In vitro antioxidant and cytotoxic capacity of *Kappaphycus alvarezii* successive extracts

Ruthiran Papitha[1], Chinnadurai Immanuel Selvaraj[1,*], V. Palanichamy[1], Prabhakarn Arunachalam[2] and Selvaraj Mohana Roopan[3,*]

[1]School of Biosciences and Technology, VIT School of Agricultural Innovations and Advanced Learning, Vellore Institute of Technology, Vellore 632 014, India
[2]Electrochemistry Research Group, Chemistry Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[3]Chemistry of Heterocycles and Natural Product Research Laboratory, Department of Chemistry, School of Advanced Science, Vellore Institute of Technology, Vellore 632 014, India

***Kappaphycus alvarezii*, marine red algae was collected from the Mandapam coastal region, Tamil Nadu, India to examine cytotoxic and redox capacity of its extracts. HPLC analysis indicates the presence of phenolic compounds in the methanolic extract. The maximum phenol content measured as mg of gallic acid standard equivalent was found to be 86.45 ± 70.72 mg/ml extract. The total flavonoid content was found to be 85.52 ± 32.57 mg/ml in the extract (using quercetin as a standard). The antioxidant potential of the extracts was determined by employing DPPH scavenging assay and antioxidant assay using the phosphomolybdenum method. Methanolic extract demonstrated efficient antioxidant capacity and DPPH radical scavenging activity. GC–MS analysis was carried out in crude extracts of *K. alvarezii.* MTT assay showed that the extracts are toxic to HeLa cells and their IC_{50} value was 1.75 mg/ml. The study shows that different extracts of *K. alvarezii* have strong antioxidant and less cytotoxic activity against HELA cells.**

Keywords: Antioxidants, cytotoxic capacity, *Kappaphycus alvarezii*, phytochemical screening.

KAPPAPHYCUS ALVAREZII is a red tropical commercial seaweed that is cultivated mainly in the Far East countries as raw material for industrial production of carrageenan[1]. The marine red–algae falls under the domain Eukaryota, kingdom Archaeplastida, phylum Rhodophyta, class Rhodophyceae, order Gigartinales, family Solieriaceae, genus *Kappaphycus*, species *alvarezii*. Carrageenan is a key ingredient in food and non-food products. It is an economically important seaweed highly in demand for its cell-wall polysaccharides[2]. Cultivation of *K. alvarezii* for commercial purposes originated in the Philippines way back in the 1960s (ref. 3). Since then, many countries such as Indonesia, Japan, Tanzania, Kiribati, Fiji, South Africa and Hawaii are cultivating this species in large/industrial scale[4]. Further, marine algae can be used as gelling and stabilizing agents in the food industry[5].

In India, seaweed cultivation was initiated at Mandapam, Tamil Nadu on the southeast coast[6]. Attempts to grow this seaweed in experimental open sea stations at three Indian localities (Palk Bay, Mandapam region and southeast Indian coast) were successful, and all the three sites were suitable for cultivation[7]. The chemical composition of these seaweeds serves as proof of their rich nutritional value, with contents such as essential amino acids, vitamins and minerals. In addition, these seaweeds possess bioactive secondary metabolites and several drug-like molecules[8]. Among the bioactive compounds separated from algae, phlorotannins is one of the most prominent secondary metabolites with a broad array of nutritional, functional and bioactive properties[9]. The phlorotannin compound in Ecklonia cava, a marine blown algae, has 6,6′-bieckol and dioxinodehydroeckol which can increase matrix metalloproteinase (MMP) inhibitory activities that may have potentials to use as an active ingredient in anti-wrinkle cosmetics[10]. The antimicrobial activities of the seaweed (*Sargassum wightii*) are associated with secondary metabolites like terpenoid derivatives of phlorotannins or phenolic lipids[11,12]. Antioxidants are a major component of seaweeds. The seaweeds are exposed continuously to free radicals and strong oxidizing agents due to reaction between sunlight and oxygen. However, the structural component of seaweeds does not experience any oxidative damage. This indicates that seaweeds are capable of developing essential defence mechanisms against oxidation. Seaweeds are recognized as an essential source of antioxidant compounds that could be suitable for defending our bodies against the reactive oxygen species formed, e.g. by our metabolism or induced by external factors[13]. Metabolism in living cells results in the production of increased amounts of reactive oxygen species (ROS) in the cell which causes cell destruction due to bimolecular oxidation[14]. The harmful effects of ROS can be neutralized by an antioxidant. Crude extracts of marine algae have demonstrated strong antioxidant properties[15,16]. Several seaweeds

*For correspondence. (e-mail: immanuelselvaraj@vit.ac.in; mohanaroopan.s@gmail.com)

have already been proven as a rich source of antioxidants[17]. In India, the lacuna in current production rate of phycocolloids from natural habitat is greater than the industrial demand. This difference between production rate and demand can be bridged through mariculture practices and by cultivating beneficial species on a commercial scale.

