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Pharmacognostical and phytochemical analysis of *Sargassum cinereum* (Turner) C. Agardh

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Abstract
Marine algae exhibit extraordinary potential due to the presence of many active ingredients. Hence, it has become mandatory to evaluate the pharmacognostical and phytochemical profile of the marine algae. *Sargassum cinereum* commonly known as brown algae is native to the coastal region of the peninsular India belonging to division, *Phaeophyceae* was focused to highlight the knowledge for the pharmacognosy basically deals with standardization, authentication and study of natural drugs to explore marine algae *S. cinereum* in pharmaceutical field for its valuable medicinal importance.

Keywords: *Sargassum cinereum*, macroscopy, microscopy, pharmacognostic studies, phytochemical analysis

1. Introduction
It is well known that phytochemicals are non-nutritive plant compounds that have defensive or disease preventive properties. Human body has a number of physiological, biochemical and enzymatic processes by which it can combat oxidative stress and does not depend solely on dietary intake of bioactive components. Previous studies did not support the fact that increase in intake of dietary antioxidants leads to increase in the bioavailability of antioxidants in the physiological system (Witztum & Steinberg (2001)) [42].

Marine macroalgae, commonly referred to seaweeds, are categorized by their pigmentation, morphology, anatomy and nutritional composition as red (*Rhodophyta*), brown (*Phaeophyta*) and green seaweeds (*Chlorophyta*) (Dawczynski *et al.,* 2007) [11]. About 250 macroalgal species have been commercially utilized worldwide and about 150 species are consumed as human food (Kumari *et al.,* 2010) [25]. Fresh and dry seaweeds are traditionally consumed as a sea vegetable in many countries and their safety has been recognized (Manivannan *et al.,* 2011) [26]. They are of high nutritional value (Jimenez Escrig & Goni Cambrodon, 1999) [22] and the quality of proteins and lipids are comparatively better than other vegetables (Dawes, 1998) [12].

Seaweeds are the excellent source of the bioactive compounds such as protein, fiber, vitamins, polyunsaturated fatty acids, macro, and trace elements, as well as important bioactive compounds (Ortiz *et al.,* 2006) [31]. They also possess some valuable medicinal components such as antibiotics, laxative, anticoagulant and anti-ulcer products (Illiopoulere *et al.,* 2002) [20]. Thus, they have been recognized as being beneficial for human and animal health (Fleurence J (1999)) [16]. However, the nutrient composition of seaweeds is different depending on species, habitats, maturity and environmental conditions (Ito and Hori (1989)) [21].

Although there is doubt on the benefit of phytochemical consumption in reducing oxidative stress, it must be noted that such compounds may have other physiological effects. Previous studies in animal models and cell culture have suggested that seaweed phytochemicals have the potential to inhibit the progression of carcinoma formation (Murakami *et al.,* 1996) [28].

The extracts from various brown algae have been used in traditional medicine in Asia (Anastyuk *et al.,* 2017) [2]. The bioactive compounds present in extract such as carotenoids, dietary fibre, protein, essential fatty acids, vitamins and minerals present in brown seaweeds are primarily used for treating diseases like cancer, Acquired immune deficiency Syndrome (AIDS) and, arthritis (Bhaskar and Miyashita (2004)) [5]. Several works have been carried out on the extracts from marine algae and numerous reports related with its *antioxidant* (Zhao *et al.,* 2006) [44], *anticancer* (Venkatpurwar *et al.,*, 2011) [40], *antiaging, antifatigue* (Guo *et al.,* 2005) [18], *anticoagulants* and *antihyperlipidemic* (Zhou *et al.,* 1990) [45], *sunscreen agent* (Bhatia *et al.,* 2011) [3], *immunomodulation* (Yoshizawa *et al.,* 1993) [43], *antitumour* and *antiviral* (Pujol *et al.,* 2007) [32] activities have been found.

Hence, this study was focused to investigate the pharmacognostical properties and phytochemical evaluation of brown seaweed *S cinereum* (Turner) C.

Agardh collected from the Mandapam coastal region (Rameshwaram coast) to discover the presence of bioactive components which can be useful for pharmaceutical applications.

2. Materials and Methods

2.1 Collection and Authentication

S. cinereum (Turner) C. Agardh was collected from Mandapam, Gulf of Mannar (Lat.09º 17'N; Long.79º 08'E), Rameshwaram coast, Ramnad, South India,Tamil Nadu, and authenticated by Dr. Palani, Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, and India.

