Research Article

Phycochemical Screening of Seaweeds from Sindhudurg District of Maharashtra

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ABSTRACT

Phycochemical screening of thirteen seaweed species from Sindhudurg District of Maharashtra was done employing qualitative and quantitative analysis. Tannins, phenols, flavonoids, alkaloids and coumarin were present in methanolic and ethanolic extracts of all the seaweeds. Phenols and flavonoids were significant in the brown seaweeds and alkaloids were prominent in the red species of seaweeds. Phycochemical tests showed positive results to carbohydrates, proteins, flavonoids in all the seaweeds. From the present study it was concluded that the phycochemical and fluorescence analysis could be used for rapid identification of seaweeds having medicinal value and bioactive compounds present in them. The data obtained in this study suggested that seaweeds possess a good nutritional as well as pharmaceutical potential.

Keywords: Fluorescence study, Phycochemical analysis, Seaweeds.

INTRODUCTION

Seaweeds produce a great variety of secondary metabolites possessing several biological activities like antiviral, antihelminthic, antifungal, antibacterial etc.[1] Phytochemical and pharmacognostical studies of a few seaweeds from India such as *Sargassum wightii, Cladophora glomerata, Ulva lactuca, Ulva reticulata, Gracilaria corticata, Kappaphycus alvarezii* etc. for their pharmaceutical studies have been carried out.[2]

Kunakeshwar is a rocky coastal region of Sindhudurg district of Maharashtra which supports a luxuriant growth of macroalgae. Biological activities of seaweed species from this region are not explored. Intention of this study is to investigate pharmacognostical properties of some seaweed through qualitative and quantitative phycochemical analysis.

MATERIALS AND METHODS

Collection of Seaweeds

Fresh seaweed species belonging to green, brown and red groups were collected during low tide from Kunakeshwar in Sindhudurg district of Maharashtra [16°40.120'N Latitude and 073°28.120' E Longitude] and brought to laboratory. A total of thirteen species were collected consisting of green seaweeds: *Ulva lactuca* Lin., *Ulva fasciata* (Delile), *Chaetomorpha antennina* (Bory de Saint-Vincent) Kutzing, *Chaetomorpha media* (C. Agardh) Kutzing and *Enteromorpha intestinalis* (Linnaeus) Nees, brown seaweeds: *Sargassum cinereum* J.Agardh, *S. ilicifolium* (Turner)C, *S.tenerrimum* J.Agardh and *Padina tetrastromatica* Hauck and red seaweeds: *Gelidiella acerosa* (Forsskål) Feldmann & G.Hamel, *Gracilaria corticata* (J. Agardh), *Acanthophora specifera* (M. Vahl) Borgesen and *Jania rubens* (Linnaeus) J.V.Lamouroux .

Fresh algal material was washed thoroughly with tap water to remove epiphytes and other contamination and then shade dried at room temperature for seven days. Then it was ground to a fine powder and stored in air tight containers in dark, away from moisture and used for analysis.

Preparation of algal extract

Two grams of seaweed powder were extracted in 20 ml distilled water or organic solvent (Ethanol/ Methanol/Acetone) for 24 hours on a rotary shaker at a constant speed (170 strokes per minute). The extract was filtered through a Buckner's funnel using Whatman No. 1 filter paper and volume of filtrate was adjusted with respective solvent. This extracts was stored in glass vials at 11°C.

Phycochemical analysis

Algal extracts were subjected to qualitative tests for the identification of various phycochemical constituents such as phenols, flavonoids, tannins, alkaloids, coumarine, saponins, quinine, carbohydrate, terpenoids, glycosides, phlobatanins, steroids and proteins following standard procedures.[3]

Pharmacognostical analysis

Fluorescence analysis of algal extracts was carried out as per Pandurangan *et al*[2]. Dry seaweed powder was mixed with conc. mineral acids (H_2SO_4, HCl and HNO_3), $FeCl_3$ (5%), KOH (5%), ethanol and acetic acid and the change in color was recorded.

Biochemical Analysis

Total carbohydrate content was estimated using Anthrone reagent and total phenolic content using Folin-Ciocateu reagent.[4] Total flavonoids were measured by the

method described by Luximon-Ramma[5] and total alkaloid content was analyzed by following method of Singh *et al*.[6]

RESULTS AND DISCUSSION

Results of qualitative analysis of seaweeds are presented in Tables 1 & 2. Flavonoids, carbohydrate and proteins were present in all the 13 seaweeds while terpenoids and glycosides were absent in all the organic as well as aqueous extracts of seaweeds. Tannin and phenol were detected in methanol, ethanol and aqueous extracted seaweeds and were absent in the acetone extracts. Saponin and phlobatannin were observed in methanol, ethanol and aqueous extracts of green and red seaweeds and absent in acetone extract and in all the extracts of brown seaweeds. Methanolic and ethanolic extracts of all the seaweeds showed the presence of coumarine and alkaloid. While steroids were observed in ethanolic extracts of green and red seaweeds. In general the phytochemical constituents were extracted at a high concentration in the ethanolic and methanolic extracts of seaweeds. Thinakaran *et al*[7] detected all secondary metabolites in petroleum ether, benzene and chloroform extracted material during phytochemical screening *of Ulva lactuca*, *Gracilaria corticata, Sargassum wightii* and *Padina tetrastromatica*. Janarthanan and Senthilkumar[8] have reported presence of alkaloids, steroids, tannins, saponins and flavonoids in petroleum ether and methanol extracts of *Sargassum wightii.*

