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Pharmacognostic standardization of chaetomorpha *antennina* and *Ulva lactuca*, green seaweeds from Gujarat coast

Dhanki Abhishek, Pande Jyoti, Donga Savan and Chanda Sumitra

Abstract
Chaetomorpha antennina (Bory) Kutzing and *Ulva lactuca* Linnaeus are two green marine seaweeds (algae) belonging to the families cladophoraceae and Ulvaceae respectively. They are known for biological activities like antibacterial, antioxidant, antiplasmodial, antifouling, etc. They ae rich in various secondary metabolites.Pharmacognostic, physicochemical and phytochemical parameters of plants are the identity of a particular plant and they are very useful to authenticate the plant under study and prevent it from adulteration and substitution. Hence, in the present study, macroscopic, microscopic, phytochemical and physicochemical parameters of both the algae were evaluated. All the standard procedures were followed. They were rich in phytoconstituents like alkaloids, steroids, cardiac glycosides and saponins. The extractive values were maximum in methanol and minimum in petroleum ether in both the algae. The different salient diagnostic features established in this study will help for proper identification and standardization of crude drug in powder form. They may also be useful in preparation of monograph of them.

Keywords: *Chaetomorpha antennina*, *Ulva lactuca*, green seaweeds, macroscopy, microscopy, phytochemical analysis, physicochemical analysis, quality control

1. Introduction
The ocean covers 70% area of the earth surface and it is the natural habitat of many plants, animals and microorganisms. Marine algae are an extremely diverse group, predominantly of aquatic plants comprising a few thousands of species; they occupy a considerable part of the littoral biomass and are the primary producers of the oceans. As compared to bryophytes and Pteridophytes, algae show comparatively high differentiation of tissue and organs. Algae can be divided into two groups' viz. macro-algae or seaweeds and micro-algae. Similar to well-developed plants, seaweeds are photosynthetic and form basic biomasses in intertidal zones. Algae have chlorophyll as their primary photosynthetic pigment which lack a sterile covering of cells around the reproductive cell. They represent wide range in size, as found in any other group in plant kingdom [1].

Approximately, 9000 seaweed species have been identified. On the basis of their pigmentation, they have been broadly classified into three main groups: brown (Phaeophyta), red (Rhodophyta) and green (Chlorophyta) seaweeds [2]. Seaweeds, one of the important marine living resources, could be termed as the futuristically promising plants. These plants have been a source of food, energy, medicine, cosmetics, feed, agricultural importance and medicines since ancient times. Man has used the sea for many years as a productive source for several economically useful materials, especially to supplement in diet because of high nutritional value [3].

About 250 macro algal species have been commercially utilized. Worldwide about 150 species are favourably consumed, as human food. Seaweeds are food source for humans especially in East Asia, it is most commonly associated with Japanese food. Seaweeds also are used to make a number of food additives such as alginates and carrageenan which is used in cooking and baking as a vegetarian alternative to gelatine. Many seaweeds are used as medicine [4]. Alginates are used in wound dressings and in the production of dental moulds and agar is widely used in microbiology to help grow bacterial cultures. Seaweeds are ingredients in toothpaste, cosmetics and paints and are used in industrial products such as paper coatings, adhesives, dyes, gels, explosives and many more. Much of the oil and natural gas used today, is formed from seaweeds, which was partially decomposed on the sea floor many millions of years ago.

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 [5]. They are source of vitamins such as A, Bl, B12, C, D and E, folic acid, pantothenic acid niacin and riboflavin, they contain some minerals such as Ca, P, Na, K [6]. Marine organisms are source materials for structurally unique natural products with pharmacological and biological activities [7]. Among the marine organisms, the macroalgae (seaweeds) occupy an important place as a source of biomedical compounds [8].

The Chlorophyceae commonly called green algae is a very large group of green algal plants important group of freshwater and marine green algae. Chlorophyceae class include approximately 425 genera and 6500 species. It is diversified class, differing greatly in their vegetative structure, distribution and methods of reproduction [1].

Chaetomorpha antennina (Bory) Kutzing is a genus of green algae in the family Cladophoraceae. Algae of this genus are made up of macroscopic filaments of cylindrical cells. The genus is characterized by its unbranched filaments, making it distinctive; its closest relatives are branching species of the genus *Cladophora*. Some biological activity reported for *C. antennina* are antibacterial activity [9], antiplasmodial activity [10], antioxidant activity [11], etc.

