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Research Article

Phytochemical Screening, Gc-Ms Analysis and Antioxidant Activity of Marine Algae Obtained from Coastal Andhra Pradesh, India

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ABSTRACT

Introduction: We have seen much research on novel compounds obtained from natural sources, but in the recent era, it's seen that there is tremendous scope for investigation on marine sources. In the past, due to a lack of technology, there was less knowledge about the treasure hidden in marine sources. Because of the latest technology, it's been an easy way of collecting marine sources and investigating them. Marine organisms are the source of highly bioactive secondary metabolites, which might help develop new pharmaceutical agents. Marine algae are classified into two main groups, i.e., microalgae and macroalgae. Microalgae include blue-green algae, bacillariophyte and dinoflagellates, whereas macroalgae, mostly known as seaweed, are divided into green, brown and red algae. **Material and methods:** For the present study, a green macroalga named *spongomorpha indica* has been selected, and it's been investigated for its physico chemical parameters, phytochemical study, GC-MS analysis and antioxidant activity. This investigation was performed to know whether this seaweed has the potential requirements for further research to see whether it's useful for healthcare. **Results:** The physicochemical parameters results obtained were in accordance with the who guidelines and the phytochemical tests revealed the presence of potent active constituents like alkaloids, steroids, tannins, flavonoids because of which the study was further extended to GCMS analysis where seven components were identified among which has the highest peak and has the highest molecular weight. And finally it was tested for antioxidant activity using four different models all the results showed best antioxidant activity, among which superoxide scavenging activity showed the best results. **Conclusion:** basing upon the results obtained it was proved that *spongomorpha indica* have potent active constituents which shows great antioxidant effect and hence the study is further proceeded to check target related activity so that it can produce best results in curing a particular disease.

Key words: *Spongomorpha indica,* Superoxide, DPPH, GCMS analysis, Physicochemical.

INTRODUCTION

In today's economic life of humans and ecosystems, the role of seaweeds is best known. Algae, mainly the macroalgae commonly known as seaweeds, are used as food, animal fodder, fertilizers, raw materials in the production of industrial products, and as natural feed for economically critical aquatic species has received attention in Thailand and in many other countries around the world such as Japan,[1] China,[2] and other Asian countries (e.g., Korea, Philippines, India).[3] Seaweeds serve as an essential source of bioactive natural substances.[4,5]

Seaweeds are classified into three main categories based on their pigmentation: Phaeophyta, Rhodophyta and Chlorophyta. Phaeophyta or brown seaweeds are brown macroalgae due to the presence of the carotenoid fucoxanthin. The primary polysaccharides present include alginates, laminarins, fucans and cellulose. Rhodophyta or red seaweeds are red-pigmented macroalgae, whereas Chlorophyta, or green seaweeds, are dominated by chlorophyll a and b, with ulvan the major polysaccharide component present in it. Seaweeds are the best sources of bioactive compounds with cytostatic, antiviral, antihelminthic, antifungal, antibacterial and many more biological activities. Seaweeds are natural renewable sources used as food, feed and fertilizer in many parts of the world. They have been screened extensively to isolate life-saving drugs or biologically active components worldwide.[6] Traditional and complementary medicine used active compounds produced by marine organisms. Many varieties of marine algae reported active compounds which can cure diseases. Due to the fewer side effects caused by drugs made of natural origin, most of the population prefers using natural-origin remedies to cure diseases.[7] Many pharmacological studies have reported that the chemical compounds produced by marine algae have different biological activities such as anti-HIV, anticancer, antimutagenic, scavenging free radicals and anti-inflammatory.[8,9] Previous studies on marine microalgae reported that over 15,000 compounds had been isolated, including sterols, fatty acids, terpenes, phenolic compounds, enzymes, alkaloids, flavonoids and polysaccharides. Recently it was reported that marine algae are a source of antioxidant compounds with free radical scavenging activity.[10]

The preliminary screening of *Spongomorpha indica* was performed in the present study to know the phytochemical constituents present. GC-MS analysis was done to identify and analyze different components present in the extract of *Spongomorpha indica*. Also, the study aimed to investigate the antioxidant potentials of *Spongomorpha indica*.

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MATERIAL AND METHODS

Selection and collection of *Spongomorpha indica*

Past literature study reveals that not much work was done on this seaweed. Still, potentially active constituents present in this seaweed can provide scientific proof of specific activity towards any disease. Hence, it's been collected from Visakhapatnam coastal area during the low tidal conditions. The collected sample was submitted to the herbarium of the botany department of Andhra University and was authenticated.

