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Pharmacognostical and phytochemical evaluation of selected seaweeds of *Rhodophyceae*

G.Adaikalaraj[1], M.Johnson[1,*], D.Patric Raja[1], N.Janakiraman[2]

[1]Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai - 627 002, Tamil Nadu, (INDIA)

[2]Department of Botany, St. Joseph's College (Autonomous), Tiruchirappalli - 620 002, Tamil Nadu, (INDIA)

E-mail: ptcjohnson@gmail.com

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ABSTRACT

The objective of the present study is to describe the pharmacognostical and phytochemical investigation of selected marine seaweeds of Rhodophyceae from the coastal areas of Manapad, Tamil Nadu state. The pharmacognostic studies were carried out in terms of morphology, anatomy, ash analysis and fluorescence analysis. Preliminary phytochemical screening of secondary metabolites was carried out by the method described by Brindha *et al*. The results of ash analysis showed the presence of more amount of total ash followed by sulphated ash in all the seaweeds compared to water soluble and acid insoluble ashes. In fluorescence analysis, most of the treatment show the characteristics of different shades of green under visible and UV light Qualitative analysis of selected seaweeds revealed the presence of steroids, triterpenoids, alkaloids, tannins, phenols, saponins, flavonoids, catechine, anthroquinone etc. in methanol and aqueous extracts. TLC studies showed different Rf values with high resolution and reproducible peaks. These studies will help the manufacturers for identification and selection of raw materials for drug production.

KEYWORDS

Pharmacognosy;
Phytochemical;
Fluorescence;
Identification;
Metabolites.

INTRODUCTION

Among the Asian countries, India is perhaps the only one that has a long record of inventories of coastal and marine biodiversity dating back to at least two centuries. In terms of marine environment, India has a coastline of about 8000 km, an Exclusive Economic Zone (EEZ) of 2.02 million km^2 adjoining the continental regions and the offshore islands and a very wide range of coastal ecosystems which are characterized by unique biotic and abiotic properties[1]. From the dawn of the history, man has been dependent on seaweed as a source of food, minerals, fertilizers and medicine In India, about 624 seaweeds have been reported with a potential of 77,000 tons per annum. The red seaweeds contribute 27%, brown 0.2% and others 72.8%[2]. Seaweeds are the only source of phytochemicals namely agar – agar, carrageenan and algin, which are extensively used in various industries such as confectionary, textiles, dairy and paper industries mostly as gelling, stabilizing and thickening agents[3]. Seaweeds also provide an excellent source of bioactive compounds such

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as carotenoids, dietary fiber, protein, essential fatty acid, vitamins and minerals[4]. They have been screened extensively to isolate life saving drugs or biologically active substances all over the world[5,6].

Marine algae serve as important resources for biologically active natural products and metabolites isolated from marine algae have bioactive efforts[7-11]. Previous research also depicts the important functional activities of marine seaweeds, such as antioxidant, antimutagen and anticoagulant effect, anti-tumor activity and an important role in the modification of lipid metabolism in the human body shown by the marine seaweeds[12]. Marine seaweeds are the most abundant source of non-mammalian anticoagulant sulfated polysaccharides in nature. They are used as antithrombotic[13], anti-adhesive[14], antitumoral[15], antiviral[16], anticoagulant, antioxidant, proangiogenic, anti-inflammatory and anthelmintic compounds[17,18]. The well known sulfated polysaccharides from red seaweeds are homogalactans[19]. The coastal region of South India, especially Tamil Nadu produces a rich vegetation of marine algae. Many studies have reported a great diversity in the macro algal community of the marine algal vegetation in this region. So far, pharmaceutical industries are giving more importance only to the compounds derived from traditional sources (soil and plants) and less importance to marine organisms[20]. However, information is lacking on the specific metabolites of marine algae in South India with potential bioactive compounds. Considering the high therapeutic efficacy of sea weeds, we carried out the pharmacognostical and phytochemical investigation on selected marine seaweeds of Rhodophyceae from the coastal areas of Manapad, Tamil Nadu state.