Ulva lactuca Linnaeus is a member of green macro algae in the class Chlorophyceae, belongs to the family Ulvaceae. *Ulva* is commonly called "sea-lettuce", is a marine alga found in the littoral zone. It is found attached to rock, stone etc., present in the intertidal zones of the sea. The genus is represented by about 30 species, of which *U. lactuca* is the most common one. These macroalgae have already been studied for antimicrobial activity [12], antioxidant activity [13], antimicrobial activities [14] and antifouling activity [15].

In the present work, an attempt has been done to lay down pharmacognostic, phytochemical and physicochemical parameters of two green algae *Chaetomorpha antennina* and *Ulva lactuca* from Gujarat coast.

2. Materials and methods

2.1 Plant collection

Two green algae viz. *Chaetomorpha antennina* (Bory) Kutzing and *Ulva lactuca* Linnaeus, were collected in November, 2017 from Dwarka and Porbandar sea coast of 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

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 [16]. Photographs at different magnifications were taken by using digital camera.

Microscopic studies

Microscopic studies were carried out by preparing thin sections of different part of different algae. The thin sections were further washed with water, mounted in glycerin for observation and confirm its lignifications (10x, 40x).

2.3 Qualitative phytochemical analysis

The qualitative phytochemical analysis of crude powder was carried out to identify different phytoconstituents [17]. The phytoconstituents analysed were alkaloids, flavonoids, phenols, saponins, tannins, cardiac glycosides, steroids, phlobatannins, triterpenes, anthocyanins, etc. The presence of specific phytochemicals was indicated with (+) sign and the absence of phytochemicals was indicated with (-) sign. The procedure followed is as described earlier [18].

2.4 Physicochemical analysis

Various physicochemical parameters evaluated were loss on drying, total ash, water soluble ash, acid insoluble ash, sulphated ash, carbonated ash, nitrated ash, etc. The procedure followed is as described earlier [18].

Extractive values

The extractive values of dried algae powder was evaluated by extracting the dry powder in 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 [18].

Fluorescence analysis

Fluorescence study of crude powder of different plants was performed as per Kokaski *et al.* [19]. A small quantity of the crude powder of different plants was placed on a grease free clean microscopic slide and 1-2 drops of freshly prepared various reagent solutions were added, mixed by gentle tilting of the slide. The slides were 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 green seaweeds *C. antennina* and *U. lactuca*, is given in Table 1. Both the seaweeds were marine in habitat. The shape of *C. antennina* was filamentous, while that of *U. lactuca* was thallus like and they were light green in color. The base of *C. antennina* was simple holdfast while that of U. *lactuca* was stalk attaching disc like holdfast. The texture of both the seaweeds was smooth and airbladder was absent.

Table 1: Organoleptic features of *C. antennina and U. lactuca*

Characters	*C. antennina*	*U. lactuca*
Habit	Marine	Marine
Shape	Filamentous	Thallus
Size	7 cm	25 cm
Color	Light green	Light green
Odour	Fishy	Fishy
Taste	Salty	Salty
Base	Holdfast	Stalking attaching disc
Blades	Absent	Thallus
Texture	Smooth	Smooth
Air-bladder	Absent	Absent

3.1 Macroscopic characteristics of *C. antennina*

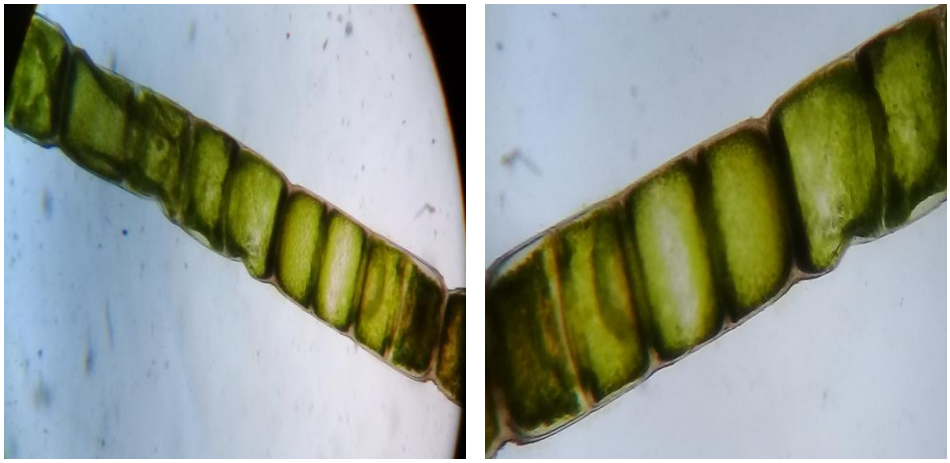
Macroscopic characteristics of *C. antennina* is given in Fig.1. *C. antennina* was green in colour, filamentous, inflexible, growing in clumps on rocky substrate about 5-7 cm in height (Fig. 1a). In some, plamella stage, a colonial aggregate of immobile non flagellated individuals, was found (Fig. 1b).