The objective of this study is to phytochemically analyse *K. alvarezii*, determine total antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, reducing power, cytotoxicity and characterize it through high-performance thin-layer chromatography (HPTLC) and gas chromatography–mass spectrometry (GC-MS). We also evaluate the toxicological properties of the algae using HeLa cell-line studies.

Materials and methods

Seaweed collection and preparation

The marine alga was gathered from the Mandapam waterfront locale (9°28′.3466″N; 79°11′.3976″E), Ramanathapuram region, Tamil Nadu, India, during low tide. The specimens were recognized and affirmed at the Central Marine Fisheries Research Institute (CMFRI), Mandapam. They were rinsed thoroughly with clear seawater to remove impurities, dirt and extraneous materials. The seaweeds were brought to the laboratory in sterile plastic bags which had seawater to prevent drying. The samples were then washed with sterile water and shade-dried (37°C) at room temperature for 10 days. The shade-dried specimens were minced and ground in a mixer grinder. The powdered samples were kept in cold storage (–20°C) until further use.

Extract preparation

The powdered materials (20 g) were extracted progressively using a Soxhlet apparatus with 250 ml petroleum ether, methanol and sterile distilled water utilizing an extractor. The extraction was done using soxhlet apparatus for 6 h at a specific temperature but not more than petroleum ether boiling point. Filtration was done for the extracts using filter paper (Whatman No. 1) and condensed in vacuum at 40°C utilizing rotovap. The concentrate was stored in a refrigerator at –20°C until further study.

Phytochemical screening

The freshly obtained crude extracts were analysed to determine the presence of different phytochemicals using standard methods[18]. Extracts were screened for carbohydrates, starch, proteins, amino acids, saponins tannins, terpenoids, flavonoids, alkaloids and phenolic compounds.

Carbohydrates: Carbohydrates were determined by taking a small quantity of the extract (crude), which was dissolved in sterile milliQ water and then filtered. The obtained filtrate was subjected to the following procedures: (a) Molisch's test: A few drops of alcoholic alpha naphthol were added followed by 2 ml of concentrated sulphuric acid. Purple colour at junction of the two layers indicates the presence of carbohydrates. (b) Fehling's test: To 1 ml of the pre-heated filtrate kept on a water bath, was added one-drop of Fehling's solutions 1 and 2. The presence of carbohydrates was determined by visualizing brick red-coloured precipitate.

Proteins: Small amount of plant extract dissolved in water was subjected to Millon's and Biuret tests. (a) Millon's test: The extract was mixed with Million's reagent. White precipitate indicates the presence of amino acids. (b) Biuret test: To the extract, 1 ml of Biuret reagent was added. Purple colour indicates the presence of proteins.

Amino acids: One millilitre of extract was mixed with ninhydrin reagent. Purple colour indicates the presence of amino acids.

Phenolic compound: The filtrate was mixed with 5% ferric chloride solution. Black precipitate in the extract indicates the presence of phenolic compound.

Tannins: A small amount of filtrate was mixed with lead acetate solution. The occurrence of white precipitates indicates the presence of tannins and phenols.

Saponins: One gram of dried, powdered sample was heated along with 10 ml of sterile water in a water bath for 10 min. The mixture in hot condition was filtered and the filtrate was allowed to cool at room temperature. The volume was made up to 10 ml with distilled water using 2.5 ml of filtrate and vortexed vigorously for 2 min (frothing indicates the presence of saponins in the filtrate).

Terpenoids: Salkowski test: Five millilitres of the extract and 2 ml of chloroform were mixed well. A layer was formed when concentrated sulphuric acid (3 ml) was added to the mixture. Formation of reddish-brown coloured precipitate at the interface shows the presence of terpenoids.

