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Research Article

Bioactive Compounds Screening and *In Vitro* Appraisal of Potential Antioxidant and Cytotoxicity of *Cladophoropsis sp*. Isolated from the Bay of Bengal

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Abstract

In recent years, seaweeds got a major attention worldwide and Bangladesh is becoming one of the raising seaweed hub. Until now, about 193 seaweed species identified in coastlines of Bay of Bengal in Bangladesh. Studies like identification of phytochemical bioactive compounds, antioxidant, cytotoxicity and antimicrobial activities are some of the trending studies among the others and may be haven't been studied yet in Bangladesh. Focusing on the trending activities, the present study showed an insight of bioactive phytochemicals and their antioxidant and cytotoxic profiles from the ethanolic and methanolic extracts of green seaweed, *Cladophoropsis sp.* isolated from Saint Martins Island, Bay of Bengal, Bangladesh. Though phytochemical screening and UV-VIS analysis we confirmed the presence of phenolics, tannins, flavonoids, steroids, alkaloids and chlorophyll a/b. The pharmacological efficacy of these bioactive compounds was further investigated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and brine shrimp lethality assay (BSLA) *in vitro*. We showed that the ethanol (preserve and preserve dry) had higher radical scavenging capacity and the methanol extract also significantly ($p < 0.001$) reduced the free radicals compared to ethanol (crude) extract. Though brine shrimp lethality assay, we also confirmed the higher cytotoxic effect of ethanol (crude) and methanol extract and a significant LC_{50} was calculated concerning to methanol extract ($p < 0.05$) compared to ethanol (crude) extract. Thus, it is plausible that *Cladophoropsis sp.*, from the Bay of Bengal, has potential antioxidant and cytotoxic activity and could be a potential source of novel bioactive compounds.

Keywords: *Cladophoropsis sp.; Bioactive Compounds; Phytochemical Screening; Antioxidant; Cytotoxicity*

Abbreviations

DPPH: 2,2-diphenyl-1-picrylhydrazyl; BSLA: Brine Shrimp Lethality Assay; UV-VIS: Ultraviolet-Visible; IC: Inhibition Concentration; LC: Lethal Concentration; AEAC: Ascorbic Acid Equivalent Antioxidant Capacity; DW: Dry Weight; SD: Standard Deviation

Introduction

Marine bio-resources are reported to have medicinal values with least side effects and seaweeds have great economic value in providing low-cost, wholesome nutrition and therapeutic protection. Seaweeds are macroscopic multicellular benthic marine algae that are

plant-like organisms and generally live attached or freely floating and grow in deep-sea areas up to 180 meter in depth and also in estuaries, black water and in shallow rocky coastal areas [1-3]. Seaweeds are highly competitive and complex species, having the advantages of strong adaptability, obliged to share a limited extent habitat and high yield, which is also considered as potential bioactive reservoir and produce complex secondary metabolites as a response to ecological stress, therefore, it is of great significance to make better use of marine algae resources [4-7].

Self-defense system is the ancestral inheritance to all living being ranged from prokaryotes to eukaryotes. Mostly, in eukaryotes, specially in human, various pathological conditions arises due to uncontrolled redox homeostasis in the body such as lipid peroxidation, DNA damage, cellular degeneration in the cells and so on [8]. Antioxidants scavenge these free radicals to assist primary protection by maintaining the redox homeostasis [9]. Many synthetic antioxidants and cytoprotective drugs are available but the key concern is the side effects. Concerning to the side effects of these synthetic drugs, scientists are looking for alternative sources of natural drugs, which would have less side effects and very precise target specific effects. In this regard, marine sources have received a great attention mostly due to its less side effects on human [10]. Seaweeds represented a virtual component of the marine ecosystems and are important natural source of marine bioactive compounds such as carotenoids, dietary fibres, protein, essential fatty acid, lipoproteins, vitamins and minerals alongside with their significant ecological role in the nature [3,11-17]. The nutrient and chemical contents vary with species, habitat, geographical location, maturity stage, sampling, ecological conditions, environmental condition, season, light, pH, turbulence, temperature etc [18-20]. So, it is plausible that marine ecosystem plays a great role for the availability of the bioactive compounds in marine seaweeds.