a) Habit **b) Plamella stage of *C. antennina***

Fig 1: Macroscopic study of *Chaetomorpha antennina* (Bory) kutzing

3.2 Microscopic characteristics

The transverse section of *C. antennina* filament is given in Fig. 2. The filament was thin cell walled with straight, unbranched structures (Fig. 2 a). The cells were box-shaped with mucilaginous thin walled single layer meristoderm (Fig. 2 b).



a) Straight unbranched threads **b) Box-shaped cells**

Fig 2: Microscopic study of *C. antennina*

3.3 Macroscopic characteristics of *U. lactuca*

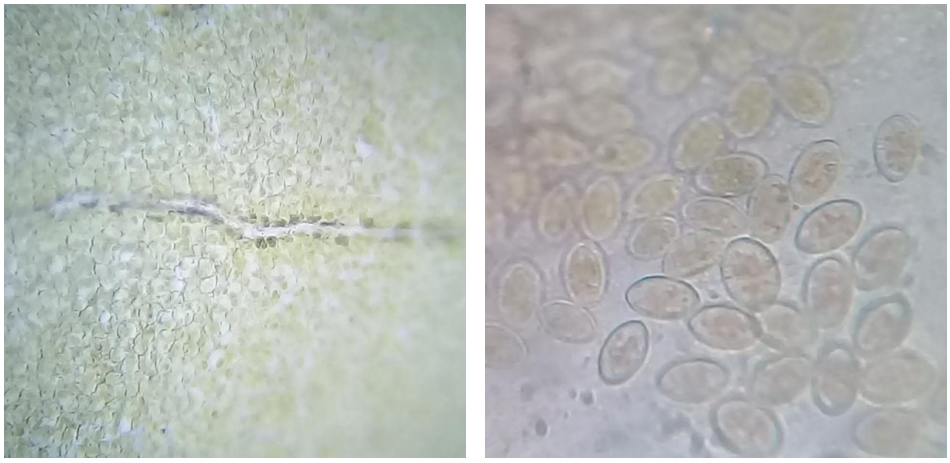
Macroscopic characteristics of *U. lactuca* is given in Fig.3. The thallus was broad, flat, light green in color and irregular in shape. The length of thallus was 15-20 cms and width was 10-15 cms (Fig. 3a). The thallus was attached to substratum with the help of holdfast; it is a form of rhizoidal outgrowth. This outgrowth comes downwards through the space present between two layers of thallus. This outgrowth come out of the thallus and make closely appressed with one another and make attachment disc (Fig. 3b).

a) Habit **b) Thallus**

Fig 3: Macroscopic study of *Ulva lactuca* Linnaeus

3.4 Microscopic characteristics of *U. lactuca*

The transverse section of *U. lactuca* thallus is given in Fig. 4a. The middle thallus was made up of two rows of cells. In the reproductive gametes, the male gametes were narrower and smaller, female gametes were slightly larger than male gametes (Fig. 4b).



a) T.S of Thallus **b) Gametangium of Thallus**

Fig 4: Microscopic study of *U. lactuca*

3.5 Qualitative phytochemical analysis

The qualitative phytochemical analysis of crude powder of *C. antennina* is given in Table 2. In *C. antennina* seaweed, saponins, alkaloids, steroids were present in maximum amount followed by cardiac glycosides, anthocyanins. Flavonoids, tannins, triterpenes were present in trace amount and other phytoconstituents were absent. The qualitative phytochemical analysis of crude powder of *U. lactuca* is given in Table 2. In *U. lactuca*, cardiac glycosides, coumarins were present in maximum amount followed by saponins, and steroids. Triterpenes and anthocyanins were present in trace amount and other phytoconstituents were absent.

The qualitative phytochemical analysis of different solvent extracts of C. *antennina* is given in Table 3. In PE solvent extract, steroids and flavonoids were present in moderate amount; alkaloids, quinones and cardiac glycosides were present in less amount, remaining phytoconstituents were absent (Table 3). In TO solvent extract, alkaloids and steroids were present in moderate amount; flavonoids, saponins, tannins, cardiac glycosides and anthocyanins were present in less amount and remaining phytoconstituents were absent. In EA solvent extract, steroids were present in maximum amount; alkaloids, flavonoids and cardiac glycosides were present in moderate amount; saponins, tannins, anthocyanins and triterpenes were present in less amount while remaining phytoconstituents were absent. In ME solvent extract, steroids, cardiac glycosides and anthocyanins were present in maximum amount; saponins and tannins were present in moderate amount; alkaloids, phenol and triterpenes were present in less amount and remaining phytoconstituents were absent. In AQ solvent extract, only saponins were present in trace amount and remaining phytoconstituents were absent.