Flavonoids: A magnesium ribbon piece and 1 ml concentrated HCL were added to the extract. A pinkish red or red colour formation indicates the presence of flavonoids.

Total seaweed phenolic content: The total phenolic content of the seaweed was estimated with slight modification of procedure proposed by Moreno *et al.*[19]. A volume of the sample – 100 µl (1 g of dry specimen, 10 ml of

acetone was added) and diluted Folin–Ciocalteu reagent (1 : 2 with sterile distilled water) – 1 ml was incubated at room temperature for 5 min. Seven per cent Na_2CO_3 (1 ml) was mixed with the above solution and incubated at room temperature for 90 min. The samples were thawed and absorbance was measured at 750 nm using a spectrophotometer along with the blank. The total phenolic content of the sample was calculated using the linear equation of a gallic acid standard curve, and the result was expressed as gallic acid equivalent in milligrams per gram of the sample based on its dry weight.

Total flavonoids: K. alvarezii extracts were used to determine total flavonoids content (TFC), with a slightly modified method of Prieto *et al.*[20]. To the extract, potassium acetate and aluminium nitrate were added. The mixture was incubated for 40 min at room temperature and then absorbance was measured at 415 nm using quercetin as the standard.

Determination of total antioxidant activity

Phosphomolybdenum method was used to measure the antioxidant activity of *K. alvarezii* samples following the procedure given by Madhumitha and Saral[21]. The assay was based on the reduction of Mo (VI) to Mo (V) and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. To 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) 0.3 ml of the extract solution was added. The reaction solution along with the tubes was incubated for 90 min at 95°C. Using a spectrophotometer (HACH 4000 DU UV–visible Spectrophotometer), the solution absorbance was measured at 695 nm against blank. Methanol (0.3 ml) was used as a blank solution. Antioxidant activity was expressed as the number of gram equivalents of ascorbic acid.

Determination of reducing power

Different concentrations of the extract (250, 500, 750 and 1000 µg/µl) were dissolved with 2.5 ml of 0.2 M phosphate buffer, pH 6.6, and equal volume of 1% potassium ferricyanide solution was added. The mixtures were incubated for 30 min at 50°C. To the mixture, 10% trichloroacetic acid was added, and centrifuged for 10 min at 2000 rpm. A mixture of 2.5 ml aliquot, 2.5 ml distilled water and 0.1% ferric chloride (0.5 ml) was taken and solution absorbance was recorded at 700 nm.

DPPH radical scavenging assay

The scavenging activity of the concentrate was evaluated utilizing DPPH (Sigma Aldrich, USA) test, with a few modifications[22]. An extract of 2.5 ml (containing 250, 500, 750 and 1000 µg/µl of the extract) was prepared using DMSO. The concentrate (2.5 ml) was blended well in methanol with 1 ml of 0.3 mM of DPPH completely. The blended solution was agitated vigorously and maintained at room temperature for 30 min. Absorbance was measured at 518 nm, and the free-radical scavenging activity was determined.

$$\text{Scavenging effect (\%)} = [1 - (\text{Absorbance of sample}/\text{Absorbance of control})] \times 100.$$

HPTLC analysis of extract for phenolic profile

One hundred milligram of the algae sample extract was dissolved in 1 ml of solvent, and centrifuged for 5 min at 3000 rpm. The supernatant was collected. Three microlitre of the sample (A) and 2 µl of the phenolic standard solution (20 mg/ml) was applied as 5 mm bands on HPTLC sheets (HPTLC silica gel 60 F254, Merck KGaA, Darmstadt, Germany) using a semiautomated HPTLC application device (Linomat 5, CAMAG, Muttenz, Switzerland). The chromatographic separations were performed inside a trough developing chamber (after saturating with solvent vapour) using a mixture of toluene–acetone–formic acid as the mobile phase (4.5 : 4.5 : 1), on a 3 × 10 cm (0.25 thick) TLC plates. The sample was allowed to run up to 90 mm. Twenty per cent sodium carbonate solution followed by Folin–Ciocalteu reagent was sprayed over the HPTLC plates. The plate was approximately dried for 5 min in warm air until the spots appeared. Visualization was performed. The images were captured under visible and light long-wave UV light (254 nm, 366 nm) using the photo documentation chamber (Camag Reprostar 3, Sigma-Aldrich, USA).

