Research Article

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EVALUATION OF PHYSICOCHEMICAL PROPERTIES OF SEAWEED, *CAULERPA RACEMOSA*

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ABSTRACT

The aim of present analytical study was to evaluate the physicochemical and standardization parameters of *Caulerpa racemosa*, a green marine algae or seaweed. This seaweed was collected and identified from Gujarat state coastal area of India. The shade dried samples of seaweed were subjected to a battery of analytical processes to determine the extractive values and various physicochemical parameters. Further, seaweed was subjected to fluorescence and elemental analysis and quantitative estimation of phytoconstituents. The HPTLC of methanolic extract of seaweed was also conducted. Overall screening of various extracts indicated the presence of carbohydrate, glycoside, alkaloids, tannins, saponin, steroid and triterpenoid. High water soluble extractive value (8.5 ± 1.28 %) indicates the possibility of presence of large quantity of water soluble constituents. The IR spectrum of seaweed showed an absorption band at 2850.88 cm^{-1} indicating the presence of aldehyde; 1745.64 cm^{-1} for ester; 1462.09 cm^{-1} for alkanes and 1018.45 cm^{-1} for alcohol ester or carboxylic acid. In this study, several elements were determined in the marine alga by using Energy Dispersion X-Ray Fluorescence. In HPTLC findings, among the various solvent systems, pet ether: acetone (8:2 v/v) mobile phase was found to be more suitable for proper compound resolution. HPTLC study has narrowed-down the identification to possible compounds like steroids, saponins and alkaloids; latter was confirmed by quantitative analysis too. The present analytical study of *Caulerpa racemosa* on physicochemical parameters, preliminary phytochemical analysis, and elemental analysis provides importance information to support further on-going studies to evaluate structure of bioactive compounds and their pharmacology.

Keywords: *Caulerpa racemosa*, seaweed, algae, physicochemical, fluorescence, phytochemical

INTRODUCTION

The natural products of seaweed and other marine organisms represent one of the new frontiers in the exploration for bioactive compounds. India is sitting on a gold mine of well-recorded and well-practiced knowledge of traditional herbal medicine. India is one of the 12 leading bio-diversity centers with the presence of over 45,000 different plant species. From this flora 15,000 to 20,000 have good medicinal values.[1] Amongst all of these herbal alternatives, algae, especially marine algae, are least explored for their medicinal properties. The identification of bioactive compounds present in marine algae is a new potential area.[2] Seaweeds or macroalgae belong to the lower plants, meaning that they do not have roots, stems and leaves. Instead they are composed of a thallus (leaf-like) and sometimes a stem and a foot. Some species have gas-filled structures to provide buoyancy. They are subdivided in three groups, the red, green and brown macroalgae.[3] Initial attempts at determining the chemistry of seaweeds were simply extensions of tried and true phytochemical techniques. Thus, easily accessible sea organisms (generally sponges and encrusting organisms such as ascidians) were collected by hand using snorkel or simple scuba systems, and then their chemical components were extracted and identified. Eventually, the modern screening programmes are motivated by the chemical ecology of this marine organisms.[4] *Caulerpa racemosa*, a large, edible green algae, is widely distributed in tropical and subtropical areas of Brazil and other countries. It is commonly known as "sea grapes" and is found in many areas of shallow sea around the world. There are a number of different forms and varieties. The present study was conducted to analyze the phytochemical properties of one of the unexplored seaweeds, *Caulerpa racemosa* (family, caulerpaceae), collected from the Indian coast. Caulerpa comes from a family of bisindole natural products, and it shows a variety of important biological activities already described in the literature, among which the important one to mention are the antitumor[5], anti-inflammatory[6] and growth regulator[7]. *Caulerpa racemosa* contains sulphated polysaccharides (SP), with anticoagulant and antiviral activity, and recently it has been shown that SP fractions from *Caulerpa racemosa* have significant antitumor activity[8]. However, to date there is a scarcity of investigations supporting the phytochemical screening of this seaweed. Therefore, we have undertaken this research to explore the phytochemical properties of this green seaweed, *Caulerpa racemosa*.

