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Pharmacognostic characterization, phytochemical and physicochemical evaluation of *Sargassum wightii* and *Padina gymnospora,* two brown seaweeds from Gujarat coast

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Abstract
Sargassum wightii belonging to the family Sargassaceae and *Padina gymnospora* belonging to the family Dictyotaceae are two brown seaweeds known for various biological activities like antibacterial, antiviral, antioxidant, anti cancer, anti-inflammatory, etc. The reported biological activities may be because of the phytoconstituents present in them. Considering their therapeutic uses, like other medicinal plants there is a need to do pharmacognostic studies of these two seaweeds. In the present work, an attempt was done to evaluate the pharmacognostic, phytochemical and physicochemical profile of *S. wightii* and *P. gymnospor*a. In pharmacognostic studies, macroscopic and microscopic studies were done which revealed their characteristic features. The phytochemcial analysis revealed the presence of alkaloids and glycosides while solvent extracts revealed the presence of flavonoids also. On the whole, *P. gymnospora* had more presence of phytochemicals than *S. wightii*. The extractive values of methanol extract and aqueous extract were maximum in both the seaweeds but were considerably more in *P. gymnopora* than *S. wightii*. The parameters evaluated in this study will be useful to maintain the identity and efficacy of these seaweeds and also prevent it from adulteration.

Keywords: Brown seaweeds, *Sargassum wightii, Padina gymnospora*, pharmacognostic analysis, phytochemical analysis, physicochemical analysis

1. Introduction
Algae are heterogeneous group of plants that can be divided into the macroalgae (seaweeds) and the micro algae. The former occupy the littoral zone while the latter is found in both bentheic and littoral habitats. Macroalgae is classified into three classes; green algae (Chlorophyta), Brown algae (Phaeophyta) and red algae (Rhodophyta) [1]. The characteristic green colour of green algae (Chlorophyta) is mainly due to the presence of chlorophyl a and b in the same proportion like higher plants. The characteristic brown colour of brown algae (Phaeophyta) is because of presence of the xanthophyll pigments and fucoxanthin; it also masks the other pigments like chlorophyll a and c, b-carotenes and other xanthophylls. The characteristic red colour of red algae (Rhodophyta) is because of presence of pigments like phycoerythrin and phycothcyanin; it also masks the other pigments like chlorophyll a, b-carotene and xanthophylls [2]. All the three classes of seaweeds or marine algae are a potential source of plentiful secondary metabolites with interesting and numerous biological activities. They may represent useful leads in the development of new drugs or pharmaceutical agents.
Brown seaweeds are used as animal feed, food ingredients and fertilizers. They are also good sources of proteins, carbohydrates, vitamins and minerals. Food reserves of brown algae are typically complex polysaccharides and higher alcohols. Many bioactive metabolites have been isolated from brown algae with different pharmacological activities such as cytotoxic and antitumor, antifungal, antifeedent, antioxidant anti-inflammatory, antiviral, hepatoprotective, algicidal, anti-diabetic, antihypertensive, nematocidal, etc. [3].
Sargassum wightii J. Agardh is one of the marine macro algae belonging to the class Phaeophyceae; it is widely distributed in tropical and temperate oceans. It belongs to the marine family Sargassaceae and order Fucales. It is called gulfweed or sea holly. It is a large, cost effectively important and ecologically dominant brown algae present in much of the tropics. It is the most diverse genus among Phaeophyta in India and is represented by 38 species. Many biological activities are reported for the genus *Sargassum*.

Some of the examples are cytotoxic activity of *Sargassum tortile* [4]; antioxidant and antiviral activities of *Sargassum micracanthum* [5]; antimicrobial and antioxidant activity of *Sargassum* spp. [6]; antioxidant activity of *Sargassum plagiophyllum* [7]; analgesic and anti-inflammatory activity of *Sargassum ilicifolium* [8]; antioxidant activity of three *Sargassum* spp. [9] and antimicrobial activity of *Sargassum johnstonii* [10].

