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ORIGINAL ARTICLE

Evaluation of antioxidant and cytotoxicity activities of polyphenol extracted from brown seaweed *Sargassum tenerrimum* biomass

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Abstract
The polyphenol compound is extracted from *Sargassum tenerrimum* with various bioactivities including antibacterial and antioxidant activity and MTT assay for cell cytotoxicity. The total phenolic content was 69.12 ± 0.24%. The *S. tenerrimum* polyphenol was found to phytochemical constituent's presence of flavonoids, saponins, tannins, phenolics, alkaloids and steroid. The antibacterial activity of polyphenol presented significant inhibition against ten human pathogen bacterial cultures such as *Proteus mirabilis*, *Klebsiella oxytoca*, *Escherichia coli*, *Bacillus cereus*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Salmonella typhi* and *Bacillus subtilis*. The in vitro antioxidant activity and MTT assay revealed that the polyphenol has anticancer activity against HeLa cells. The polyphenol compound was characterized through HPLC.

Keywords Polyphenol · Phytochemical · Cytotoxicity · HPLC

1 Introduction

The marine environment is rich in bioactive secondary metabolites, many of which have structural characteristics that are not seen in terrestrial natural products [1]. Bioactive substances such as polyphenols, carotenoids and polysaccharides are abundant in seaweeds. These bioactive chemicals can be used in functional foods, medications and cosmetics because they provide customers with health benefit [2]. Polyphenolic chemicals found in catechins, flavonols and glycosides in methanol extracts of seaweeds have been discovered to exhibit antioxidant and antibacterial activity in vitro [3]. Phenolic chemicals are a major predictor of a food's antioxidant capacity [4]. Because of their rheological qualities as gelling and thickening agents, seaweeds are abundant sources of polyphenol, making them some of the most desirable additions in the food business (e.g., carrageenan). Polyphenols are known to have a variety of biological activities, including anticoagulant, antiviral and immuno-inflammatory properties, which could be used in nutraceutical/functional food and cosmetic or cosmeceutical and pharmaceutical products [5].

Polyphenolic chemicals can be found in a wide variety of foods. Due to the presence of numerous hydroxyl (OH) groups in polyphenolic compounds, they are likely to have a radical scavenging impact or, in certain cases, an oxidative effect as a generator of reactive oxygen species. Polyphenolic compounds have been shown in animal studies to have favourable health effects due to their antioxidant capabilities and inhibitory involvement in various phases of tumour formation. Polyphenolic content of teas, wines, cacaos, fruits and vegetables has been reported plenty of findings. In comparison to terrestrial plants, seaweeds may

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have a higher concentration of flavonoid catechins [6]. The effect of antioxidant naturally occurring phenolic components on the protection of cardiovascular diseases and cancer, as well as age-related degenerative brain illnesses, has been examined earlier [7, 8]. Hence, the present investigation was undertaken to value of the *Sargassum tenerrimum* from Mandapam coast of Gulf of Mannar region South east coast of Tamil Nadu, India and to study the isolation, characterization of bioactive compounds and screening the active principle against antimicrobial and antioxidant activity of different solvent extract of *Sargassum tenerrimum*.

2 Materials and methods

2.1 Extraction of crude polyphenol compound from *S. tenerrimum*

The algae was cleaned with sterile distilled water, dried in the sun, chopped into small pieces and pulverized in a mixer grinder. It was kept at room temperature in an airtight polypropylene container. In a Soxhlet extractor, 100 g of *S. tenerrimum* was extracted for 6 h with 500 ml of methanol (2:1). The entire extract was filtered, and the filtrate produced was concentrated to dryness under reduced pressure. For subsequent study, the concentrated extract was used as the seaweed polyphenol compound [9].

2.2 Estimation of total phenolic content

A phenolic content of methanolic extracts was estimated by the method of Senevirathene et al. [10].

2.3 Phytochemical analysis

The components analysed for were terpenoids, anthraquinones, flavonoids, saponins, tannins, alkaloid, cardiac glycosides, steroid and balsam [11].

2.4 Antibacterial activity

In vitro antibacterial test was performed using a panel of ten human pathogenic strains which include *Proteus mirabilis*, *Klebsiella oxytoca*, *Escherichia coli*, *Bacillus cereus*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa Vibrio cholerae*, *Salmonella typhi* and *Bacillus subtilis* which were obtained from the laboratory in Department of Microbiology, Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu, India.

