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Pharmacognostical study and phytochemical evaluation of brown seaweed *Sargassum wightii*

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PEER REVIEW

Peer reviewer

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Comments

This is an interesting study in which the pharmacognostical and phytochemical evaluation of *S. wightii* can provide useful information for the identification and authentification of the plant.
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ABSTRACT

Objective: To explore the pharmacognostical and phytochemical properties of *Sargassum wightii*.
Methods: The qualitative microscopy, phytochemical screening, physicochemical evaluation and fluorescence analysis of the plant were carried out according to the standard procedure recommended in the WHO guidelines.
Results: Macroscopic study showed that plants were dark brown, 20−30 cm in height, leaves were 5−8 cm length, shape: linear to ovate, apex: midrib in conspicuous and having the entire, serrate margin. Microscopic evaluation of the transverse section of the leaf, stem, air bladder, receptacles showed the presence of epidermis layer followed by thick cuticle, conducting strand, mesophyll and possessed antheridia or oogonia at the swollen terminal portions. The different extracts of *Sargassum wightii* showed the presence of steroids, alkaloids, phenolic compounds, saponins and flavonoids with varied degree.
Conclusions: Various pharmacognostical parameters evaluated in this study help in the identification and standardization of the of the seaweed *Sargassum wightii*.

KEYWORDS
Sargassum wightii, Microscopy, Brown weed, Phytochemical screening

1. Introduction

Sargassum, one of the marine macro algae belonging to the class Phaeophyceae, is widely distributed in tropical and temperate oceans. It belongs to the marine family Sargassaceae and order Fucales. It is a large, cost−effectively important and ecologically dominant brown algae present in much of the tropics. It is found to be the most diverse genus among Phaeophyta in India and is represented by 38 species. *Sargassum wightii* (*S. wightii*) is one of the important species belonging to the genus *Sargassum* and wide range of bioactive properties has been reported from this species[1]. 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 and its antioxidant activity[2]; indicateing 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

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pharmacognostical study and phytochemical constituents of *S. wightii* using macroscopy, microscopy, fluorescence analysis and phytochemical screening method.

2. Materials and methods

2.1. Chemicals

All the chemicals used were of analytical grade and purchased from the Himedia Lab Pvt. Ltd. Mumbai, India and SD Fine Chem. Limited Mumbai, India.

2.2. Plant collection and authentication

The fresh seaweed of *S. wightii* was collected from Intertidal area of Gulf of Mannar, Mandapam coastal regions, Tamilnadu, south east coast of India. (Lattitude 9°15′ N, Longitude 78°58′ E), authenticated by the Dr. M. Ganesan, Scientist of Marine Algae Research Station, CSMCRI (Central Salt & Marine Chemicals Research Institute) Mandapam Camp, Tamilnadu, India and Lr. Dr. D. Stephan, Department of Botany, American College, Madurai, Tamil Nadu, India.

2.3. Processing of collected plant sample

The collected seaweeds were cleaned well with sea water to remove all the extraneous matter such as epiphytes, foreign particles, sand particles, pebbles and shells. The collected seaweeds were then thoroughly washed with tap water followed by distilled water. The whole plant was shade dried at room temperature. It was powdered to get No. 40 mesh size particle. The powder was stored in refrigerator for further study.

2.4. Macroscopic analysis

The macroscopical analysis included the evaluation of organoleptic characters and external features of the various parts of selected plant materials. The following macroscopic characters for the fresh leaves were noted for size, shape, colour, surfaces, venation, margin, base, lamina, texture, odour and taste[3,4].

2.5. Microscopic analysis

Microscopic evaluation was conducted in both qualitative and quantitative studies of whole plant of *S. wightii*[5,6].

In this study transverse section and powder microscopy of leaf was carried out. Staining procedure was used as per standard procedures. The staining reagents used for staining procedure were phloroglucinol and concentrated hydrochloric acid (1:1). Various characters were identified and studied.

The collected plant material was air-dried for two weeks and then powdered using mortar and pestle. The powder obtained was stored in air tight for use in phytochemical analysis and determination of pharmacopoeial standards.