Preparation of extract

The collected crude sample was washed thoroughly, shade dried and the extract was prepared by the Soxhlet extraction method. Initially, 10 kgs of the crude sample were kept for maceration separately with three solvents, i.e., ethyl acetate, hexane and methanol: water (70:30) for About 48 hours. The solvent was collected, evaporated under steam distillation, and concentrated until a thick, greasy like consistency mass was obtained. The three extracts were separately tested for active constituents. The dried extract was kept in the refrigerator at 4°C till future use.

Physicochemical parameters[11-20]

The physicochemical parameters used in this study are to analyze the extract's quality and purity. For example, the Ash value helps determine the authenticity and purity of drugs, and also these values are also crucial for quantitative standards. The extracts were evaluated for physicochemical parameters like a loss on drying, total ash, insoluble acid ash, water-insoluble ash and different extractive values according to the official methods described in the Indian Pharmacopeia and WHO guidelines regarding quality control methods for medicinal plant materials. The following physicochemical parameters evaluated studied plant materials were given below:

Determination of ash values

To determine the quality and purity of crude drugs, especially in powder form Ash values are very helpful. The objective of incinerating the seaweed is to remove all traces of organic matter, which may otherwise interfere in an analytical determination. On incineration, crude drugs typically leave ash, usually consisting of carbonates, phosphates, and silicates of sodium, potassium, calcium and magnesium.

Determination of total ash

About 3 grams (accurately weighed) of seaweed powder was taken in a silica crucible which was incinerated and considered previously. It was incinerated by gradually increasing the heat, not exceeding red heat (450°c), until it was free from carbon, cooled down, and weighed. The percentage of ash has been calculated concerning the air-dried powder. The procedure was repeated five times to get constant weight. The obtained weights were calculated by using the following formula:

$$\text{Ash\%} = \frac{\text{Weight of ash}}{\text{Weight of dry powder}} \times 100$$

Determination of water-soluble ash

Boil the total ash obtained in the above process for 5 minutes with 25ml of water. In a Gooch crucible or in an as-less filter paper, insoluble matter was collected, washed shed with hot water, and incinerated to constant weight at a low temperature. The weight of insoluble matter was removed from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated concerning the air-dried drug. The weights obtained were calculated by using the following formula:

$$\text{Water-soluble ash\%} = \frac{\text{Weight of ash residue}}{\text{Weight of dry powder}} \times 100$$

Determination of acid insoluble ash

The total ash for 5 minutes was boiled with 25 ml of dilute hydrochloric acid, in a Gooch crucible or on an ash-less filter paper. The insoluble matter was collected, washed with hot water, ignited, and weighed.

The percentage of acid-insoluble ash was calculated with reference to the air-dried drug. The weights obtained were calculated by using the following formula:

$$\text{Acid insoluble ash \%} = \frac{\text{Weight of acid-insoluble ash}}{\text{Weight of dry powder}} \times 100$$

Determination of sulphated ash

The silica crucible was heated until it turned red for 10minutes, allowed to cool in desiccators, and weighed.1g of substance was accurately weighed and was transferred to the crucible. At first, it was ignited gently until the whole substance was thoroughly charred. Then the residue was cooled and mixed with 1 ml concentrated sulfuric acid, heated gently until white fumes no longer evolved, and ignited at 800°c =/- 25 until all black had disappeared.

The ignition was conducted in a place isolated from air currents. The crucible was cooled, and to this, a few drops of concentrated sulfuric acid was added and heated. It was Ignited as before, allowed to cool and weighed. Repeated the process until two successive considers did not differ by more than 0.5mg. Calculate the percentage of sulfated ash with reference to the air-dried drug. The weights obtained were calculated by using the following formula:

$$\text{Sulphated ash\%} = \frac{W3-w1}{W} \times 100$$

Since w1 = crucible weight

W2 = crucible wt. + sample

W3 = final wt. of crucible + sample

W = sample taken

Loss on drying

The loss on the drying test measures the amount of water and volatile matter present in a sample when the sample is dried under specified conditions. The weighing bottle was dried for about 30min, cooled in the desiccators, and weighed again accurately. 5gms of the sample was taken into a weighing bottle, and the sample was spread as a thin layer of less than 5mm and weighed accurately and placed in oven-dried at a temperature of about 10 degrees for 1/2hour and cooled and weighed until a constant weight was obtained. The weights obtained were [calculated by using the following formula:

$$\text{Loss on drying \%} = \frac{\text{Initial weight – final weight}}{\text{Final weight}} \times 100$$