Another genus *Padina* belongs to the class Phaeophyceae and order dictyotales. *Padina gymnospora* (Kutzing) Sonder belongs to the family Dictyotaceae. Its common name is funnelweed. It has a wide range of bioactive properties like anticoagulant activity [11] (Silva, 2005); haemagglutinating and cytotoxic activity [12]; antimicrobial and hemolytic activity [13]; antioxidant activity [14]; Plant growth promoting effect [15]; Would healing property [16]; synthesis of platinum nanoparticles [17]; cytotoxic activity [18, 19]; antimicrobial activity [20] and antibacterial activity [21].

Interest in natural remedies for all ailments is increasing day by day. Medicinal plants are traditionally used since ages and their usage and popularity is increasing more and more merely because they are with less side effects, easily available, economic and affordable by all people. Hence, standardization and quality control parameters must be laid down for each plant used as a source of natural drug. It will ensure the authenticity and efficacy of the drug and prevent it from malpractices like adulteration and substitution. The most simplest and easiest method is pharmacognostic study which involves macroscopic, microscopic and powder study, physicochemical, phytochemical and flouroscence analysis. Sea weeds like medicinal plants are known for many biological activities. Hence it is important and necessary to do pharmacognostic studies of marine algae. There are some reports of pharmacognostic study of algae for eg. pharmacognostical and phytochemical investigation of selected marine seaweeds of Rhodophyceae [22]; pharmacognostic and phytochemical evaluation of *Sargassum wightii* from Gulf of Mannar, Mandapam coastal regions Tamilnadu is reported by Devi *et al*., [23]; while pharmacognostic and phytochemical studies of *Sargassum ilicifolium* from Rameshwaram coast, Chennai is reported by Sumithra and Arunachalam [24]. We have reported pharmacognostic study of two green algae *Chaetomorpha antennina* and *Ulva lactuca* [25] and in the present work we report pharmacognostic study of two brown algae *Sargassum wightii* and *Padina gymnospora*.

2. Materials and Methods

2.1 Plant Collection

Two brown algae viz. *Sargassum wightii* J. Agardh and *Padina gymnospora* (Kutzing) Sonder were collected in November, 2017 from Gujarat, India. The algae were washed thoroughly under tap water, shade dried and homogenized to fine powder and stored in closed container for further studies.

2.2 Pharmacognostic Study

2.2.1 Macroscopic Studies

Pharmacognostic study was done by organoleptic evaluation. The morphological features of different parts of the algae were observed under magnifying lens. Macroscopic characters were studied using standard methods [26]. Photographs at different magnifications were taken by using digital camera.

2.2.2 Microscopic studies

Microscopic studies were carried out by preparing thin sections of different part of both the algae. The thin sections were washed with water, mounted in glycerin and its lignifications were confirmed (10x, 40x) [27].

2.3 Qualitative phytochemical analysis

The dried powder of both the algae was subjected to qualitative phytochemical analysis to identify for the presence of phytoconstituents like alkaloids, flavonoids, phenols, saponins, tannins, cardiac glycosides, steroids, phlobatannins, triterpenes and anthocyanins [28]. The presence or absence of phytochemicals was indicated with (+) sign and (-) sign respectively. The procedure followed is as described earlier [25].

2.4 Physicochemical analysis

2.4.1 Ash Values

The dry powder of both the algae was subjected to physicochemical analysis as per WHO guidelines [29]. The physicochemical parameters evaluated were loss on drying, total ash, acid-insoluble ash, water-soluble ash, sulphated ash, carbonated ash, nitrated ash and extractive values. The details of the procedure followed is as described earlier [30].

2.4.2 Extractive values

The extractive values of dried algae powder was assessed by extracting the dry powder in five solvents of different polarity. The solvents used were petroleum ether (PE), toluene (TO), ethyl acetate (EA), methanol (ME) and water (AQ). The procedure followed is as described earlier [31].

2.5 Fluorescence analysis

Fluorescence study of dried powder of both the algae was carried out as per the method described by Kokaski *et al.*, [32]. A small quantity of the dried powder of algae was placed on a microscopic slide and few drops of freshly prepared various reagents were added and mixed by gentle tilting of the slide. The slides were then placed inside the UV chamber and observed in visible light, short (wave length 254 nm) and long (wave length 365 nm) ultra violet radiations. The colours observed by applications of different reagents in different wave lengths were recorded.

