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Research Article

SCREENING OF PHYCOCHEMICAL CONSTITUENTS QUALITATIVELY AND QUANTITATIVELY CERTAIN SEAWEEDS FROM GULF OF MANNAR BIOSPHERE RESERVE

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ABSTRACT
The six seaweeds namely *Ulva lactuca* (L), *Caulerpa racemosa* C. *Agardh, Sargassum wightii* Greville, *Padina tetrastomatica* Hauck, *Gracilaria corticata var. cylindrica* J. Ag., *and Acanthophora spicifera* (vahl.) Boergesen, collected from the Gulf of Mannar were screened for the presence of Phycochemical constituents like primary and secondary metabolites both qualitatively and quantitatively. The present investigation revealed that saponin and polyphenol were absent in the acetone extract of seaweeds and other extracts posses all the phycoconstituents. Maximum percentage of protein (210.31±6.3 mg/g), carbohydrate (317.11±9.51mg/g) and phenol (3.02±0.09mg/g) were recorded in *Sargassum wightii* whereas lipid (81.95±2.45mg/g), anthocyanin (0.202±0.006mg/g) in *Padina tetrastomatica* and tannin (35.87±1.07 mg/g) in *Acanthophora spicifera.* Phycochemical analysis of these seaweeds revealed the presence of potential pharmaceutical activity.

Keywords: Marine macroalgae, Screening, phycochemicalconstituents, Gulf of Mannar.

INTRODUCTION

Seaweeds are one of the important constituents of the primary producers and contribute substantially to the carbon budget of the coastal ecosystem. Further, they provide habitat and food to a variety of invertebrate species and also play a significant role in their nutrient recycling[1]. The source of these nutrients is fishes, aquatic plants and aquatic animals. Among them, seaweeds are fascinating and diverse group of organisms thriving in the earth oceans and possessing treasures for the benefit of human welfare. The phycocolloid align in all brown algae, carrageenan and agar in many red algae aggressively trap metallic ions. The seaweeds containing such compound are able to remove heavy metals from food and body, excrete metals out in the stool[2].

Seaweeds have been used by human as medicine and food for at least 13,000 years. Over the past several decades, seaweeds and their extracts have generated an enormous amount of interest in the pharmaceutical industry as the fresh source of bioactive compounds with immense medicinal potential. Seaweeds are rich in antioxidant such as carotenoids, pigments, polyphenols, enzymes and diverse functional polysaccharides[3].

Seaweeds are a part of stable diet in the orient as they are nutrionally rich materials due to a much lesser extent in the rest of the world[4]. The mineral nutrients present in seaweeds are divers and the main elements being iodine and calcium. The chemical composition of seaweed varies with species, habitat, and maturity and environmental conditions[5]. Seaweeds excellent source of Vitamin A, B1, B2, C, D and E. Quality of protein and lipid in seaweeds are most acceptable for consumption compared to other vegetables mainly due to their high content in essential amino acid and relatively high level of unsaturated fatty acid[6]. In contrast to terrestrial plant materials, very few researches have been contributed on the antioxidant potential of marine seaweeds. Reports on the antioxidant properties of seaweed extracts from India are limited[7]. Seaweed produced various types of antioxidant to counteract environmental stresses. Therefore, seaweed is a potential source of novel antioxidant[8].

The seaweeds are main source of drugs for treatment of diseases but also of new and novel structures with useful biological activity. In folk medicines, seaweeds have been used for a variety of remedial purposes such as eczema, gallstone, gout, scrofula, cooling agent for fever, menstrual trouble, renal problem, scabies etc[9, 10]. Marine flora and fauna are reported to have a wide spectrum of interesting biological properties[11]. However, although review of literature revealed that unlike the terrestrial plants, the marine plants especially the seaweeds have not been studied from the pharmacognosy point of view. Hence, the present investigation is to screen the seaweeds quantitatively and qualitatively for their Phycochemicals.

MATERIALS AND METHODS

The seaweeds namely *Ulva lactuca* Linnaeus, *Caulerpa racemosa* (R. Brown ex Turner) C. Agarth, *Sargassum wightii* Greville, *Padina tetrastomatica* Hauck, *Gracilaria corticata* var. *cylindrica* J. Ag., and *Acanthophora spicifera* (vahl) Boergesen were collected in the month of September 2011, during the low tide period in the early morning time from Kanyakumari to Pudumadam at south east coast of Tamil Nadu, Gulf of Mannar region.