Swelling index

Under specified conditions, the swelling index is the volume in ml taken up by the swelling of 1gm of herbal material. As specified, its determination was based on the addition of water or a swelling agent. About 1gm of the powdered drug was taken in a glass stoppered measuring cylinder and added water of about 25ml and shaken repeatedly for about 1hour and allowed to stand it for 24hours and volume in ml is read is measured. The procedure was repeated two times. The weights obtained were calculated by using the following formula:

$$\text{Swelling index\%} = \frac{\text{Final weight} - \text{initial weight}}{\text{Final weight}} \times 100$$

Foaming index

The ability to produce foam of an aqueous decoction of herbal material and extracts is measured using a foaming index. About 1gram of the powdered drug was taken into a 500ml conical flask, and 100ml of boiling water was added to it and maintained at moderate boiling for 30 min; collected and filtered into a 100ml conical flask. The decoction was taken into ten stoppered test tubes add it in successive portions of 1ml, 2ml,3ml,…….10ml. The volume was adjusted to 10ml, and the test tubes were stoppered and shaken for 15 seconds lengthwise, two shakes per second, and allowed to stand for 15 mins and the length of foam was measured.

The foaming index was calculated using the following formula:

Foaming index = 1000/a

Where a= the volume in ml of the decoction used to prepare the tubes, dilution foaming to a height of 1cm was observed.

Extractive values

5gms of an air-dried coarse powder of drug macerated with 40 ml of solvents (hexane, ethyl acetate, methanol and methanol: distilled water (70:30) in a glass stoppered conical flask with frequent shaking for 6hours and then allowed to stand for two days, then filtered carefully, taking care against loss of solvent. The filtrate was evaporated in a silica crucible to dryness on a water bath and then dried at 105 for 6 hours, cooled in a desiccator for 30mins, and weighed without delay. The weights obtained were calculated by using the following formula:

$$\text{Extractive values \%} = \frac{\text{Residue weight}}{\text{Weight of the sample}} \times 100$$

Crude fiber content

2grams of ground material were extracted with ether or petroleum ether to remove fat (initial boiling temperature 35-38°c and final temperature 52°c). An extraction might be omitted if the fat content was below 1%. After extraction with ether,2gms of dried material was boiled with 200ml of sulphuric acid for 30mins with bumping chips. The whole liquid was filtered through muslin and washed with boiling water until the washings were no longer acidic. 200ml of sodium hydroxide solution was added and heated for 30min. Filtered through muslin cloth again and washed with 25ml of boiling 1.25% H2SO4, three 50ml portions of water, and 25ml alcohol. The residue was removed and transferred to the ashing dish (pre-weighed dish W1). The residue was dried for 2 hours at 130 ±2°C. Cool the dish in desiccators and weigh (W2). Ignite for 30min at 600±15°C. Cool in desiccators and reweigh (W3). The weights obtained were calculated by using the following formula:

$$\text{Crude fiber content \%} = \frac{(W2-W1) - (W3-W1)}{\text{Weight of the drug sample}} \times 100$$

Since W1= Empty China dish weight.

W2= Powder weight after oven-dried and cooled

W3= Powder weight after igniting and cooled

Foreign organic matter

Weigh 200gms crude sample accurately and evenly spread into a thin layer. The sample was inspected with the unaided eye or used a 6x lens, and the foreign organic matter was removed manually altogether as much as possible. Finally, it was Weighed, and the weight of the drug taken determined the percentage of foreign organic matter. The maximum samples have to be used for coarse or bulky drugs.

PHYTOCHEMICAL SCREENING OF *SPONGOMORPHA INDICA*

Preliminary phytochemical analysis was carried out for three different solvent-based extracts of *Spongomorpha Indica* i.e., hexane, ethyl acetate and hydro methanolic extracts by applying standard procedures.[21-24]

Detection of alkaloids

The three different extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids by the following tests.

Mayer's test

Filtrates were treated with Mayer's reagent. The Mayer's reagent is prepared by dissolving Mercuric chloride (1.358g) in 60ml of water and potassium iodide (5g) in 10ml of water. The two solutions were mixed and the volume was made upto 100ml of water. The formation of yellow color on the addition of Mayers reagent to filtrates determines the presence of alkaloids.

Wagner's test

Filtrates were treated with Wagner's reagent. The formation of brown/ reddish brown precipitate indicates the presence of alkaloids. Wagner's reagent was prepared by dissolving iodine (1.2g) and potassium iodide (2g) in 5ml of water and the volume is made up to 100ml with distilled water.

Detection of flavonoids

Lead acetate test

Extracts were treated with a few drops of lead acetate solution. The formation of a yellow color precipitate indicates the presence of flavonoids.

H2SO4 test

Extracts were treated with a few drops of H2SO4. The formation of orange color is a indication for the presence of flavonoids.