MATERIAL AND METHODS

Collection and Identification of Seaweed

Seaweed, *Caulerpa racemosa* was collected from the Gujarat coast (west) of India. Further, it was washed thoroughly, kept for overnight to remove dirt and unnecessary material. Then it was subjected for shade drying then prepared into samples to preserve its phytochemical properties. Since, this particular seaweed type is very common in Gujarat coastal area; the collected

seaweed sample was authenticated by a local commercial seaweed based organization.

Preparation of Extracts

Coarse powders (50 g) of marine alga was individually extracted with sufficient quantity of different solvents *viz.* ether, chloroform, ethyl acetate, acetone, ethanol, methanol and water for 48 h by maceration and then filtered to obtain respective extracts. The extracts in different solvent were collected separately and volume reduced under low pressure. 25 ml of the each extract was used to determine the percentage extractive values in different solvents. The remaining extract was stored in air tight glass container at 4-8°C for further analysis.

Preliminary Phytochemical Investigation

Preliminary phytochemical investigation of marine alga extracts in different solvents was performed to detect the phytoconstituents like; alkaloid, amino acid, carbohydrate, glycoside, mucilage, tannin, starch, saponin, steroid, triterpenoid and flavonoid.[9,10]

Determination of Physicochemical Parameters

The shade dried seaweed *Caurlepa racemosa* was subjected for determination of physicochemical parameters such as foreign organic matter, loss on drying, total ash content, acid insoluble ash, water soluble ash, ethanol soluble extractives, water soluble extractives, according to methods described in Indian Pharmacopoeia and WHO guidelines on quality control methods for medicinal plants materials.[11-13] IR spectra (KBr disc) were obtained with a JASCO FTIR 420 spectrophotometer.

Fluorescence Analysis

The dried algae was subjected to fluorescence analysis. It was treated separately with 1 N of HCl, HNO_3, H_2SO_4, NaOH, KOH, alcoholic NaOH, alcoholic KOH and ammonia against normal and ultra-violet light (254 nm). Color reaction of hexane, chloroform, ethyl acetate, methanol and water extract was also observed in normal light and UV light (254 nm).[14]

Elemental Analysis

The element content of the seaweed samples was analyzed by atomic absorption spectrophotometry (AAS) for Ca, K, Mg and Na[15]. Inductively coupled plasma optical emission spectrometry (ICP-OES) was used for determination of Cu, Fe and Zn[15], gravimetric method for P and chloride analyzer for Cl. Analyses of two toxic elements, namely Pb and Cd, was conducted by flame atomic absorption spectrophotometer according to the methods of Evan[16] and Suddendorf[17].

Quantitative Estimation of Phytoconstituents

Polysaccharides Extraction

The seaweed was dried in an electric oven at a temperature not exceeding 40°C, and obtained in a dry powdered form. It was then extracted by cold water extraction method. The same extraction process was repeated twice for exhaustive extraction of polysaccharides; then combined the aqueous extract slowly while stirring four volumes of ethanol 95 %. The precipitate obtained by centrifugation was washed several times with ethanol. The polysaccharides was then vigorously stirred in absolute acetone, filtered and then dried in vacuum desiccators over anhydrous calcium chloride.[18]

Alkaloid Estimation

5 g of marine algae powder was extracted with 10 % acetic acid in ethanol and allowed to stand for 4 h. This was filtered and concentrated on a water bath to one-quarter of its original volume. Concentrated ammonium hydroxide solution was added drop wise to the extract until the solution becomes basified; thereafter it was partitioned with chloroform for alkaloid separation. The total alkaloid residue was dried and weighed.[19]

HPTLC Fingerprinting

In the present study, HPTLC of methanol extract of *Caulerpa racemosa* was performed by using mobile phase pet ether:acetone 8:2 v/v, which showed good separation of the phytoconstituents from the point of application. The chromatogram was observed in UV chamber at 245 nm in absorbance and at 336 nm in fluorescence modes.[20]