3. Results

Organoleptic characteristics of both brown seaweeds *S. wightii* and *P. gymnospora* is given in Table 1. Both the seaweeds were marine in habitat. The shape of *S. wightii* was well branched and erect while that of *P. gymnospora* was fan shaped. The base of *S. wightii* was simple holdfast while that of *P. gymnopora* was modified rhizoidal base. The texture of *S. wightii* and *P. gymnospora* were rough. The airbladder was present in *S. wightii* and it was spherical in shape while airbladder was absent in *P. gymnospora*.

Table 1: Organoleptic features of *S. wightii* and *P. gymnospora*

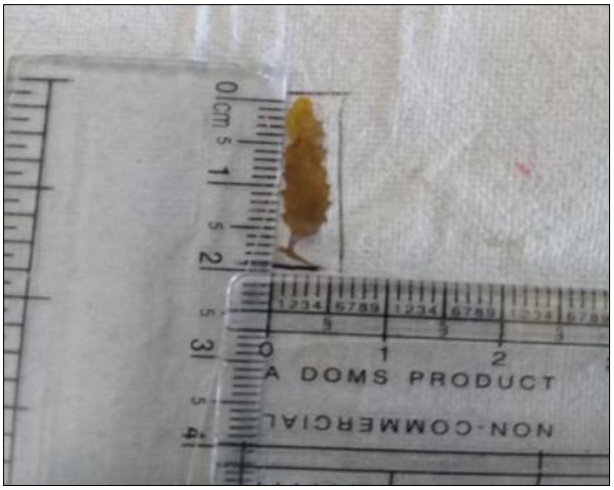
Characters	*S. wightii*	*P. gymnospora*
Habit	Marine	Marine
Shape	Well branch and erect	Fan-shape blades
Size	20 cm	7 cm
Color	Dark brown	Olive brown
Odour	Fishy	Fishy
Taste	Salty	Salty
Base	Holdfast	Rhizoidal base
Blades	Leaf like	Concentric rows of hair
Texture	Rough	Rough
Air-bladder	Spherical	Absent

3.1 Macroscopic characteristics of *S. wightii*

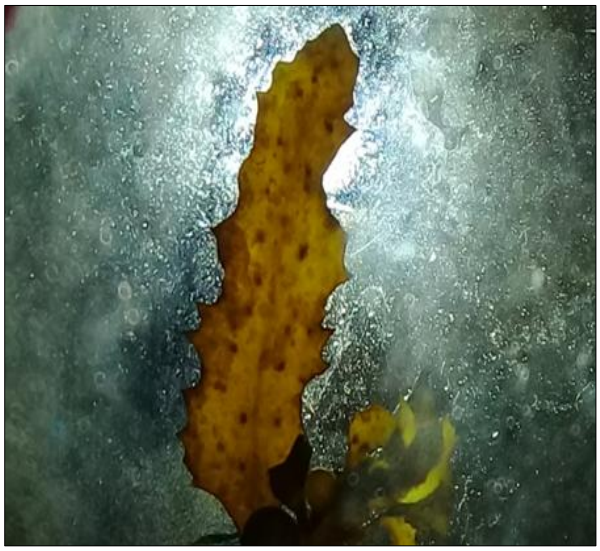
Macroscopic characteristics of *S. wightii* is given in Fig.1. The thallus was plant like structure, brown in colour, branched and erect. It is differentiated into cylindrical main axis and basal holdfast (Fig. 1a). The thallus was differentiated into various parts like receptacle, laterals and spherical air-bladders (Fig. 1b). The laterals were of two types: primary laterals and secondary laterals. Primary laterals were developed in large number from the main axis, radially arranged and unlimited growth. The primary lateral bears secondary laterals, which was leaf like structure (Fig. 1c). The secondary laterals were simple, flat and broad shaped, apex was acute and midrib was absent. The average size of secondary laterals were 1-2 cm in length and 0.2-0.6 cm width (Fig.1d).