2.5 In vitro antioxidant activity

The antioxidant activity of *S. tenerrimum* polyphenol in total antioxidant activity, Reducing power, Hydrogen peroxide assay, DPPH, and ABTS [12–14].

2.6 In vitro anticancer activity of polyphenol compound

2.6.1 MTT assay

The cytotoxicity of the polyphenol compound was investigated using Hela cells utilizing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Trypsinization was used to collect the cultivated Hela cells, which were then pooled in a 15-ml tube. The cells were then plated at a density of 1×10^5 cells/ml cells/well (200 l) in a 96-well tissue culture plate in DMEM medium containing 10% FBS and 1% antibiotic solution for 24–48 h at 37 °C in DMEM medium containing 10% FBS and 1% antibiotic solution. In a serum-free DMEM medium, the wells were rinsed with sterile PBS and treated with various concentrations of the polyphenol sample. Each sample was reproduced three times, and the cells were cultured for 24 h at 37 degrees Celsius in a humidified 5% CO_2 incubator. MTT (20 µl at 5 mg/ml) was added to each well after the incubation period, and the cells were incubated for another 2–4 h until purple precipitates were visible under an inverted microscope. Finally, the medium was aspirated out of the wells together with MTT (220 µl) and rinsed with 1X PBS (200 µl). DMSO (100 µl) was also added to dissolve formazan crystals, and the plate was agitated for 5 min. Using a micro-plate reader (Thermo Fisher Scientific, USA), the absorbance of each well was measured at 570 nm, and the % cell viability and IC_{50} value were computed using GraphPad Prism 6.0 software (USA).

2.7 HPLC analysis of polyphenol compound

The polyphenol compound was studied with a HPLC C18 system column (LC- 10VP Shimadzu).

3 Results and discussion

3.1 Yield of the crude extract

The crude polyphenol compound was extracted from 100 g of *S. tenerrimum* powder using hot water, and the dry weight of the crude polyphenol compound was found to be 12 g, respectively. Erwan et al. [15] report that in the extraction of

phlorotannins, a simple process based on the utilization of water and organic solvent combinations is used. Separating methods based on both the polarity and the molecular size of substances are used to purify and fractionate crude extracts.

3.2 Estimation of total phenolic content

The total phenolic content present in the polyphenol compound was found to be 69.12 ± 0.24% of phenolic content present in *S. tenerrimum*. Similarly, Vijayabaskar and Shiyamala [16] evaluated that the phenolic content of *T. ornata* was determined to be the highest (43.72 1.63 mg GAE/g extract).

3.3 Phytochemical analysis

The present investigation brings out the *S. tenerrimum* phytochemical constituents presence of flavonoids, saponins, tannins, Phenolics, alkaloids and steroid is shown in Table 1. Gopalan et al. [17] looked into the alkaloids, terpenoids, flavonoids, tannins, polyphenols, saponins, cardiac glycosides and quinines found in *G. corticata*. According to Heo et al. [18], marine seaweeds are high in polyphenolic substances such catechins, flavonols and phlorotannins. Bromophenols, phenolic acids and flavonoids make up the majority of the phenolic chemicals found in green and red algae. The primary polyphenolic secondary metabolites found only in maritime brown seaweeds are phlorotannins, a series of complex polymers of phloroglucinol (1,3,5-trihydroxybenzene).

3.4 Antibacterial activity of polyphenol compound

The *S. tenerrimum* polyphenol compound high activity in 18 mm of inhibition zone against *Streptococcus pyogenes* and minimum of 8 mm of inhibiting zone against *Salmonella typhi* is shown in Table 2. Rajasulochana et al. [19] determined the antibacterial activity of the experimental brown algae. The brown algae showed maximum antibacterial activity of *E. coli* (2.2 ± 0.063) and *Staphylococcus aureus* (6.2 ± 0.128), respectively. Smullen et al. [20] evaluated that the antimicrobial assay of the polyphenol compound was related to their chemical structure and ester sulphate groups. Vijayabaskar and Shiyamala [16] reported that the *S. wightii* and *T. ornate* methanolic extracts were tested against a variety of human pathogenic microorganisms. The discovery suggests that *T. ornata* methanol extracts could be used as a valuable source of antibacterial activity in the pharmaceutical business.