Description of study area

Gulf of Mannar

The Gulf of Mannar region lies between India and Srilanka on the Indian Part; it extends from Kanyakumari to Pudumadam along the main land coast to a distance of about 170 nautical miles. The maximum east to west length up to the meeting point of the Indo-Srilanka maritime boundary is about 75 nautical miles. The Gulf of Mannar is spread over on the Indian side along the longitude from 78°08′E to 79°30′E and along the latitude from 8°35′ to 9°25′.

Six station *viz.,* Pudumadam, Kilakkarai, Tuticorin, Tiruchendur, Idinthakarai and Kannyakumari lying in the Gulf of Mannar were selected for the present study (Fig. 1).

Station 1: Pudumadam

Pudumadam (Lat. 0.9°16′N; Long. 79°12′E) is a coastal fishing village of Ramanathapuram district, Tamil Nadu. This station is less polluted with industrial waste disposal.

Station 2: Kilakarai

Kilakarai (Lat. 9°15′N; Long. 79°11′E) is also coastal fishing village of Ramanathapuram district, Tamil Nadu. This station is polluted with industrial waste disposal.

Station 3 : Tuticorin

Tuticorin (Lat. 8°45′N; Long. 78°10′E) is also harbour town, the most of the ships discharge oil and the area is slightly polluted. The shore is mainly sandy without crops.

Station 4: Tiruchendur

Tiruchendur (Lat. 8°30′N; Long. 78°8′E) is a coastal town it is a pilgrim place. The shore is mainly sandy. A few sand stone rocks situated above low water level which are having rich seaweed growth. These rocks are completely sub merged during high tide and are just exposed during low tide. Collection of marine algae was made from this place during extreme low, spring tides.

Station 5: Idinthakarai

Idinthakarai (Lat. 8°10′N; Long. 77°43′E) is a small coastal village. The coast extends about 2000 m from north east to south east direction. The coast is formed with sand stones or beach rocks and located 14 km. north of Kanyakumari.

Station 6: Kanyakumari

Kanyakumari (Lat. 9°11′N; Long. 79°24′E) is southern boundary of peninsular India, where Indian Ocean, Arabian sea and Bay of Bengal meet together. Shore of the Kanyakumari is a rocky coast and the rocks get exposed during low tide to a greater extended which provide a good substance for the luxuriant growth of diversified algal flora.

Collection and Preparation of the sample:

The seaweeds were handpicked and washed thoroughly with seawater to remove all the impurities, sand particles and epiphytes. It was kept in an ice box containing slush ice, transported to the laboratory and washed thoroughly with tap water to remove the salt on the surface of the material. The water was drained off from the seaweed material was spread on blotting paper to remove excess water, shade dried separately and powdered.

Preparation of the organic extract of the sample and phycochemical analysis:

The screenings of phycochemical seaweeds powder sample were used. 50 gram of powder was weighed and successively extracted with 250 ml of different solvent such as petroleum ether, benzene, chloroform, acetone and methanol with increasing order of polarity by soxhlation for 6 hours. All the extracts of the six seaweeds were subjected to qualitative tests for the identification of various Phycochemical constituents as per the standard procedures [12, 13, 14].

Qualitative and quantitative analysis of phycochemical:

Screening test for the presence of phycochemical:

The powdered samples were analyzed for quantification of protein[15], carbohydrate[16], lipid[17], tannins [18] and phenol[19]. Fresh seaweeds were analyzed for anthocyanin pigments[20]. All the above mentioned biochemical's were estimated using SL 27 Elico spectrophotometer.

RESULT

The present investigation revealed that saponin and polyphenol were absent in the acetone extract of seaweeds and other extracts posses all the phycoconstituents (Table 1-3). Maximum percentage of protein (210.31±6.3 mg/g), carbohydrate (317.11±9.51mg/g) and phenol (3.02±0.09mg/g) were recorded in *Sargassum wightii* whereas lipid (81.95±2.45mg/g), anthocyanin (0.202±0.006mg/g) in *Padina tetrastomatica* and tannin (35.87±1.07 mg/g) in *Acanthophora spicifera* were observed (Table-4). Phycochemical analysis of these seaweeds revealed the presence of potential pharmaceutical activity.