a) Habit

b) Plant parts



c) Size of lateral



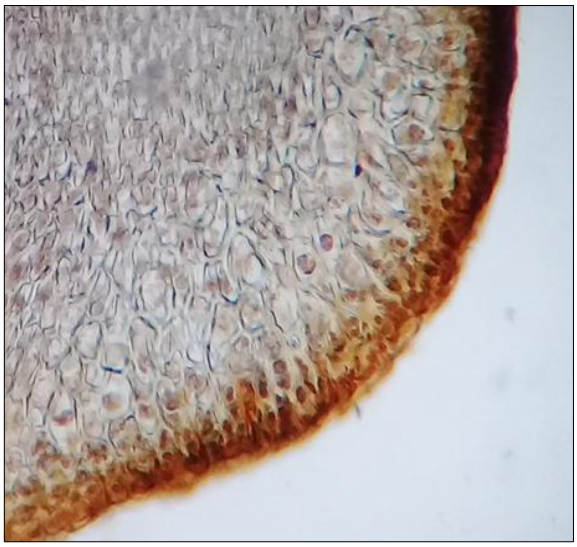
d) Lateral

Fig 1: Macroscopic study of *Sargassum wightii* J. Agardh

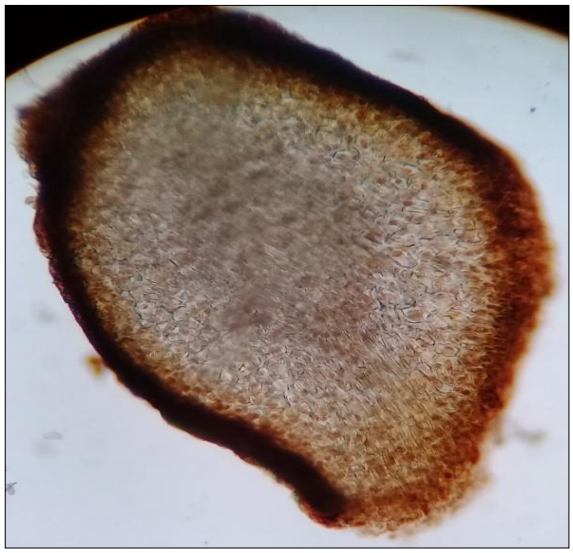
3.2 Microscopic characteristics of *S. wightii*

The transverse section of *S. wightii* main axis is given in Fig. 2. The outer most layer of meristoderm was dark brown in colour, made up of small, closely packed two to three layers of meristmatic cells (Fig. 2a). The main axis was differentiated into three regions meristoderm, cortex and medulla. The cortex region was made up of of 6-8 layers with similar type of parenchymtous cells. The medulla was present in centre, it was narrow, elongated and double walled cells (Fig. 2b). The single layer mucilagionous envelope was present on outer surface of meristoderm of air blader, which

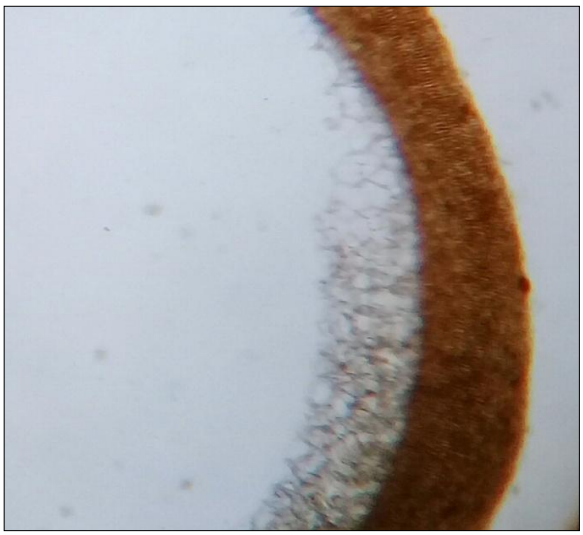
was brown in colour (Fig. 2c). The cortex of air blader was of few layers with paranchymatous tissue, in the centre medulla was absent but a hollow cavity was present, which was filled with air and gases (Fig. 2d).