2.1.1 Processing of collected sample

The seaweeds collected were cleaned with seawater to remove dirt and debris along with epiphytes, sand particles, and shells. Then, the seaweeds were washed with running tap water more than 5 times followed by distilled water and drained completely and dried at room temperature. The resulting dried material was coarsely powdered (passing through 40 size sieve) and utilized for further pharmacognostic and phytochemical studies.

2.1.2 Preparation of extract

Brown seaweed extract was prepared by adding methanol to round bottom flask followed by the addition of 50 g of seaweed powder, and was subjecting to soxhlet apparatus at 64°C for 72 hours. The extract (representing both lower polar, polar and non-polar components) of brown seaweed were pooled together and evaporated under reduced pressure using rotary flash evaporator (Superfit, India). The crude extract was quantified and further analysis.

2.2.1 Macroscopic analysis

The organoleptic properties like shape, color, dimension, uprights or creeping and taxonomy of *S. cinereum* were studied.

2.2.2 Microscopic analysis

Qualitative and quantitative microscopic evaluation was conducted on entire plant *S. cinereum*. In this study, microscopic evaluation of air bladder, stem, (O'Brien *et al.,* 1964) and a transverse section of the leaf were carried out using Toluidine blue-O dye and the morphology was studied.

2.2 Physico-chemical constants

The procedures were followed according to Indian Pharmacopoeia and WHO guidelines for evaluating the physio-chemical constants.

2.3.1 Determination of Ash content

Dried and coarsely powdered *S. cinereum* were subjected to the determination of total ash, water-soluble ash, sulfated ash, and acid insoluble ash by standard procedure.

2.3.2 Loss on Drying

Loss on drying is the loss of mass demonstrated as percent w/w and can be determined by the following procedure.

About 2 g of powder was weighed and transferred to a dry stoppered weighing bottle. The weight of the bottle and sample was taken accurately. After removing the stopper, the bottle containing sample was placed in an oven for 1 hour at 120° C. After 1 hour the bottle was removed and cooled in a desiccator and weighed by replacing the stopper. This was ontinued until the difference between two successive weighing is not more than 0.25%.

2.3.3 Inorganic mineral analysis

A study of inorganic constituents of the plant is of interest to researchers in several fields, such as in the healthcare industry (Sizer, 2000). Therefore, the seaweed *S. cinereum* was subjected to inorganic mineral analysis.

2.4 Phytochemical screening

1% w/v stock concentration of each extract along with controls were tested for the presence of active phytochemicals such tannins, alkaloids, phytosterols, triterpenoids, flavonoids, cardiac glycosides, anthraquinone glycosides, saponins, carbohydrates, proteins, amino acids and fixed oils & fats by following standard methods as briefed below:

1. Tannin

Ferric chloride Test: Few drops of 5% ferric chloride solution was added to 2 ml of the methanol extract of *S. cinereum*. Formation of a blue color indicates the presence of hydrolyzable tannins.

Gelatin Test: Five drops of 1% gelatin containing 10% sodium chloride was added to 1 ml of the methanol extract of *S. cinereum*. Formation of white precipitate confirms the test.

2. Alkaloids

Approximately 40 mg of the methanol extract of *S. cinereum* was dissolved in 5 ml of distilled water. Further 2M HCL was added until an acid reaction occurred and filtered. The filtrate was tested for the presence of alkaloids as detailed below.

Dragendorff's Test: 2 ml of above filtration, 1 ml of Dragendorff's reagent was added along the side of the test tube. Formation of orange or orange reddish brown precipitate indicates the test as positive.

Mayer's Test: 1 ml of the filtrate a drop or two of Mayer's reagent was added along the sides of test tube. A white or a creamy precipitate confirms the test as positive.

Hager's Test: 1 ml of the filtrate, a drop of Hager's reagent was added. The emergence of yellow precipitate indicates the test as positive.

Wagner Test: 2 drops of Wagner's reagent was added to 1ml of the filtrate along the sides of the test tube. The development of yellow or brown precipitate confirms the test as positive for alkaloids.

3. Phytosterols

Liebermann-Burchard's Test: The methanol extract of *S. cinereum* (2 mg) was dissolved in 2 ml of acetic anhydride, heated to boiling, cooled and then 1 ml of conc H_2SO_4 was added along the side of the test tube. Brown ring formation at the junction and the turning of the upper layer to dark green color confirms the test for the presence of phytosterols.

4. Triterpenoids

Salkowski Test: Approximately 2 mg of dry extract was shaken with 1 ml of chloroform and a few drops of conc H_2SO_4 was added along the side of the test tube. A red-brown color formed at the cross point indicates the test as positive for triterpenoids.