Cladophoropsis sp., marine green algae, can reproduce, associated with sponge tissue, typically grows on intertidal to shallow subtidal rocky coastlines throughout the tropics and subtropics like Bay of Bengal and also found on coral reefs. Most of the *Cladophoropsis sp*. shows temperature tolerance and found at 15ºC to 36ºC but shows highest growth at 25ºC [21-25]. Various pharmacological properties like antimicrobial [25-28], cytotoxic [29,30], antioxidant [31,32] of this genus were previously reported, which were isolated from different parts of the ocean with biodiversity. There is no report on the bioactive properties of *Cladophoropsis sp.*, originated from the Bay of Bengal of Bangladesh, a highly diversified continental shelf due to its geographical location. There are at least 193 seaweed species in Bangladesh and a minimum of 140 of them are found in the St. Martin's Island [33].

The aim of this study was to inspect the bioactive compounds and to gauge the antioxidant and cytotoxic assays to decode the pharmacological effects and phytochemical inspection of *Cladophoropsis sp.* from the Bay of Bengal of Bangladesh. Though numerous studies have shown the pharmacological importance of this species, which have been done in different continental shelf of the world, there still remains bountiful scope for further research due to its ecological biodiversity. So far, for the first time a pursuit was taken to condone the antioxidant and cytotoxic effect of its ethanolic and methanolic extracts in South Asian territory. Accordingly, we disclose herein the potent antioxidant and cytotoxic properties of *Cladophoropsis sp.* to further establish the scientific basis of this species from the Bay of Bengal.

Materials and Methods

Sample collection and processing

Seaweed samples were collected in late November 2019 from the shallow water on the eastern side of Chera Island (Chera Dwip), which is an indwelled extension of St. Martin's Island. They were then cleaned with clean seawater and completely immersed in 50% ethanol for preservation. The collected seaweed was subsequently identified to be *Cladophoropsis* sp. based on its morphology [34]. Around one month later, the seaweeds were taken out of ethanol, washed with filtered water and segmented followed by air drying and oven drying at 37^{0}C, then ground to powder with mortar and pestle. The powder was stored at -20^{0}C until further use.

Preparation of extracts

2.0 mg of *Cladophoropsis* sp. tissue powdered sample was soaked in 20 mL of 50% ethanol and 70% methanol, separately, with occasional shaking at 150 rpm for 3 days. After subsequent occasional shaking, the resultant extracts were filtered through a Whatman No.1 filter paper and the filtrate extracts were stored at -20^{0}C until further analysis. Extracts prepared in this way were referred to as EtOH (Soak) and MeOH (Soak), respectively. The remaining 50% EtOH preserve solution were filtered by Whatman No.1 filter paper and kept at -20^{0}C. This extracts denoted as 50% EtOH (Preserve and Preserve Dry). A portion (20 ml) of all filtered extracts were evaporated in a dry oven at 40^{0}C and were weighed. The obtained concentrations were 3.25 mg/ml (for both 50% EtOH extract and 70% MeOH extract) and 10.5 mg/ml for 50% EtOH preserve solutions. The dried extracts were stored at -20^{0}C.

Phytochemical screening

Qualitative phytochemical tests for the identification of phenolic compounds, tannins, alkaloids, steroids, steroidal glycosides, flavonoids and saponins were performed using previously described methods [35-38]. Phytochemical screening of the extracts was performed with following tests: phenolic compounds with lead acetate test, tannins with ferric chloride test, alkaloids with Mayer's test, steroids and glycosides with Salkowski's test, flavonoids with alkaline reagent test and saponins with frothing test.

UV-VIS spectrum analysis

The extracts were examined under UV-VIS spectrum analysis and the absorption maxima of compounds with a wide range of wavelength were determined according to [39-42]. To detect the UV-Visible spectrum profile, different extracts were examined under visible and UV light for proximate analysis. For UV-Visible spectrophotometer analysis, the extracts were 10 times diluted with corresponding solvent and centrifuged at 3000 rpm for 10 min and filtered through Whatman No.1 filter paper. Corresponding blanks were also prepared. The extracts were scanned to get spectrum of compounds compared with their corresponding blank at wavelength ranging from 190.0 to 1100.0 nm in a medium scanning speed, single scanning mode, auto sampling interval, slit width 1.0 and the threshold was 0.001 using UV-Visible Spectrophotometer (Shimazdu UV-1900 Spectrophotometer Series). The corresponding blanks were balanced during each baseline correction. The distinctive peaks values were then recorded to detect the corresponding compound. Each and every analysis was repeated twice for the spectrum confirmation.