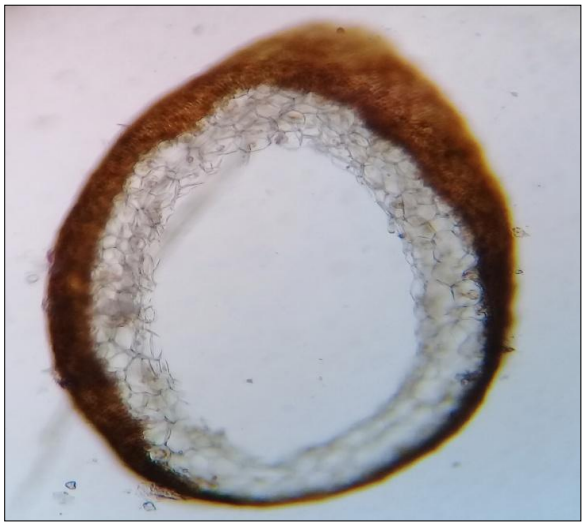
a) T.S. of main axis with meristoderm



b) T.S. of main axis



c) T.S. of air-bladder with meristoderm



d) T.S. of air-bladder

Fig 2: Microscopic study of *S. wightii*

3.3 Macroscopic characteristics of *P. gymnospora*

Macroscopic characteristics of *P. gymnospora* is given in Fig. 3. *P. gymnospora* are brown colour seaweeds, blades were fan-shaped, thick, deposition of limestone, distal margin of blades was circinately rolled which protects the apical margin (Fig. 3a). The average size of blade was 7-9 cm in length and 6-8 cm in width (Fig. 3b).

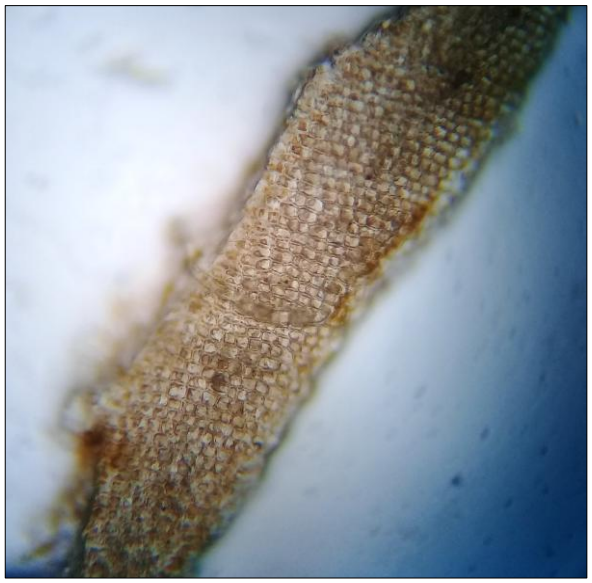
a) Habit

b) Thallus

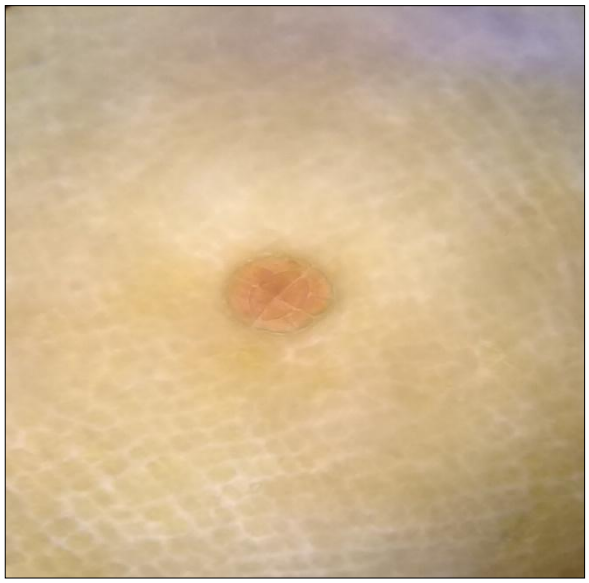
Fig 3: Macroscopic study *Padina gymnospora (Kutzing) Sonder*

3.4 Microscopic characteristics of *P. gymnospora*

The transverse section of *P. gymnospora* thallus is given in Fig. 4. The blade was dorsiventral in nature. It was divided into a basal stalk cell and upper antheridial cell. The antheridial cells were multicellular, brown in colour, small, multi-chambered body; each cell produces a single antherozoid for sexual reproduction (Fig. 4a). The other section of thallus showed tetrasporangium with four tetraspores, it may occur singly or in groups on upper and lower surfaces (Fig. 4 b).



a) T.S. of thallus with antheridia



b) T.S. of thallus with tetrasporeangium

Fig 4: Microscopic study of *P. gymnospora*

3.5 Qualitative phytochemical analysis

The qualitative phytochemical analysis of crude powder of *S. wightii* and *P. gymnospora* is given in Table 2. In *S. wightii*, alkaloids were present in maximum amount; coumarins were present in moderate amount; while saponins and cardiac glycosides were present in trace amount. The other phytoconstituents were absent. In *P. gymnospora*, cardiac glycosides were present in maximum amount; flavonoids, steroids and coumarins were present in moderate amount while alkaloids, saponins, tannins and anthocyanins were present in trace amount. The other phytoconstituents were absent.