5. Flavonoids

Shinoda test: 1 ml of the methanol extract of *S. cinereum* a few magnesium turnings and five drops of conc HCL was

added in dropwise. A pink, scarlet, crimson red or occasionally green to blue color appears after few minutes confirms the test.

Alkaline reagent test: 1 ml of the methanol extract of *S. cinereum* and 5 drops of 5% NaOH was added. An increase in the intensity of yellow color becomes colorless with the addition of a few drops of 2M HCL indicating the presence of flavonoids.

Lead acetate test: A few drops of 10% lead acetate was added to 1ml of the methanol extract of *S. cinereum* resulting in the formation of yellow precipitation confirms the presence of flavonoids.

6. Saponins

Foam Test: 5 ml of the extract was taken in a test tube and shaken well for five minutes. A formation of stable foam confirms the test.

Olive oil test: Few drops of olive oil was added to 2ml of the extract and shaken well. Formation of soluble emulsion confirms the test.

7. Cardiac glycosides

Keller -Killiani test: 1mg of the dry extract 4 ml of glacial acetic acid and a few drops of 5% ferric chloride solution was added together. To this 0.5 ml of Conc H_2SO_4 was added along the side of the test tube carefully. The formation of blue color in acetic acid layer confirms the test.

8. Anthraquinone glycosides

Hydroxyanthraquinone Test: add a few drops of 10% KOH in 1 ml of the methanol extract. Formation of red color confirms the test.

9. Test for carbohydrates

Molisch's test: 1 ml of the methanol extract few drops of 1 % α- naphthol and 2-3 ml conc H_2SO_4 was added along the sides of the test tube. The reddish violet or purple ring formed at the junction of two liquids confirms the test.

Barfoed's test: 2ml of Barfoed's reagent was added to 2 ml of the methanol extract and kept in under water bath at 90°C for 1 min. Formation of red precipitate indicates the presence of monosaccharides.

Seliwanoff's test: 3 ml of Seliwanoff's reagent was added to 1 ml of the methanol extract and heated on a water bath for one minute. The formation of rose red color confirms carbohydrates.

Fehling's test: Dissolved 2 mg dry extract in 1 ml of distilled water and added 1ml of Fehling's (A+B) solution, soaked and heated on water bath for 10 minutes. The brick red precipitate formed confirms the test.

10. Test for proteins

Biuret test: 2 ml of the methanol extract 5 drops of 1% copper sulfate solution and, 2 ml of 10% NaOH was added and mixed thoroughly. Formation of purple or violet color confirmed presence of proteins.

11. Test for amino acids

Millon's test: 5 drops of Millon's reagent was added to 1 ml of the methanol extract and heated on a water bath for 10 min, cooled and 1% of sodium nitrite solution was added. Appearance of red color confirmed the test.

12. Fats and fixed oils

In 5 drops of the sample, 1 ml of 1% copper sulfate solution and a few drops of 10% sodium hydroxide were added. The appearance of clear blue solution confirmed the test.

3. Result & Discussion

Sargassum cinereum, a brown algal species which is considered as a rare species is characterized by the presence of golden brown xanthin pigment (fucoxanthin) imparting a color variation. *Sargassum* is abundant in warmer areas and often attached to rocks (lithophytes).

The marine specimen *S. cinereum* C. Agardh selected for the proposed work was collected from Mandapam coast, Rameshwaram, India and authenticated. In order to study the therapeutic value of this species, Pharmacognostic parameters like microscopic observation, morphology, extractive values, and physicochemical parameters like a loss on drying, ash values, inorganic constituents and mineral analysis were done based on the standard procedures available.

Macroscopic characters

S. cinereum Turner C. Agardh

Plants with short, stout main axis generally brown or dark green in colour bearing terate, smooth, primary branches at their upper part, beset with secondary branches and branchlets; basal leaves membranaceous, oblong, about 2 – 3 cm long, 5 – 8 mm broad, rounded at the apices and dentate at the margins; leaves of the branchlets lanceolate, 2-3 cm long, 3 mm borad, cuneate at the base. The vesicles are in spherical shape about 4 mm diameter, obovate, rounded, and usually mucronate at the apices, subcylindrical.

Microscopy

Transverse section of *S. cinereum* **(**Metcalfe and Chalk 1979)

Anatomy of thallus

The cells in the leaf are differentiated into epidermis, cortex, and medulla. Cortex is mainly distributed in wings with few cells thick in the middle region. Inner to the epidermis, vertically oblong cylindrical cells with dense accumulation of chromophores was noticed (Figure 1 & 2).

