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Phytochemical evaluation of marine algae from various coastal regions of Tamil Nadu, India for beneficial bioactive compounds

Krishnamoorthy Sivakumar, Sudalayandi Kannappan and Ghanshyam N Jha

Abstract
Characterization of phytochemicals from the marine algae provides the development of new drugs against disease-causing agents. Lately, researchers have described the marine algae for its sources like minerals, bioactive compounds, and vitamins, which could be used for animals as well as humans for their health care applications. In the present study, the marine diatom of *Skeletonema costatum,* marine macro algae such as *Ulva fasciata* and *Kappaphycus alvarezii* were collected from the different coastal areas of Tamil Nadu and screened for phytochemicals. The marine diatom *S.costatum* was mass cultured, shade dried, then the macro algae also shade dried and later used for extraction using ethyl acetate. The shade dried algal powders and crude extracts were used for phytochemical analysis through SEM-EDAX and TLC methods. SEM-EDAX analysis has shown the total elemental profiles such as C, O, Na, Mg, Si, Cl, K,Ca, etc from each alga whereas, the TLC methods have exposed the various non-volatile compounds such as R_f value of 0.02 to 0.98 from the marine algae. Based on these analyses, it could be concluded that the marine algae of *S.costatum, U. fasciata* and *K. alvarezii* have exhibited its various bioactive compounds, which may be useful for animal and human health care products, especially against pathogenic diseases.

Keywords: Bioactive compounds, marine algae, phytochemical analysis, SEM-EDAX, TLC

Introduction
The marine environment is considered as one of the major explored habitats due to its diversity in chemical and biological resources [1]. Among the marine sources, algae are the most valuable sources with structurally diverse bioactive compounds, which are attributed to combat various diseases [2]. Though, there are significant attention has been given to different marine organisms, yet algae species are considered as potential sources for valuable materials which include high nutritive values of protein, lipid, polysaccharide, mineral, vitamin contents, etc [3]. The primary or secondary metabolites produced by marine algae as potential sources for bioactive compounds of interest in the pharmaceutical industry and many of these substances have been demonstrated to possess remarkable biological activities [4]. Recently, marine algae have become popular food items, and using them as regular diet is increased. Besides, the significance of algal food on human health is still unclear. In another way, marine algae were reported to produce a wide variety of bioactive secondary metabolites such as antimicrobial, antifeedant, antihelmintic and cytotoxic agents. The various bioactive substances included were alkaloids, polyketides, cyclic peptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols [5]. Marine macro algae are known as the producers of bioactive compounds with higher biological activity [6]. Marine resources are an unmatched reservoir of biologically active natural products, many of which exhibit structural features that have not been found in the terrestrial organism [7]. Hence, attention on marine algae in developing novel metabolites against various diseases is being enhanced recently [5, 8].
Scanning Electron Microscopy (SEM) was used in the morphological analysis of marine algae. SEM imaging provides detailed images of the microstructure that enlarges those from the stereo and optical microscopy. Energy Dispersive X-rayAnalysis (EDAX) technique is used for identifying the elemental profile of marine algal species. The EDAX analysis system performs as an integrated feature of an SEM. The biodeterioration of *Ulva fasciata* on coastal concrete structures was analyzed by SEM-EDAX. It was reported that the effect of metabolic activity of macro algae *U. fasciata* on concrete was verified through its varying mineral contents [9]. In other macro algae *Chaetomorpha antennina,* the biodeterioration on concrete was analyzed by SEM-EDAX [10]. SEM-EDAX analyses of macro algae *Sargassum wightii* (Brown algae) and *Gracilaria corticata* (Red algae) were evaluated and found various trace

elements [11]. Francavilla *et al.* [12] reported about natural porous agar materials extracted from macro algae *Gracilaria gracilis*, then characterized by SEM-EDAX and obtained elemental composition with calcined samples. The microalgae *Chlorella vulgaris* was subjected to SEM-EDAX, which was isolated from the polluted environment and compared with the strains from the unpolluted environment. Further, the ability to the uptake of lead was observed from *C. vulgaris* which was isolated from a polluted environment [13]. Also, the phytochemical proportion of eight elements which were in order, as C>O>S>Mg>Na>Ca>Si>K of red algae *G. corticata* was studied using SEM-EDAX recently [14].