Brine shrimp lethality assay (BSLA)

Brine shrimp lethality assay was performed using previously described methods [43-45] with few modifications. In brief, artificial seawater was made by dissolving 37g sodium chloride 1L of sterile distilled water and adjusting its pH between 8.25 and 8.5 by adding 1.0N sodium hydroxide (NaOH). 200 mg of brine shrimp (*Artemia salina*) eggs were hatched to produce nauplii in 1L of this water for 24 hours under strong aeration in a vessel illuminated by a 60 watts bulb. Lethal capacity of EtOH (Crude) and MeOH (Crude) extracts were determined for 5 different concentrations (0.065, 0.13, 0.191, 0.26 and 0.325 mg/mL.) and the other 2 extracts, EtOH (Preserve and preserve dry) were determined at 10 different concentrations (0.105, 0.21, 0.315, 0.42, 0.525, 0.63, 0.735, 0.84, 0.945 and 1.05 mg/mL) in triplicates into separate test tubes. The organic solvent of the extracts was completely evaporated and added with 5 ml artificial seawater. 10 nauplii were added to each test tube and the final volume was adjusted to 5.0 mL by adding artificial seawater. After 24 hours, number of dead nauplii was counted with the aid of a magnifying glass. Mortality (%) was calculated using the following formula:

$$\text{Mortality (\%)} = \frac{number\ of\ dead\ nauplii}{number\ of\ dead\ nauplii + number\ of\ alive\ nauplii}\ x\ 100$$

Cytotoxicity of the positive control, potassium dichromate ($K_2Cr_2O_7$), was determined in the same way using final concentrations of 0.009, 0.018, 0.027, 0.036, 0.045, 0.054, 0.063, 0.072, 0.081 and 0.09 mg/mL in triplicates. 50% ethanol and 70% methanol were used as

negative controls.

DPPH scavenging activity

DPPH scavenging activity was assayed using a previously described method [46,47] with slight modifications. In brief, 4 mg of DPPH (Cat. No.: sc-202591, Santa Cruz Biotechnology, USA) was dissolved in 100 mL of 95% methanol. Antioxidant activities of EtOH (Crude) and MeOH (Crude) extracts were determined for 3 different concentrations (0.542, 0.667 and 0.813 mg/ml) and those of the other 2 extracts, EtOH (Preserve and preserve dry) were determined at 7 different concentrations (0.125, 0.188, 0.25, 0.313, 0.375, 0.438 and 0.5 mg/ml). In each case, 1.5 ml aliquot of the each sample, after dilution, was mixed with 1.5 ml of DPPH solution. Three replicates were prepared for each dose level. L-Ascorbic acid was used as positive control. L-ascorbic acid (#cat: 23006, CAS no: 50-81-7, Sisco Research Laboratories Pvt. Ltd., India) was dissolved in distilled water. Standard curve was generated using radical scavenging activities of L-ascorbic acid at various concentrations (at the concentrations of 0.00015, 0.0015, 0.01, 0.015 and 0.015625 mg/ml) utilizing the aforementioned protocol. These mixtures were kept in the dark at room temperature for 1 hour. Then absorbance was measured at 517 nm using a UV-VIS spectrophotometer (model: UV-1900, Shimadzu). All determinations were performed in triplicates. The free radical scavenging activity or % of inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \left(1 - \frac{A_{sample} - A_{blank}}{A_{control} - A_{blank}}\right) \times 100$$

Where A_{sample} = Absorbance of reaction in presence of the sample (sample dilution + DPPH solution)

$A_{control}$ = Absorbance of control reaction (sample solvent + DPPH solution)

A_{blank} = Absorbance of blank for each sample dilution (sample dilution + DPPH solvent).

Ascorbic acid equivalent antioxidant capacity (AEAC) of each extract was calculated using the following formula:

$$\frac{IC_{15} of\, ascorbic\ acid (mg/ml)}{IC_{15} of\, sample (mg/ml)} * 10^5 = \frac{Xmg\ of\, ascorbic\ acid\ equivalent}{100g\, of\, dry\ weight}$$

Statistical analysis

The significance of data was calculated using two tail paired/independent t-test with unequal variance. LC_{50} was also calculated using Probit Analysis. All the triplicate data were expressed as Mean±SD as appropriate. The limit of significance was set at $p < 0.05$.