DISCUSSION

The results of the Phycochemical screening are. Petroleum ether, benzene and methanol extracts of *Ulva lactuca* and *Gracilaria corticata* showed the presence of all the fourteen secondary metabolites. Whereas the chloroform and acetone extracts of these two seaweeds did not answer for phenols. Extracts of *Caulerpa racemosa, Sargassum wightii* and *Padina tetrastomatica* obtained in the petroleum ether, benzene and chloroform answered for all the phycochemical. However, the methanol and acetone extracts of *Caulerpa racemosa, Sargassum wightii* and *Padina tetrastomatica* did not exhibit the presence of phycophenols. Interestingly in the case of *Acanthophora spicifera,* all Phycochemical were present in all the organic extracts except acetone. Phenol was absent in the acetone extract of *Acanthophora spicifera.* Saponin was absent in the acetone extract of all the six seaweeds.

Noticeable variation in the quantity of many of the biochemical contents among the six seaweeds. Highest amount of protein, carbohydrate and phenols was recorded in the brown seaweed *Sargassum wightii.* The other brown seaweed *Padina tetrastomatica* showed maximum value of lipid and anthocyanin content. Highest amount of tannin was recorded in the red seaweed *Acanthophora spicifera.*

It has been reported that the presence of phycoconstituents such as flavonoids, tannins and polyphenols help in preventing a number of disease through free radical scavenging activity[21]. Antitumor and antioxidant properties have been attributed to the flavonoids based on *invitro* and *invivo* studies both in humans and in animals[22]. These phenolics which include phenol, tannin and flavonoids have been found to be present in appreciable amount in all the six seaweeds.

The presence of anthocyanin, a class of flavonoids in the seaweeds gains significance because of the following facts. Anthocyanin protects tiny blood vessels from free radical damage and stimulates the formation of healthy connective tissue[23]. Since anthocyanin helps in regenerating rodopsin, a purple pigment needed for the night vision and adaptation to light, a number of anthocyanin preparations are flooding the market and the secondary metabolites phenolics are found to play a greater role in the maintenance of the human body[24]. The presence of various secondary metabolites in these seaweeds is a clear indication of their pharmaceutical potential. The secondary metabolites may be useful in containing infection, act as hypolipomic and hypoglycemic agents, reduce blood pressure and regulate cholesterol levels[25].

As per the earlier reports, seaweeds contain large amount of polysaccharides but less amount of protein and amino acids[26]. The total Phenol content of edible Irish brown seaweeds, *Himanthalia elongata* was found to be at higher level[27]. Studies on brown seaweed, Sargassum wightii showed the presence of steroid and flavonoids. The seaweeds are low in fats but contain vitamins and bioactive compounds such as

terpenoids and sulphated polysaccharide a potential natural antioxidant which are not found in land plants property[28]. Seagrasses are also rich source of secondary metabolites particularly phenolic compound[29]. Nature has been a source of medicinal agent for thousand of years and an impressive number of modern drugs have been isolated from natural sources[30]. Hence these biochemical characters make the seaweeds nutraceutical in nature and therefore important as food supplement in order to give good health and disease resistant. More detailed pharmagonostic study of these seaweeds is necessary.

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Table 1: Phycochemical screening of green seaweed extracts

Solvent	Seaweed	Alkaloids	Terpinoids	Steroids	Coumarin	Tannin	Saponin	Flavonoids	Anthroquinine	Quinine	Polyphenols	Proteins	Carbohydrates	Catachin	Glycosides
Petroleum	*U. lactuca*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C. racemosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Benzene	*U. lactuca*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C. racemosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chloroform	*U. lactuca*	+	+	+	+	+	+	+	+	+	-	+	+	+	+
	C. racemosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acetone	*U. lactuca*	+	+	+	+	+	-	+	+	+	-	+	+	+	+
	C. racemosa	+	+	+	+	+	-	+	+	+	-	+	+	+	+
Methanol	*U. lactuca*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C. racemosa	+	+	+	+	+	+	+	+	+	-	+	+	+	+

+ Present, _ Absent *U. lactuca = Ulva lactuca; C.racemosa= Caulerpa racemosa*

Table 2: Phycochemical screening of brown seaweed extracts

Solvent	Seaweed	Alkaloids	Terpinoids	Steroids	Coumarin	Tannin	Saponin	Flavonoids	Anthroquinine	Quinine	Polyphenols	Proteins	Carbohydrates	Catachin	Glycosides
Petroleum	***S. wightii***	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	P. tetrastomatica	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Benzene	***S. wightii***	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	P. tetrastomatica	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chloroform	***S. wightii***	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	P. tetrastomatica	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acetone	***S. wightii***	+	+	+	+	+	-	+	+	+	-	+	+	+	+
	P. tetrastomatica	+	+	+	+	+	-	+	+	+	-	+	+	+	+
Methanol	***S. wightii***	+	+	+	+	+	+	+	+	+	-	+	+	+	+
	P. tetrastomatica	+	+	+	+	+	+	+	+	+	-	+	+	+	+