Chloroform and methanol extracts of micro algae species such as *Chlorella*, *Haematococcus*, *Ulothrix*, *Chlorococcum*, *Scenedesmus*, *Rivularia* and *Scytonema* were developed in Thin Layer Chromatography (TLC) using ethyl acetate, methanol, and water as mobile phase. The lipids compounds were separated by iodine vapor and visualized [15]. The identification of fatty acid and triglyceride by TLC and R_f (Retention factor) values were calculated from the extracts of micro algae *Scandasmus dimorphus* [16]. Macro algae *Ulva compressa*, *Portieria hornemannii*, *Gracilaria crassa*, *Dictyota bartyrensiana*, *Hydroclathrus clathratus*, *S. wightii* and *Halimeda macroloba* extracts were developed in TLC. The separated compounds were viewed under visible and UV (240 and 300 nm), further compound fractions were identified by R_f values [17]. The extracts of various algae such as *C. vulgaris*, *Rhizoclonium hieroglyphicum* and mixed algae were analyzed for bioactive compounds. The results are compared with various standards of fatty acids and methyl esters by TLC [18]. The column chromatography, separated fractions of macro algae species such as *Jania rubens*, *U. fasciata* and *Sargassum vulgare* extracts were characterized by TLC and *U. fasciata* exhibited the strongest antimicrobial activity with R_f values of active fractions were matched with standards [3]. Solvent extracted compounds from brown, red, and green algae such as *U. fasciata*, *Caulerpa racemosa*, *Dictyota cervicomis*, *Hypnea musciformis*, etc were separated by TLC and major bands showed corresponding to glycolipids like sulfoglycolipids and glycosyldiacylglycerols [19]. Recently, the TLC profile of *S. wightii* was exhibited to have three distinct phenolic spots from the methanolic extract with different R_f values of 0.172, 0.534, and 0.810, and steroids profile displayed only one distinct spot with the R_f value 0.068 [20]. Also, brown seaweed *Himanthalia elongata* extract was separated by TLC and found many compounds including pigments based on R_f values [21]. Therefore, it is important to study on different marine algae from various locations through phytochemical analysis and to determine their bioactive potential to facilitate and further to identify the various individual biomolecules for beneficial purposes in the food and medicinal sectors.

Material and Methods

Algae collection

In the present study the micro and macro algae such as *Skeletonema costatum*, *U. fasciata* and *Kappaphycus alvarezii* were collected from different locations of Tamil Nadu, India, and used for phytochemical evaluation.

Micro alga collection

Sampling was carried out using 10 µm plankton net at various places in the backwaters of Muttukadu (Latitude 12.806°N; Longitude 80.248°E), Chennai, India.

Isolation of *S. costatum*

The collected algae samples were stored in a modified F/2 medium for enrichment [22]. After reaching the maximum exponential phase, the enriched algae samples were subjected to the dilution method to get the uni-algal *S. costatum* [23, 24, 25]. Using an aseptic technique dispensed the 9.0 ml of the medium into each of ten test tubes with sterile 10 ml pipettes. Test tubes were labeled from 10^{-1} to 10^{-10} indicating the dilution factor. One ml of enriched sample was aseptically added to the first tube (10^{-1}) and mixed gently. Then 1.0 ml of this dilution was taken out and added to the next tube (10^{-2}), mix gently. This procedure was repeated for the remaining tubes until the last dilution (10^{-3} to 10^{-10}). The test tubes were shaker incubated, under controlled temperature and light conditions as follows; the temperature at 25–28°C and illumination at 6000 Lux (12 h in light and 12 h in dark) respectively. The algae were examined microscopically (Light microscope, Optika, Italy). After 2-4 weeks a small amount of algae sample was withdrawn aseptically from each dilution. A uni-algal culture was observed in many of the higher dilution tubes from 10^{-6} to 10^{-10}. If tubes contained 2 to 3 different species, then it was micromanipulated (using a capillary pipette) and obtained uni-algal *S. costatum*. The isolated *S. costatum* test tubes were inoculated in fresh medium for enrichment. The contents of the test tubes were transferred into 50 ml conical flask for continuous cultivation. The maximum exponential phase was obtained between 8-10 days. Seawater with a salinity of 32 PSU (Practical salinity Unit) was filtered by using a filter bag (5 µm) and sterilized for preparing algal medium and pH was maintained at 7.8 to8.2. The conical flask was kept either in a shaker incubator or provided the continuous filtered (0.45 µm) aeration. The axenic *S. costatum* was obtained by use of an antibiotic (Ampicillin, Hi-Media, India; 100 mg of ampicillin was dissolved in 1.0 ml 50% methanol (v/v); filter sterilized by 0.2 µm membrane filter, Pall Corporation, USA) as 500 µl per liter of *S. costatum* was maintained through in-door algae laboratory.

For large scale production of *S. costatum* 100 L of FRP tanks were used and itwas arranged in an out-door shed by side covered by a greenhouse net and plastic roof atthe top. The grown stock was used as an inoculum for mass culture. For efficient growth of alga, commercially available fertilizers namely, ammonium sulfate, super-phosphate and urea were added in the ratio of 100 g; 10g, and 10g respectively for 100 L of seawater [26]. For 100 L of seawater, 2 L of inoculum was added for culturing in the tank. Continuous and vigorous aeration was provided to algae which keeps the algae always in suspension and helpful for uniform distribution of nutrients in the medium. The cells were collected using a micron net (10 µm). The collected algae were shade dried, then stored at 4°C, later used for extraction.

Estimation of density and biomass of *S. costatum*

Microalgal biomass was harvested by filtering the algae through filtering equipment (Tarsons, India) using filter paper (Whatman filter paper No.1, 0.45 cm dia.) and also by centrifugation at 3000 rpm/10 min. The cell pellets were washed twicewith distilled water. The collected pellets were dried by hot air oven at 80ºC/40 minand the weight was determined gravimetrically (g/l). The density of algae from the culture system was determined by cell counts using the Sedgewick counting chamber under a light microscope [27]. The biomass of micro algae was estimated by the standard method of Strickland and Parsons [28]. Ten ml of *S. costatum*

was filtered by using a filtering system (Tarsons, India) fitted with a 4.5 cm diameter GF/C filter paper (Pall Corporation, USA) by applying low suction. Before filtering the sample, a thin bed of magnesium carbonate (2 ml) was made for effective filtration. After the filtration, the filter paper was removed by using clean forceps and ground with 90% acetone using Pestle and Mortar. The ground samples are transferred to screw-cap test tubes and covered by using a black cloth and incubated in the refrigerator for 24 h. The contents were reground with 90% acetone and centrifuged at 3000 rpm/ 10 min. The optical density (OD) was measured at different wavelengths of 630 nm, 645 nm, and 665 nm respectively for chlorophyll 'a' estimation [26].