2.6. Physicochemical analysis

Physicochemical analysis of the whole plant material was determined according to the WHO guidelines and the official methods[7]. In the physicochemical analysis various parameters such as ash values, extractive values, loss on drying were calculated.

2.7. Preliminary phytochemical screening

The preliminary phytochemical screening of the whole plant extract was mainly done for the evaluation of various phytoconstituents such as steroids, tannin and glycosides *etc*. present in the plant[5-7].

2.8. Fluorescence analysis

Fluorescence analysis is one of the most important parameter for the evaluation of the quality, strength and purity of the selected plant material. The powdered whole plant material was analysed under the three regions of light like daylight and UV region after treatment with various organic/inorganic reagents[8].

3. Results

3.1. Macroscopic characteristics (Morphology)

The macroscopical characters such as colour, odour, taste, shape, margin, apex, base and surface of *S. wightii* plant were observed and shown in Figure 1 and Table 1.

Figure 1. *S. wightii* plant.

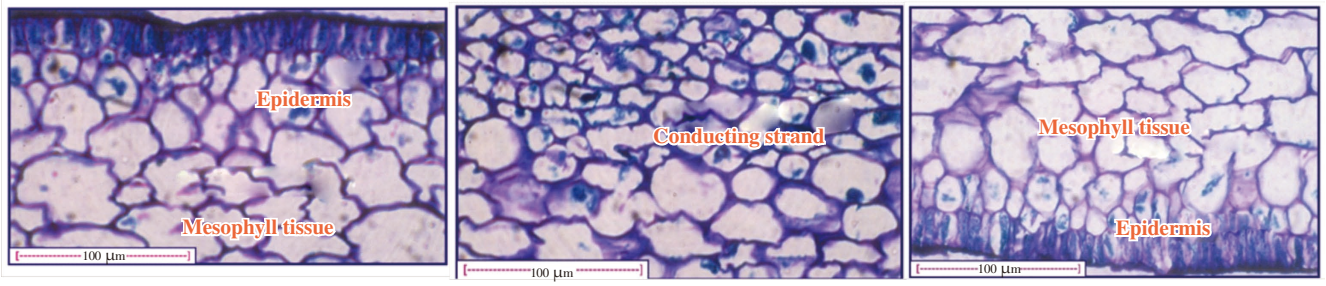

Figure 2. The structure of mesophyll tissue (40×).
A: TS of leaf showing upper epidermis with mesophyll tissue, B: Conducting strand, C: TS of Leaf showing lower epidermis with mesophyll tissue.

Table 1
Macroscopical characters of *S. wightii* leaf.

Macroscopical characters	Observation
Plant colour	Dark brown
Plant height	20–30 cm
Condition	Fresh
Size	Length 5–8 cm, width 2.9 mm
Shape	Linear to ovate
Apex	Midrib in conspicuous
Margin	Entire, serrate margins flat sometimes recurved or inflated
Base	Tapering
Colour	Dark brown
Odour	No characteristic odour
Taste	No taste
Holdfast	Well marked, discoid, conical, terete or flattened contains
Main axis	Shape–spherical
Vesicles	Stalk–small, length 5–8 mm

3.2. Microscopic characteristics

3.2.1. Leaf microscopy

The leaf like appendages were flat, wide and thick. No differentiation in to adaxial and abaxial sides. It had wide epidermis with thick cuticle. The cells were vertically oblong rectangular (20 µm thick) and uniform all along the surface. Median part was wide, smaller, angular and slightly thick walled cell mass called conducting strand. The mesophyll tissue around the conducting strand were longer, thin walled and polyhedral in outline. Marginal part was semi circular in shape containing parallel files of horizontally oblong, rectangular mesophyll tissue which is shown in Figure 2.

3.2.2. Axis (stem) microscopy

Flat, spindle shaped with wider 1–2 mm thick central part having thin cuticle and less distinct epidermis, wide mesophyll. The end part was 400 µm thick, tapering and blunt. Epidermis of the stem was 20 µm thick and cuticle was dark. The inner cells were polyhedral, small, and compact. Central core cells were smaller, slightly thick walled and rhomboidal in outline and were called as conducting strand extended up to the marginal portion which is shown in Figure 3.