+ Present, _ Absent *S. wightii = Sargassum wightii; P. tetrastomatica = Padina tetrastomatica*

Table 3: Phycochemical screening of red seaweed extracts

Solvent	Seaweed	Alkaloids	Terpinoids	Steroids	Coumarin	Tannin	Saponin	Flavonoids	Anthroquinine	Quinine	Polyphenols	Proteins	Carbohydrates	Catachin	Glycosides
Petroleum	*G. corticata*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	A. spicifera	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Benzene	*G. corticata*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	A. spicifera	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chloroform	*G. corticata*	+	+	+	+	+	+	+	+	+	-	+	+	+	+
	A. spicifera	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acetone	*G. corticata*	+	+	+	+	+	-	+	+	+	-	+	+	+	+
	A. spicifera	+	+	+	+	+	-	+	+	+	-	+	+	+	+
Methanol	*G. corticata*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	A. spicifera	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ Present, _ Absent *G. corticata = Gracilaria corticata; A. spicifera = Acanthophora spicifera*

Table 4: Phycochemical content (mg/g) of six seaweed species

Seaweed	Protein (mg/g f.wt.)	Carbohydrate (mg/g f.wt.)	Lipid (mg/g f.wt.)	Tannin (mg/g f.wt.)	Phenol (mg/g f.wt.)	Anthocyanin (mg/g f.wt.)
Ulva lactuca	134.31±4.06	294.59±8.83	57.94±1.73	28.01±0.84	0.52±0.1	0.005±0.0001
Caulerpa racemosa	80.46±2.41	172.08±5.16	32.27±0.96	26.95±0.80	0.44±0.01	0.029±0.0008
Sargassum wightii	201.31±6.3	317.11±9.51	73.92±2.21	30.89±0.92	3.2±0.09	0.082±0.002
Padina tetrastomatica	140.53±4.21	284.65±8.53	81.95±2.45	27.68±0.83	1.85±0.05	0.202±0.006
Gracilaria corticata	161.68±4..85	295.18±8.85	40.19±1.20	30.94±0.92	0.46±0.01	0.018±0.0005
Acanthophora spicifera	68.86±2.06	144.62±4.32	39.41±1.18	35.87±1.07	0.51±0.01	0.030±0.0009

Values are mean SD; sample size (n) = 3.

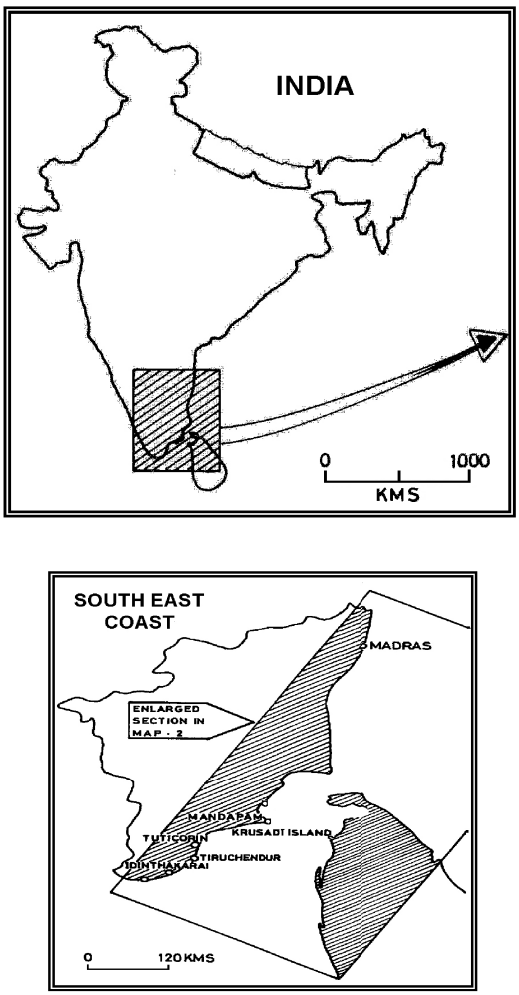

Fig. 1. Map showing the study areas

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