Macro algae collection

Macro alga *U. fasciata* was collected with a knife, from all substratessuch as rock, plant, wood, etc from the intertidal zone of Tuticorin (Latitude 8.7874°N; Longitude 78.1983°E) region, Tamil Nadu. The *K. alvarezii* was collected in the intertidal zone of Mandapam (Latitude 9.2886°N; Longitude 79.1329°E) region, Ramanathapuram District, Tamil Nadu. The algae were washed in freshwater (1% KMnO4, w/v) to eliminate epiphytes, sand, and other extraneous matters and then shade dried. The dried algae were weighed, pulverized using a mechanical grinder separately, and subjected to extraction.

Solvent extraction method

The solvent extraction method recovers almost all the compounds from the algae powder. The organic solvent, ethyl acetate was used for the algae powdered such as *S. costatum*, *U. fasciata* and *K. alvarezii*. These powders were extracted separately at 30°C, called as "Cold extraction method". The extract was prepared by taking 1.0 g ofshade-dried powder then mixed with 10.0 ml of solvent and shaker incubated at 30°C/96 h at 50 rpm. Then the extract was filtered by Whatman filter paper No.1, rotaryevaporated (30°C) under vacuum, and stored at 4°C for further use. The resultant extract was liquefied with 5 mg/ml of 30% (v/v) DMSO (Dimethyl Sulfoxide) and used for analysis [29].

Phytochemical characterization of marine algae

The pulverized marine micro and macro algae species like *S. costatum*, *U. fasciata* and *K. alvarezii* powders were ascertained for a morphological and elemental profile by SEM–EDAX analysis. The preliminary phytochemical characterization of crude algal extracts was carried out by TLC.

Morphological observations and surface analysis by using SEM-EDAX

Marine algae such as *S. costatum, U. fasciata* and *K. alvarezii* were characterized using scanning electron microscopy and energy dispersive X-ray analysis (SEM-EDAX). The morphological characteristics were observed using SEM and the determination of trace elements by surface analysis was done using EDAX. The mass cultured micro alga *S. costatum* sample was filtered by using micron net (10 µm). It was dehydrated by air-drying under a shade and dried in an oven at 60ºC/ 4 h to remove moisture. Dried samples were ground into a fine powder using pestle and mortar. The powdered algal samples were stored in vacuum desiccators in airtight containers until further analysis. The macro algae *U. fasciata* and *K. alvarezii* leaves were collected and thoroughly washed with ample water to remove adhered materials associated with algae. The cleaned algae leaves were shade dried and further dried in an oven at 60ºC for 4 h to remove moisture. Dried samples were pulverized using a mechanical grinder and then ground in pestle and mortar to obtain a fine powder. The powdered algal samples were stored in vacuum desiccators in airtight containers for further analysis. Sample preparation was carried out as described by Abirami *et al.* [30].

The dehydrated algae were used for the SEM-EDAX analysis, for these algae samples (1 mg) separately were gold-coated at 10^{-3} mm Hg in the sputter coat apparatus before SEM observations and EDAX analysis. The SEM microphotographs were recorded using Hitachi VP-SEM S-3400N, Japan with an accelerating voltage of 30 kV, at high vacuum (HV) mode and Secondary Electron Image (SEI). The maximum magnification possible in this equipment was 300000 times with a resolution of X 500 for all samples. The semi quantification elemental analysis to identify the percentage of the weight of major and minor elements present in the samples was done using the EDAX Thermo Scientific™ NORAN™ System 7 X-ray Microanalysis System, USA. The SEM photographs and corresponding EDAX spectrum were taken for all samples and one typical microphotograph and spectrum of the algae sample were taken. The percentages of the weight of elements present in all dehydrated algae samples were presented [9, 31].

TLC analysis of crude *S. costatum, U. fasciata* and *K. alvarezii* extracts

The preliminary phycochemical characterization of crude extracts obtained from *S. costatum, U. fasciata* and *K. alvarezii* algae was analyzed by thin-layer chromatography (TLC). The determination of a compound on a TLC is usually described in terms of its relative mobility or R_f value. R_f value is a unique value for each compound under the same conditions. The retention factor or Rf is defined as the distance traveled by the compound divided by the distance traveled by the solvent. In TLC, the mixtures of compounds were separated based on their differences in solubility and partition co-efficient in a binary solvent system. TLC of algae extracts was performed on a silica gel sheet (20×20 cm with 0.2 mm thickness (Merck, Germany). Further, the analytical TLC was carried out with a cut size of 2×7 cm from the above commercially available sheets. The plate was air-dried and activated through heating at 105°C in an oven for 1 h. An aliquot of each crude algal extract (5 µl at 5 mg/ml) was spotted (not wider than 4 – 5 mm) separately to above 1 cm of the base of the TLC plate and allowed to dry for a few minutes. Afterward, the plate was developed with the solvent system of Toluene/ Ethyl Acetate with the various ratios of 6:4, 7:3, 8:3, and 9:3 (v/v) as mobile phase in a previously saturated glass chamber with eluting solvents for 15 to 30 min at room temperature (RT). The developed plate was dried under normal air and the spots were visualized under visible light, UV irradiation (254 and 365 nm), and were exposed for 5 min in a sealed chamber containing vapor from solid iodine. The R_f values of isolated compounds were calculated and analyzed [21, 32].