Detection of steroids

Liebermann- Burchard test

2ml of acetic anhydride was added to 0.5g of the prepared extracts, each with 2ml of H2SO4. if their is a color change from violet to blue or green in some samples is a indication for the presence of steroids

Detection of terpenoids

Salkowski's test

Chloroform and concentrated sulphuric acid (3ml) were carefully added to 0.2g of the extract of the whole sample to form a layer. the presence of terpenoids is determined by a reddish-brown coloration of the inner face.

Detection of anthroquinones

Borntrager's test

0.2g of the extract was boiled with 10% Hydrochloric acid for a few minutes in a water bath. It was filtered and allowed to cool. the same volume of chloroform was added to the filtrate. A few drops of 10% ammonia was added to the mixture and heated. Anthraquinones presence can be indicated by the formation of pink color.

Detection of phenols

Ferric chloride test

The formation of bluish-black color when the extracts are treated with 5% ferric chloride solution indicates the presence of phenol.

Lead acetate test

The formation of a yellow color precipitate when the extracts were treated with few drops of lead acetate solution indicates the presence of phenolic compounds.

Detection of saponins

Froth test

Formation of frothing (appearance of creamy stable and persistent tiny bubbles) the extract was shaken with 5ml of distilled water appearance of tiny bubbles indicates the presence of saponins.

Detection of tannins

Ferric chloride test

A small quantity of extract was mixed with water and heated in a water bath. The mixture was filtered to which 0.1% ferric chloride was added. A dark green color formation indicates the presence of tannins.

Detection of carbohydrates

Fehling's test

0.2gm filtrate from each extract was boiled in a water bath with 0.2ml of Fehling solutions A and B. The presence of sugars can be determined by the indication of red precipitation. Fehling's solution A is prepared by Copper sulphate (34.66g) dissolved in distilled water and made up to 500ml using distilled water. Fehling' solution B is prepared by Potassium sodium tartrate (173g) and sodium hydroxide (50g) dissolved in water and made up to 500ml.

Detection of oils and resins

Spot test

Apply test on a filter paper. If it develops a transparent appearance like a stain on the filter paper, indicates the presence of oils and resins.

GC-MS analysis

The components present in the extract were separated using Helium as carrier gas at a constant flow of 1 ml/min, Clarus 680 GC was used to employ a fused silica column packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μm df) and During the chromatographic run, the injector temperature was set at 260°C. The 1μL of extract sample was injected into the instrument; the oven temperature was as fixed to 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; ionization mode electron impact at 70 eV, a scan time of 0.2 sec, and scan interval of 0.1 sec—the fragments from 40 to 600 Da. The spectrums of the components were compared with the database of the spectrum of known compounds stored in the GC-MS NIST (2008) library. Measurement of peak areas and data processing were carried out by Turbo-Mass OCPTVS-Demo S.P.L. software.

Antioxidant activity

Antioxidants are compounds that inhibit oxidation. Free radicals can be produced through a chemical reaction named Oxidation, thereby leading to chain reactions that may damage the cells of an organism. Well-known antioxidants include various enzymes and other substances, such as beta carotene, vitamin C and vitamin E, which can counter the damaging effects of oxidation. Antioxidation terminates these chain reactions, such as thiols or ascorbic acid (vitamin C). To balance the oxidative stress, animals and plants maintain complex systems of overlapping antioxidants such as enzymes (e.g., superoxide dismutase and catalase) and glutathione, produced internally in a human system or the dietary antioxidants such as vitamin C and Vitamin E. Antioxidants may reduce the risk of cancer. The progression of age-related macular degeneration is slowed down with the help of antioxidants. This process is called Antioxidant activity.

Reducing power assay[25]

The above sample, including extract with Ascorbic acid solutions, was spiked with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was kept in a 50°C water bath for 20min. The resulting solution was then cooled rapidly, spiked with 2.5ml of 10% trichloroacetic acid, and centrifuged at 3000rpm for 10 min. The supernatant liquid (5ml) was then mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride. The absorbance was detected at 700nm after reaction for 10min. The higher the absorbance represents, the more substantial the reducing power. The reducing power assay was expressed as ascorbic acid equivalent per gram of dry weight basis.

DPPH activity[26,27]

DPPH radical scavenging activity was carried out by adding 1.0 ml of 100.0 μM DPPH solution in methanol; an equal volume of the sample in methanol of different concentrations was added and incubated in the dark for 30 minutes. It was observed for colour change change in terms of absorbance using a spectrophotometer at 514 nm. To the control tube, 1.0 ml of methanol instead of the test sample was added. The different concentration of ascorbic acid was used as reference compound. The percentage of inhibition was calculated from the equation.