The results of fluorescence analysis are presented in Table 3. These studies revealed specific colours of seaweed extracts in presence of UV and visible light. In green seaweeds green and greenish black, in brown seaweeds brown and black and in red seaweeds brownish orange and red colours were observed under visible and UV light (260 nm) respectively. These tests are used to confirm the purity of samples in Pharmacognosy. In green seaweeds dark green and green and brown seaweeds dark red and brown colour have been observed in fluorescence studies.[2&9]

Table 1: Qualitative phycochemical screening of seaweeds

Seaweed	Alkaloids				Coumarine				Tannins				Saponins				Flavonoids				Quinine				Carbohydrates			
	M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac
Ulva lactuca	+	+	-	+	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+	-	+	+	-	+	+	+	+
Ulva fasciata	+	+	-	+	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+	-	+	+	-	+	+	+	+
Chaetomorpha media	+	+	-	+	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+	-	+	+	-	+	+	+	+
Chaetomorpha antennina	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+	-	+	+	-	+	+	+	+
Enteromorpha intestinalis	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+	-	+	+	-	+	+	+	+
Sargassum cinereum	+	+	-	-	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Sargassum ilicifolium	+	+	-	-	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Sargassum tenerrimum	+	+	-	-	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Padina tetrastromatica	+	+	-	-	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Gelidiella acerosa	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+	+
Gracilaria corticata	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+	+
Acanthophora specifera	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+	+
Jania rubens	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+	+

+ present, - absent M - Methanol , E - Ethanol, Aq - Aqueous, Ac - Acetone

Table 2: Qualitative phycochemical screening of seaweeds

Seaweed	Terpenoids				Phenols				Glycosides				Phlobatanins				Steroids				Proteins			
	M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac	M	E	Aq	Ac
Ulva lactuca	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-	-	+	-	-	+	+	+	+
Ulva fasciata	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-	-	+	-	-	+	+	+	+
Chaetomorpha media	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-	-	+	-	-	+	+	+	+
Chaetomorpha antennina	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-	-	+	-	-	+	+	+	+
Enteromorpha intestinalis	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-	-	+	-	-	+	+	+	+
Sargassum cinereum	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Sargassum ilicifolium	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Sargassum tenerrimum	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Padina tetrastromatica	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Gelidiella acerosa	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	+	+	+
Gracilaria corticata	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	+	+	+
Acanthophora specifera	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	+	+	+
Jania rubens	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	+	+	+

+ Present, - absent, M - Methanol, E - Ethanol, Aq - Aqueous, Ac - Acetone.

Table 3: Fluorescence analysis of seaweeds

Seaweed Tests	Conc. H_2SO_4		Conc. HCl		Conc.HNO_3		5%$FeCl_2$		Ethanol		Acetic acid		5%KOH	
	Visible	UV	Visible	UV	Visible	UV	Visible	UV	Visible	UV	Visible	UV	Visible	UV
Ulva lactuca	Gr	Br	Gr	Bw	PGr	Bw	PGr	Bl	Gr	Bl	Gr	Bl	Gr	Bl
Ulva fasiata	OGr	Bw	DGr	Bl	Gr	Bl	Gr	Bl	YwGr	RdBw	YwGr	DBw	YwGr	DBw
Chaetomorpha media	Gr	Bw	PGr	Bw	DGr	GrBl	OGr	Gr	OGr	Bw	OGr	Bw	OGr	Bw
Chaetomorpha antennina	OGr	Bw	DGr	Bl	Gr	Bl	PGr	Bw	YwGr	Bw	YwGr	RdBw	PGr	GrBw
Enteromorpha intestinalis	PGr	Bw	Gr	Bl	DGr	Bl	Gr	Bl	DGr	GrBl	DGr	Bl	DGr	Bl
Sargassum cinereum	Bw	Bl	DGr	Bl	DGr	Bl	Bw	Bl	Bw	Bl	Bw	Bl	BwOr	Bl
Sargassum ilicifolium	Bw	Bl	GrBl	Bl	GrBl	Bl	Bw	Bl	Bw	Bl	Bl	Bl	BwOr	Bl
Sargassum tenerrimum	Bw	Bl	Gr	Bw	Gr	Bw	DGr	Bl	Bl	Bl	Bw	Bl	BwOr	Bl
Padina tetrastromatica	Bw	Bl	GrBl	Bl	Gr	Bl	DBw	Bl	Bl	Bl	Bl	Bl	Bw	Bl
Gelidiella acerosa	BlRd	Bw	Or	Rd	Or	Rd	Bw	Bl	BwBl	Bl	Or	Rd	DOGr	Bl
Gracilaria corticata	GrBl	Bw	BwOr	Rd	Or	Rd	Bw	Bl	Bw	Bl	BwOr	Rd	As	Wh
Acanthophora specifera	RdBw	Bw	BwOr	Rd	Or	Rd	Bw	Bl	BwBl	Bl	BwOr	Rd	Bl	GrBl
Jania rubens	As	Wh	Pk	Rd	Pk	Rd	GrBl	Bl	Pk	Rd	Bl	Rd	As	Wh