Table 2: Qualitative phytochemical analysis of crude powder of *S. wightii* and *P. gymnospora*

S. No	Phytochemicals	*S. wightii*	*P. gymnospora*
1	Alkaloids		
	(a)Mayer's reagent	-	+
	(b)Wagner's reagent	+++	-
	(c)Dragondroff's reagent	-	+
2	Phenols	-	-
3	Flavonoids	-	++
4	Saponins	+	+
5	Tannins	-	+
6	Phlobatannins	-	-
7	Steroids	-	++
8	Cardiac glycosides	+	+++
9	Anthocyanins	-	+
10	Triterpenes	-	-
11	Quinones	-	-
12	Leucoanthocyanins	-	-
13	Coumarins	++	++

(+++) more amount, (++) moderate amount, (+) less amount, (-) absent

The qualitative phytochemical analysis of different solvent extracts of *S. wightii* is given in Table 3. In PE solvent extract, steroids were present in moderate amount; flavonoids, saponins and cardiac glycosides were present in less amount and remaining phytoconstituents were absent. In TO solvent extract, alkaloids, flavonoids and steroids were present in moderate amount, saponins and cardiac glycosides were present in less amount and remaining phytoconstituents were absent. In EA solvent extract, alkaloids, flavonoids, saponins and steroids were present in moderate amount; cardiac glycosides were present in less amount and remaining phytoconstituents were absent. In ME solvent, alkaloids, flavonoids, saponins, steroids and cardiac glycosides were present in moderate amount while remaining phytoconstituents were absent. In AQ solvent extract, saponins and quinones were present in less amount while remaining phytoconstituents were absent.

Table 3: Qualitative phytochemical analysis of *S. wightii* in different solvent extracts

S. No	Test	PE	TO	EA	ME	AQ
1.	Alkaloids					
	(a)Mayer's test reagent	-	-	-	-	-
	(b)Wagner's test reagent	-	++	++	++	-
	(c)Dragondroff's reagent	-	-	-	-	-
2.	Phenols	-	-	-	-	-
3.	Flavonoids	+	++	++	++	-
4.	Saponins	+	+	++	++	+
5.	Tannins	-	-	-	-	-
6.	Phlobatannins	-	-	-	-	-
7.	Steroids	++	++	++	++	-
8.	Cardiac glycosides	+	+	+	++	-
9.	Anthocyanins	-	-	-	-	-
10.	Triterpenes	-	-	-	-	-
11.	Quinones	-	-	-	-	+
12	Leucoanthocyanins	-	-	-	-	-
13	Coumarins	-	-	-	-	-

The qualitative phytochemical analysis of different solvent extracts of *P. gymnospora* is given in Table 4. In PE solvent

extract, steroids and cardiac glycosides were present in moderate amount; flavonoids were present in less amount while remaining phytoconstituents were absent. In TO solvent extract, flavonoids were present in maximum amount; alkaloids, steroids and cardiac glycosides were present in moderate amount, tannins were present in less amount and remaining phytoconstituents were absent. In EA solvent extract, flavonoids, steroids and cardiac glycosides were present in maximum amount; alkaloids were present in moderate amount while tannins and triterpenes were present in less amount and remaining phytoconstituents were absent. In ME solvent extract, steroids and cardiac glycosides were present in moderate amount; alkaloids and saponins were present in less amount and remaining phytoconstituents were absent. In AQ solvent extract, saponins were present in moderate amount and quinones were present in less amount while remaining phytoconstituents were absent.

