

Pharmacognostic Characterization and Phytochemical Evaluation of Brown Seaweed *Hydroclathrus Clathratus* (C.Agardh) M.Howe

T. Vimala¹, T. V. Poonghuzhali²

¹Research Scholar, Dept. of Botany, Queen Mary's College, Chennai- 600004

²Associate Professor, Dept. Of Botany, Queen Mary's College, Chennai-600004.

Abstract: *Objective:* To explore the pharmacognostical and phytochemical properties of *Hydroclathrus clathratus*. *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, 6-10cm flat, yellow brown sponge-like clump of very porous, chain-like tissue. Thallus is irregularly globular. The plant exhibits two layers of tissue as seen in transactional view. The outer zone consists of radially elongated, cylindrical, less compact palisade cells. Inner to the palisade layer is a narrow zone of three layers of wide, polygonal compact thin walled parenchyma cells. The different extracts of *H.clathratus* showed the presence of tannins, saponins, flavonoids, alkaloids, phenols, cardiac glycosides, terpenoids, Coumarins, steroids, phytosteroids, Anthroquinones, Phlobatannins. Physico-chemical and fluorescence analysis of the seaweed was also recorded. *Conclusions:* Various pharmacognostical parameters evaluated in this study help in the identification and standardization of the of the seaweed *Hydroclathrus clathratus*.

Keywords: *Hydroclathrus clathratus*, brown seaweed, microscopy, phytochemical screening, pharmacognosy

1. Introduction

Seaweeds are the potential renewable marine sources. About 6000 species of seaweeds have been identified all over the world, of which 844 species have been reported in Indian coasts. Marine organisms are source material for structurally unique natural products with pharmacological and biological activities (Faulkner, 2001). New compounds isolated from marine sources has been increasing steadily in recent decades (Blunt, Copp, Keyzers, Munro, & Prinsep, 2012). Seaweeds are fresh sources of bioactive compounds with immense medicinal potential which have attracted the attention of pharmaceutical industries (Pietra, 1997). Seaweeds have been found to be rich in secondary metabolites that include alkaloids, glycosides, saponins, tannins, flavonoids, steroids which are of immense medicinal value and useful in broad spectrum of biological activities (Eluvakkal et al., 2010). They are an excellent source of vitamins such as A, B1, B12, C, D and E, riboflavin, niacin, pantothenic acid and folic acid as well as minerals such as Ca, P, Na, K (Dhargalkar & Pereira, 2005).

Several species of seaweeds have also been found to produce or contain polysaccharides, glycoproteins or other secondary metabolites with antibacterial (Manivannan et al., 2011), antifungal (Cox, et al., 2010), antiviral (Wijesinghe and Jeon, 2012), anti-inflammatory (Ananthi et al., 2010), antidiabetic (Gokce and Haznedaroglu, 2008), antioxidant (Chew et al., 2008) and cytotoxic activities (Chia et al., 2015). Among all the types of seaweeds highest phytochemical content have been reported from brown seaweeds (Seafoodplus, 2008).

The Phaeophyceae or brown seaweeds are a large group of multicellular algae, and they play an important role in marine environments both as food, and for the habitats they

form. Most brown seaweeds contain the pigment fucoxanthin and various pheophycean tannins which are responsible for the distinctive greenish-brown colour as the name indicated (Wijesinghe and Jeon, 2011). Worldwide there are about 1,500 species of brown seaweeds and they produce vast numbers of useful active components (Davis et al. 2003; Reddy and Urban 2009). Some species are of sufficient commercial importance, such that they have become subjects of extensive research in their own right.

H.clathratus, a brown marine alga, has been used for centuries in traditional cuisine and medicine of island countries such as Hawaii. *H.clathratus* is known to possess anticancer, anti-herpetic, anti-inflammatory and anti-coagulant properties and is now used as a mineral supplement in cosmetics and as soil-additive (fertilizer) for its high concentration of micronutrients (seaweed industry association, 2011). Extracts of *H.clathratus* have antiviral (Wang et al., 2007), antitumour (Awad et al., 2009; Jayasooriya et al., 2011) and antimicrobial (Kantachumpoo et al., 2010; Arunkumar and Sivakumar, 2012) activity. The present study was aimed to explore the pharmacognostical study and phytochemical constituents of *H.clathratus* 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.