EXPERIMENTAL

Collection and preparation of plant material

Seaweeds belonging to the members of Rhodophyceae viz. *Gracilaria verrucosa* (Hudson), *Hypnea musciformis* (Wulf) Lamour, *Enatiocladia prolifera* (Grev.) falk, *Gracilaria ferugosoni*, *Gelidium* species and *Gracilaria verrucosa* var. were collected by handpicking from the coast of Manapad, Tamil Nadu, India. The collected samples were cleaned well with seawater to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The samples were then thoroughly washed with tap water followed by distilled water. For drying, washed seaweeds were blotted on the blotting paper and spread out at room temperature in shade. Shade dried samples were grounded to fine powder using homogenizer. The powdered samples were then stored in refrigerator for further use.

Morphological and anatomical studies

For morphological studies, herbaria were made and macroscopic characters such as colour, size, height, nature and texture were studied. For anatomical studies, collected seaweeds were preserved in 3% formalin.

Physico-chemical parameters

Various ash types and fluorescence analysis were determined by following the standard method[21]. For fluorescence analysis, the plant powder was treated with different chemical reagents and subjected to fluorescence analysis in visible/daylight and UV light.

Phytochemical analysis

For hot extraction, the powdered materials (5 g) were extracted successively with 250 mL of methanol and Distilled water using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whattman filter paper (No.1) and then concentrated in vacuum at 40ºC using Rotary evaporator. The residues obtained were stored in a freezer -20ºC until further tests. For cold extraction, 2 g of air dried powder of sample was extracted with 50 ml of solvents viz., methanol and Distilled water for 72 h. The sample was kept in dark for 72 h with intermittent shaking. After incubation, the solution was filtered through filter paper and the filtrate was collected (crude extracts). The different extracts were tested for steroids, triterpenoids, reducing sugars, alkaloids, phenolic compounds, saponins, tannins, flavonoids, catechin, anthroquinone and aminoacids. Phytochemical screening of the extracts was carried out according to the standard method[22]. TLC studies were carried out by the Harborne method[23].

RESULTS AND DISCUSSION

Morphological studies

Gracilaria verrucosa

Plants bushy, texture firmly flesly, colour dull purplish, 0.5-2.0 mm diameter, repeatedly dividing, dichotomously branched, having a tendency to be flattened with pseudoparenchymatous tissue. Thallus up to 8cm long, hold fast discoid.

Hypnea musciformis

Thalli fleshly, growing up to 8-30 cm in length with numerous short branches; longer branches incurved at the tip, functioning like "tendrils" of higher plants; tendrils curved and sickle shaped making contact with other branches of the same plant or with other algae; distinct hold-fast absent; plants attaching themselves to the substrate by means of hooked branches, branches developing irregularly from all sides of the body; branchlets (ramuli) spur like, 2-15 mm long, arising from all over the body; ramuli numerous in fertile plants, lesser in number in sterile plants.

Enatiocladia prolifera

Thallus erect up to 5 cm, small branched; arising in single and forms a small discoid hold fast. Reddish, single branch, assimilator nine, assimilator size 0.8 mm, upper branch cylindrical, fresh specimen firm in texture and light to dark red.

Gracilaria ferugusoni

Thallus is highly branched terminal node, Reddish green colour, height of the plant 12cm, assimilator many, size of the assimilator 3.5 cm, hold fast discoid.

Gelidium species

Thallus is often pinnately branched; uniaxial construction is most clearly seen at the apex. Broadly flattened axis and in size from about 1cm tall, fleshy texture, height of the plant 17 cm, size of the assimilator 5 cm, hold fast are discoid shaped.

Gracilaria verrucosa var.

Thalli are usually highly branched; flattened, long filamentous, dichotomously branched, height may be 15 cm, assimilator 3.2 cm, assimilator length 4 cm, holdfast large disc shaped.