Table 4: Qualitative phytochemical analysis of *P. gymnospora* in different solvent extracts

S. No	Test	PE	TO	EA	ME	AQ
1.	Alkaloids					
	(a)Mayer's reagent	-	-	-	-	-
	(b)Wagner's reagent	-	++	++	+	-
	(c) Dragondroff's reagent	-	-	-	-	-
2.	Phenols	-	-	-	-	-
3.	Flavonoids	+	+++	+++	-	-
4.	Saponins	-	-	-	+	++
5.	Tannins	-	+	+	-	-
6.	Phlobatannins	-	-	-	-	-
7.	Steroids	++	++	+++	++	-
8.	Cardiac glycosides	++	++	+++	++	-
9.	Anthocyanins	-	-	-	-	-
10.	Triterpenes	-	-	+	-	-
11.	Quinones	-	-	-	-	+
12	Leucoanthocyanins	-	-	-	-	-
13	Coumarins	-	-	-	-	-

(+++) more amount, (++) moderate amount, (+) less amount, (-) absent

3.6 Physicochemical Analysis

The physicochemical parameters of the crude powder of *S. wightii* is given in Table 5. The moisture content was 12%. The total ash was 34.83% while water soluble ash was 12.5% and acid insoluble ash was 7.5%. The sulphated ash was 39.33%. The carbonated ash was 37.66% The nitrated ash was 35.16%. The maximum soluble extractive value was found in ME solvent (3.44%) while the minimum soluble extractive value was found in PE solvent (1.01%). The water soluble extractive value was 3.01%. The physicochemical parameters of the crude powder of *P. gymnospora* is given in Table 5. The moisture content was 10%. The total ash was 35% while water soluble ash was 19.16% and acid insoluble ash was 7%. The sulphated ash was 55%. The carbonated ash was 36.83%. The nitrated ash was 49.16%. The maximum soluble extractive value was found in ME solvent (6.36%) while the minimum soluble extractive value was found in TO solvent (0.58%). The soluble extractive value was 9.85%.

Table 5: Physiochemical parameters of *S. wightii* and *P. gymnospora*

S. No	Parameters	*S. wightii* % value (w/w)	*P. gymnospora* % value (w/w)
1	Loss on drying	88	90
2	Total ash	34.83	35.00
3	Water soluble ash	12.5	19.16
4	Acid insoluble ash	7.5	7.00
5	Sulphated ash	39.33	55.00
6	Nitrated ash	35.16	49.16
7	Carbonated ash	37.66	36.83
8	Petroleum ether soluble extractive value	1.01	0.90
9	Toluene soluble extractive value	1.42	0.58
10	Ethyl acetate soluble extractive value	1.95	0.90
11	Methanol soluble extractive value	3.44	6.36
12	Water soluble extractive value	3.01	9.85

3.7 Fluorescence analysis

The fluorescence analysis of crude powder of both brown seaweeds are given in Tables 6-7. In the present study dried powder of seaweeds was treated with a number of different reagents which showed characteristic fluorescence at 254 nm and 365 nm wave length. The crude powder showed different colors at both the wave lengths. For both the algae, the colours observed were black, light black, brown, dark green, brownish red and reddish brown.

Table 6: Fluorescence analysis of *S. wightii*

S. No.	Treatment	Under visible light	Under uv light short wave length (254 nm)	Under uv light long wave length (365 nm)
1	1N NaOH (Aq)	Black	Black	Black
2	1N NaOH (alc)	Brown	Black	Brown
3	Ammonia	Dark green	Black	Black
4	Petroleum ether	Brown	Black	Brown
5	50% HCl	Black	Black	Brown
6	50% H_2SO_4	Brown	Black	Black
7	Ethyl-acetate	Brown	Black	Brown
8	Ethyl-alcohol	Brown	Black	Black
9	Methanol	Brown	Black	Light black
10	50% KOH	Black	Black	Black
11	50% HNO_3	Brownish red	Black	Black
12	Acetic acid	Brown	Black	Reddish brown
13	Iodine in water	Brown	Black	Brown
14	$FeCl_3$	Brown	Black	Black