The qualitative phytochemical analysis of different solvent extracts of *U. lactuca* is given in Table 4. In PE solvent extract, alkaloids, saponins and steroids were present in less amount while remaining phytoconstituents were absent. In TO solvent extract, alkaloids, flavonoids, saponins and quinones were present in less amount; steroids and triterpenes were present in moderate amount and remaining phytoconstituents were absent. In EA solvent extract, alkaloids, flavonoids, saponins, steroids and quinones were present in less amount and remaining phytoconstituents were absent. In ME solvent extract, saponins were present in moderate amount; flavonoids, steroids and quinones were present in less amount and remaining phytoconstituents were absent. In AQ solvent extract, alkaloids and saponins were present in less amount and remaining phytoconstituents were absent.

Table 2: Qualitative phytochemical analysis of crude powder of *C. antennina and U. lactuca*

S. No	Phytochemicals	*C. antennina*	*U. lactuca*
1	Alkaloids		
	(1) Mayer's reagent	++	-
	(2) Dragondroff's reagent	+++	-
	(3) Wagner's reagent	++	-
2	Flavonoids	+	-
3	Tannins	+	-
4	Phlobatanins	-	-
5	Saponins	+++	++
6	Steroids	+++	++
7	Cardiac glycosides	++	+++
8	Triterpenes	+	+
9	Anthocyanins	++	+
10	Phenols	-	-
11	Coumarins	-	+++
12	Leucoanthocyanins	-	-
13	Quinones	-	-

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

Table 3: Qualitative phytochemical analysis in different solvent extracts of *C. antennina*

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	-	-	-	-	-

Table 4: Qualitative phytochemical analysis in different solvent extracts of *U. lactuca*

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	-	-	-	-	-

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

3.6 Physicochemical analysis

The physicochemical parameters of the crude powder of *C. antennina* is given in Table 5. The moisture content was 7%. The total ash was 34.75% while water soluble ash was 33.16% and acid insoluble ash was 5.66%. The sulphated ash was 45.33%. The carbonated ash was 48.5%. The nitrated ash was 43%. The maximum soluble extractive value was found in ME solvent (9.81%) while minimum soluble extractive value was found in PE solvent (0.98%). The water soluble extractive value was 22.84%.

The physicochemical parameters of the crude powder of *U. lactuca* is given in Table 5. The moisture content was 14.75%. The total ash was 16.25% while water soluble ash was 10.30% and acid insoluble ash was 11.50%. The sulphated ash was 53%. The carbonated ash was 53.33%. The nitrated ash was 49.83%. The maximum soluble extractive value was found in ME solvent (3.47%) while the minimum soluble extractive value was found in TO solvent (0.45%). The water soluble extractive value was 20.1%.

Table 5: Physiochemical parameters of *C. antennina and U. lactuca*

S. No	Parameters	*C. antennina* % value (w/w)	*U. lactuca* % value (w/w)
1	Loss on drying	7.00	14.75
2	Total ash	34.75	16.25
3	Water soluble ash	33.16	10.30
4	Acid insoluble ash	5.66	11.50
5	Sulphated ash	45.33	53.00
6	Nitrated ash	43.00	49.83
7	Carbonated ash	48.50	53.33
8	Petroleum ether soluble extractive value	0.98	1.56
9	Toluene soluble extractive value	2.15	0.45
10	Ethyl acetate soluble extractive value	1.12	0.84
11	Methanol soluble extractive value	9.81	3.47
12	Water soluble extractive value	22.84	20.1

3.7 Fluorescence analysis

The fluorescence analysis of crude powder of both green seaweeds is given in Tables 6-7. The dry 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.