2.2 Plant collection and authentication

Fresh, matured and healthy seaweed was collected from Kilakarai (09° 13' 45" N - 078° 46' 32" E) of Gulf of Mannar region of Tamilnadu coast. The samples were collected in the month September, 2016 during spring tide. The seaweed was transported to laboratory for further analysis. The alga collected was identified as *Hydroclathrus clathratus* and was authenticated by Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India (Certificate No.BSI/SRC/5/23/2016/Tech./1425) and the voucher specimen of the plant was deposited in the Department of Botany, Queen Mary's College, Chennai, Tamilnadu, India for further reference.

2.3. Processing of collected plant sample

The sample was gently rinsed with fresh water to remove salt, sand and epiphytes, epizoonas, animal castings, calcareous and other adhering detritus matters. The seaweed was then shade dried at room temperature for a span of one week to prevent photolysis and thermal degradation, under a continuous stream of air flow. The completely dried seaweed material was weighed and powered in a mechanical grinder and then stored at -20 °C for further analysis. One kilogram fresh seaweed yielded approximately 120g of dry weight.

2.4. Macroscopic analysis

The macroscopical analysis included the evaluation of organoleptic characters and external features of the various parts of selected plant materials (Evans, 2002).

2.5. Microscopic analysis

Microscopic evaluation was conducted in both qualitative and quantitative studies of whole plant of *H.clathratus* (Khandelwal, 2007). In this study transverse section and powder microscopy of entire plant was carried out. The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was by customary procedure (Johansen, 1940). Staining procedure was used as per standard procedures. The sections were stained with Toluidine blue as per the method published by O'Brien et al. (1964).

Various characters were identified and studied. The dry powder of seaweed was studied for determination of pharmacopoeial standard.

2.6. Physico-Chemical Evaluations

The physico-chemical analysis like ash values, extractive values, loss on drying were calculated according to the methods prescribed in Indian Pharmacopoeia (Anonymous 1996).

2.7. Preliminary phytochemical screening

Preliminary phytochemical screening of methanol extract of *H.clathratus* (MEHC) was carried out to detect the

phytoconstituents using standard conventional protocols (Harborne, 1998).

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. Fluorescence analysis of dried and powdered seaweed was carried out according to the procedure described by (Kirtikar *et al.*, 2005) by using the reagents and viewed in day light and ultraviolet radiations. The colours and fluorescence observed by application of different reagents in different radiations were recorded.

3. Result

3.1 Macroscopic Characters

Hydroclathrus clathratus (C. Agardh) Howe is a brown seaweed, appearing as a 6-10cm yellow brown sponge-like clump of very porous, chain-like tissue. Thallus is irregularly globular (Figure 1). The organoleptic characters observed are given in Table. 1.



Figure 1: *H.clathratus* whole plant

Table 1: Macroscopical characters of *H.clathratus*

Macroscopical Characters	Observation
Plant colour	Dark brown
Plant height	6-10cm flat
Condition	Fresh
Size	6-10cm
Shape	irregularly globular
Odour	Fishy smell
Taste	No taste
Apperance	Perforated, sponge-like clump

3.2 Microscopic Characters

In sectional view, the septa are circular with undulate outline (Fig.2.1, 2.2, 2.3, 2.4). There is a central wide air-chamber enclosed by thick cylindrical tissue. The circular structure of the plant body measures 1.6mm to 2mm in thickness. The section enclosing the air-chamber is up to 130µm thick. The plant exhibits two layers of tissue as seen in transactional

view. The outer zone consists of radially elongated, cylindrical, less compact palisade cells. The palisade cells are 30µm long. The terminal part of the palisade cells is darkly stained. Inner to the palisade layer is a narrow zone of three layers of wide, polygonal compact thin walled parenchyma cells. No specific cell inclusions are seen with inner zone of cells.