RESULTS AND DISCUSSION

Morphological Evaluation of *Caulerpa racemosa*

The organoleptic studies indicated the useful diagnostic features of *Caulerpa* species. It has a uniaxial siphonous thallus mostly divided into a creeping axis (stolon) with rhizoids and erect shoots (fronds) either nude, leaf-like or with grape- or feather-like ramuli. *Caulerpa racemosa* has erect fronds up to 9 -13 cm high bearing un-crowded vesiculate ramuli that are radially arranged. Fronds are slightly inflated above the attachment to the stolon which are fixed to the substrate by thin short rhizoids.[21]

Preliminary Phytochemical Test for Extracts

Preliminary phytochemical test was performed for the identification of different class of chemical constituents present in *Caulerpa racemosa.* Results of preliminary phytochemical screening are compiled in Table 1. Overall screening of extract indicated the presence of carbohydrate, glycoside, alkaloids, tannins, saponin, steroid and triterpenoid.

Physicochemical Evaluation

The physicochemical parameters are assisting in determining the purity and quality of the drug. The powdered drug was evaluated for its physicochemical parameters like foreign matter, loss on drying, total ash, acid insoluble ash and different extractive values (Table 2). Foreign matters were found to be 0.26 ± 0.09 %. High water soluble extractive value (8.5 ± 1.28 %) has been noted in comparison to the alcohol (6.5 ± 1.03 %), petroleum ether (0.15 ± 0.05 %), chloroform (2.2 ± 0.15 %) and ethyl acetate (1.5 ± 0.02 %). It indicates the possibility of considerable amount of polar compounds and presence of large quantity of water soluble constituents such as sugar, glycosides, phenolics and tannins in the seaweed. Loss on drying turned out to be 8.62 ± 2.04 and 1.25 ± 0.71 % in wet and dry condition respectively. As ash value is useful in determining

authenticity and purity of drugs and also these values are important quantitative standards. Content of total ash was found to be15.51 ± 3.16 % to high content of carbonates, phosphates, silicates and silica. This is also in accordance with moderate content of acid insoluble and water soluble ash 7.14 ± 1.20 % and11.80 ± 3.03 %, respectively. The total ash is particularly important in the evaluation of purity of drugs, the presence of or absence of foreign inorganic matter such as metallic salts or silica (Table 3). Infrared spectroscopy provides useful information of functional group present in respective extracts. The IR spectrum of marine alga *Caulerpa racemosa* showed an absorption band at 2850.88 cm^{-1} indicating the presence of aldehyde; 1745.64 cm^{-1} for ester; 1462.09 cm^{-1} for alkanes; 1018.45 cm^{-1} for alcohol ester or carboxylic acid.

Fluorescence Analysis of Drug Powder and Extracts

The result of fluorescence studies of *Caulerpa racemosa* using different reagents are given in Table 4 and that of the extracts is compiled in Table 2. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Some constituents show fluorescence in the visible range in day light. The UV light produces fluorescence in many medicinal plants (e.g. alkaloids like berberine), which is not visible in day light.[14]