Results

Cell density and biomass of *S. costatum*

In the present study, marine micro algae *S. costatum* was cultured in both indoor and outdoor systems. The maximum cell density and biomass were calculated forin-door and out-door systems. The daily growth in terms of cell density and biomass asof chlorophyll 'a' concentration of *S. costatum*

were estimated. *S. costatum* showed maximum growth and biomass, as it produced dark brown colored bloom with amaximum cell density. The growth in terms of cell density of *S. costatum* in the in-doorsystem was ranged between 63,356 and 8,12,000 cells/ml. The maximum cell density was noticed on 9th day whereas the minimum was on 1st day. The *S. costatum* biomass in terms of chlorophyll 'a' concentration was ranged between 0.051 mg/10ml and 0.619mg/10ml. The maximum biomass was showed on 9th day, while minimum density was observed on the first day. After 9th day, the declining phase was observed. The cell density of *S. costatum* in the out-door system was ranged from 2,63,000 to 30,06,000 cells/ml with maximum density obtained on 7th day whereas minimum on 1st day. Inthe out-door system, the maximum biomass (2.163 mg/10ml) was obtained on 7thday. However, the minimum biomass (0.219 mg/10ml) was estimated on the first day.

Phytochemical characterization of marine algae by SEM-EDAX

SEM-EDAX of *S. costatum*

The morphological characteristics of shade dried and pulverized *S. costatum* were observed using SEM. It was found that the spindle structures on both sides of cells, exhibited about the uniform size of particles ~7 µm (Figure 1). The trace elements were determined by surface analysis using EDAX. EDAX of *S. costatum* powder revealed a totally 8 elements such as C, O, Na, Mg, Si, Cl, K and Ca. The higher level of elements reported such as Silicon (31.41%), Oxygen (28.53%), Chlorine (14.90%), etc. The EDAX spectrum of *S. costatum* was exhibited in Figure 2. The elements profile of *S. costatum* exhibited by EDAX was given in Table 1.

SEM-EDAX of *U. fasciata*

The shade dried and powdered *U. fasciata* was viewed under SEM and observed for the particle size of ~50 µm in one dimension as a morphological characteristic (Figure 1). The surface analysis of *U. fasciata* powder was done by EDAX and found its elements profile. EDAX of *U. fasciata* showed 11 elements in total such as C, O, Na, Mg, Al, Si, S, Cl, K, Ca, and Br. The elements at a higher level were observed as Oxygen (35.60%), Sulfur (17.75%), Calcium (13.42%), etc. The trace elements of *U. fasciata* were observed by EDAX in Table 2. The EDAX spectrum of *U. fasciata* was revealed in Figure 2.

SEM-EDAX of *K. alvarezii*

In the SEM image of shade dried and powdered *K. alvarezii* was shown an almost uniform particle size of ~100 µm in one dimension (Figure 1). The elements profile of *K. alvarezii* powder was determined by EDAX as its surface analysis. *K. alvarezii* exhibited a total of 8 elements such as O, Na, Mg, Si, S, Cl, K, and As by EDAX, of which Chlorine (39.25%), Potassium (36.90%), Oxygen (15.22%), etc were exhibited higher percentages. *K. alvarezii* revealed an EDAX spectrum is shown in Figure 2. The elements of *K. alvarezii* were observed by EDAX in Table 3.