The characteristic microscopic features are found out using cross section and are displayed in Figure 1 and 2. The results of ash analysis of different seaweeds are given in TABLE 1. The results showed the presence of more amount of total ash followed by sulphated ash in all the seaweeds compared to water soluble and acid insoluble ashes. The fluorescence analysis of different seaweeds treated with various chemical reagents is tabulated in TABLE 2. The colour of the extracts from organic and inorganic solvents was observed both under ordinary and UV light. There is little difference between extracts and the light sources. As green plants, most of the treatment show the characteristics of different shades of green under visible and UV light.

The results of the phytochemical analysis were tabulated in TABLE 3. The presence of phenols, protein, amino acids and reducing sugar were observed in all the seaweeds of Rhodophyceae. Steroids were present only in methanol extract of all seaweeds except *Gracilaria verrucosa* var. Triterpenoids were found in all seaweeds except aqueous extracts of *Gracilaria verrucosa* and *Gelidium* species. Catechins and anthroquinones were present only in methanol hot extract of *Enatiocladia prolifera*. TLC analysis is performed in order to obtain high resolution and reproducible peaks. The seaweeds showing different Rf values are illustrated in TABLE 4.

The observations shown in the present morphological studies were similar to previous studies reported viz. *Gracilaria denticulata*[24] and *Gracilaria Vieillardii*[25]. Similarly, the present anatomical studies correlate with previous studies viz. *Hypnea musciformis*[26] and *Gracilaria aculeata*[27]. The physico-chemical evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs[28]. The ash value of the drug gives an idea of the inorganic composition and other impurities present in the plant species[29]. This provides a basis to identify the quality and purity of the drug[30]. 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[31].

Preliminary phytochemical screening of selected seaweeds revealed the presence of steroids, triterpenoids, alkaloids, tannins, phenols, saponins, flavonoids

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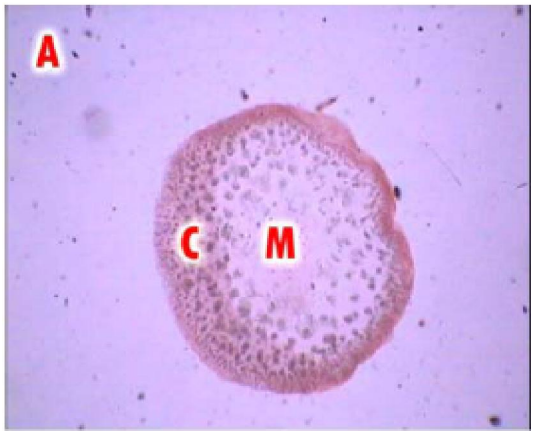