Elemental Analysis

Knowledge of the elemental content of natural drugs is very important since many trace elements play an important role in the formation of active constituents responsible for the therapeutic properties. In addition, some of these elements are vitally important for various metabolic processes in the human body. They are closely linked to growth and health of human being.[22] The elements in the marine drugs is listed in Table 5. In this study, a total of fifty elements such as (Al, Ca, Fe, Mn, K, Mg, Na, Cu, Cr, Zn, Ni, Br, Rb and Zr) were determined in the marine plant by using Energy Dispersion X-Ray Fluorescence. The mean concentration of various metals in the marine sample was shown in Table 5. Al, Na, Ca Mg, K, Fe and SiO_2 were found to be ranged from 0.034-22.06 g/100 g; whereas Cl, S, Br and S were found to be moderate in concentration. But P_2O_5, Mn, Sr, Zr and I found in trace amount. Heavy metals like Cr, Co, Ni, Cou, Zn, As and Se were found to be in the range of 0.5-30 ppm. Thus the marine drug specie was rich in Na, Ca, Si, Al, Fe K and Cl but low in P_2O_5, Co, Mn, Sr, etc. As for the trace elements, copper plus zinc contents were found within the range of 14.7-25.1 ppm. Most of the trace elements present in the marine drug are heavy metals (As, Cd, Cu, Hg, Pb, Zn). The harmful toxic elements in the marine drugs were As (2.9 ppm) and Cd (1.3 ppm), which are at trace concentrations. Ca, Al, K Na, Mg were found in major concentrations in these plants. It is known that potassium is necessary for muscle contraction for the synthesis of some proteins and as an enzyme cofactor. Ca is the main constituent of the skeleton and is important for regulating many vital cellular activities such as nerve and muscle function, hormonal actions, blood clotting and cellular mortality. Calcium concentrations were in the range 14.92 %. The high concentration of potassium in plants is needed for many essential processes including enzyme activation, photosynthesis, water use efficiency, starch formation and protein synthesis. Potassium participates actively in the maintenance of the cardiac rhythm and in constipation. Kanneez *et al.*[23] stated that magnesium in plant lowers the cholesterol level. Magnesium plays an important role in regulating muscular activity of heart rhythm and also magnesium is important cofactor of converting blood glucose into energy.[24] Copper is an essential nutrient that plays an important role in the production of hemoglobin, myelin, collagen and melanin.[25] Iron (Fe) is an essential element for human beings and animals and is an essential component of hemoglobin. It facilitates the oxidation of carbohydrates, protein and fat in order to control body weight, which is very important factor in diabetes. The iron concentrations varied from 1.363 %. Cr, Mn and Zn have important roles in the metabolism of cholesterol as well as heart diseases. It is important in the utilization of glucose. According to Zayed[26] and Perry[27], the presence of Cr and Mn in plants may be correlated with therapeutic properties against diabetic and cardiovascular diseases. The concentrations of Cr and Zn varied from 20-25 ppm. Zinc is the component of more than 270 enzymes[28] and its deficiency in the organism is accompanied by multisystem dysfunction. Besides, Zn is responsible for sperm production. Silicon (17.26 %) is also another important element to prevent the hardening of veins and arteries. Chloride works with sodium and potassium, which carry an electrical charge when in dissolved body fluids and to regulate the pH in the body. Chloride is also important to digest the food properly and absorbs many elements. Ni is required in minute quantity for body as it is mostly present in the pancreas and hence plays an important role in the production of insulin. Its deficiency results in the disorder of liver[23] and the daily intake shouldn't exceed 1.0 mg since beyond this level is toxic.

HPTLC

HPTLC gives better choice of analysis as it can handle several samples of divergent nature and composition by several analysts at the same time. Alcoholic extracts of marine alga was studied for finger printing pattern using HPTLC. The plate was developed under optimization techniques for proper resolution of chemical compounds and tried in several solvent systems like pet ether: acetone (8:2 v/v) , pet ether: acetone (7:3 v/v), hexane: ethyl acetate (8:2 v/v), and hexane: ethyl acetate (7:3 v/v) as mobile phase in Camag twin trough TLC chamber with lid up-to 8 cm. Among these solvent systems pet ether: acetone (8:2 v/v) mobile phase was found to be more suitable for proper compound resolution. The plate was scanned at 366 nm using Scanner 3. Four different colored peaks were detected and showed *Rf* at 0.31, 0.50, 0.7 and 0.99 which represented % area as 25.74, 11, 10.74 and 13.20 respectively, as shown in Figure 1 & 2 and Table 6. After spraying with Liebermann–Burchard reagent only one pink colored compound was identified

which may be steroid or saponins class of compound. When another plate with same mobile phase was sprayed using Dragendorff's reagent only one compound was identified which may belongs to alkaloid.