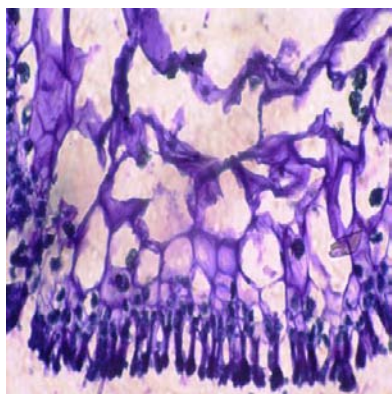
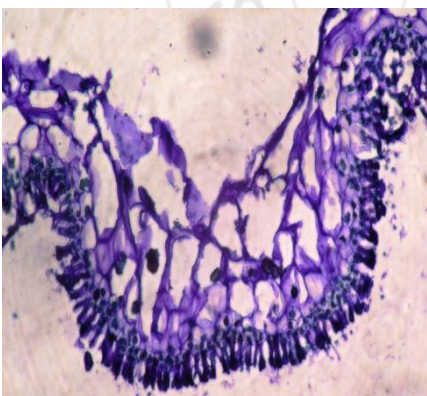
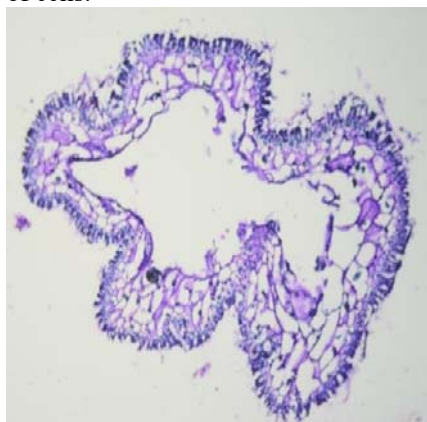


Figure 2.1, 2.2, 2.3, 2.4: Transverse section of thallus

Table 2: Physico-chemical analysis of *H.clathratus*

Parameters		Values (% w/w)
Extractive values	Hexane	0.22
	Ethyl Acetate	1.2
	Methanol	1.4
	Water	6.4
Total Ash		3.84
Acid insoluble ash		1.42
Water soluble ash		2.64

Table 3: Phytochemical screening of various extracts of *H.clathratus*

Phytochemical tests	Hexane	Ethyl acetate	Methanol	Aqueous
Tannins	+	+	+	-
Saponins	+	+	-	-
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Phenols	+	+	+	+
Glycosides	-	-	-	-
Cardiac Glycosides	-	+	+	-
Terpenoids	+	+	+	+
Quinones	-	-	-	-
Coumarins	+	+	+	-
Steroids	+	-	+	-
Phytosteroids	+	+	+	-
Anthroquinones	-	-	+	-
Phlobatannins	-	-	+	-

3.4. Physico-Chemical Evaluations

The results of the physico-chemical analysis are shown in Table.2.

3.5 Phytochemical screening

By preliminary phytochemical screening of fourteen different chemical compounds (steroids, alkaloids, phenolic groups, saponins, tannin, flavonoids, anthraquinone, coumarins, phytosteroids, glycosides, cardiac-glycosides, terpenoids, quinones) were tested in four different extracts of *H.clathratus*. Thus, out of 56 tests for compounds, only 33 gave positive results and the remaining 23 gave negative results. The positive results showed the presence of tannins, saponins, flavonoids, alkaloids, phenols, cardiac glycosides, terpenoids, Coumarins, steroids, phytosteroids, Anthroquinones, Phlobatannins. Glycosides and quinones did not show in any of the extracts.

Flavonoids, Phenols, Alkaloids, Terpenoids showed the maximum presence in all the four different extracts. Coumarins and phytosteroids were present in 3 different extracts. Among the four extracts, methanolic extract showed maximum number (11) compounds followed by hexane and ethyl acetate (9 compounds) extracts. Aqueous extract showed the minimum number (4) compounds (Table 3).