Table 6: Fluorescence analysis of *C. antennina*

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)	Green	Black	Brown
2	1N NaOH (alc)	Green	Black	Black
3	Ammonia	Light green	Black	Brown
4	Petroleum ether	Green	Black	Brown

5	50% HCl	Green	Black	Brown
6	50% H_2SO_4	Green	Black	Red
7	Ethyl acetate	Green	Black	Red
8	Ethyl alcohol	Green	Black	Red
9	Methanol	Green	Black	Red
10	50% KOH	Green	Black	Black
11	50% HNO_3	Yellowish green	Black	Brown
12	Acetic acid	Brown	black	Red
13	Iodine in water	Green	Black	Brown
14	$FeCl_3$	Green	Black	Black

Table 7: Fluorescence analysis of *U. lactuca*

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)	Green	Black	Brown
2	1N NaOH (alc)	Green	Black	Brown
3	Ammonia	Light green	Black	Brown
4	Petroleum ether	Grren	Black	Brown
5	50% HCl	Green	Black	Black
6	50% H_2SO_4	Dark green	Black	Reddish brown
7	Ethyl acetate	Green	Black	Red
8	Ethyl alcohol	Green	Black	Brown
9	Methanol	Green	Black	Light black
10	50% KOH	Green	Black	Green
11	50% HNO_3	Light green	Black	Brown
12	Acetic acid	Green	Black	Reddish brown
13	Iodine in water	Green	Black	Brown
14	$FeCl_3$	Light green	Black	Black

4. Discussion

Marine seaweeds are ecologically important and have been used as food and medicines from time immemorial. Today various species of marine algae provide not only food but also produce extracts for commercial uses such as agar, carrageenans and alginates. The algae are used in numerous foods, pharmaceutical, cosmetic and industrial applications. Seaweeds have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with immense medicinal potential [20]. It is very important to lay down standardization and quality control parameters for plants under study so that they may be correctly identified and prevent from adulteration and substitution when they are in drug form. Such studies will ensure its identity, quality and efficacy [21]. The parameters which are reported here can be considered as distinctive enough to identify and decide the authenticity of the drug in herbal industry and this can be useful in the preparation of monograph for its proper identification. Hence, the present study may be valuable to supplement information in respect to its identification, authentication and standardization of both the studied green seaweeds. Similar pharmacognostic studies are reported for some algae by other researchers [22-24].

In *C. antennina*, the macroscopic evaluation showed plant body to be green in colour, filamentous and unbranched. The microscopic evaluation showed the filaments to be box-shaped cells with thin walls, straight and unbranched structures. In *U. Lactuca*, the macroscopic evaluation showed the thallus was green in colour and irregular in shape; it was attached with holdfast. The microscopic study showed the middle thallus was two rows of cells. The male gametes and female gamates were present in gametangium. Specific macroscopic and microscopic studies for different plants is reported [25-28].

The qualitative phytochemical analysis was done in crude powder and various solvent extracts of *C. antennina* and *U. lactuca*. Both the green algae were rich in alkaloids, steroids, cardiac glycosides and saponins. These phytoconstituents are well known for antimicrobial, antioxidant, diuretic, antimalarial, anticancer activity [29, 30]. Such studies thus give an idea regarding their use for a particular biological activity. The presence of phytoconstituents in different solvents was also different which simply implies that solvent also plays an important extracting various phytoconstituents. Such results are also reported for various plants [31-33].

The physicochemical analysis of dried powder of *C. antennina* and *U. lactuca* was evaluated by using different parameters like loss on drying, total ash, water soluble ash, acid insoluble ash, sulphated ash, nitrated ash and carbonated ash. In *C. antennina* seaweed, the extractive value was maximum in methanol (9.81%) and minimum in petroleum ether (0.98%). In *U. lactuca*, the extractive value was maximum in methanol (3.47%) and minimum in toluene solvent (0.45%). The physicochemical parameters like different ash values, moisture content and extractive values are plant specific and they help to ensure purity of the drug and also prevent adulteration. The ash values measure the inorganic impurities present including silica and sand. Thus, it is one of the parameter to check contamination and adulteration [34, 35]. The extractive values give an idea about particular constituent present in a particular solvent and help the researcher for further analysis [36].

Fluorescence is a necessary parameter for first line standardization of crude drugs. Light rich in short wavelengths is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substances which are not visibly fluorescent in day light. The fluorescence analysis is useful and a necessary parameter for qualitative assessment of crude drugs. It is an important parameter for pharmacognostic evaluation of crude drugs [26]. Similar fluorescence analysis is reported for other algae [37, 38].

5. Conclusion

The pharmacogonostic parameters like organoleptic, macroscopic and microscopic studies; physicochemical, phytochemial and fluorescence analysis of *C. antennina* and

U. lactuca evaluated in this work will ensure its identity, quality and efficacy. The parameters which are reported here can be considered as distinctive enough to identify and decide the authenticity of the drug in herbal industry and this can be useful in the preparation of monograph for its proper identification. They also will help to prevent adulteration and substitution of these seaweeds in crude drug form. Hence, the present study may be valuable to supplement information in respect to its identification, authentication and standardization of studied two green seaweeds.

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