Specific reagent (phenolic solution) was sprayed over the developed plates and dried well in a hot-air oven at 100°C for derivatization. Using the photo-documentation chamber, the plates were again photo-documented in visible light and UV 366 nm mode. The chromatographic results obtained were analysed using HPTLC software, winCATS version 1.3.4 (CAMAG, Muttenz, Switzerland). The table, display and densitogram of the peak were obtained after the analysis.

Gas chromatography–mass spectrometry analysis

The algal samples were shade-dried completely and ground in a mixer mill (MM 400; RETSCH, Germany) to obtain a fine powder. The powdered sample (5 mg) was weighed and transferred into the test tube. To this, petroleum ether (15 ml) was added. Sonication was done for the test tubes containing sample solution for 5 min. Using 0.2 µm nylon membrane, the sample solution was filtered and then injected into the GC column.

GC–MS analysis of the sample was carried out by using Perkin Elmer-Clarus 680 model with the specific column

Elite 5MS ((30 m × 0.25 mm ID and 250 μm film thickness, 95% dimethylpolysiloxane) operating in an electron impact mode at 70 eV with helium as a carrier gas at a flow rate of 1 ml/min. The injection volume was 1 μl (split ratio 10 : 1). The injector temperature was set at 250°C and the oven temperature was programmed at an initial temperature of 60°C for 2 min, raising at 10°C/min to 300°C and maintained at that temperature for 6 min and a total run time of 32 min. The carrier gas was helium. The ion source and transfer temperatures were 230°C. Sample ionization was electron impact and was analysed by positive mode. The spectrum obtained after GC–MS analysis was interpreted and compared with the National Institute Standard and Technology (NIST) compounds library. The GC studies were carried out at sophisticated instrumentation facility (SIF), Vellore Institute of Technology, Vellore.

Cytotoxicity assay

To estimate the cell cytotoxicity level of the algal extract, MTT assay was performed in HeLa cell lines. The mitochondrial succinate dehydrogenase in the living cell transforms and forms purple formzan subsidiary from yellow tetrazolium bromide, which can be measured colorimetrically. The NAD(P)H-dependent cellular oxidoreductase enzymes are capable of reducing 3-(4,5-dimethylthiazol 2y1)-2,di-phenyl tetrazolium bromide salt resulting in the formation of formazan, which is purple in colour and the measure of resultant formazan can be related to the percentage of live cells[23,24]. In the 96-well plate, cell lines were seeded with 5×10^3 HeLa cells in 100 μl of Dulbecco's Modified Eagle's Medium (DMEM) with 10% foetal bovine serum (FBS) and incubated overnight at 37°C with 5% CO_2 condition. Next, 10 ml of DMEM was used to dissolve 10 mg of the algal extract. From the above stock solution, the sample extract was serially diluted with DMEM–10% FBS. One hundred microlitres of these diluted samples was added to the cells in triplicate. Negative control set for this experiment was 100 ml of DMEM alone and internal positive control used for the assay was 5 μg/ml doxorubicin. A blank was run without any cells. The above treatments were incubated at 37°C/5% CO_2 for 48 h. After 48 h, to each well 5 mg/ml MTT (20 μl) in PBS was added. It was incubated for 4 h at 37°C/5% CO_2. The medium was well aspirated and to each well 200 μl of dimethyl sulphoxide (DMSO) was added. The optical density (OD) was measured at 570 nm using a microplate reader. The cytotoxicity was measured using inhibition of growth and inhibition percentage was estimated as follows

$$\% \text{ Inhibition} = 100 - [(\text{Mean OD for test sample}/\text{Mean OD for the control}) \times 100].$$

Results and discussion

Qualitative phytochemical analyses were performed to detect the presence of various phytocompounds. The algae were extracted with methanol, petroleum ether and sterile water. Standard procedures were followed to test for the presence of primary metabolites such as carbohydrates, proteins, amino acids and secondary metabolites such as phenolics, tannins, saponins, terpenoids and flavonoids. Table 1 shows the results of the phytochemical analysis.