3.2.3. Air bladder

The air bladder had wide central chamber enclosed by a thin cylinder of cells. The unsheathing cylinder had shallow ridges and furrow at certain places. The ridged portion was 250 µm thick and narrow part was 750 µm wide. The bladder has thick cuticle and district, darkly stained epidermal layer of 15–20 µm thick. The mesophyll tissue consists of 5–7 layers of tabular, compact thin walled cells. The cells were sheathed parallel to the surface of the bladder which is shown in Figure 4.

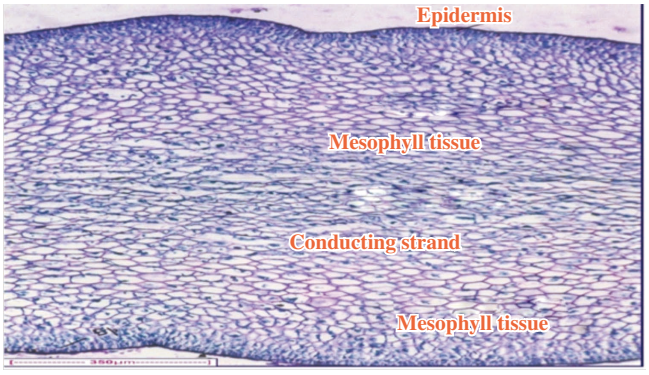

Figure 3. TS of axis–central portion enlarged (10×).

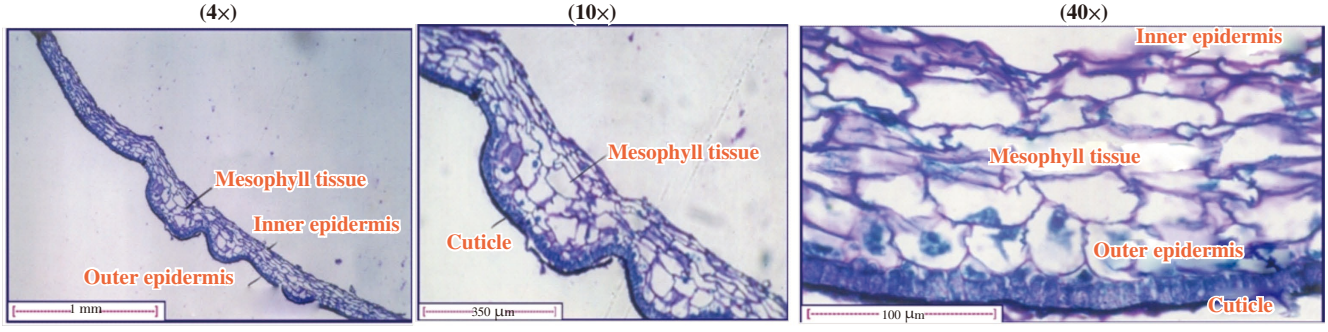

Figure 4. TS of air bladder showing central wider part with two lateral narrow portions.

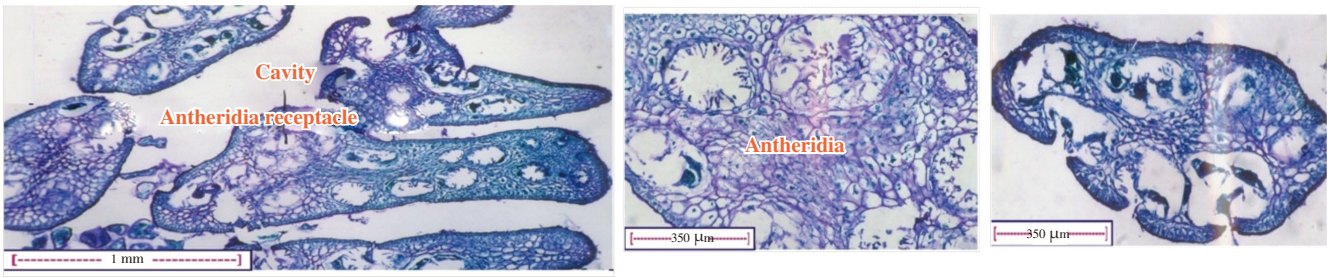

Figure 5. Receptacles.
A: Receptacles (4×), B: Antheridia receptacles(10×), C: Oogonia receptacles (10×).