[(Absorbance of control - Absorbance of test)/ Absorbance of control)] ×100. The IC50 value was calculated using Graph pad prism 5.0.

Superoxide radical scavenging activity[28]

The superoxide radical scavenging activity of the test sample was studied using the method of Lee with slight modifications. Superoxide radicals are generated in phenazine methosulphate (PMS) - (Nicotinamide

adenine dinucleotide (NADH) systems by oxidation of NADH and assayed by the reduction of Nitro Blue Tetrazolium (NBT). 200.0 µl of test samples of different concentrations were taken in a series of test tubes. Superoxide radical was generated by 1.0 ml of Tris-HCl buffer (16.0 mM, pH-8.0), 1.0 ml of NBT (50.0 µM),1.0 ml NADH (78.0 µM) solution and 1.0 ml of PMS (10 µM). The reaction mixture was incubated at 25°C for 5 min and the absorbance at 560 nm was measured. A control tube containing Tris-HCl buffer was also processed in the same way without a test sample. Different concentrations of ascorbic acid were used as a reference compound.

Nitric oxide radical scavenging activity[29]

Nitric oxide radical scavenging activity was measured by spectrophotometry method. 1 ml of Sodium nitroprusside (5 mmol) in phosphate buffer (pH 7.4, 0.1 M) was mixed with different concentrations of the extract (100 – 500 microgram/ml in phosphate buffer (pH 7.4, 0.1 M). The tubes were incubated at 250C for two hours. At the end of the second hour, 1.5 ml of the reaction mixture was removed and diluted with 1.5 ml of Greiss reagent (1% sulphanilamide, 2% o-phosphoric acid, 0.1% of naphthyl ethylenediamine dihydrochloride) The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was measured at 546 nm. The Control tube contains all chemicals except plant extract.

Evaluation of total antioxidant capacity of the extract[30]

Phosphomolybdenum method was used to determine the total antioxidant capacity of the selected extract. The main mechanism

Table 1: Physicochemical parameters results.

S.No	Parameters	Values obtained (% w/w)
1	Total ash	38.33
2	Water soluble ash	9.8
3	Acid insoluble ash	14
4	Sulphated ash	13.59
5	Loss on drying	11.58
6	Swelling index	11.11
7	Foaming index	<100
8	Crude fiber content	1.5
9	Foreign organic matter	1

Table 2: Extractive values results.

S.No	Extractive values	Values obtained (% w/w)
1	Hexane soluble extractive values	0.8
2	Acetone soluble extractive values	2.4
3	Ethyl acetate soluble extractive values	1.6
4	Methanol soluble extractive value	9.6
5	Methanol : distilled water (70:30) soluble extractive value	13.6

Table 3: Phytochemical screening results.

Phytochemical tests	Observations	Extracts		
		Hexane	Ethyl acetate	Hydroalcoholic (70%methanol and 30% water)
Alkaloids				
Mayer's test	Cream color	–	–	+
Wagner's test	Reddish brown solution/ precipitate			
Flavonoids				
Lead acetate test	Yellow orange	–	+	+
H_2SO_4 test	Reddish brown/ orange color precipitate			
Steroids				
Liebermann-buchard test	Violet to blue or green color formation.	+	+	+
Terpenoids				
Salkowski test	Reddish brown precipitate	+	+	+
Anthroquinones				
Borntragers test	Pink colour	-	-	+
Phenols				
Ferric chloride test	Deep blue to black colour formation	–	+	+
Lead acetate test	White precipitate			
Saponin	Stable persistent	-	+	-
Tannin	Brownish green/ blue black	-	+	+
Carbohydrates	Yellow/brownish/blue/green color	+	+	+
Oil and resin	Filter paper test	+	+	+
Gums and mucilage		+	+	+

Table 4: **GCMS results.**

Si. No	CAS	Name of the Compound	Molecular Formula	Molecular Weight	Retention Time	Peak Area (%)
1	39682-48-9	1,6-ANAHYDRO-3,4-DIDEOXY-.BETA.-D-GLUCO-HEXOPYRANOSE	C6H10O3	130	19.050	1.881
2	35305-79-9	CYCLOPROPANEPENTANOIC ACID,2-UNDECYL-,METHYL ESTER, TRANS-	C20H38O2	310	20.180	4.128
3	900143-83-9	DECYL TRIFLUUOROACETATE	C7H16O	116	20.411	5.049
4	90526-63-5	2,3-EPOXYHEXANOL	C6H1202	116	20.631	7.615
5	13205-57-7	1-METHYLDODECYLAMINE	C13H29N	199	21.011	32.780
6	646-30-0	NONDECANOIC ACID	C19H38O2	298	22.126	47.006
7	41446-54-2	4-TRIDECENE,(Z)-	C13H26	182	24.187	1.541

