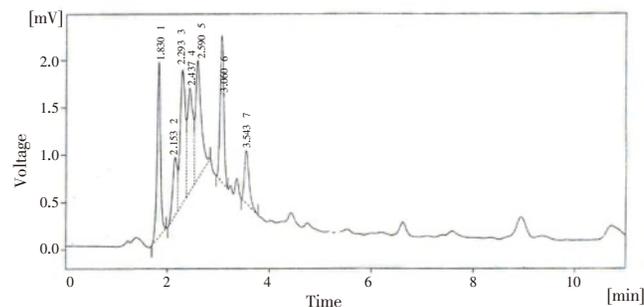


Fig. 1: HPLC chromatogram of the chloroform extract of *S. wightii*

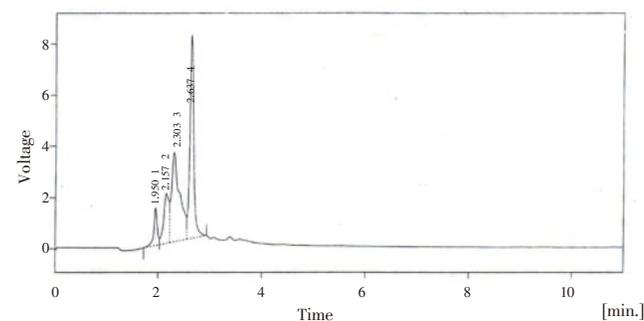


Fig. 2: HPLC chromatogram of the benzene extract of *S. wightii*

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The crude powder of *S. wightii* was passed into the FTIR and the functional groups of the components were separated based on its peak ratio. The results of FTIR analysis showed different peaks at 3315.41, 2362.64, 1668.31, 1400.22, 1110.92, 752.19, 653.82 and 603.68 respectively. It confirmed the presence of functional groups such as amides, phosphorus compound, alcohols, phenols and halogen compounds etc (Fig. 3).

4. Discussion

Plant substances continue to serve as the viable source of drugs for the world population and several plant-based drugs are in extensive clinical use. For the past few decades, several plants have been widely used for the treatment of various diseases due to their antioxidant properties. It is

a real fact that the importance of marine organisms as a source of new substances is growing. The metabolic and physiological capabilities of marine organisms that allow them to survive in complex habitat types provide a great potential for production of secondary metabolites which are not found in terrestrial environments. Thus, marine algae are among the richest sources of known and novel bioactive compounds [15].

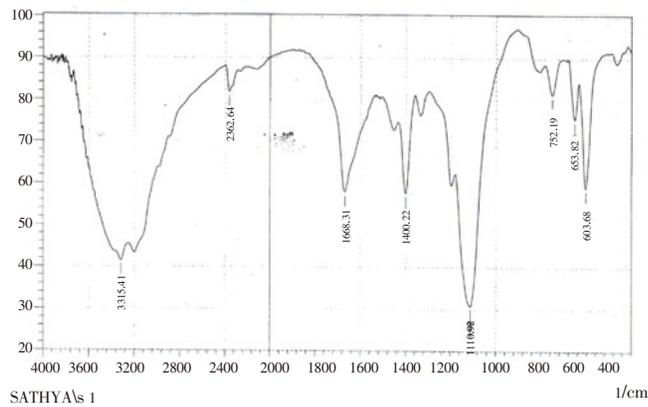


Fig. 3: FT-IR spectrum for crude powder of *S. wightii*

The results of the phytochemical analysis of various solvent extracts revealed the presence of various secondary metabolites with varied degree. Phenolic compounds are widely distributed in the plant kingdom and have been reported to have several biological activities including antioxidant properties. Earlier reports revealed that marine seaweed extracts, especially polyphenols have antioxidant activity [16, 17]. Alkaloids are commonly found to have antimicrobial properties against both Gram-positive and Gram-negative bacteria [18]. Flavonoids are known as nature's tender drug which possesses numerous biological and pharmacological activities. Recent reports of antiviral, anti-fungal, antioxidant, anti-inflammatory, antiallergenic, antithrombic, anticarcinogenic, hepatoprotective and cytotoxic activities of flavonoids have generated interest in studies of flavonoid containing plants [19, 20]. Steroids may serve as an intermediate for the biosynthesis of downstream secondary natural products and it is believed to be a biosynthetic precursor for cardenolides in plants. Marine algae have shown to be good source of unsaponifiable, non toxic sterols that have medicinal value [21, 22]. Saponins possess numerous biological properties which include antimicrobial, anti-inflammatory, anti-feedent and hemolytic effects [23]. The presence of phenolics, alkaloids, flavonoids, steroids and saponins, in the crude extracts of *S. wightii* suggest that seaweeds can be used as antimicrobial (anti-viral, anti-fungal and anti-bacterial), anti-parasitic, anti-inflammatory, anti-feedent, antioxidant, antiallergenic, anti-thrombic, anti-carcinogenic and anti-ulcer agents in the near future.

The fluorescence analysis is adequately sensitive and enables the precise and accurate determination over a satisfactory concentration range without several time-consuming dilution steps prior to analysis of pharmaceutical samples [24]. A number of studies were carried out for the separation of phenolics and steroids using TLC [25, 26]. They employed the Rf values to distinguish the seaweeds from other species and adulterant. In the present study also we developed the TLC profile for *S. wightii* which can be applied to distinguish between each other. HPLC identification test are required to confirm the presence of the active constituents and potential adulterant in ayurvedic drugs [27]. In the present study, the HPLC profile for *S. wightii* exhibited novel markers in standardization as useful analytical tools to check not only the quality of the powder but also the presence of adulterants in ayurvedic drugs. The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation [14]. The crude powder of *S. wightii* showed different peaks which confirmed the presence of functional groups such as amides, phosphorus compound, alcohols, phenols and halogen compounds.

Seaweeds known as medicinal are rich in secondary metabolites which include alkaloids, phenols, flavonoids, saponins, steroids and related active metabolites are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. Recently, a number of studies have been reported on the phytochemistry of seaweeds across the world [28–31]. Thus the present studies on phytochemical analysis of *S. wightii* can help the manufacturers for identification and selection of raw materials for drug production.

Conflict of interest statement

We declare that we have no conflict of interest.

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