3.2.4. Receptacles

The receptacles were short, repeated branched clusters of branches. They contained wide circular cavities which were surrounded by one or two layers of rectangular cells. From the inner surface of the cavities arise short branched bodies which possess antheridia or oogonia at the swollen terminal portions (Figure 5A). The antheridial branches were club shaped and had dark inclusions (Figure 5B). The oogonial branches have spherical terminal part possessing spherical oogonia and short thin stalk (Figure 5C). The chamber bearing the reproductive branches breaks open to liberate the gametes.

3.4. Physicochemical analysis

The results of the phytochemical analysis is shown in Table 2.

Table 2
Physicochemical constant of whole plant of *S. wightii*.

Parameters		Values % w/w
Total ash values		3.81
Acid insoluble ash values		1.39
Water soluble ash values		2.26
Extractive values	Petroleum ether soluble	0.20
	Chloroform soluble	1.40
	Ethanol soluble	2.40
	Water soluble	1.62
	Methanol soluble	2.78
Extractive values	Petroleum ether soluble	0.40
(successive solvents)	Chloroform soluble	1.67
	Ethanol soluble	2.81
	Water soluble	2.46
	Methanol soluble	2.98
	Loss on drying	7.26

3.5. Phytochemical screening

Preliminary phytochemical screening of the whole plant extract mainly revealed the presence of the phytoconstituents such as carbohydrates, tannins, flavonoids and steroids. The petroleum ether extract showed only the presence of steroids (Table 3).

3.6. Fluorescence analysis

The results of fluorescence analysis are shown in Table 4.

Table 3
Phytochemical screening of various extracts of *S. wightii*.

Tests	Petroleum ether	$CHCl_3$	Ethanolic	Aqueous	Methanolic
Alkaloids					
Mayers reagent	–	–	+	–	–
Dragendorff's reagent	–	–	+	–	–
Hager's reagent	–	–	+	–	–
Wagner's reagent	–	–	+	–	–
Purine alkaloid murexide test	–	–	–	–	–
Carbohydrates					
Molisch's test	–	–	–	–	–
Fehling's test	–	–	–	–	–
Benedict's test	–	–	–	–	–
Glycosides					
Anthraquinone glycosides					
Borntrager's test	–	–	–	–	–
Modified borntager's	–	–	–	–	–
Cardiac glycoside					
Keller killiani test	–	–	–	–	–
Raymond test	–	–	–	–	–
Legal's test	–	–	–	–	–
Cyanogenetic glycoside	–	–	–	–	–
Coumarin glycoside	–	–	–	–	–
Sterols					
Sallkowski's test	–	+	+	–	+
Libermann–burchard	–	+	+	–	+
Saponins	–	+	–	–	–
Tannins					
$FeCl_3$ test	–	–	–	+	–
Gold beater's skin test	–	–	–	+	–
Proteins and free amino acids					
Million's test	–	–	–	–	–
Biuret test	–	–	–	–	–
Ninhydin test	–	–	–	–	–
Mucilages	–	–	–	–	–
Terpenoids	–	–	–	–	–
Flavonoids					
Shinoda test	–	+	–	–	–
Alkali test	–	+	–	–	–
Acid test	–	+	–	–	–
Zn–HCl test	–	+	–	–	–
Volatile oil	–	–	–	–	–
Fixed oils	–	–	–	–	–

Table 4
Fluorescence analysis of whole plant of *S. wightii*.