Table 5: **Antioxidant results.**

Sl.No	Concentration µg/ml	Reducing Power assay		DPPH assay		Superoxide Radical Scavenging Assay		Nitricoxide radical Scavenging assay	
		% Inhibition	IC_{50}	% Inhibition	IC_{50}	% Inhibition	IC_{50}	% Inhibition	IC_{50}
1	50	22.94	76.72	40.03	93.52	22.65	162.96	38.10	109.83
2	100	49.25		49.87		34.32		51.21	
3	150	70.13		67.21		47.12		55.65	
4	200	82.98		81.34		51.02		64.98	

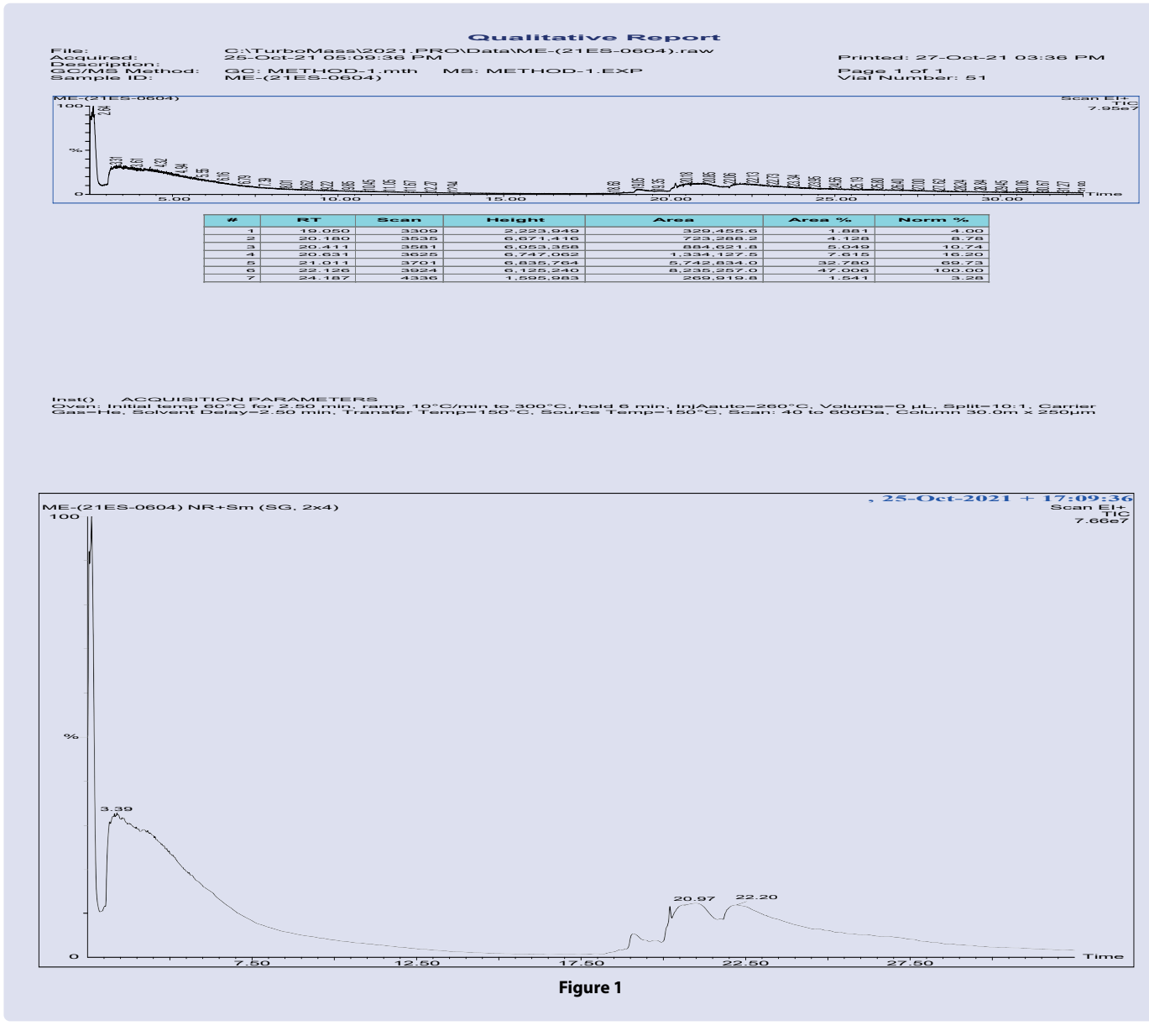

Figure 1

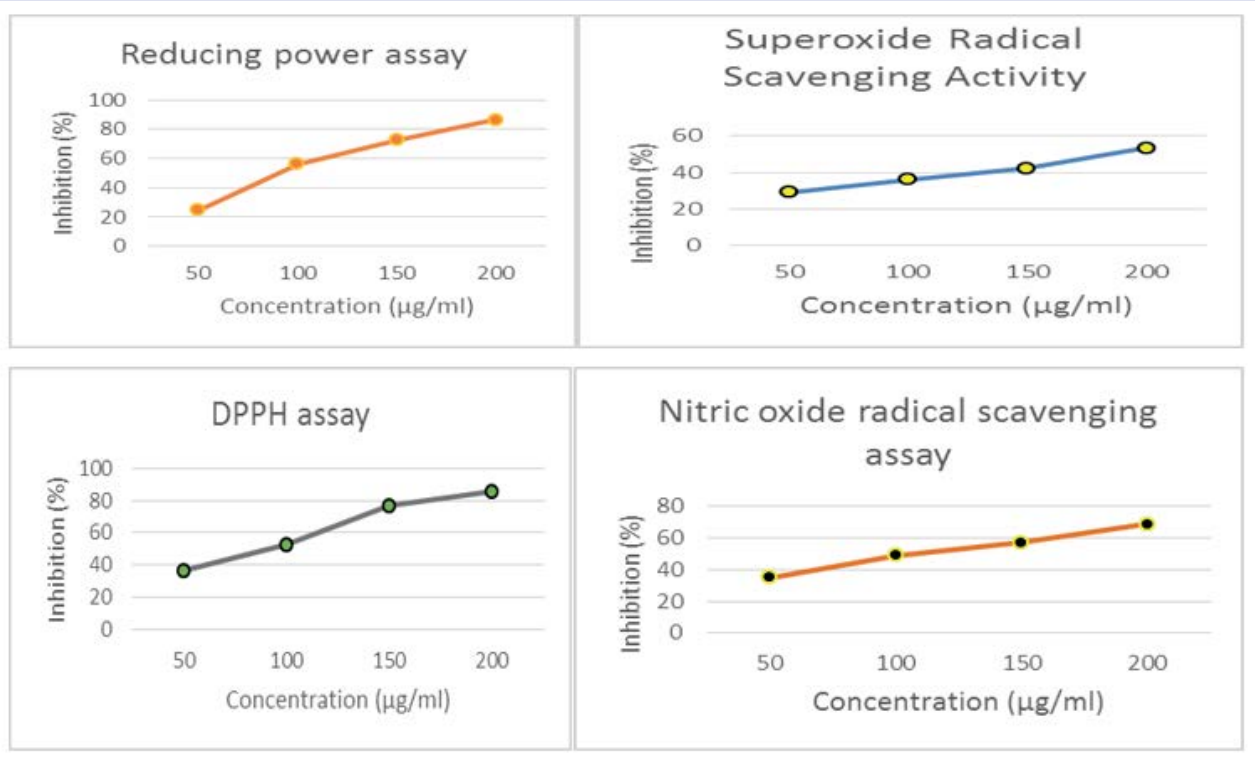

Figure 2

involved in this process is the reduction of Mo (VI) to Mo (V) by the antioxidant compounds present in the extract and formation of a green Mo (V). An aliquot of sample solution (0.1ml) containing a reducing species in DMSO was combined in an Eppendorf tube with 1ml of reagent solution (0.6M Sulphuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95 °C for 90min. The samples were cooled to room temperature, and the absorbance of each solution was measured at 695nm. The total antioxidant capacity was expressed as mM equivalent of ascorbic acid.

RESULTS AND DISCUSSION

The physicochemical parameters and extractive values obtained were according to the Indian pharmacopeia and WHO guidelines standards. The table 1 and 2 shows all the results observed and recorded during the study.

The phytochemical screening for the selected sample was done by using three different solvent systems i.e; hexane, ethyl acetate and hydroalcoholic (hydro methanolic) mentioned in table 3. And it was observed that except saponins all other active compounds were available in hydro methanolic extract of *spongomorpha indica* compared to the remaining two solvent derived extracts. From the results obtained we can study that hydro methanolic extract produced more compounds compared to the other solvent based extracts showing the active compounds like alkaloids, phenols, steroids, tannins etc which are essential medicinal compounds. And hence, for further studies hydro methanolic extract was selected.

The GCMS results shown in Table 4 and Figure 1 revealed the presence of seven different compounds. The compounds exhibited have a wider range in their nature. Nondecanoic acid was observed to exhibit largest peak area of about 47.006% with retention time 22.126 whereas compound Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-with highest molecular weight 130 and retention time 19.050.