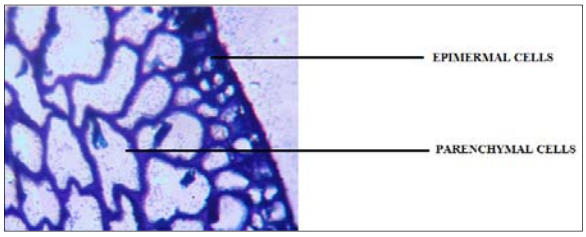

Fig 1: Anatomy of Thallus 400X (*S. cinereum*)

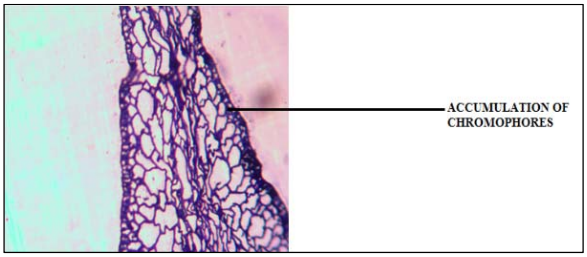

Fig 2: Whole transverse section of Thallus 100X (*S. cinereum*)

Stem

Axis is differentiated into meristoderm, cortex and medulla. The outermost layer is made up of closely-packed, small cells called Meristoderm. The cells aremeristematic in nature and filled with chromatophores. It is also covered by a layer of cuticle. This layer is also known as the epidermis, and due to the presence of chromatophores, its function is photosynthetic. Below the meristoderm, there is a region of cortex which is made up of many parenchymatous cells. Cells are polygonal in shape and bear intercellular spaces. Cells are filled with reserve food, and so it is also known as storage tissue. Centre of the axis is occupied by a thick-walled region or medulla. Cells of the medulla are narrow and elongated. Lateral walls of medulla bear some scalar form thickenings (Figure 3).

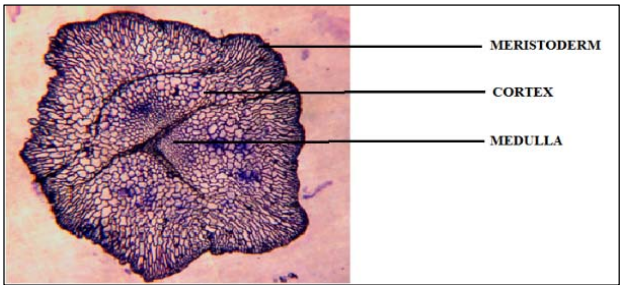

Fig 3: Transverse section of stem 100X (*S. cinereum*)

Air bladder

The air bladder is circular in section with wide empty central space which has shallow ridges on the surface. The bladder has a thick epidermal layer of square cells with inner compact parenchymal cells with no cell inclusion (Figure 4 & 5).

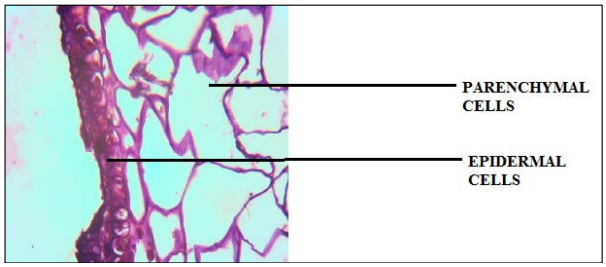

Fig 4: Anatomy of Air bladder 400X (Sargassum cinereum)

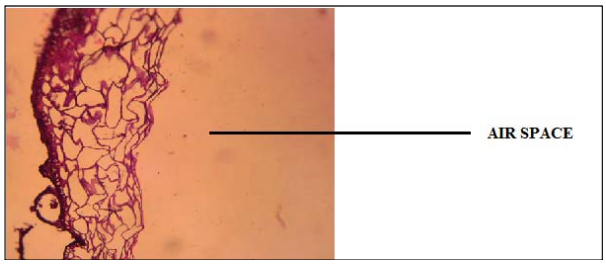

Fig 5: Anatomy of Air bladder 100X (*S. cinereum*)

Yield of extraction

The percentage yield of extracts obtained from *S. cinereum* using Methanol as solvent was about 27 g from 100 g of powdered sample. The yield of methanol extract showed methanol is a good solvent to extract bioactive compounds from living organism (Ahmed *et al.,* 2016) [1]. This is due to the physical characteristic of methanol which has the ability to dissolve both polar and non-polar compounds (Table 1) (Sunar *et al.,* 2009) [39].