Results

Phytochemical screening

Phytochemical screening is the preliminary aspect in evaluating bioactivity of seaweeds, since the constituents vary qualitatively and not only from species to species but also in different samples of the same species depending upon various solvent, factors and storage conditions. Various phytochemical analysis revealed the presence of tannins, phenolic compounds and steroids both in 50% ethanol preserve and preserve dry extracts and only a lower amount of steroids were observed in 50% ethanol and 70% methanol extracts of *Cladophoropsis* sp (Table 1).

The UV-Visible absorption spectrum was recorded to depict the efficacy of *Cladophoropsis sp.* seaweed extract. The qualitative UV-

Tested compounds	(50%) EtOH			(70%) MeOH
	50% EtOH	Preserve	Preserve dry	
Tannins	-	+++	+++	-
Phenolic compounds	-	+++	+++	-
Steroids	+	+++	+++	+
Glycosides	-	-	-	-
Flavonoids	-	-	-	-
Alkaloids	-	-	-	-
Saponins	-	-	-	-

Table 1: *Qualitative analysis of the phytochemicals of Cladophoropsis sp extracts.*
(-): Not Detectable; (+): Low Quantities; (+++): High Quantities.

Visible spectrum profile of different extract of *Cladophoropsis sp.* was selected at wavelength from 190.0 to 1100.0 nm due to sharpness of the peaks and proper baseline. The UV-Visible absorption spectrum analysis showed the presence of chlorophyll a, chlorophyll b, terpenoids, steroids, flavonoids and phenolic compounds as like phytochemical screening study (Figure 1A-1D and table 2).

50% EtOH		70% MeOH		50% EtOH Preserve		50% EtOH Preserve Dry		Descriptions
Wavelength	Abs	Wavelength	Abs	Wavelength	Abs	Wavelength	Abs	
908.00	0.002	–	–	–	–	–	–	*
664.00	0.007	664.00	0.019	665.00	0.032	665.00	0.032	Chlorophyll a/Alkaloids
–	–	609.00	0.006	605.00	0.014	605.00	0.014	Chlorophyll b
400.00	0.030	402.00	0.071	401.00	0.156	402.00	0.156	Terpenoids
280.00	0.104	272.00	0.152	279.00	0.607	279.00	0.605	Steroid/Flavonoids
195.00	0.707	198.00	1.114	198.00	3.651	198.00	3.643	Phenolics

Table 2: *Absorbance with corresponding wavelengths of various extracts.*
**: Compounds on relevant spectrum are unidentified.*