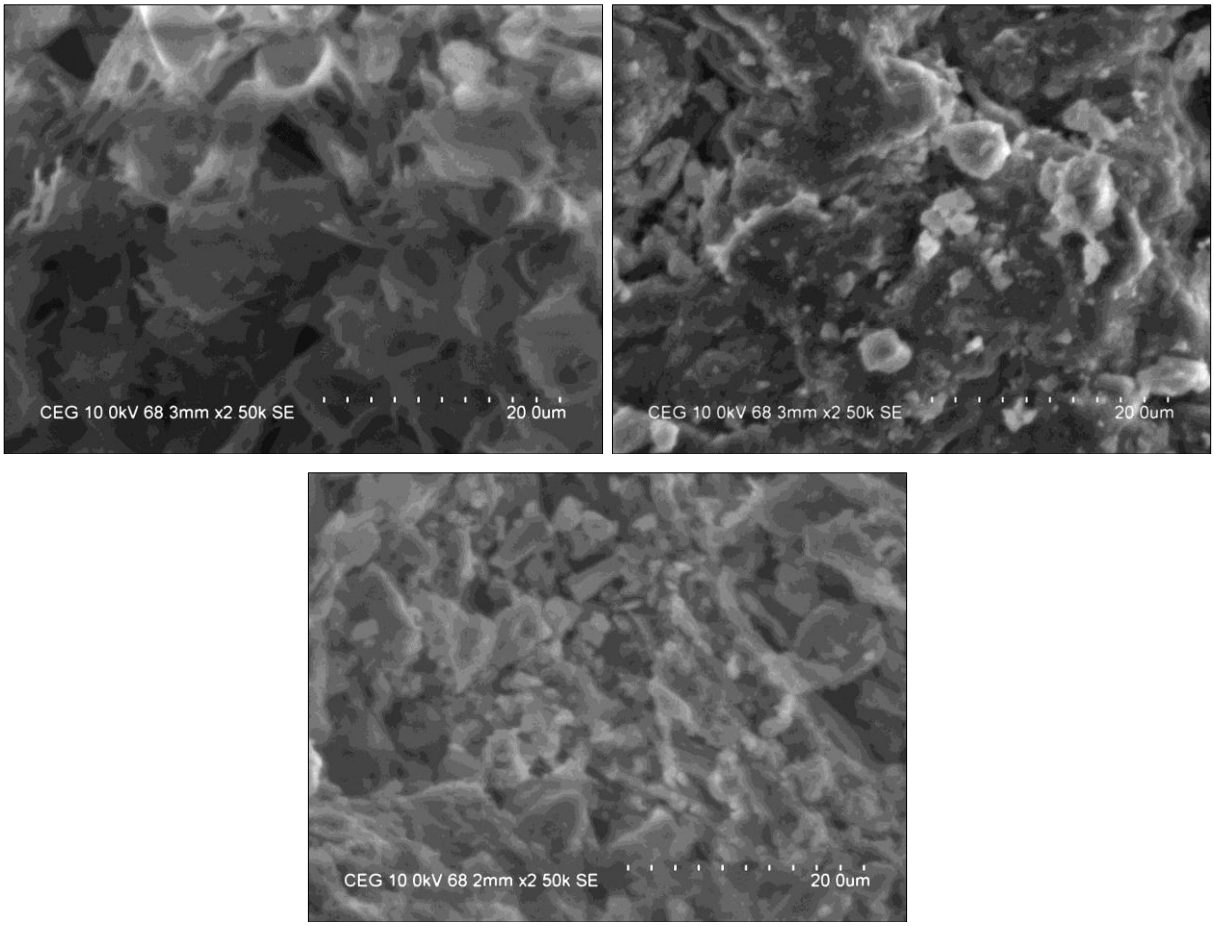

Fig 1: SEM image of marine algae *S. costatum, U. fasciata* and *K. alvarezii* respectively.

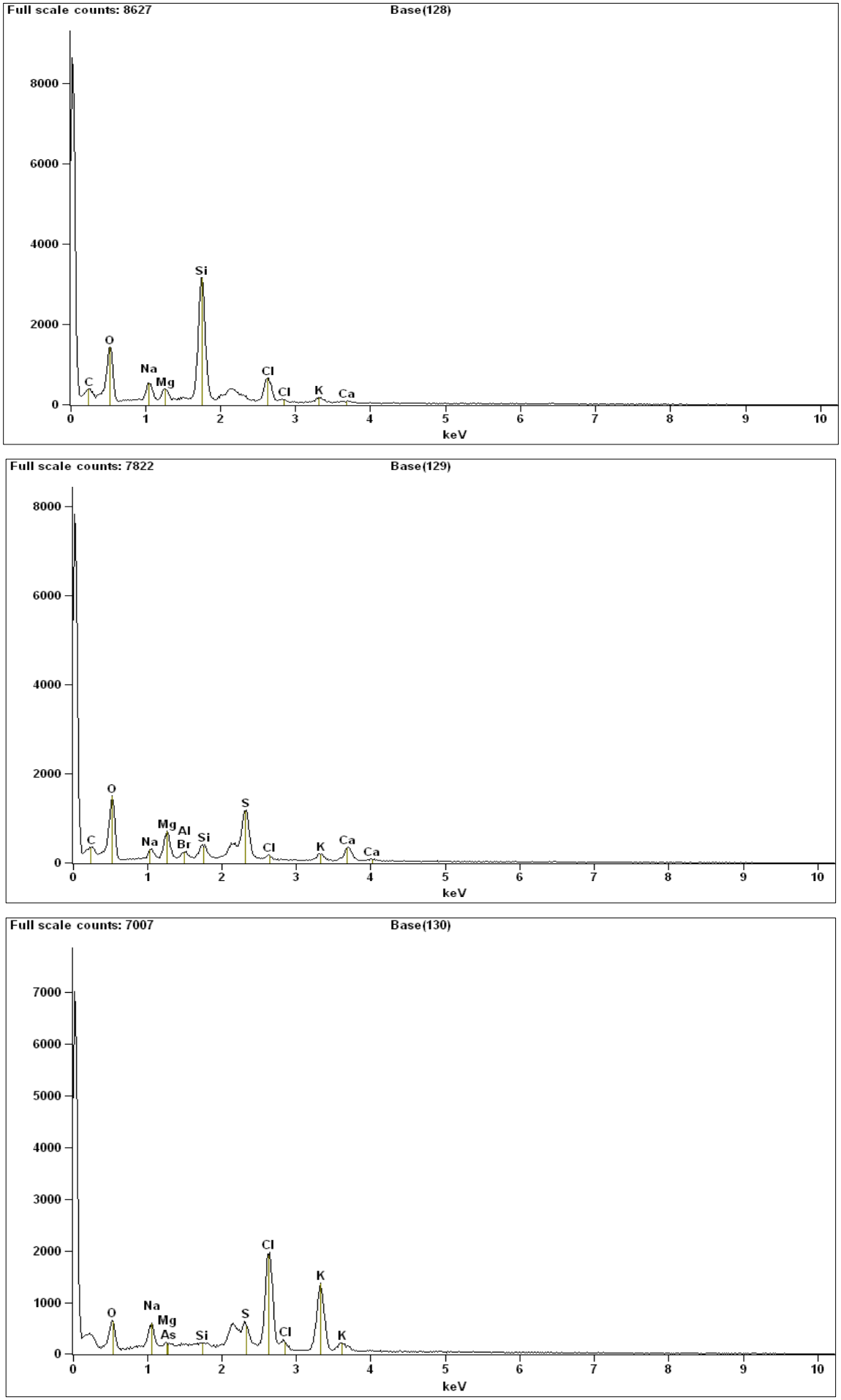

Fig 2: EDAX spectrum of *S. costatum*, *U. fasciata* and *K. alvarezii* respectively.