Treatment	Visible light	UV light
Powder+50% H_2SO_4	Black	Greenish black
Powder+Conc. H_2SO_4	Brown	Greenish brown
Powder+50% HCl	Black	Greenish black
Powder+Conc. HCl	Black	Greenish brown
Powder+50% HNO_3	Dark red	Yellowish brown
Powder+Conc. HNO_3	Dark red	Yellowish brown
Powder+10% NaOH	Brown	Green
Powder+5% $FeCl_2$	Greenish black	Black
Powder+5% KOH	Greenish black	Black
Powder+C_2H_5OH	Black	Blackish brown
Powder+CH_3COOH	Black	Black
Powder+1 N HCl	Black	Blackish brown
Powder+1 N NaOH+C_2H_5OH	Dark red	Yellowish brown

Conc.: Concentration.

4. Discussion

To ensure reproducible quality of herbal medicines proper control of starting material is almost essential. The first step towards ensuring quality of starting material is authentification followed by creating numerical values of standards for comparison. Pharmacognostical parameters for easy identification like leaf constituents, microscopy and physicochemical analyses are few of the basic protocol for standardisation of herbals[9]. The information obtained from the preliminary phytochemical screening will reveal the useful finding about the nature of the drug. The total ash value, extractive value will be helpful in identification and authentification of the plant material[10,11]. The pharmacognostical and phytochemical evaluation of *S. wightii* can provide useful information for the identification and authentification of the plant.

The results of the phytochemical investigation of various solvent extracts revealed the presence of various secondary metabolites with varied degree. Recent reports of antiviral, anti-fungal, antioxidant, anti-inflammatory, antiallergenic, anti thrombic, anti carcinogenic, hepatoprotective and cytotoxic activities of flavonoids have generated interest in studies of flavonoid containing plants[12,13], and these flavonoids are known as nature's tender drug which possess numerous biological and pharmacological activities. 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[14,15]. Saponins possess numerous biological properties which include antimicrobial, anti-inflammatory, anti-feedent and haemolytic effects[16]. Steroids are 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[17,18]. Alkaloids are commonly found to have antimicrobial properties against both Gram-positive and Gram-negative bacteria[19]. Recently, a number of studies have been reported on the phytochemistry of seaweeds across the world[20-23]. The presence of secondary metabolites which include alkaloids, flavonoids, polyphenols, steroids and saponins in the crude extracts of *S. wightii* suggest that seaweeds can be used as antimicrobial, anti-parasitic, anti-inflammatory, antifeedent, antioxidant, antiallergenic, anti-thrombic, anticarcenogenic and anti-ulcer agents, have great medicinal value and have been extensively used in the drug and pharmaceutical industry.

Conflict of interest statement

The authors have no conflict of interest.

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Comments

Background

The pharmacognostical studies have a vital role in the herbal research which helps to identify and also to fix a standard for the herbal material. Therefore study of pharmacognostical and phytochemical properties of brown seaweed *S. wightii* helps to set up a basic standard to do any future research on this species.

Research frontiers

In the pharmacognostical studies the anatomy of *S. wightii* was observed systematically. By studying the histological charecters of leaf, stem, air bladder and receptacles through transverse section, the entire anatomy of *S. wightii* was reported.

Related reports

Pharmacognostical reports were given as per WHO guidelines. So it may be used as a standard for future

reference. The reports of preliminary phytochemical studies support the antioxidant activity in the presence of flavanoids.

Innovations and breakthroughs

Phytochemical screening revealed the presence of the phytoconstituents such as carbohydrates, tannins, flavonoids and steroids. The results of the phytochemical investigation of various solvent extracts revealed the presence of various secondary metabolites in varied degree.

Applications

Pharmacognostical studies of the plant may be used as a standard for the future raw material. It also helps to analyze the purity and authenticate the future raw material. Fluorescence analysis may also used to identify the powdered material. The phytochemical analysis helps to justify the traditional uses of this algae.

Peer review

This study helps to ensure reproducible quality of herbal medicines for proper control of starting material which is almost essential. The pharmacognostical parameters help for easy identification like leaf constituents. Microscopy and physicochemical analyses are few of the basic protocols for standardisation of herbals. The preliminary phytochemical screening revealed the useful finding about the nature of the drug. The total ash value, extractive value will be helpful in identification and authentification of the algae. The pharmacognostical and phytochemical evaluation of *S. wightii* can provide useful information for the identification and authentification of the plant.

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