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Research Article

Exploring the Bioactive Potential of Marine Algae: Insights from Phytochemical Analysis, GC-MS Profiling, and Antioxidant Evaluation

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ABSTRACT

Introduction: While there has been a lot of research on novel compounds derived from natural sources, there is now considerably greater opportunity for study when it comes to marine sources. The immense wealth concealed in marine sources was less known in the past because of a lack of technology. The most recent technological advancements have made gathering and researching marine sources simple. Highly bioactive secondary metabolites that may aid in the development of novel pharmacological drugs are found in marine creatures. The two primary categories of marine algae are macroalgae and microalgae. While macroalgae, also referred to as seaweed, are classified into green, brown, and red algae, microalgae comprise blue-green algae, bacillariophyte, and dinoflagellates. **Material and methods:** *Spongomorpha indica*, a green macroalga, has been chosen for this study, and its physicochemical parameters, phytochemical analysis, GC-MS analysis, and antioxidant activity have all been examined. The purpose of this study was to determine whether more research on this seaweed's potential benefits for medical purposes is necessary. **Results:** The phytochemical tests indicated the presence of potent active constituents like alkaloids, steroids, tannins, and flavonoids; as a result, the study was further extended to GCMS analysis, where seven components were identified, the highest peak and molecular weight of which are all in accordance with WHO guidelines. The physicochemical parameter results were also in line with WHO guidelines. Ultimately, antioxidant activity was assessed using four distinct models, and all results demonstrated a significant amount of antioxidant activity, with superoxide scavenging activity demonstrating the best results. **Conclusion:** Based on the results, it was determined that *Spongomorpha indica* contains potent active ingredients with significant antioxidant effects. As a result, the study is now being conducted to examine target-related activity to determine the most efficient way to cure a specific condition.

Keywords: Antioxidant, *Spongomorpha indica,* GCMS analysis, Phytochemical.

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INTRODUCTION

Seaweeds play a well-known function in the economic interactions between humans and ecosystems in modern times. Algae, particularly the macroalgae known as seaweeds, are utilized in many countries around the world, including Thailand, Japan[1], China[2], and other Asian nations (such as Korea, Philippines, and India), as well as for food, animal feed, fertilizers, raw materials for the production of industrial products, and as natural feed for aquatic species that are economically important[3]. Seaweeds are a vital source of naturally occurring bioactive compounds [4,5]. Based on pigmentation, seaweeds are divided into three primary groups: Phaeophyta, Rhodophyta, and Chlorophyta. Brown macroalgae, or phaeophyta, are distinguished by the presence of fucoxanthin, a carotenoid. The main polysaccharides found include cellulose, alginates, laminarins, and fucans. Red-pigmented macroalgae are known as Rhodophyta, while green seaweeds, or Chlorophyta, are mostly composed of chlorophyll a and b, with ulvan being the main polysaccharide component. With cytostatic, antiviral, antihelminthic, antifungal, antibacterial, and many other biological actions, seaweeds are the best source of bioactive chemicals.

In many places of the world, seaweeds provide a naturally occurring, renewable source of food, feed, and fertilizer. They have undergone rigorous screening around the globe to separate biologically active ingredients or life-saving medications[6]. Marine organisms produce active chemicals that are used in alternative and traditional medicine. Active chemicals found in several types of marine algae have been shown to have medicinal properties. The majority of people prefer adopting natural remedies to treat illnesses since they have fewer adverse effects from natural origin drugs[7]. The chemical compounds produced by sea algae have been shown in numerous pharmacological studies to exhibit a variety of biological actions, including anti-inflammatory, anti-HIV, anticancer, and antimutagenic properties[8, 9]. More than 15,000 chemicals, including sterols, fatty acids, terpenes, phenolic compounds, enzymes, alkaloids, flavonoids, and polysaccharides, have been identified from marine microalgae, according to earlier research. It was recently revealed that antioxidant molecules with the ability to scavenge free radicals can be found in marine algae[10].

Hence the study was aimed at providing scientific evidence for the claim of antioxidant efficacy possessed by the marine algae. In this work, *Spongomorpha indica* was selected for the study and performed a preliminary screening to determine the phytochemical components that were present in the algae. The chemical constituents present in the *Spongomorpha indica* extract were identified and

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confirmed by GC-MS analysis. The study also aimed to understand more about *Spongomorpha indica's* potential as an antioxidant agent and correlate the chemical constituents present to it.

MATERIAL AND METHODS

Methods

Field investigation: A field investigation was conducted in and around Visakhapatnam coastal line with the support of Dr. Mohan Narasimharao (field botanist), Retired Professor, Department of Marine, Andhra University in order to trace out the macro algae selected.