Quantitative Estimation of Phytoconstituents

Quantitative estimation indicates that *Caulerpa racemosa* has higher percentage yield of alkaloid and total polysaccharides was recorded as 3.20 ± 0.78 and 10.02 ± 1.11 %, respectively (Table 3).

Table 1: Preliminary phytochemical tests for different extracts of marine algae *Caulerpa racemosa*

Class of phytoconstituents	Pet. ether extract	Alcohol extract	Water extract
Alkaloids	-	++	+
Carbohydrates	-	-	++
Glycosides	-	-	+
Tannin / Phenolics	-	+	++
Flavonoids	-	-	-
Steroid / triterpenoids	+	+	-
amino acids	-	-	-
Saponins	-	-	+
Mucilage	-	-	+

(Where, – absent; + present; ++ intense present)

Table 2: Extractives values of marine algae *Caulerpa racemosa*

Solvents	% Extractive value	Color
Ether	0.15	Yellowish green
Chloroform	2.2	Yellowish green
Ethyl acetate	1.5	Yellowish green
Acetone	2.1	Dark green
Methanol	6.6	Dark green
Ethanol	4.5	Dark green
Water	8.5	Dark green

Table 3: Results of physicochemical parameters of marine algae *Caulerpa racemosa*

Parameters	Results (% w/w) n = 3 Mean ± SEM
Ash values	
Total ash	15.51 ± 3.16
Acid insoluble ash	7.14 ± 1.20
Water soluble ash	23.80 ± 5.03
Extractives values	
Ether	0.15 ± 0.05
Alcohol	6.5 ± 1.03
Water	8.5 ± 1.28
Loss on Drying	
On wet basis	8.62 ± 2.04
On Dry basis	1.25 ± 0.71
Foreign Organic matter	0.26 ± 0.09
Total polysaccharides	10.02 ± 1.11
Total alkaloid content	3.20 ± 0.78

Table 4: Fluorescence analysis of marine algae *Caulerpa racemosa*

Powder + solvents	Observation under	
	Normal Light	UV light 366 nm
Dry Powder	off-white	Fair Yellow
Powder + Water	Yellow	Dark brown
Powder + HCl	Yellowish green	Dark red
Powder + HNO_3	Red	Dark grey
Powder + H_2SO_4	Dark brown	Purple
Powder + NaOH	Yellowish green	Blackish brown
Powder + KOH	Green	Blackish brown
Powder + Alc. NaOH	Green	Dark grey
Powder + Alc. KOH	Dark green	Dark grey
Powder + Ammonia	Khaki Dark	Purple