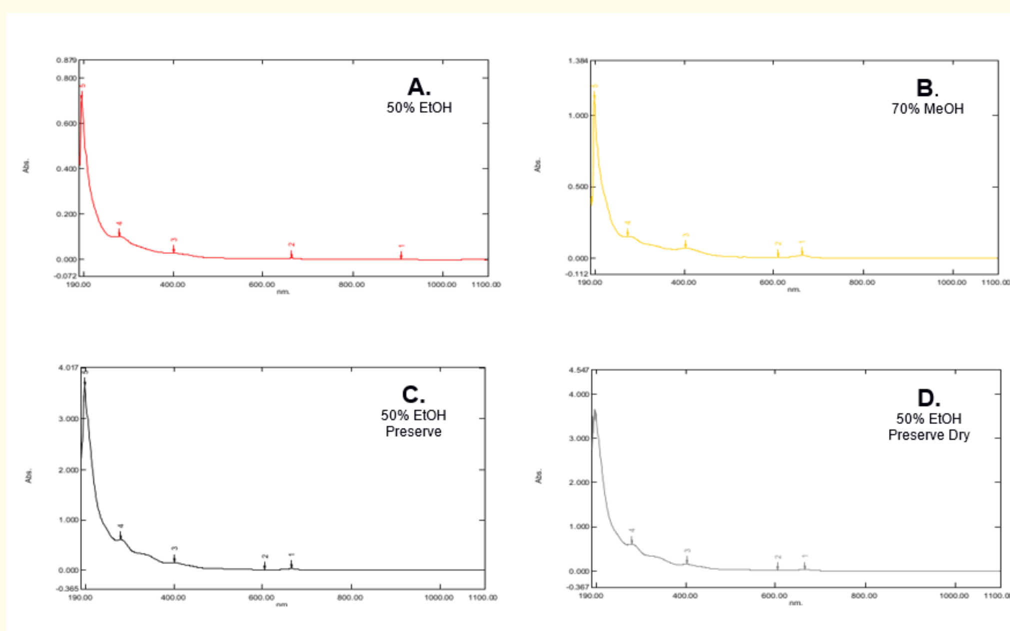

Figure 1: *UV-VIS spectrums of various extracts.*

DPPH scavenging activity

The DPPH radical scavenging activity was evaluated at different concentrations of various extracts of *Cladophoropsis sp.* Highest DPPH scavenging activity (46.557 ± 1.425% of inhibition) was observed for EtOH (Preserve Dry) at 0.5 mg/mL (Table 3), which is similar with the EtOH (Preserve) group but significantly higher % of inhibition were observed at concentrations of 0.125, 0.188 and 0.25, when comparison were made between EtOH (Preserve) and EtOH (Preserve Dry) groups. Furthermore, highest and significant % of inhibition was recorded (15.133 ± 1.082*) when comparison was done between the EtOH (Crude) and MeOH extracts (Table 3). None of the extracts caused 50% of inhibition. So, IC_{15} was determined. We used both linear regression and best-fitting models (4- and 5-parameter logistic regression for extracts) for determining IC_{15}. Values obtained from linear regression remained within the 95% confidence interval for IC_{15} estimated by 4- or 5-parameter logistic regression in all cases. 50% EtOH (Crude) extract shows lowest DPPH radical scavenging activity at a highest IC_{15} values of 4.0347 ± 3.04 mg/mL, while Ascorbic Acid exhibit highest DPPH radical scavenging activity at a lowest IC_{15} values of 0.00062 mg/mL.

Sample	Concentration (mg/mL)	% inhibition	IC_{15} (mg/mL)	AEAC (mg ascorbic acid equivalent/100g)
50% EtOH (Crude)	0.542	1.826 ± 0.286[a]	4.035 ± 3.04	27.11
	0.667	3.362 ± 0.87[b]		
	0.813	4.593 ± 3.164[c]		
70% MeOH (Crude)	0.542	11.364 ± 0.923***[a]	0.819 ± 0.052	75.87
	0.667	12.508 ± 0.714**[b]		
	0.813	15.133 ± 1.082*[c]		
50% EtOH (Preserve)	0.125	16.592 ± 0.652[d]	0.016 ± 0.017	4209.27
	0.188	31.183 ± 1.753*[e]		
	0.25	32.311 ± 1.556*[f]		
	0.313	34.37 ± 0.714		
	0.375	37.27 ± 0.153		
	0.438	39.222 ± 0.54		
	0.5	46.221 ± 0.449		
50% EtOH (Preserve Dry)	0.125	19.186 ± 0.935**[d]	0.0424 ± 0.024	2140.29
	0.188	27.305 ± 0.232[e]		
	0.25	28.368 ± 0.248[f]		
	0.313	31.535 ± 1.337		
	0.375	34.868 ± 2.491		
	0.438	40.512 ± 5.379		
	0.5	46.557 ± 1.425		
L-Ascorbic Acid	0.15×10^{-3}	3.69		
	1.5×10^{-3}	28.35		
	10×10^{-3}	68.53		
	15×10^{-3}	92.09		
	15.625×10^{-3}	95.17		

Table 3: *DPPH radical scavenging activity of Cladophoropsis sp. extracts.*
Two tail paired T-test has been done. Comparison were made among a, b, c, d, e, and f group.
**p < 0.05, **p < 0.01 or ***p < 0.001 were considered as significant.*