Selection and Collection of *Spongomorpha indica*

Previous literature review indicates that this seaweed has not received significant attention. However, the presence of potentially active components in this seaweed could provide scientific evidence regarding their specific usefulness against any kind of disease. Due to this, it was collected in low tide in the coastal region of Visakhapatnam. The herbarium of sample that had been collected was submitted to the Department of Botany, Andhra University and was authenticated.

Preparation of Extract

After collecting the crude sample, it was carefully cleaned, dried in the shade, and the Soxhlet extraction method was used to prepare the extract. Initially, 10 kg of the crude sample was macerated separately for 48 h in three different solvents: water, ethyl acetate, and petroleum ether. After collecting, the solvent was evaporated using steam distillation and concentrated to produce a mass with a thick, greasy-like consistency. The active ingredients in each of the three extracts were examined individually. The dried extract was stored at 4°C in the refrigerator for future study.

Physicochemical Parameters

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 such as Moisture content (loss on drying method), total ash, insoluble acid ash, water-insoluble ash, Swelling index, foaming index, extractive values, Crude fibre content and Foreign organic matter according to the official methods described in the Indian Pharmacopeia and WHO guidelines related to quality control methods for medicinal plant materials[11-20].

Phytochemical Screening of *Spongomorpha Indica*

Preliminary phytochemical analysis, for presence of Alkaloids, Glycosides, Tannins, Flavonoids, Phenols, Oils, Steroids and Terpenoids was carried out for three different solvent-based extracts of *Spongomorpha indica* i.e., hexane, ethyl acetate, and hydro methanolic extracts by following standard procedures[21-24].

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 namedOxidation , 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.

Reducing Power Assay

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[25].

DPPH activity

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 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[26,27]. 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

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 was used as a reference compound[28].

Nitric Oxide Radical Scavenging activity

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[29].

Evaluation of Total Antioxidant Capacity of the extract

Phosphomolybdenum method was used to determine the total antioxidant capacity of the selected extract. The main mechanism 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[30].

Table 1: Physicochemical parameters results.

S.No	Parameters	Values obtained (% w/w)
1	Total ash	36.83
2	Water soluble ash	8.9
3	Acid insoluble ash	13
4	Sulphated ash	12.67
5	Loss on drying	12.47
6	Swelling index	12.15
7	Foaming index	<100
8	Crude fibre content	1.04
9	Foreign organic matter	1

Table 2: Extractive values results.

S.No	Extractive values	Values obtained (% w/w)
1	Ether soluble extractive values	0.6
2	Acetone soluble extractive values	3.2
3	Ethyl acetate soluble extractive values	2.1
4	Methanol soluble extractive value	9.8
5	Water: $CHCl_3$(95:5) soluble extractive value	14.3

RESULTS AND DISCUSSION

Physicochemical parameters results

Extractive values results

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

Phytochemical screening results

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 III. 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.

GC-MS Results

The GCMS results shown in Table IV and Figure I 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 where as compound Cyclopropanepentanoic acid, 2-undecyl-,methyl ester, trans-with highest molecular weight 130 and retention time 19.050 .

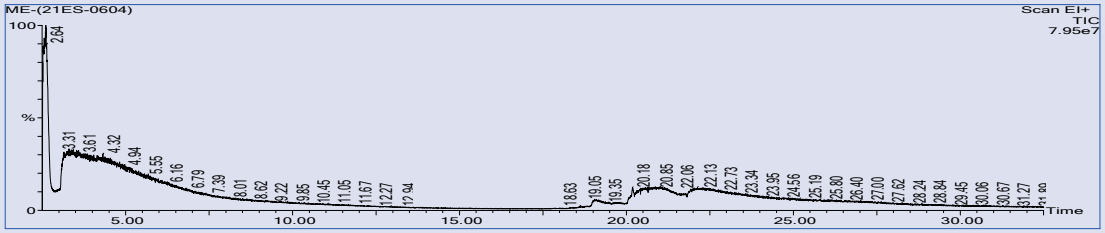
Table 3: Phytochemical screening results.

Phytochemical tests	Observations	Extracts		
		Hexane	Ethyl acetate	Hydroalcoholic (70%methanol and 30% water)
Alkaloids Mayer's test Wagner's test	Cream color Reddish brown solution/ precipitate	–	–	+
Flavonoids Lead acetate test H_2SO_4 test	Yellow orange 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 Lead acetate test	Deep blue to black colour formation 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		+	+	+

Qualitative Report

File: C:\TurboMass\2021.PRO\Data\ME-(21ES-0604).raw
Acquired: 25-Oct-21 05:09:36 PM
Description:
GC/MS Method: GC: METHOD-1.mth MS: METHOD-1.EXP
Sample ID: ME-(21ES-0604)