Table 1 Phytochemical analyses of polyphenol compound

Sl. no	Phytochemical	*S. tenerrimum*
1	Terpenoids	+
2	Anthraquinones	-
3	Flavonoids	+
4	Saponins	+
5	Tannin	+
6	Alkaloids	+
7	Cardiac glycosides	+
8	Steroids	+
9	Balsam	-

Table 2 Antibacterial activity of polyphenol against pathogens

S. no	Test organisms	Tetracycline (mm)	Polyphenol (mm)
1	*Proteus mirabilis*	22	9
2	*Klebsiella oxytoca*	20	15
3	*Escherichia coli*	21	17
4	*Bacillus cereus*	18	12
5	*Streptococcus pyogenes*	24	18
6	*Staphylococcus aureus*	25	14
7	*Pseudomonas aeruginosa*	23	11
8	*Vibrio cholera*	17	10
9	*Salmonella typhi*	18	8
10	*Bacillus subtilis*	20	16

3.5 In vitro antioxidant activity of polyphenol compound

The antioxidant activity of polyphenol compound was present in total antioxidant capacity, reducing power, hydrogen peroxide scavenging activity, DPPH and ABTS shown in Table 3. Riaz et al. [21] found that the total antioxidant of *Gardenia jasminoides* polyphenolic components may be represented in terms of ascorbic acid equivalents. In a dose-dependent way, total antioxidant activity was raised. According to Valdes [22], the polyphenol molecule found in seaweed can be a possible source of antioxidants with both protective and beneficial properties. Their bioactivity has been mostly attributed to their direct antioxidant, radical scavenging and anti-inflammatory activities for a

Table 3 In vitro antioxidant activity of polyphenol compound

Sl. no	Antioxidant	Polyphenol (%)
1	Total antioxidant capacity	88.11 ± 0.25
2	Reducing power	76.45 ± 0.31
3	Hydrogen peroxide scavenging activity	82.72 ± 0.13
4	DPPH	65. 87 ± 0.14
5	ABTS	70.25 ± 0.18

long time. In the reducing power antioxidant experiment, increased absorbance indicates that materials are capable of donating hydrogen atoms in a dose-dependent way [23, 24]. Audibert et al. [25] investigated the antioxidant activity of pure extracts of phlorotannin fractions and discovered that low molecular weight phlorotannins had increased antioxidant activity, which declined as the polymerization process progressed. Czochra and Widensk [26] have been investigated that the measurement of H_2O_2 scavenging activity is one of the useful methods of responsible the ability of antioxidants to decrease the level of pro-oxidants in H_2O_2. Similarly, Vijayabaskar and Shiyamala [16], the DPPH, *T. ornata* had a high activity (84.27 2.17% scavenging activity on DPPH) when compared to ordinary Gallic acid. In DPPH radical scavenging antioxidant experiments, Riaz et al. [21] reported considerable free radical scavenging capabilities when compared to conventional ascorbic acid. Kajal et al. [27] worked that the ABTS of the MeOH extracts/fractions of the red algae at 0.6 µg/ml are recorded. Among *H. musciformis* MeOH extracts recorded significantly higher ABTS (19.6%), *H. valentiae* (14.9%) and *J. rubens* (8.7%), respectively. The ethyl acetate fraction of *S. marginatum* was shown to have the highest antioxidant activity (39.62 mg ascorbic acid equivalent/g extract) by Sachindra et al. [28].

3.6 In vitro anticancer activity of polyphenol

3.6.1 MTT assay for cell cytotoxicity

To investigate the effect of polyphenol on colon cancer cell proliferation, Hela cells were grown for 24 h at various concentrations of polyphenol (10, 20, 40, 60, 80 and 100 g/ml), and the vitality of the cells was determined using the MTT assay (Table 4 and Fig. 1). By using an inverted microscope, morphological alterations in Hela cells were observed, indicating that polyphenol reduced the viability of Hela cells at various concentrations (10, 20, 40, 60, 80 and 100 g/ml), as shown in Table 5 and Fig. 2. The presence of both viable cells was demonstrated in Hela cells treated with 10 and 20 g/ml polyphenol. Hela cells treated with 40 and 60 g/ml polyphenol revealed the presence of both viable and non-viable cells. A lower number of viable cells were seen in cells treated with 80 g/ml polyphenol. Figure 3 shows that a 100 g/ml polyphenol treatment resulted in a higher