The total phenolic content was measured using methanol extract of *K. alvarezii* by spectrophotometry utilizing Folin–Ciocalteau reagent. The extract contained a total phenolic content of 86.45 ± 70.72 mg/ml, which was expressed as gallic acid equivalent in milligrams per gram of the sample based on its dry weight. To calculate total flavonoid content in *K. alvarezii* extracts, aluminium chloride method was used. The amount of flavonoid was determined spectrophotometrically. The flavonoid content was found to be 85.52 ± 32.57 mg/ml, which was expressed equivalent to that of quercetin.

The total antioxidant activity of *K. alvarezii* was determined using the phospho-molybdenum method. Based on the increased concentration, antioxidant activity was found to increase. The methanol extract of *K. alvarezii* showed increasing antioxidant activity with increase in extract concentration along with standard ascorbic acid, which could be attributed to its antioxidant activity. A concentration of 1000 μg/ml was found to have higher antioxidant activity as represented in Table 2.

The reductive capacities of different extracts of *K. alvarezii* were determined based on their concentration. On the basis of the reducing power of each compound, the solution (yellow colour) changes to various shades starting from green and then blue. The reductive capacity of the algal extract was found to be elevated with increasing concentration of the extracts which can be compared with the standard ascorbic acid (Table 3).

The DPPH assay is a quick and economical method for the determination of the potential antioxidant activity of different available natural products. According to the

Table 1. Phytochemical analysis of *Kappaphycus alvarezii* extracts with different solvents

Phytochemical and tests	Petroleum ether	Methanol	Aqueous
Carbohydrate (Molisch's test)	–	–	++
Proteins (biuret)	++	++	++
Amino acids (ninhydrin)	++	++	–
Phenolics (ferric chloride)	++	++	–
Tannins (lead acetate)	++	++	–
Saponins	–	–	++
Terpenoids (Salkowski test)	–	++	++
Flavonoids (Shinoda)	++	++	++

Table 2. Percentage antioxidant activity of *K. alvarezii* extracted with different solvents

Concentration (µg/ml)	Standard (ascorbic acid)	Methanol	Petroleum ether	Water or sterile distilled water (H_2O)
250	56.17 ± 0.26	44.45 ± 0.25	39.88 ± 0.24	21.76 ± 0.42
500	68.10 ± 0.29	52.70 ± 0.27	41.50 ± 0.25	34.35 ± 0.31
750	72.80 ± 0.28	67.12 ± 0.26	52.70 ± 0.27	41.58 ± 0.61
1000	81.21 ± 0.31	74.24 ± 0.33	62.34 ± 0.28	53.86 ± 0.25

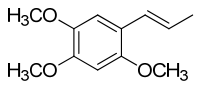
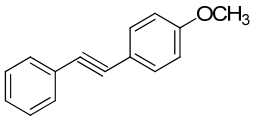
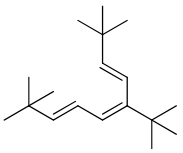
Table 3. Percentage reducing activity of *K. alvarezii* extracted with different solvents

Concentration (µg/ml)	Standard (ascorbic acid)	Methanol	Petroleum ether	Water or sterile distilled water (H_2O)
250	19.38 ± 0.91	15.28 ± 0.12	12.50 ± 0.14	8.48 ± 0.82
500	31.28 ± 0.18	25.39 ± 0.12	21.17 ± 0.91	15.33 ± 0.12
750	45.17 ± 0.21	42.43 ± 0.24	33.22 ± 0.14	29.40 ± 0.12
1000	69.76 ± 0.27	65.78 ± 0.29	55.72 ± 0.22	48.62 ± 0.26

Table 4. DPPH radical scavenging activity (%) of *K. alvarezii* extracted with different solvents

Concentration (µg/ml)	Standard (gallic acid)	Methanol	Petroleum ether	Water or sterile distilled water (H_2O)
250	18.50 ± 0.12	13.45 ± 0.92	8.82 ± 0.62	5.56 ± 0.59
500	28.45 ± 0.12	23.68 ± 0.14	18.72 ± 0.12	13.33 ± 0.92
750	47.67 ± 0.28	43.24 ± 0.28	22.45 ± 0.95	25.81 ± 0.13
1000	62.70 ± 0.31	54.78 ± 0.29	47.68 ± 0.26	41.54 ± 0.21

Table 5. Chemical composition of *K. alvarezii* extracted with petroleum ether identified by GC–MS