Table 1: Elements profile of *S. costatum* (EDAX).

Element	NetCounts	Weight %	Atom %
C	1129	13.31	22.88
O	12328	28.53	36.84
Na	4036	4.57	4.10
Mg	2345	1.78	1.51
Si	34001	31.41	23.10
Cl	8581	14.90	8.68
Cl	395	---	---
K	1745	4.04	2.13
K	0	---	---
Ca	510	1.46	0.75
Ca	0	---	---
Total		100.00	100.00

Table 2: Elements profile of *U. fasciata* analyzed by EDAX.

Element	NetCounts	Weight %	Atom %
C	995	10.22	18.07
O	12484	35.60	47.24
Na	1862	2.52	2.33
Mg	5734	5.07	4.43
Al	691	0.70	0.55
Si	4249	4.56	3.44
S	10933	17.75	11.75
S	0	---	---
Cl	1346	2.65	1.59
Cl	343	---	---
K	2120	5.43	2.95
K	0	---	---
Ca	4153	13.42	7.11
Ca	0	---	---
Br	973	2.09	0.55
Br	0	---	---
Total		100.00	100.00

Table 3: Elements profile of *K. alvarezii* (EDAX).

Element	NetCounts	Weight %	Atom %
O	4983	15.22	28.57
Na	3927	4.37	5.70
Mg	430	0.32	0.39
Si	1195	1.03	1.10
S	2148	2.71	2.54
S	0	---	---
Cl	25386	39.25	33.26
Cl	1249	---	---
K	16971	36.90	28.35
K	0	---	---
As	110	0.20	0.08
Total		100.00	100.00

Phytochemical characterization of marine algae by TLC

TLC of *S. costatum*

TLC of *S. costatum* extract showed various sizes of compounds and it was viewed under visible and UV. In visible light, a total of 8 compounds showed and were found as green colored. Further, TLC sheets were examined in UV light with two different nm (254 nm and 365 nm). The short UV wavelength of 254 nm exhibited green and blue colored compounds, 18 in total. But, UV long wavelength of 365 nm showed many colored compounds such as pink, red, brown, blue, and grey of 18 compounds in total. Besides, iodine derivates of TLC sheet were examined under visible light and observed 13 compounds with green and yellow-colored. The R_f values for all sizes of compounds of *S. costatum* extract were calculated and presented in Figures 3 and 4.

TLC of *U. fasciata*

In TLC analysis of *U. fasciata* exhibited varying sizes of 10 compounds with green and light brown colored under visible light. Then it was examined under UV 254 nm showed 11 compounds in green color. But, under UV 365 nm it exhibited14 compounds with many colors like pink, red, rose, brown, and grey. Moreover, only 10 compounds of iodine derivatives of *U. fasciata* extract were observed under visible light with slight yellow, green, and slightly brown color. The compounds of *U. fasciata* extract were calculated by R_f values and presented in Figures 3 and 4.

TLC of *K. alvarezii*

K. alvarezii revealed only 4 compounds in slight brown color under visible light by TLC. But in UV 254 nm, the extract showed 11 compounds in total with many of them blue colored except a few exhibited slight brown color. Ten compounds were observed under UV 365 nm with colors such as blue, grey, light yellow, and white. Further, it was

examined for iodine derivatives under visible light and found 8 compounds with slight yellowish-brown colored. The R_f values of compounds of *K. alvarezii* extract was calculated and showed in Figure 3 and 4.