Table 5: Elemental analysis of marine algae *Caulerpa racemosa*

Symbol	Element	Concentration %	Abs. Error
SiO_2	Silicon	17.26 %	0.04
Al_2O_3	Aluminum	3.871 %	0.042
K_2O	Potassium	1.256 %	0.014
CaO	Calcium	14.92 %	0.04
TiO_2	Titanium	0.2275 %	0.0048
Na_2O	Sodium	22.06 %	0.57
MgO	Magnesium	2.536 %	0.092
P_2O_5	Phosphorus	0.5713 %	0.0084
MnO	Manganese	0.03499 %	0.00082
Fe_2O_3	Iron	1.363 %	0.004
S	Sulfur	11910 ppm	40
Cl	Chlorine	115100 ppm	100
V	Vanadium	< 27 ppm	-27
Cr	Chromium	21.2 ppm	2
Co	Cobalt	< 13 ppm	-10
Ni	Nickel	11.5 ppm	0.9
Cu	Copper	14.7 ppm	0.9
Zn	Zinc	25.1 ppm	0.8
Ga	Gallium	2.3 ppm	0.5
Ge	Germanium	< 0.5 ppm	0
As	Arsenic	2.9 ppm	0.4
Se	Selenium	1.1 ppm	0.3
Br	Bromine	469.1 ppm	1.7
Rb	Rubidium	16.9 ppm	0.6
Sr	Strontium	851.6 ppm	1.7
Y	Yttrium	4.1 ppm	0.3
Zr	Zirconium	15 ppm	3.1
Nb	Niobium	< 1.1 ppm	-1.1
Mo	Molybdenum	7.4 ppm	0.9
Ag	Silver	< 2.0 ppm	0
Cd	Cadmium	1.3 ppm	0.5
Sn	Tin	18.1 ppm	1.4
Sb	Antimony	9 ppm	1.5
Te	Tellurium	14.4 ppm	1.4
I	Iodine	55.3 ppm	3.7
Cs	Cesium	56 ppm	10
Ba	Barium	< 2.0 ppm	0
La	Lanthanum	< 2.0 ppm	0
Ce	Cerium	< 2.0 ppm	0
Er	Erbium	< 5.1 ppm	0
Yb	Ytterbium	< 2.0 ppm	0
Hf	Hafnium	< 2.5 ppm	-1.2
Ta	Tantalum	< 2.6 ppm	-1.9
W	Tungsten	< 1.0 ppm	0
Hg	Mercury	< 1.0 ppm	0
Tl	Thallium	2.4 ppm	0.4
Pb	Lead	10.9 ppm	0.7
Bi	Bismuth	2 ppm	0.5
Th	Thorium	4.8 ppm	0.6
U	Uranium	5.6 ppm	0.5

Table 6: Retention factor (R_f), area under curve and % area of all peaks of alcoholic extracts of marine algae *Caulerpa racemosa using HPTLC**

Peak	Max R_f	Max height	Max %	Area	% Area
1	0.04	366.4	62.02	3577.8	39.28
2	0.31	140.4	23.77	2345.0	25.74
3	0.50	21.3	3.60	1001.8	11.00
4	0.77	23.8	4.03	982.6	10.79
5	0.99	38.9	6.58	1202.2	13.20

*Solvent system: petroleum ether: acetone (8:2 v/v)

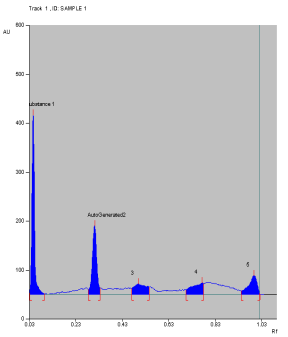

Figure 1: Chromatogram of alcoholic extracts of marine algae *Caulerpa racemosa*; solvent system petroleum ether: acetone (8:2 v/v)

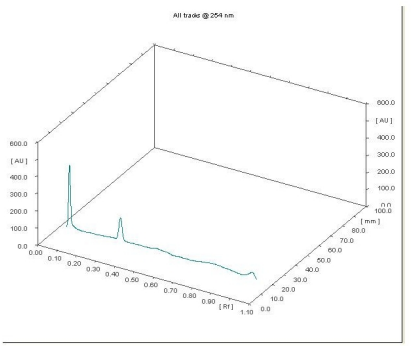

Figure 2: 3 D chromatogram of alcoholic extracts of marine algae *Caulerpa racemosa*; solvent system petroleum ether: acetone (8:2 v/v)

CONCLUSION

The present analytical study of *Caulerpa racemosa* on physicochemical parameters, preliminary phytochemical analysis, and elemental analysis provides important information which may be helpful in authentication and quality control of raw material. HPTLC fingerprint enables a particular plant to be identified and distinguished from closely related species. In 21st century, Ayurveda cannot be continued as in the age-old conventional manner. It has to accept the new challenges and be prepared to answer the queries of the modern system about the quality and efficacy of the herbal drugs and also how these plants are cultivated, collected, processed, preserved and used. Further studies are going on this seaweed in order to isolate, identify, characterize and elucidate the structure of bioactive compounds along with its pharmacological activity.

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