Retention time	Compound	Structure	Area %	Molecular formula
14.528	1,2,4-trimethoxy-5-(prop-1-en-1yl)benzene		81.006	$C_{12}H_{16}O_3$
15.219	1-methoxy-4-(phenylethynyl)benzene		6.044	$C_{15}H_{12}O$
30.590	(3E, 5E, 7E)-5-(tert-butyl)-2,2,9,9-tetramethyldeca-3,5,7-triene		12.950	$C_{18}H_{30}$

dosage, methanol extracts of brown algae *K. alvarezii* showed potent antioxidant activity. The petroleum ether extracts of *K. alvarezii* provided increased amounts of phenolic compounds. There was increased scavenging activity according to the increased concentration of various extracts along with reference to the standard gallic acid (Table 4). Zones with blue and brown were visible in the tracks at light mode, as it was visualized after derivatization from the chromatogram, which was confirmed with the help of phenolic standard (Figures 1–4).

For GC–MS analysis, the in-built NIST05.LIB library was used for comparison and the compounds were determined by comparing standard mass with their recorded mass spectra. Using GC–MS software system (Turbo-Massver 5.4.2) and the literature data, we analysed and confirmed the mass spectra by determining their similarities with retention indices. Table 5 shows the GC–MS analysis results. Three compounds were detected in methanol ether extract of *K. alvarezii*. The results revealed that 1,2,4-trimethoxy-5-(prop-1-en-1yl)benzene (81%) was the major component in the extract. The minor compounds revealed by GC–MS analysis were 1-methoxy-4-(phenylethynyl)benzene (6%) and (3E, 5E, 7E)-5-(tert-butyl)-2,2,9,9-tetramethyldeca-3,5,7-triene (12.9%).

Table 6. Absorbance level in the MTT assay

	1	2	3	4	5	6	7	8
A	0.015	0.819	2.041	1.8041	1.758	1.942	2.171	2.171
B	0.021	0.241	2.171	1.711	1.784	2.028	2.061	2.211
C	0.013	0.371	1.829	1.657	1.891	2.128	2.061	2.241
D	2.211	0.318	1.901	1.635	1.775	2.135	2.248	1.531
E	2.283	0.402	2.037	1.671	1.963	2.102	2.232	
F	2.281	0.311	1.831	1.640	1.981	2.049	2.091	
G	0.592	0.482	1.768	1.671	1.891	2.131	2.162	
H	0.769	1.959	1.867	1.641	2.173	2.159	2.181	

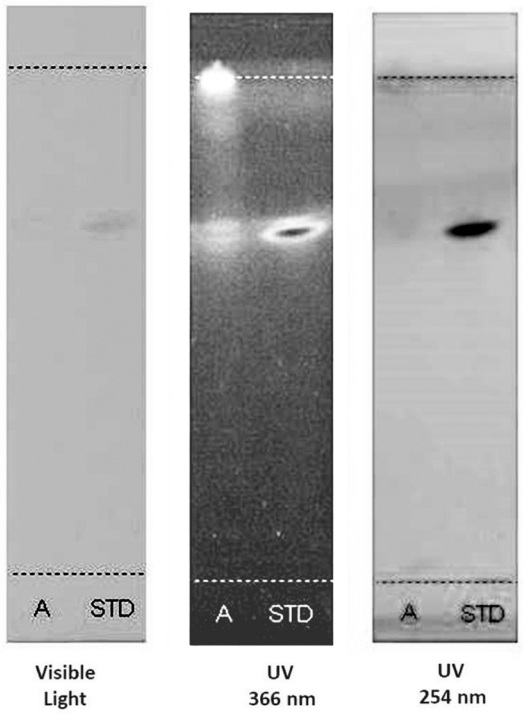


Figure 1. Chromatogram of *Kappaphycus alvarezii* sample showing phenolic profile before derivatization. A, Extract sample; STD, Phenol standard.

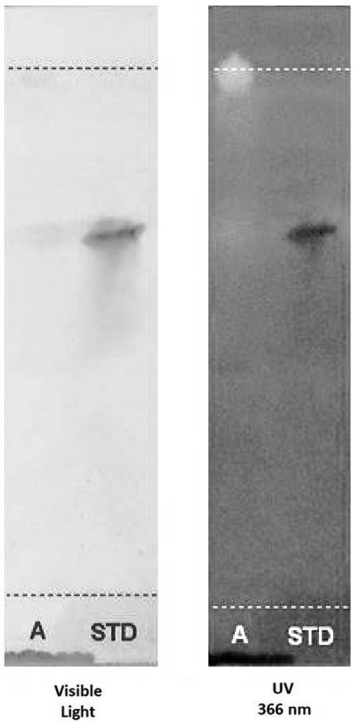


Figure 2. Chromatogram of *K. alvarezii* sample showing phenolic profile after derivatization. A, Extract sample; STD, Phenol standard.