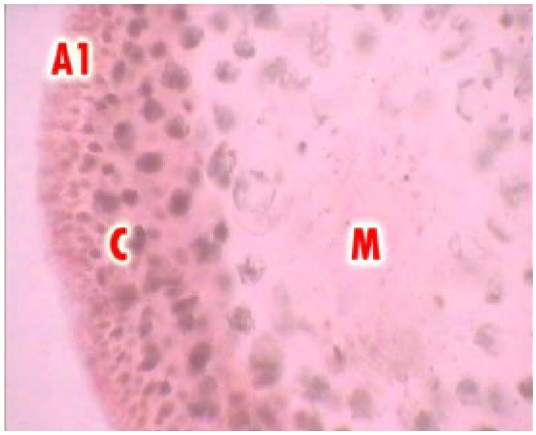

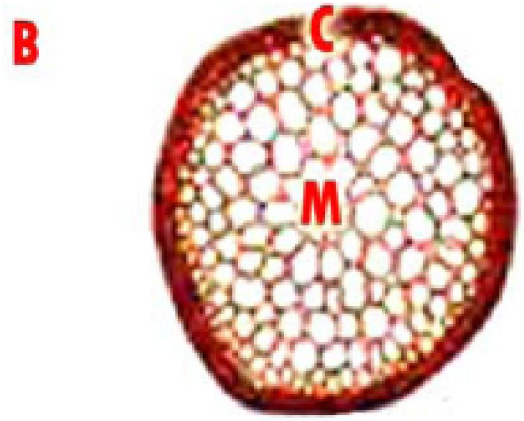

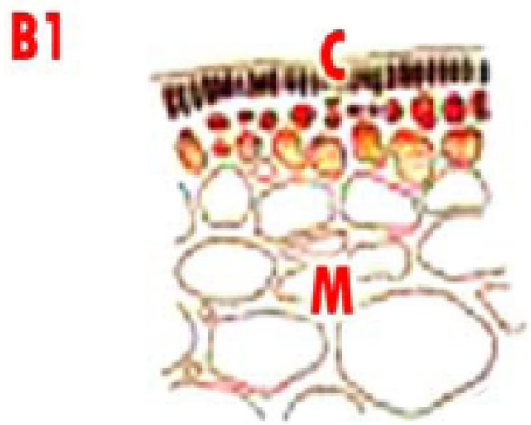

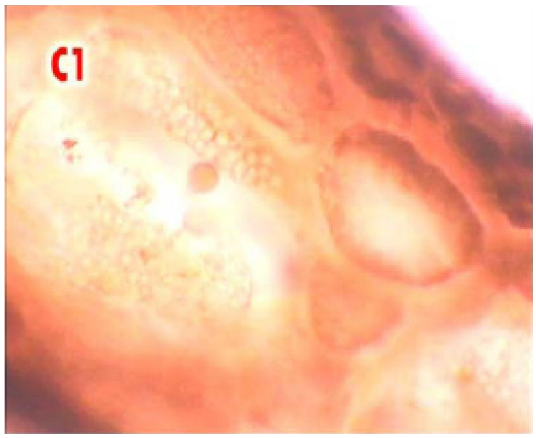

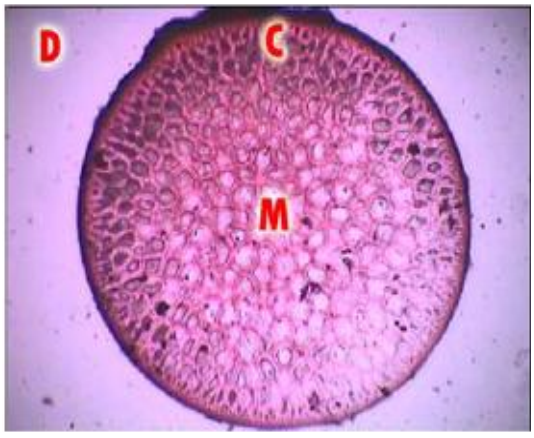

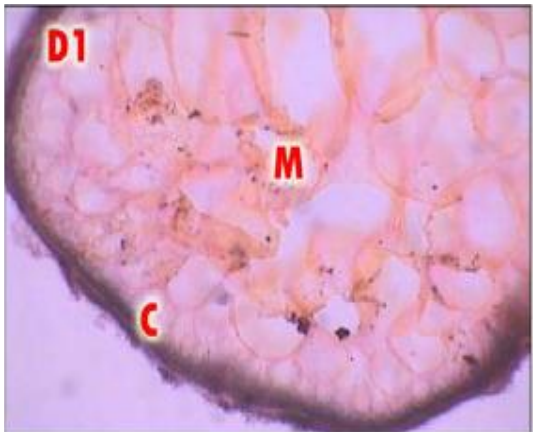

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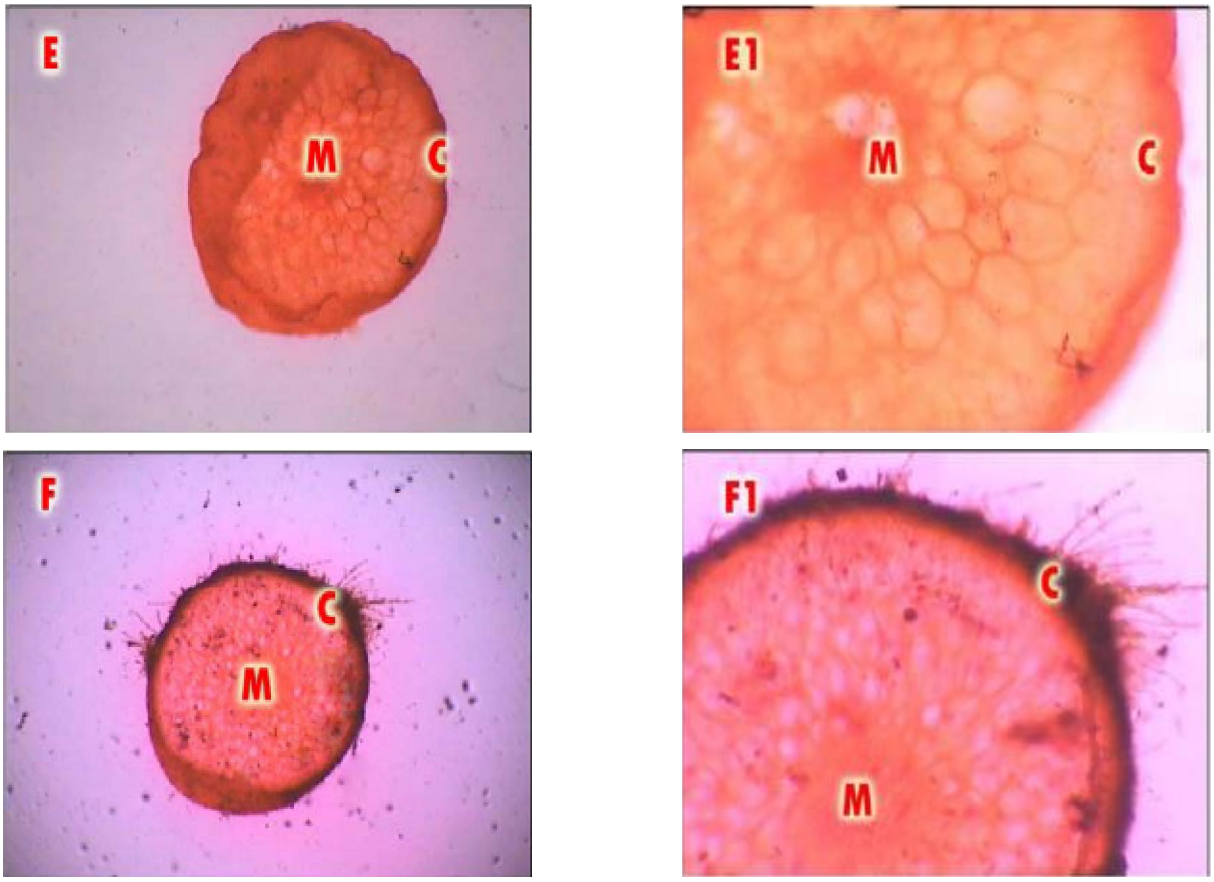

A - *Gracilaria verrucosa* (Outer view); A1 - *Gracilaria verrucosa* (A portion enlarged); B - *Hypnea musciformis* (Outer view); B1 - *Hypnea musciformis* (A portion enlarged); C - *Enatiocladia prolifera* (Outer view); C1 - *Enatiocladia prolifera* (A portion enlarged); D - *Gracilaria ferugosoni* (Outer view); D1 - *Gracilaria ferugosoni* (A portion enlarged); E - *Gelidium* species (Outer view); E1 - *Gelidium* species (A portion enlarged); F - *Gracilaria verrucosa* var. (Outer view); F1 - *Gracilaria verrucosa* var. (A portion enlarged); M – Medulla; C - Cortex

Figure 1 : C.S. of assimilator of selected seaweeds of Rhodophyceae

TABLE 1 : Ash analysis of selected seaweeds of Rhodophyceae

S. No	Parameters	*Gracilaria verrucosa*	*Hypnea musciformis*	*Enatiocladia prolifera*	*Gracilaria ferugusoni*	*Gelidium* species	*Gracilaria verrucosa* var.
1.	Total ash	2.4	2.1	2.5	2.1	2	2.2
2.	Water soluble ash	0.3	0.3	0.2	0.3	0.3	0.3
3.	Acid insoluble ash	0.9	0.7	0.8	0.6	0.4	0.5
4.	Sulphated ash	1.2	1.1	1.5	1.2	1.3	1.2