Table 1: Yield of the extract *S. cinereum*

Parameters	
Solvent	Methonal
Amount of sample (g)	100 g
Boiling point	64.7°C
Total hours of extraction	72 hrs
Yield (g)	27 g
Colour of extract	Dark brown greenish colour

Physiochemical constituents and phytochemical analysis

The Physiochemical constants are listed in table 2. The total ash value, the methanol crude extractive value will be helpful in identification and authentication of the brown seaweed *S. cinereum* and to scrutinize adulterants from original species of biological importance. Phytochemicals like tannins, alkaloids, steroids, phenolic compounds, terpenoids were highly present in the extract (Table 3).

Table 2: Physio-chemical constants of *S. cinereum*

S. No	Parameters	Percentage (%)
1	Total ash	19.78
	Water soluble ash	6.80
	Acid insoluble ash	10.73
	Sulphated ash	0.87
2	Loss on drying	4.03

Table 3: Phytochemical analysis of *S. cinereum*

S. No	Phytochemicals	(+/-) 70% Methanolic extract
1	Alkaloids	+
2	Amino acid	-
3	Carbohydrates	-
4	Proteins	-
5	Glycosides	-
6	Phenolic compounds	+
7	Flavonoids	+
8	Terpenoids	+
9	Steroids	+
10	Saponins	+
11	Tannins	+
12	Oils & fats	-
13	Resins	-

Inorganic analysis

The methanol extract of *S. cinereum* showed the presence of 12 inorganic minerals (Table 4). Inorganic analysis showed the presence of calcium and iron in milligram level and other minerals were in microgram level showing that the species is free from toxic level of minerals. All the parameters were noted for pharmacognostic importance and utilized to differentiate the adulterant and original species.

Table 4: Inorganic analysis of *S. cinereum*

S. No	Inorganic compounds	Weight of the samples (g)
1	Cadmium	0.007 µg
2	Calcium	0.81 mg
3	Chromium	0.0005 µg
4	Copper	0.198 µg
5	Iron	1.38 mg
6	Lead	0.0001 µg
7	Magnesium	0.091 mg
8	Nickel	0.003 µg
9	Zinc	0.032 µg
10	Phosphorus	0.07 µg
11	Potassium	2.979 µg
12	Sodium	2.003 µg

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 authentication followed by creating numerical values of standards for comparison. Pharmacognostical parameters furnish an easy understanding the basic protocol for standardization of medicinal plants and identification like plant constituents, microscopy and physicochemical analysis. The information obtained from the preliminary phytochemical screening will reveal the useful finding about the nature of the drug. The pharmacognostical and phytochemical evaluation of *S. Cinereum* can provide useful information for the identification and authentication of the seaweed.

The results of the phytochemical investigation of methanol solvent extract revealed the presence of various secondary metabolites. Recent reports of *antiviral, anti-fungal, antioxidant, anti-inflammatory, antiallergenic, anti thrombic, anticarcinogenic, hepatoprotective* and c*ytotoxic activities* of flavonoids have generated interest in studies of flavonoid containing plants (Cushnie and Lamb, 2005 & De Sausa *et al.,* 2007) [10] 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 Saponins possess numerous biological properties which include *antimicrobial, anti-inflammatory, anti-feedent* and *haemolytic* effects. Polyphenols have antioxidant activity (Chandhini *et al.,* 2008 & Ganesan *et al.,* 2008) [8]. Steroids are believed to be a biosynthetic precursor for cardenolides in plants. Marine algae have shown to be good source of unsaponifiable, nontoxic sterols that have medicinal value (Sanchez *et al.,* 2004) [35]. Alkaloids are commonly found to have antimicrobial properties against both Gram-positive and Gram-negative bacteria (Cowan 1999) [9]. Recently, a number of studies have been reported on the phytochemistry of seaweeds across the world (Selvin and Lipton (2004), Fayaz *et al.,* 2005, Somepalli *et al.,* 2007) [37, 15, 38]. The presence of secondary metabolites which include alkaloids, flavonoids, polyphenols, steroids and saponins in the crude extracts of S. cinereum suggest that the seaweeds can be used as antimicrobial, anti-parasitic, antiinflammatory, antifeedent, antioxidant, antiallergenic, antithrombic, anticarcinogenic and anti-ulcer agents, have great medicinal value and have been extensively used in the drug and pharmaceutical industry.

Conclusion

Morphological and phytochemical analysis of any plant material is essential for the identification and authentication of individual species. Therefore in this study we have carried out the macroscopic as well as microscopical analysis of the morphology of *S. cinereum* (Turner) C. Agardh species. Further analysis of phytochemical constituents gave us the preliminary information regarding the pharmaceutical application of brown seaweed *S. cinereum*.

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