The antioxidant activity of hydroalcoholic extract of *spongomorpha indica* was studies using the different models i.e; Reducing power assay, DPPH assay, Superoxide radical scavenging assay, Nitric oxide radical scavenging assay shown in Table 5 and Figure 2. In all the four assays it is seen that the percentage inhibition of the extract was directly proportional to concentration of the extract. *Spongomorpha indica* showed a significant dose dependent reduction in case of DPPH radical in the DPPH assay model. The highest IC50 value was seen in the superoxide radical scavenging activity. Total antioxidant activity of hydroalcoholic extract of *spongomorpha indica* found to be 153.

CONCLUSION

By thorough study of past literature about *spongomorpha indica* it was observed that these seaweeds are used as food in many countries and is mainly used as a fodder in aquaculture. And there has been only few medicinal studies performed. In the present study, the extraction process was done using three solvent systems i.e.; hexane, ethyl acetate and methanol:water and extracts were obtained screening for active constituents. Basing upon results obtained i.e.; containing tannins, alkaloids, phenols, steroids etc the study was further proceeded with hydro-methanolic extract. GCMS results revealed the presence of seven compounds with different nature present in the hydro methanolic extract, it also showed significant antioxidant potential with the four different assays performed. Thus, further study is continued to check the medicinal potentiality of this extract having important active compounds which can be helpful treating targeted disease which could be helpful for the mankind.

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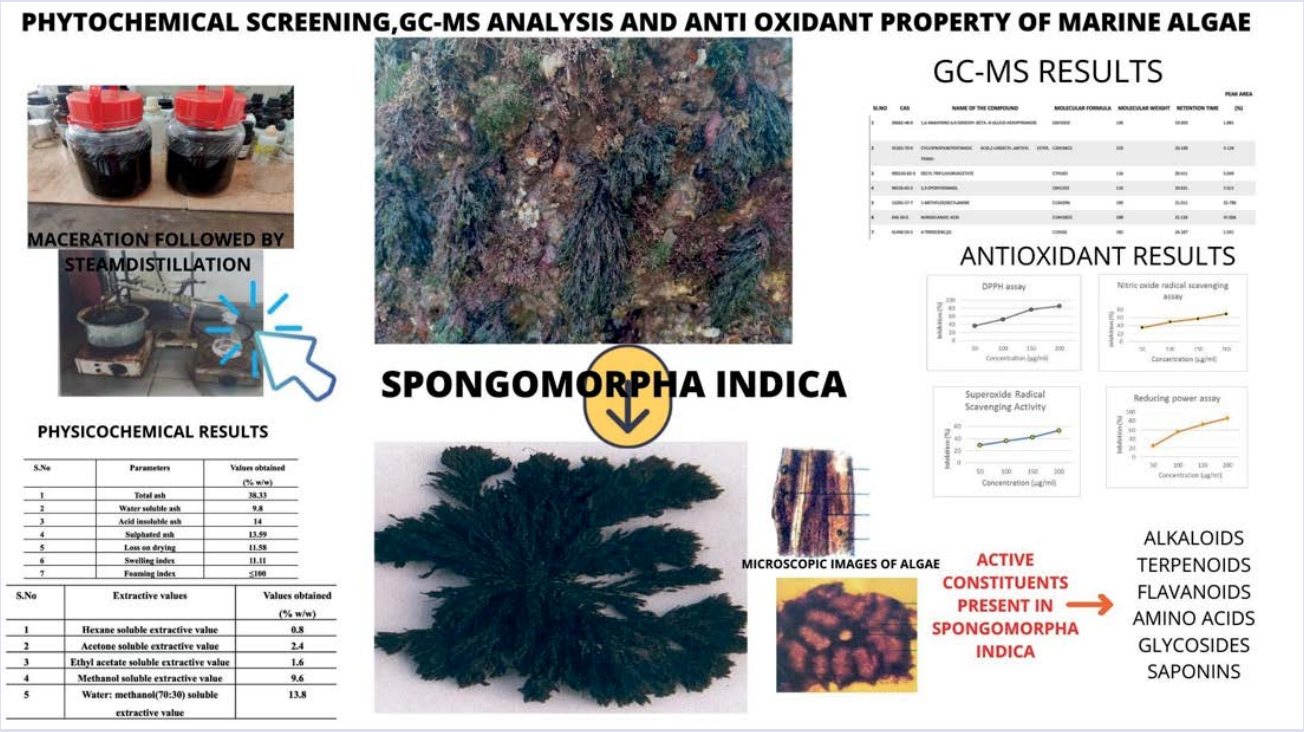
GRAPHICAL ABSTRACT



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