TABLE 2 : Fluorescence analysis of selected seaweeds of Rhodophyceae

S. No	Solvents	*Gracilaria verrucosa*		*Hypnea musciformis*		*Enatiocladia prolifera*		*Gracilaria ferugusoni*		*Gelidium* species		*Gracilaria verrucosa* var.	
		Visible light	UV light	Visible light	UV light	Visible light	UV light	Visible light	UV light	Visible light	UV light	Visible light	UV light
1.	Acetone	Light green	Pale green	Light green	Green	Light green	Green	Pale green	Light green	Light green	Fluorescence green	Golden yellow	Light green
2.	Aqueous NaOH	Yellowish green	Light green	Brown	Golden green	Brown	Green	Yellowish green	Green	-	Fluorescence green	Light yellow	Light green
3.	50% H_2SO_4	-	Pale green	Black	Dark blue	Black	Dark blue	Pale yellow	White	Blackish brown	Brown	-	Pale green
4	1N HCl	Dark brown	Dark green	Green	Golden green	Green	Fluorescence green	Dark brown	Dark blue	Dark brown	Blue	-	Pale green
5.	Ethanol	Dark green	Fluorescence green	Yellow	Fluorescence green	Yellow	Fluorescence green	Light yellow	Fluorescence green	Light green	Green	Light green	Fluorescence green

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S. No	Solvents	*Gracilaria verrucosa*		*Hypnea musciformis*		*Enatiocladia prolifera*		*Gracilaria ferugusoni*		*Gelidium* species		*Gracilaria verrucosa* var.	
		Visible light	UV light	Visible light	UV light	Visible light	UV light	Visible light	UV light	Visible light	UV light	Visible light	UV light
6.	Methanol Hot	Dark green	Yellowish green	Light green	Yellow green	Brown	Light green	Yellowish green	Green	Green	Fluorescence green	Dark green	Fluorescence green
7.	Methanol Cool	Green	Fluorescence green	Light green	Yellow green	Dark green	Fluorescence green	Dark green	Yellowish green	Light green	Fluorescence green	Yellowish green	Fluorescence green
8.	Aqueous Hot	Brown	Light green	Light brown	White	Reddish brown	Green	-	Light green	Pale	Light green	Light brown	White
9.	Aqueous Cool	Black	Light blue	Light brown	White	Brown	Blue	Black	Blue	-	Light green	Black	Light blue

TABLE 3 : Preliminary phytochemical analysis of selected seaweeds of Rhodophyceae

S. No	Name of the species	Name of the extract	Steroids	Triterpenoids	Reducing sugar	Alkaloids	Phenols	Flavonoids	Catechins	Saponins	Tannins	Anthroquinone	Protein	Amino acids
1.	*Gracilaria verrucosa*	M - Sox	+	+	+	+	+	+	-	+	+	-	+	+
		M - Col	+	+	+	-	+	+	-	+	+	-	+	+
		A- Sox	-	-	+	-	+	+	-	+	+	-	+	+
		A-Cool	-	-	+	+	+	+	-	+	+	-	+	+
2.	*Hypnea musciformis*	M- Sox	+	+	+	-	+	-	-	+	+	-	+	+
		M-Cols	+	+	+	-	+	-	-	+	+	-	+	+
		A- Sox	-	+	+	+	+	+	-	+	-	-	+	+
		A-Cool	-	+	+	+	+	+	-	+	+	-	+	+
3.	*Enatiocladia prolifera*	M- Sox	+	+	+	-	+	-	+	+	-	+	+	+
		M-Cool	+	+	+	-	+	+	-	+	+	-	+	+
		A- Sox	-	+	+	-	+	+	-	-	+	-	+	+
		A-Cool	-	+	+	-	+	+	-	-	+	-	+	+
4.	*Gracilaria Ferugusoni*	M- Sox	+	+	+	-	+	+	-	+	+	-	+	+
		M-Cool	+	+	+	+	+	+	-	+	+	-	+	+
		A - Sox	-	+	+	-	+	+	-	-	+	-	+	+
		A-Cool	-	+	+	+	+	+	-	-	+	-	+	+
5.	*Gelidium* species	M- Sox	+	+	+	+	+	+	-	-	+	-	+	+
		M-Cool	+	+	+	+	+	+	-	-	+	-	+	+
		A- Sox	-	-	+	+	+	+	-	-	+	-	+	+
		A- Cool	-	-	+	+	+	+	-	-	-	-	+	+
6.	*Gracilaria verrucosa* var.	M- Sox	-	+	+	-	+	+	-	-	+	-	+	+
		M-Cool	-	+	+	-	+	+	-	-	+	-	+	+
		A- Sox	-	+	+	-	+	+	-	-	+	-	+	+
		A- Cool	-	+	+	-	+	+	-	-	+	-	+	+