Methanolic extract of *Cladophoropsis sp.* possessed potential cytotoxicity

For the cytotoxicity assay, we performed brine shrimp lethality assay (BSLA) using various concentrations of different extracts of *Cladophoropsis sp.* The lethal capacity by 50% EtOH (Crude) and 70% MeOH (Crude) extracts of the green algae *Cladophoropsis sp.* was evaluated at different concentrations of the extracts. The 70% MeOH (Crude) extract of *Cladophoropsis sp.* exhibited relatively higher lethal activity (96.7 ± 5.77%) compared to 50% EtOH (Crude) extract (43.3 ± 5.774%) at a higher concentration of 0.325 mg/mL, while $K_2Cr_2O_7$ exhibited the highest lethal activity (100%) at a lower concentration as 0.081 mg/mL. as expected (Table 4 and figure 2A). Concerning to same solvent extraction methods, the highest level of cytotoxicity were observed at a concentration of 1.07 mg/mL of 50% EtOH (Preserve) (90 ± 0.0%), and 50% EtOH (Preserve Dry) (93.3 ± 5.774%) (Table 4 and figure 2B), though there was no significant difference between these two groups regarding the LC_{50} value (Figure 2C). LC_{50} was estimated at 95% CI using LW1949 package [48] and also calculated by Probit regression analysis. On the other hand, 70% MeOH (Crude) extract showed highest lethal activity with LC_{50} values of 0.17 ± 0.009 mg/mL, while $K_2Cr_2O_7$ exhibited lethal activity at a lowest LC_{50} values of 0.033 ± 0.004 mg/mL (Figure 1C). Standard plot using various concentrations of $K_2Cr_2O_7$ showed almost a linear regression line (Figure 1D). Thus, the tested concentrations provided more precise information about the cytotoxicity of methanolic extracts of *Cladophoropsis sp.*

Extracts	Concentration (mg/mL)	Mortality (%) (Mean ± SD)	Probit Value
50% EtOH (Crude)	0.065	3.3 ± 5.774[a]	3.1661
	0.13	10 ± 0.0[b]	3.71845
	0.191	16.7 ± 5.774[c]	4.03258
	0.26	26.7 ± 5.774[d]	4.37707
	0.325	43.3 ± 5.774[e]	4.83211
70% MeOH	0.065	16.7 ± 5.774*[a]	4.03258
	0.13	30 ± 0.0***[b]	4.4756
	0.191	46.7 ± 5.774***[c]	4.91635
	0.26	73.3 ± 5.774**[d]	5.62293
	0.325	96.7 ± 5.774**[e]	6.83392
50% EtOH (Preserve)	0.105	3.3 ± 5.774	3.16609
	0.21	10 ± 0.0g	3.71845
	0.315	16.6 ± 5.774	4.03258
	0.421	20 ± 0.0	4.15838
	0.525	23.3 ± 5.774	4.27209
	0.63	30 ± 0.0h	4.4756
	0.735	46.7 ± 5.774***f	4.91635
	0.84	63.3 ± 5.774	5.3407
	0.945	80 ± 0.0j	5.84162
	1.05	90 ± 0.0	6.28155
50% EtOH (Preserve Dry)	0.105	3.3 ± 5.774	3.16609
	0.21	10 ± 0.0	3.71845
	0.315	13.3 ± 5.774	3.88923
	0.421	16.7 ± 5.774	4.03258
	0.525	26.7 ± 5.774	4.37707
	0.63	30 ± 0.0	4.4756
	0.735	36.7 ± 5.774f	4.65931
	0.84	60 ± 0.0	5.25335
	0.945	80 ± 0.0	5.84162
	1.05	93.3 ± 5.774	6.50109

Potassium Dichromate	0.009	6.7 ± 5.774	3.49891
	0.018	23.3 ± 5.774	4.27209
	0.027	30 ± 10	4.4756
	0.036	46.7 ± 5.774	4.91635
	0.045	63.3 ± 5.774	5.3407
	0.054	70 ± 0.0	5.5244
	0.063	86.7 ± 5.774	6.11077
	0.072	93.3 ± 5.774	6.50109
	0.081	100 ± 0.0	#Value!
	0.09	100 ± 0.0	#Value!
No mortality was observed in the Negative controls			

Table 4: *Cytotoxicity profile of Cladophoropsis sp. extracts.*
*Independent T-test has been done. Comparison between 50% EtOH & 70% MeOH; 50% EtOH preserve and 50% EtOH preserve dry, which corresponds to a, b, c, d, e, and f. *p < 0.05, **p < 0.01 or ***p < 0.001 were considered as significant.*