MTT assay is considered as the fastest and highly accurate colorimetric approach that can be used to determine growth and cytotoxicity of the cells, particularly for new drug development. The mitochondrial activity was measured by enzymatic reaction through the reduction of MTT to formazan and this determines the integrity of the cell membrane (Table 6). In China, marine algae is being used as a traditional medicine to treat initial stages of malignancy (cancer). In this study, the methanolic extract of *K. alvarezii* was tested against HeLa cell lines to determine the viability of the cells using MTT assay. Strong cytotoxic activity was found with IC_{50} value of 1.75 mg/ml (Table 7).

Based on the above results, it can be observed that methanol and petroleum ether have the potential to extract considerably higher quantity of the effective compounds than other solvents. Although soxhlation was done along with the various solvents, preliminary tests revealed that the polar phytochemicals were dissolved in the matched polar solvent. This is because there is no possibility of unique solvent or solvent mixture to solubilize all antioxidant compounds[25]. The commonly used solvents to extract phenolics from brown and red seaweeds are methanol, ethanol, butanol, acetone, chloroform and water[26]. Standard methods of estimation were used to identify and quantify the components present in *K. alvarezii* which will facilitate to explore it further. It was observed that the algal extracts showed significantly higher antioxidant activity, good reducing capability and DPPH activity in methanolic extract of the algae with increasing concentrations when compared to the other solvents. It was found that *K. alvarezii* algae is a good source of phenolic compounds. Earlier reports indicate that the phenolic compounds do activate enhanced free radical

Table 7. Results of cytotoxic activity on HeLa cells with methanol extract of *K. alvarezii* at different concentration

Concentration of *K. alvarezii* extracts	Mean absorbance	Blank	Inhibition %
5 mg/ml	0.310	0.294	86.27
2.5 mg/ml	0.398	0.382	82.37
1.25 mg/ml	2.057	2.033	8.90
1.0 mg/ml	1.922	1.899	14.88
500 µg/ml	1.822	1.797	19.30
250 µg/ml	1.724	1.700	23.64
200 µg/ml	1.648	1.627	27.01
150 µg/ml	1.690	1.674	25.15
100 µg/ml	1.816	1.800	19.57
50 µg/ml	1.945	1.929	13.86
25 µg/ml	2.047	2.031	9.34
12.5 µg/ml	2.130	2.114	5.66
6.25 µg/ml	2.121	2.105	6.23
3 µg/ml	2.097	2.081	6.06
1.5 µg/ml	2.190	2.174	3.01
0.75 µg/ml	2.171	2.155	3.85
0.375 µg/ml	1.994	1.972	11.69