Printed: 27-Oct-21 03:36 PM
Page 1 of 1
Vial Number: 51



#	RT	Scan	Height	Area	Area %	Norm %
1	19.050	3309	2,223,949	329,455.6	1.881	4.00
2	20.180	3535	6,671,416	723,288.2	4.128	8.78
3	20.411	3581	6,053,358	884,621.8	5.049	10.74
4	20.631	3625	6,747,062	1,334,127.5	7.615	16.20
5	21.011	3701	6,835,764	5,742,834.0	32.780	69.73
6	22.126	3924	6,125,240	8,235,257.0	47.006	100.00
7	24.187	4336	1,595,983	269,919.8	1.541	3.28

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 60°C for 2.50 min, ramp 10°C/min to 300°C, hold 6 min, InjAauto=260°C, Volume=0 µL, Split=10:1, Carrier Gas=He, Solvent Delay=2.50 min, Transfer Temp=150°C, Source Temp=150°C, Scan: 40 to 600Da, Column 30.0m x 250µm

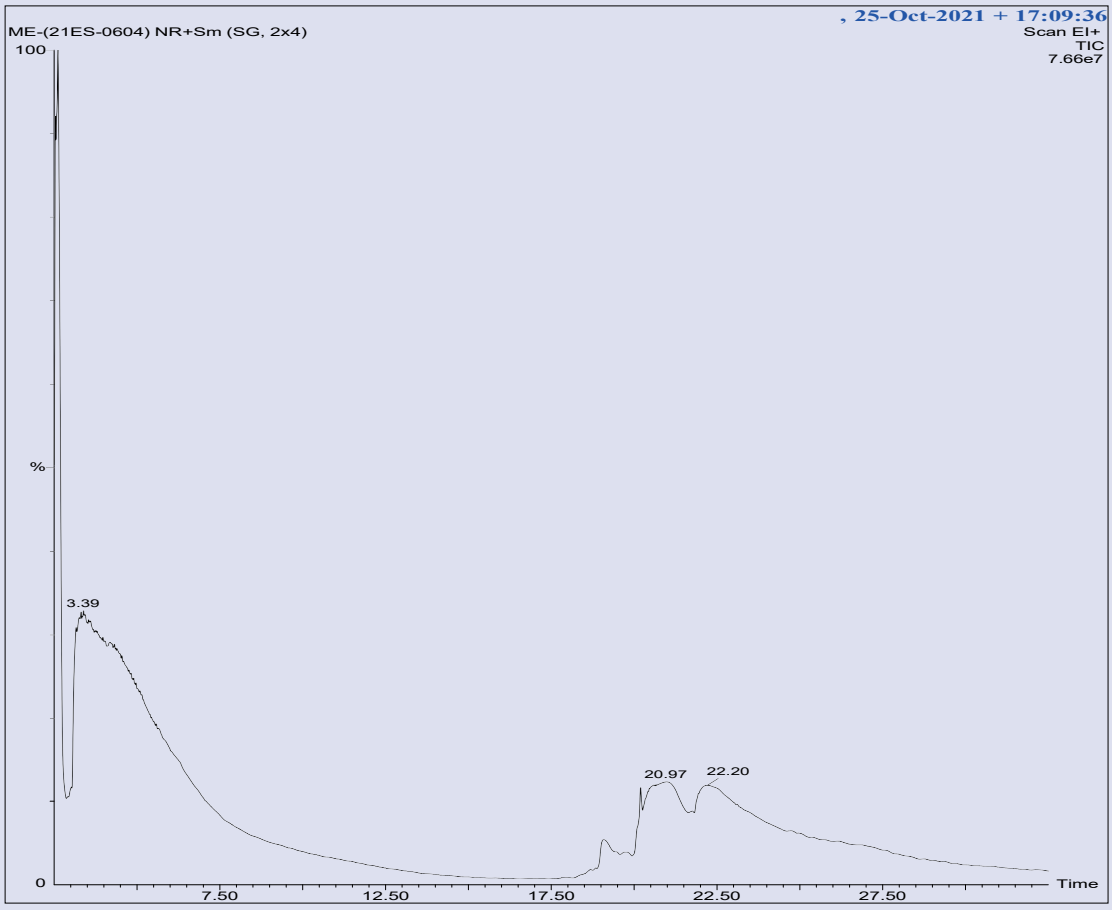


Figure 1

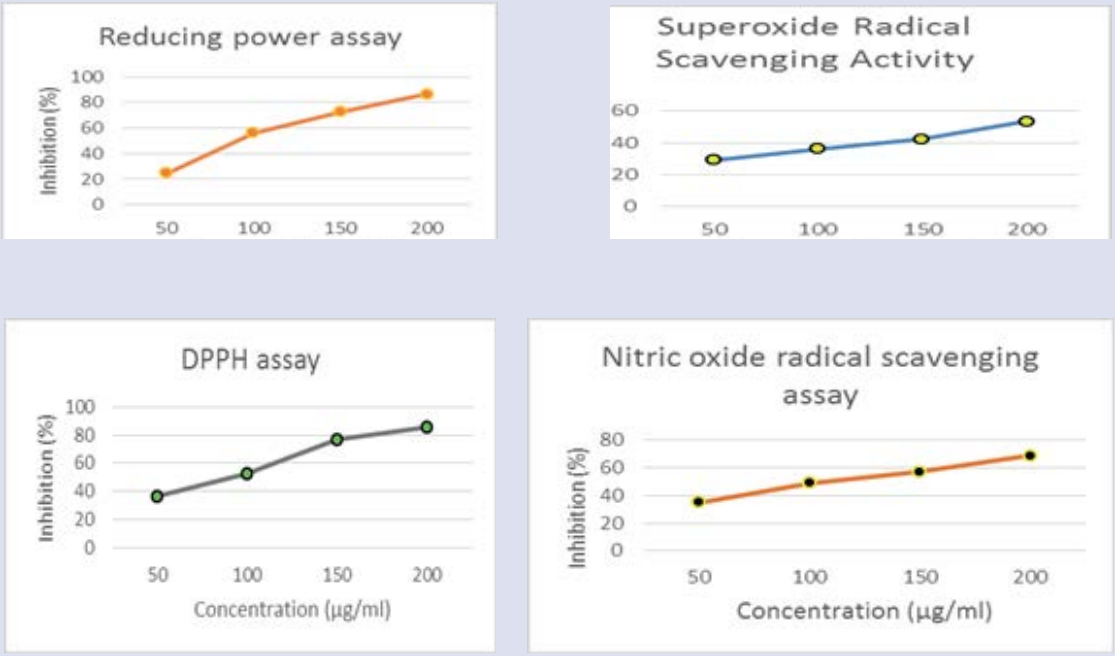


Figure 2

Table 4: Gc-Ms Results.

S.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	$C_6H_{10}O_3$	130	19.050	1.881
2	35305-79-9	CYCLOPROPANEPENTANOIC ACID,2-UNDECYL-,METHYL ESTER, TRANS-	$C_{20}H_{38}O_2$	310	20.180	4.128
3	900143-83-9	DECYL TRIFLUUOROACETATE	$C_7H_{16}O$	116	20.411	5.049
4	90526-63-5	2,3-EPOXYHEXANOL	$C_6H_{12}0_2$	116	20.631	7.615
5	13205-57-7	1-METHYLDODECYLAMINE	$C_{13}H_{29}N$	199	21.011	32.780
6	646-30-0	NONDECANOIC ACID	$C_{19}H_{38}O_2$	298	22.126	47.006
7	41446-54-2	4-TRIDECENE,(Z)-	$C_{13}H_{26}$	182	24.187	1.541

Table 5: Antioxidant results.

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

Antioxidant results

Total antioxidant activity

The antioxidant activity of hydroalcoholic extract of *Spongomorpha indica* was studied using the different models i.e; Reducing power assay, DPPH assay, Superoxide radical scavenging assay, Nitric oxide radical scavenging assay shown in Table v and Figure II. 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.