Table 4: Fluorescent analysis of powdered *H.clathratus*

Treatment	Visible Light	UV Light 366nm
Dry Powder	Dark Brown	Greenish brown
Powder + 50% H ₂ SO ₄	Black	Greenish black
Powder + conc. H ₂ SO ₄	Brown	Greenish brown
Powder + 50% HCl	Black	Greenish black
Powder + conc. HCl	Black	Greenish black
Powder + 50% HNO ₃	Brown	Yellowish brown
Powder + conc. HNO ₃	Brown	Yellowish brown
Powder + 1 N HCl	Black	Blackish brown
Powder + 5% KOH	Greenish black	Black
Powder+ 10%NaOH	Brown	Greenish brown

3.6. Fluorescence analysis

The results of fluorescence analysis are shown in Table 4. The characteristic fluorescent properties or colours emitted by the powdered sample of *H.clathratus* before and after treating with various reagents were recorded. The powdered thallus appeared brown under visible light and greenish brown under ultraviolet radiation. After treating with various reagents such as H₂SO₄, HCl, HNO₃, KOH, NaOH, under visible light, the sample showed shades of brown and black. However, under ultraviolet radiation, 10% sodium hydroxide and concentrated sulphuric acid the sample showed greenish brown shade, whereas, in 50% H₂SO₄, 50% HCl and conc. HCl, the sample showed greenish black shade. Sample treated with nitric acid showed yellowish brown and KOH showed black.

Discussion

Standardization is an important tool for herbal drugs in order to establish their identity, purity, safety and quality. In order to standardize a drug, various macroscopic, physicochemical analyses, phytochemical analysis, fluorescence analysis are done. The quantitative determination of some pharmacognostical parameters is useful for setting standards for crude drugs. Pharmacognostical parameters for easy identification like leaf constituents, microscopy and physicochemical analyses are few of the basic protocol for standardisation of herbals (Dinakaran et al., 2011). Preliminary phytochemical screening will reveal the nature of the drug and physico-chemical analysis will be helpful in identification and authentication of the plant material (Kumar et al., 2011).

Both macroscopic and microscopic studies can provide useful information for the identification and authentication of *H.clathratus*. The physico-chemical evaluation of drugs is an important parameter in detecting adulteration or improper handling of drugs. It can serve as a valuable source of information and provide appropriate standards to establish the quality of this seaweed in future study or application (Subramanian Sampathkumar and Ramakrishnan, 2011)

The phytochemical screening revealed the presence of tannins, saponins, flavonoids, alkaloids, phenols, cardiac glycosides, terpenoids, Coumarins, steroids, phytosteroids, anthroquinones, phlobatannins. Phenols and flavonoids are widely distributed in seaweeds and have been reported to exhibit several biological activities including antioxidant activity (Chandini et al., 2008). Alkaloids are commonly

found to have antimicrobial properties against both Gram-positive and Gram-negative bacteria (Cowan, 1999). Saponins possess numerous biological properties which include antimicrobial, anti-inflammatory, anti-feedent and hemolytic effects (Xu et al., 2000). The presence of these secondary metabolites suggests that the seaweed might be of medicinal importance and can be used as anti-microbial, anti-parasitic, anti-inflammatory, anti-oxidant agents.

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 (Pimenta et al., 2006). 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 *H.clathratus* in its crude form.

4. Conclusion

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus in recent years there have been an emphasis in standardization of medicinal plants of therapeutic potential. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken (Anonymous 1996).

After present investigation it can be concluded that the physico-chemical and phytochemical studies of *Hydroclathrus clathratus* yielded a set of qualitative and quantitative pharmaco-botanical parameters or standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies (Senthil Kumar et al., 2006).

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Author Profile

T.Vimala is Research Scholar, Department Of Botany, Queen Mary'S College, Chennai-600004

T.V.Poonghuzhali is Associate Professor, Department Of Botany, Queen Mary'S College, Chennai-600004.