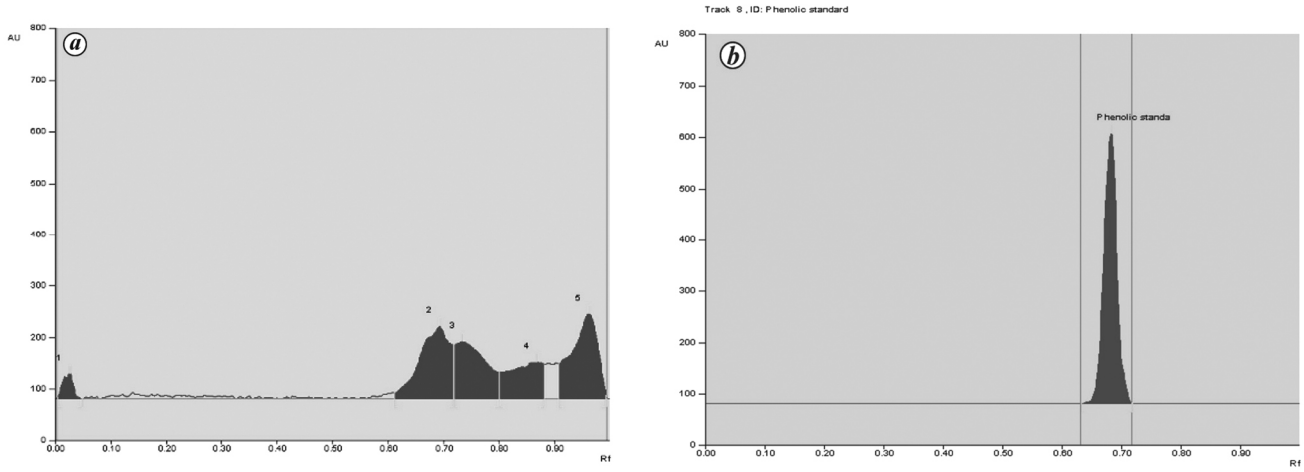


Figure 3. ***a***, HPTLC track A – plant sample A showing peak densitogram on display scanned at 254 nm. ***b***, HPTLC track STD – phenolic standard peak densitogram on display scanned at 254 nm.

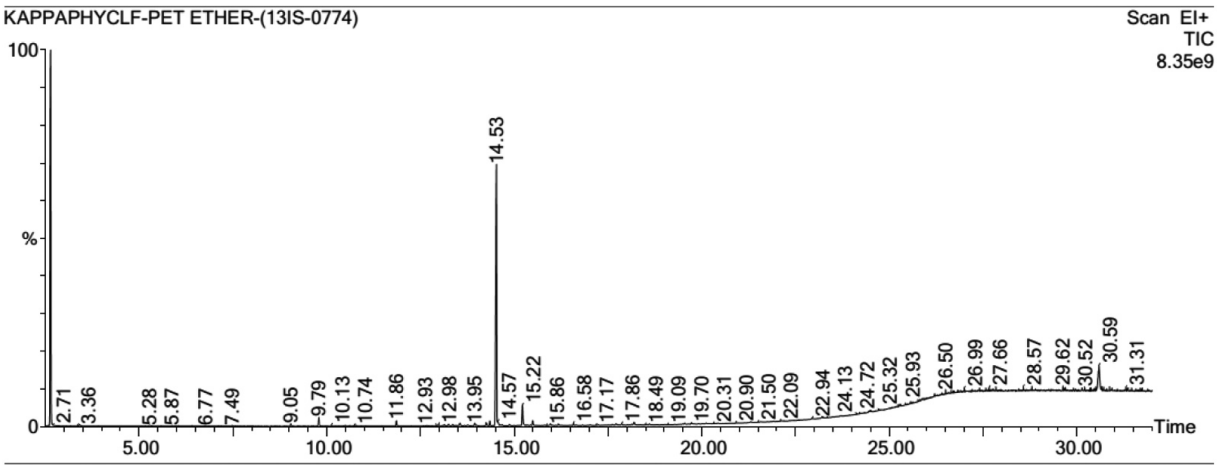


Figure 4. GC-MS chromatogram of *K. alvarezii.*

scavenging activity[27]. Previous studies report that *K. alverazii* and *K. striatum* extracts showed the presence of antioxidants, which induced apoptosis (10.2 ± 2.4%) and necrosis (2.4 ± 1.1%) respectively, among the cancer cell lines[28]. A study indicated that different solvent fractions obtained from total (methanolic) extract exhibit higher antioxidant activities compared to the total extract in edible marine algae with significantly higher phenolic compounds[29]. Higher percentage of phenolic compounds in plants was reported to be anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, reduces cardiac failure and helps in vasodilation, which helps in reducing blood pressure[30]. The seaweed extracts were effective in scavenging hydroxyl radicals to prevent degradation of deoxyribose substrate in a dose-dependent manner. These seaweed extracts are able to prevent deoxyribose damage associated with the direct binding of iron to deoxyribose and the subsequent attack by hydroxyl radicals generated via the Fenton reaction[31].

Conclusion

The present study showed that extracts with various solvents of *K. alvarezii* have a good range of phytochemicals. Presence of higher radical scavenging activity and reducing capability in the extract can be directly correlated to the higher phenolic content. The cytotoxic study as well as HPTLC, GC-MS analysis have provided valuable information. Further studies may help in identifying and purifying specific anti-tumour compounds for potential use in cancer therapy.

Conflicts of interest: The authors declare that they have no conflict of interest.

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