Spongomorpha indica is rich in flavonoid and phenolic compounds. The presence of flavonoids and hydrophilic polyphenolic compounds could function as a major antioxidant, which helps the algae resist oxidative stress [31-33]. The presence of phenolic and flavanoids contributes to the antioxidants potential of *Spongomorpha*[34]. These results were in agreement with previous studies that found the antioxidants properties due to the presence of chemical constituents like flavonoids and phenolic compounds[35,36].

CONCLUSION

The use of natural and plant-based products is a useful tool to fight against free radicals due to their few or no side effects. Marine algae have already been used as a food supplement and antioxidants and currently, research on the health benefits of various types of Algae is

gaining huge interest. This study demonstrated that hydroalcoholic extract of *Spongomorpha sp*. possessed higher Antioxidant compared to other extracts prepared. Moreover, when the extracts were screened for Antioxidant activity, were reducing power and DPPH assay were significantly affected by different concentrations of hydroalcoholic extracts of *Spongomorpha sp*. Seven different compounds were found in the hydroalcoholic extract according to the GC-MS data, which also demonstrated the extract's strong antioxidant capability in the four antioxidant assays that were carried out. This study demonstrated the antioxidant activity of *Spongomorpha sp*. which is due to the presence of several active potent Antioxidant chemicals such as phenols and flavonoids. Furthermore, it's chemical composition make it an ideal therapeutic agent in novel drugs as well as nutritional supplements.

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