As=Ash; Bl=Black; BlRd= Blackish red; Bw = Brown; BwBl= Brownish black; BwOr=Brownish orange; DBw= Dark brown; DGr=Dark green; DOGr=Dark Olive green; Gr = Green; GrBl=Greenish black; GrBw=Greenish brown; Or= Orange; OGr= Olive green; PGr=Pale green; Pk=Pink; Rd=Red; RdBw=Reddish brown; Wh=White; YwGr= Yellowish green.

Amount of various biochemical compounds in seaweeds is given in Table 4. Carbohydrate content was high in green seaweeds maximum being in *Chaetomormpha media* (152.76 mg/g) and minimum was recorded in brown seaweed *Padina tetrastromatica* (68.12 mg /g). In green and red seaweeds phenols were less (about 1.0 mg/g) except *Enteromorpha intestinalis* and *Gracilaria corticata* which had a higher amount. Flavonoids were in the range of 0.3 to 0.4 mg/g in all the green and red seaweeds. Highest amount of phenols and flavonoids was recorded in the species of *Sargassum*. A higher alkaloid content in red seaweeds (*Gracilaria corticata* and *Acanthophora spicifera)* was significant as compared to brown and green seaweeds.

High carbohydrate content has been reported in seaweeds *Caulerpa racemosa, Acanthophora spicifera, Enteromorpha intestinalis, Ulva rigida* and *Sargassum wightii* by various workers.[10-11] High values of total phenols and flavonoids for brown seaweed *Sargassum wightii* have been shown by Seenivasan.[12] Alghazeer *et al*[13] noticed a high content of alkaloid in *Dictyopteris membranacea* and lowest content in *Gelidium latifolium.* Many reports revealed the presence of alkaloids in marine algae and some of them have been investigated for their biological activity.[14]

The presence of secondary metabolites such as alkaloids, flavonoids and phenols in crude extracts of seaweeds indicated presence of antimicrobial, antioxidant properties. Flavonoids in general and alkaloids in red species were remarkable in the present study. These species can be further worked out to extract their active metabolites. It has been reported that the most active principles in seaweeds are alkaloids, flavonoids and phenols which may be accountable for the antimicrobial actions of the particular plant. Detailed studies on the isolation and characterization of these bioactive materials are required.

Table 4: Biochemical constituents in seaweeds

Seaweed	Carbohydrates	Phenols	Flavonoids	Alkaloids
Ulva lactuca	135.00 ± 0.001	1.05±0.005	0.331±0.002	2.96±0.002
Ulva fasiata	130.00 ±0.001	1.34±0.002	0.363 ±0.005	4.44±0.002
Chaetomorpha media	152.76 ±0.001	1.25±0.005	0.427±0.005	5.28±0.002
Chaetomorpha antennina	150.76 ±0.002	1.70±0.002	0.405±0.005	5.76±0.002
Enteromorpha intestinalis	87.50±0.008	2.56±0.001	0.405±0.005	5.59±0.001
Sargassum cinereum	71.80 ±0.141	2.60±0.002	0.608±0.070	5.50±0.035
Sargassum ilicifolium	88.20 ±0.141	2.60±0.002	0.544±0.002	5.44±0.028
Sargassum tenerrimum	112.00 ±0.070	2.54±0.070	0.534±0.002	5.64±0.035
Padina tetrastromatica	68.12 ±0.070	2.32±0.070	0.480±0.070	5.58±0.056
Gelidiella acerosa	95.62 ±0.005	1.09±0.002	0.373±0.001	4.43±0.001
Gracilaria corticata	80.00 ±0.015	2.50±0.002	0.341±0.004	9.60±0.002
Acanthophora specifera	70.00 ±0.005	1.01±0.002	0.427±0.004	9.07±0.005
Jania rubens	75.00 ±0.001	1.31± 0.002	0.416±0.001	3.31±0.002

Values are expressed in mg/g, ± values represents SD**.**

CONCLUSION

Fluorescence analysis can be used as the effective marker in identifying authentic sample from its adulterant. The phytochemical screening and pharmacognostical analysis of seaweeds suggested their antioxidant and antimicrobial potential.

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