TABLE 4 : TLC separation of selected seaweeds of Rhodophyceae

S. No	Species	Rf value
1.	*Gracilaria verrucosa*	0.9069
2.	*Hypnea musciformis*	0.9302
3.	*Enatiocladia prolifera*	0.8727
4.	*Gracilaria ferugusoni*	0.8837
5.	*Gelidium* species	0.9454
6.	*Gracilaria verrucosa* var.	0.836

etc. in methanol and aqueous extracts. There are numerous reports of bioactive compounds derived from macroalgae with a broad range of biological activities, such as antibacterial[32], antivirals[33], antitumorals[34], anticoagulant[35] and antifouling[36] agents. Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae[37]. Considering their great taxonomic diversity, investigations related to the search of

new biologically active compounds from algae can be seen as an almost unlimited field. Among the different compounds with functional properties, antioxidants are the most widely studied. The important role of antioxidants in human health has been demonstrated thus increasing the interest in such products and their demand by consumers[11]. Brazilian red algae have been found to have phenolic substances. *Kappaphycus alvarezzi* has nutritive and antioxidant property; different parts of the thalli are also known to differ in their antimicrobial potential[38]. Similarly, some microalgae contain excrete pharmacologically active compounds. For example, *Gymnodinium* sp. and *Gonyaulax* sp. produce an alkylguanidine compound that affects the central nervous system. Brominated bi-indoles of *Rivularia firma* show pharmacological activity. *Gracilaria lichenoides*, a red alga, excretes prostaglandins[39]. Seaweeds are known to contain reactive antioxidant molecules such as ascorbate and glutathione (GSH) when fresh, as well as secondary metabolites, including carotenoids (α- and β-carotene, fucoxanthin, astaxanthin), mycosporine like amino acids (mycosporine glycine) and catechins (e.g. epigallocatechin), gallate, phlorotannins (e.g. phloroglucinol), eckol and tocopherols (α-, χ-, δ-tocopherols)[40]. Brown algal polyphenols and phlorotannins work as antioxidants and antibacterial compounds[41]. Thus, the bioactive compounds detected in methanol and aqueous extracts of selected seaweeds are documented to possess various medicinal properties and health promoting effects such as antibacterial, antifungal, antiviral, antihelminthic, cytostatic, antitumorals, anticoagulant and antifouling agents in the near future. A number of studies were carried out for the phytochemical characterization using TLC[42,43]. They employed the R_f values to distinguish the plants from other species and adulterant. In the present study also we developed the TLC profile for different seaweeds which can be applied to distinguish between each other. Thus, these studies on pharmacognostical and phytochemical analysis of different seaweeds can also help the manufacturers for identification and selection of raw materials for drug production.

CONCLUSION

The parameters which are reported here can be considered as distinctive enough to identify and decide the authenticity of the drug in herbal industry/trade and this can be useful in the preparation of herbal monograph for its proper identification. Hence the present study may be valuable to supplement information in respect to its identification, authentication and standardization.

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