Table 7: Fluorescence analysis of *P. gymnospora*

S. No	Treatment	Under visible light	Under uv light short wave length (254 nm)	Under uv light long wave length (365 nm)
1	1N NaOH (Aq)	Brown	Black	Black
2	1N NaOH (alc)	Brown	Black	Black
3	Ammonia	Dark green	Black	Black
4	Petroleum ether	Brown	Black	Brown
5	50% HCl	Brown	Black	Black
6	50% H_2SO_4	Black	black	Reddish brown
7	Ethyl-acetate	Black	Black	Red
8	Ethyl-alcohol	Brown	Black	Red
9	Methanol	Brown	Black	Light black
10	50% KOH	Dark green	Black	Brown
11	50% HNO_3	Dark green	Black	Black
12	Acetic acid	Brown	Black	Reddish brown
13	Iodine in water	Brown	Black	Brown
14	$FeCl_3$	Black	Black	Black

4. Discussion

The importance of marine algae as sources of functional ingredients has been well recognized due to their valuable health benefits. Algae are used in a number of industries and produce useful products. These products include food (Spirulina for example), pharmaceuticals and medicines, animal feed (algae cake), fertilizer (algae cake) and fuel. They are used as pharmaceuticals, nutraceuticals, cosmetics and aquaculture purpose. They are used as biofilters to remove nutrients and other pollutants from waste waters, to assay water quality, as indicators of environmental change and in space technology. Algae can be used to make Biodiesel, Bio ethanol and bio butanol and by some estimates can produce vastly superior amounts of vegetable oil as compared to terrestrial crops grown for the same purpose.

Keeping in view the broad and wide use of seaweeds in various applications and reported biological activities, it is of paramount importance to lay down quality control parameters and standardization becomes obligatory. Such studies will ensure quality, purity and authenticity of crude drugs. Pharmacognostic studies of medicinal plants is well documented [33-35] but pharmacognostic studies of marine seaweeds is scanty. Hence, in the present investigation, pharmacognostic studies were done for two brown seaweeds *S. wightii* and *P. gymnospora*. Such studies should be done before any other parameters are evaluated; as also recommended by WHO [36]. The diagnostic features established in this study will ensure quality control and authentication of crude drugs of these algae.

The various solvent extracts of the algae revealed the presence of phytoconstituents like flavonoids, steroids and cardiac glycosides in more amount followed by alkaloids and saponins which clearly indicated that they can be therapeutically used. They may be a source of natural antioxidants and hence can be successfully used to treat diseases and disorders caused by oxidative stress. Phytochemical analysis in different organic solvents with varying polarity is reported by Deyab *et al*., [37] in *Dictyota dichotoma*, a brown seaweed. Qualitative and quantitative analysis of six seaweeds from Gulf of Mannar is reported by Thinakaran *et al.,* [38]. Other examples are phytochemical analysis in different solvent extracts of roots of *Tephrosia purpurea* [39]; stems of *Hamelia patens* [40] (Surana and Wagh, 2016); aerial parts of *Butea monosperma* [41] (Vaidya and Pandita, 2017).

Various physicochemical parameters evaluated revealed moisture content of 10-12 %; ash values were about 35 %, acid insoluble ash was about 7 % while all three ashes (sulphated, nitrated and carbonated) were quite high. Most seaweed has more ash contents than terrestrial plants and animal products [42]. Aqueous and methanol soluble extractive values were comparatively much higher than other solvent extracts indicating clearly that polar compounds were more. This is in accordance with other plants reported in literature [43, 45].

Fluorescence analysis revealed different colour with different reagents in normal visible light and UV light. For both the algal powder, some reagents showed one colour in visible light which changed to another colour in UV light (Tables 6-7). Similar results were reported for leaf and bark of *Adenanthera pavonina* [46]; for leaf and stem of *Jatropha gossypiifolia* [47]. Fluorescence analysis can be used as a tool

to identify the authenticity of the algae under study.

5. Conclusion

The seaweeds like other marine organisms are a rich source of phytochemcials that may be used as drugs as such or they may be starting material for the development of new drugs. Hence it is very important to have quality control parameters for each and all seaweeds. The characteristic feature of each seaweed must be laid down so quality, efficacy and authenticity can be maintained. The same has been attempted in the present work and the pharmacognostic, phyto and physicochemical parameters and fluorescence analysis done are the diagnostic features of *S. wightii* and *P. gymnopora*. These characters will be their identity and it will help them from getting adulterated and also in compliance of a suitable monograph.

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