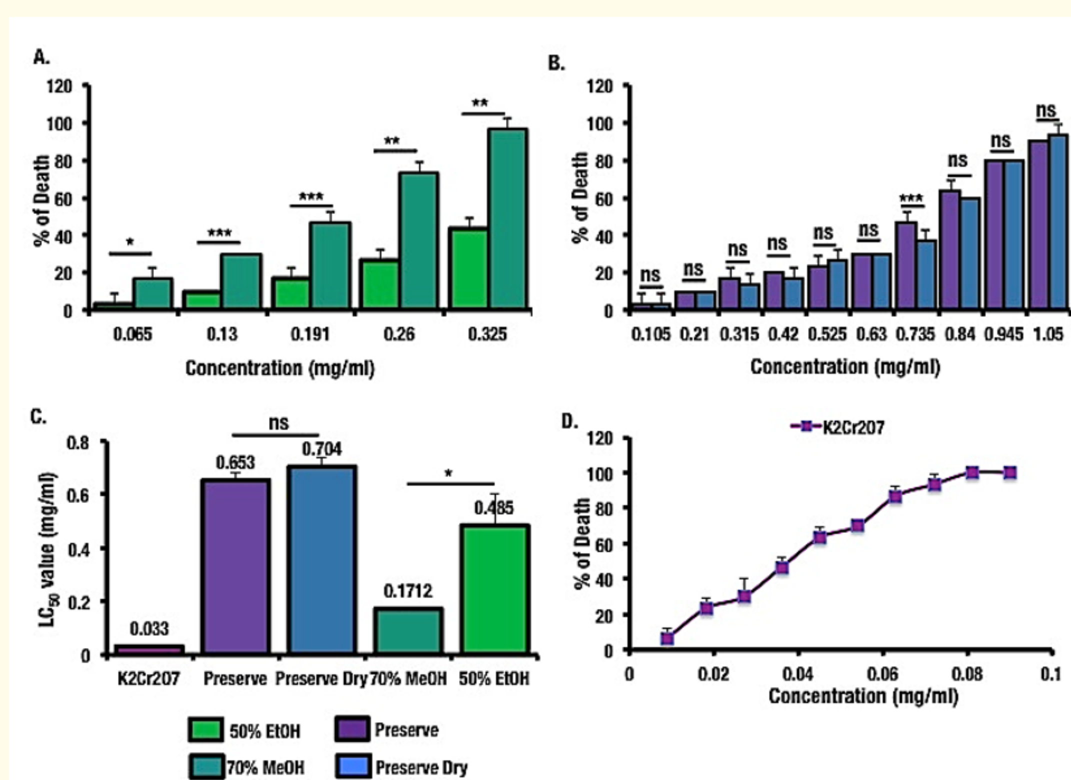

Figure 2: *Cytotoxicity profiles of Cladophoropsis sp. ethanol and methanol extracts. A. The % of death of the nauplii due to ethanol and methanol extracts. B. The % of death of the nauplii due to ethanol (Preserve) and ethanol (Preserve Dry) extracts. C. LC50 values of standard and various other extracts. D. Standard curve with K2Cr2O7. Two tailed T-test was performed and *p < 0.05, **p < 0.01 and ***p < 0.001 were considered statistically significant.*

Discussion

In the present study, we performed a pharmacological screening of one of the marine seaweeds, *Cladophoropsis sp.* in relation to its antioxidant scavenging capacity as well as cytotoxicity. In relation with this study, at the beginning, we also performed a bioactive compound screening through phytochemical screening and UV-VIS analysis. We confirmed through phytochemical screening the presence of tannins, phenolics and steroids in ethanolic extracts of *Cladophoropsis sp*. Presence of phenolics supported the previous finding where different species from *Cladophoropsis* were studied [31]. Steroids were found in all kinds of extracts, which is a novel finding of this phytochemical screening study. Furthermore, we extended our study for further confirmation through UV-VIS study and confirmed the presence of phenolics, terpenoids, steroids, flavonoids, alkaloids, chlorophyll a/b in ethanol (preserve, preserve dry) and methanol extracts, based on the absorption spectrum (Figure 1 and table 2). Thus, we confirmed the presence of bioactive compounds such as tannins, phenolics, flavonoids, terpenoids and steroids in the seaweed extracts of *Cladophoropsis sp*. We further clarified the antioxidant and cytotoxic properties of this species.