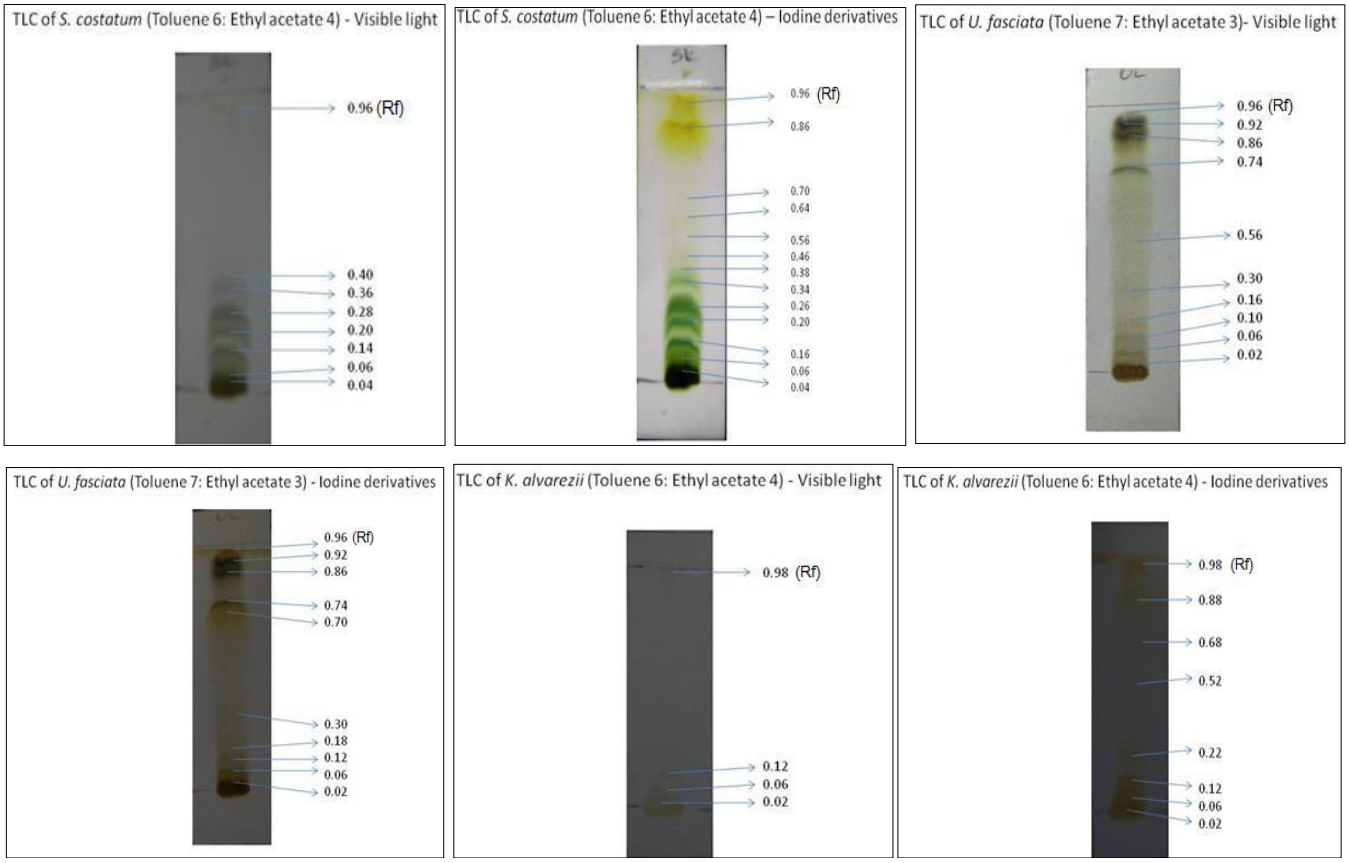

Fig 3: TLC based detection of compounds of algal extracts, visualized under visible light.