Seaweeds are considered to be a rich source of antioxidants [49]. The DPPH radical scavenging capacity was used in this study by using various concentrations of ethanolic and methanolic extracts of the green algae *Cladophoropsis sp*. We found significant DPPH scavenging activity concerning to various different extracts (Table 3). In a previous study, *Cladophoropsis sp.* significantly augmented peroxide value (POV) and showed potential antioxidant activity in 80% MeOH extracts [31]. In our study, we showed that the 70% MeOH extract of *Cladophoropsis sp.* (15.133 ± 1.082%) exhibited relatively higher DPPH activity at a concentration of 0.813 mg/mL. compared to crude ethanol extract. The 90% ethanolic extract of *Cladophoropsis sp.* showed the highest reducing power with concentration of 1.48 ± 0.04 mg/mL and 79.29% lipid peroxidation inhibition after 10 days were reported [32]. We showed that 50% EtOH extract of *Cladophoropsis sp.* inhibited as minimal as (4.593 ± 1.082%) at a concentration of 0.813 mg/mL but the 50% EtOH (preserve) and the 50% EtOH (preserve dry) exhibited at a higher DPPH activity (46.221 ± 0.449% and 46.557 ± 1.425%, respectively) (Table 3), when the concentration was 0.5 mg/mL. The IC_{15} value was the lowest in case of 50% EtOH (preserve) extract, which was statistically significant ($p < 0.05$) compared to EtOH (preserve dry) extract. The AEAC of the extracts of *Cladophoropsis sp.* was increased (50% EtOH preserve > 50% EtOH preserve dry > 70% MeOH > 50% EtOH) with decreased IC_{15} values. Presence of phenolic compounds, tannins and flavonoids in the extracts may account for their antioxidant activity [50,51].

Concerning to the antioxidant activity, flavonoids, (a large group of naturally occurring plant polyphenolic compounds including flavones, flavonols, isoflavones, flavonones and chalcones), play a significant role to scavenge the free radicals. The capability to interact with protein phosphorylation and the antioxidant, iron chelating, and free radical scavenging activity may account for the antioxidant profile of flavonoids [52-55].

Antioxidant activities of different phenolic alkaloids, such as, oleracein A, oleracein B and oleracein E, was also reported based on their inhibitory effect on hydrogen peroxide-induced lipid peroxidation in rat brain homogenates [56]. The phenolic contents of the extract can also scavenge hydrogen peroxide by donating electrons and thereby neutralizing it to water [57]. Based on the previous report, it can be said that phenolic compounds are effective hydrogen donors, which add the property as good antioxidants [58]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing super oxide anion ($O_2^{-\bullet}$), hydroxyl radical or peroxy radicals, quenching singlet and triplet oxygen or decomposing peroxides [59]. Polyphenolic contents appear to function as good electron and hydrogen atom donors, and therefore, be able to terminate radical chain reaction involved in lipid peroxidation by converting free radicals and reactive oxygen species to more stable products. Thus, the antioxidant activity of *Cladophoropsis sp.* extracts might be attributed to these modes of activity due to their flavonoid and alkaloid contents.

In brine shrimp lethality assay, all extracts showed cytotoxicity, but the highest level of cytotoxicity were observed in case of methanol, ethanol (preserve) and ethanol (preserve dry) extracts (Table 4), which is similar with the previous reports [29,60-62]. In our study, 70%

MeOH extract of *Cladophoropsis sp.* exhibited significantly ($p < 0.01$) higher lethal activity (96.7% ± 5.774%) at a concentration of 0.325 mg/mL compared to crude ethanol extract, which is similar with another group [30]. The LC50 value also supported the highest lethal activity (0.1712 ± 0.009 mg/ml) of 70% MeOH extract (Figure 2C). Presence of phenolic compounds and steroids in the extracts may account for their toxicity to brine shrimps [63,64]. Generally, cytotoxic compounds in seaweeds, including *Cladophoropsis sp.*, can act as chemical defense against herbivores [65].

Conclusion

In short, we confirmed the presence of various bioactive compounds in various extracts of *Cladophoropsis sp.* isolated from the Bay of Bengal and explored the potential antioxidant and cytotoxicity. In our phytochemical screening study, the highest quantity of phenolic compounds and tannins ensured the possible presence of antioxidant, anti-inflammatory, anti-allergenic, antithrombotic, anti-carcinogenic and hepatoprotective activities of *Cladophoropsis sp*. Furthermore, for the first time we also disclosed the presence of steroids in this species, which will open a new window to explore the cellular signaling system by using *Cladophoropsis sp.* as a source of steroids.

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Conflict of Interest

We have no conflicts of interest to disclose in this study.

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