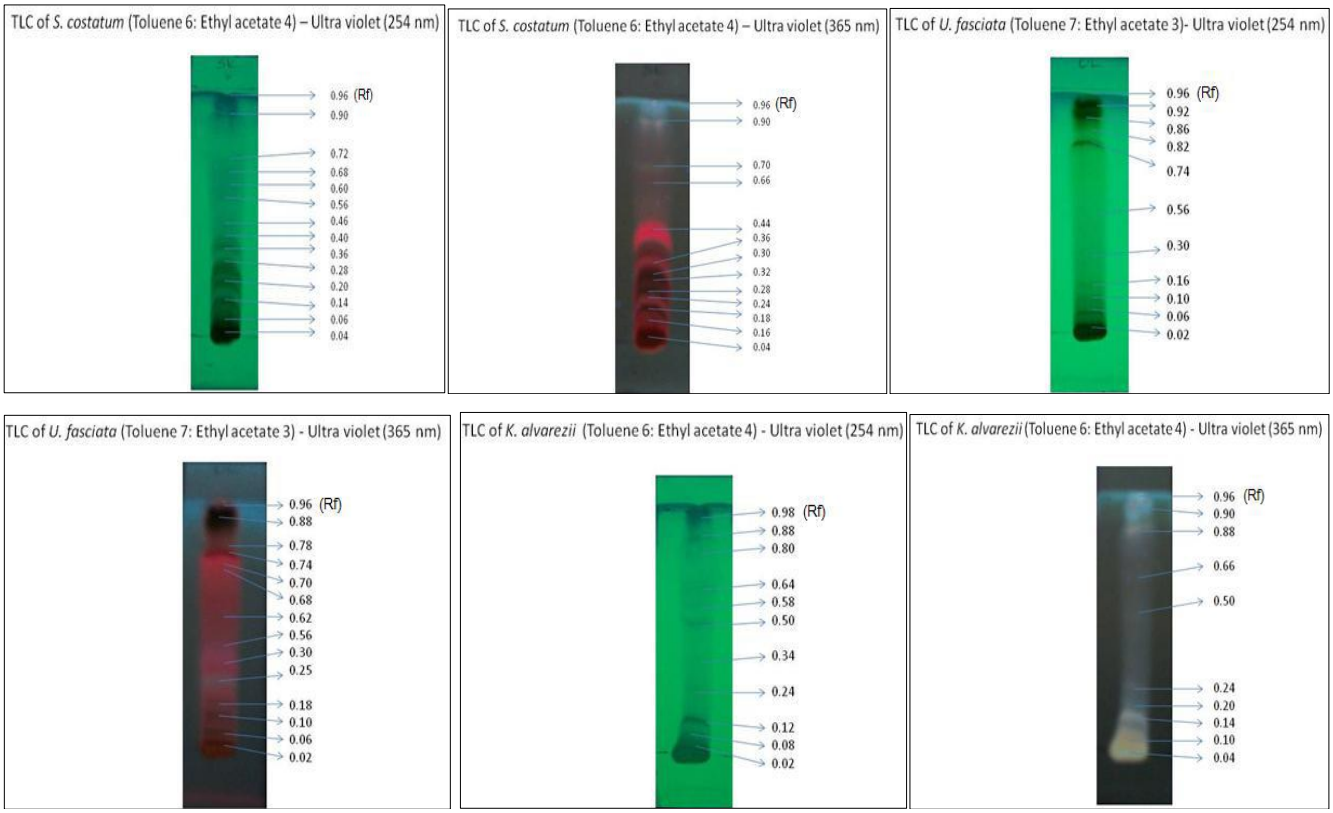

Fig 4: TLC based detection of compounds of algal extracts, visualized under UV light.

Discussion

Phytochemical characterization of marine algae

Marine algae can produce a variety of biological products, which are not produced by terrestrial plants. This may be a thrust area, which is a good starting point the investigating unique compounds from marine algae for controlling human and animal diseases [33]. The lipid, protein, and carbohydrate composition of algal biomass is usually quantified using a diverse range of techniques, involving chemical extraction followed by gravimetric determination, spectrophotometry, or mass spectroscopy [34]. Therefore, in the present study, the phytochemical characterization of marine algae such as *S. costatum, U. fasciata,* and *K. alvarezii* was evaluated using SEM-EDAX, TLC, FTIR, and GC-MS.

Phytochemical characterization of marine algae using SEM-EDAX

SEM-EDAX enables to determine the elemental composition of the cell wall, surface morphology, and to trace the distribution of metal ions [35, 36]. The surface morphology of algae was done using SEM inseveral studies. The morphology of macro algae *Sargassum filipendula* [37], *Enteromorpha* sp. [36] was analyzed using SEM before and after the acidic and metal treatment. The present investigation was also performed with SEM for morphological features particularly its structure and particle's size of *S. costatum, U. fasciata* and *K. alvarezii.* Similarly, SEM was also demonstrated to observe the morphology of *U. fasciata* [9], *Padina tetrastromatica, Gracilaria edulis* and *U. reticulata* [30] before and after treatment.

Under SEM-EDAX, SEM is a closely related electron probe of EDAX, which is designed primarily for producing electron images but can also be used for element mapping and even point analysis, if an X-ray spectrometer is added. Thus, it is considerable overlapin the functions of these instruments. EDAX uses the X-ray spectrum and is emitted by a solid sample bombarded with a focused beam of electrons to obtain a localized chemical analysis. Analysis can be performed in qualitative (involves the identificationof the atoms in the spectrum) and quantitative way (determination of the concentrations of the elements present). In the present study, many elements and their proportions were found by EDAX from the algal samples. Hence, this result was compared withelemental (EDAX) analysis in micro algae such as *Spirulina platensis* [38] and in macro algae *U. fasciata* [9], *U. reticulata* [30], *Gracilaria gracilis* [12], *S. wightii* and *G. corticata* [11].

Phytochemical characterization of marine algae using TLC

The initial screening of bioactive compounds from the algal extracts was demonstrated by TLC. Sudhakar *et al.* [39] reported that preliminary phytochemical characterization of macro algae *S. wightii, Sargassum ilicifolium, Sargassum longifolium, Padina* sp. and *Turbinaria* sp. were done by TLC without standards. The extracts of micro algae such as *C. vulgaris, Rhizoclonium hieroglyphicum* and mixed algae were subjected to TLC and found various compounds with standards [18]. But, the present study was proceeded only to separate the compounds from the algal extracts without comparing it with standard compounds. The macro alga *Sargassum subrepandum* compounds were separated and found differentcolor bands under visible and UV [40]. Isolation and partial characterization of bioactive compounds from brown alga, *H. elongata* was done by TLC and exhibited different R_f values under UV and visible including several pigment compounds [21]. Grayburn *et al.* [41] reported that analysis of *Cladophora* sp. and *Rhizoclonium* by TLC showed various proportions of free fatty acids and triglycerides from the extracts. The separated lipids including triglycerides were visualized by exposure to iodine [42]. Inaddition, purified fractions of macro algae species *Janiarubens, U. fasciata* and *S. vulgare* extracts were characterized by TLC, and R_f values of active fractions were compared with standards [3]. The present study was also agreed with the above findings. *S. costatum, U. fasciata,* and *K. alvarezii* extracts showed many pigmented compounds under visible, UV and iodine derivatives. For several compounds, their native fluorescence can be used for visualization, which is excited by UV light. This allows not only the determination of the R_f value but also often enables a further qualitative assignment [43]. Based on R_f values all the samples showed the presence of triglycerides but there may be other compounds of higher molecular weight which creates TLC spot bulky.

Conclusions

Phytochemical profiles of marine algae were determined by SEM-EDAX and TLC analyses. SEM-EDAX analysis exhibited about size and shape of algal powder with elemental composition in percentage. TLC plates showed various compounds as color, pigment's R_f value under visible and UV. These analyses may facilitate to know various major functional groups like polysaccharides, lipids, and protein, etc, and non-volatile compounds from the above algae.

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