Table 4 Determination of cytotoxicity of polyphenol on Hela cells by MTT assay

Sl. no	Tested sample concentration (µg/ml)	OD value at 570 nm
1	Control	0.587
2	10 µg/ml	0.482
3	20 µg/ml	0.417
4	40 µg/ml	0.371ss
5	60 µg/ml	0.284
6	80 µg/ml	0.210
7	100 µg/ml	0.149

Table 5 Determination of cytotoxicity of polyphenol on Hela cells viability

Sl. no	Tested sample concentration (µg/ml)	Cell viability (%)
1	Control	100 ± 0.12
2	10 µg/ml	81.88 ± 0.12
3	20 µg/ml	77.95 ± 0.12
4	40 µg/ml	60.64 ± 0.12
5	60 µg/ml	49.68 ± 0.12
6	80 µg/ml	36.45 ± 0.12
7	100 µg/ml	25.89 ± 0.12

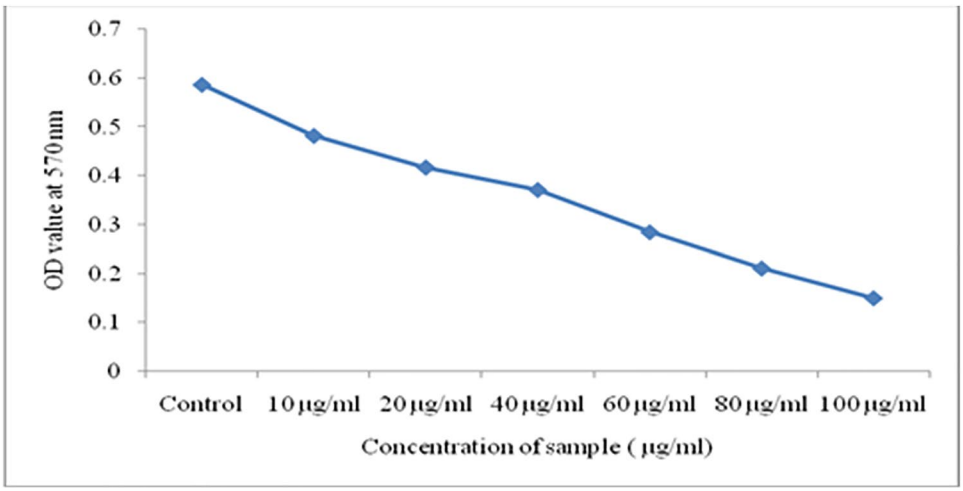

Fig. 1 Determination of cytotoxicity of polyphenol on Hela cells by MTT assay

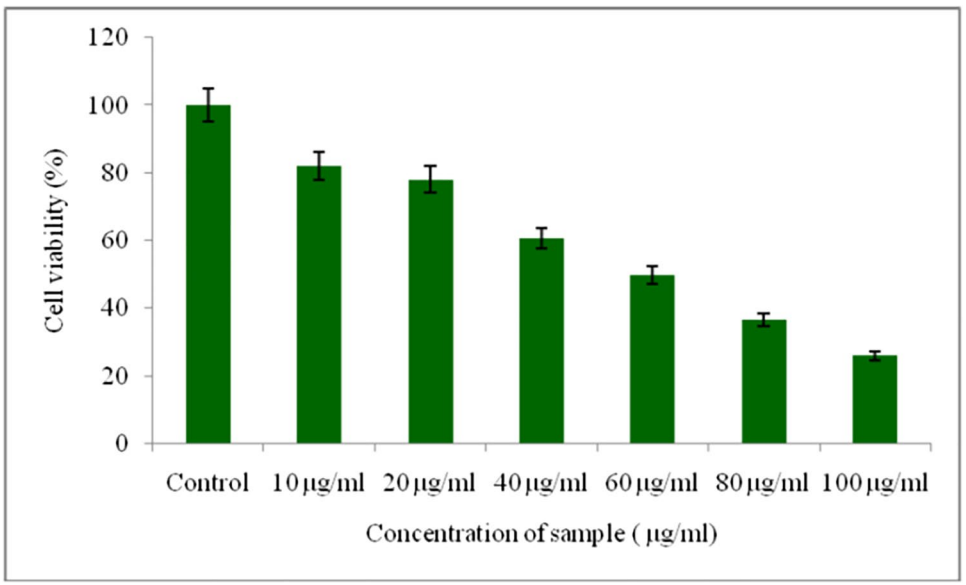

Fig. 2 Determination of cytotoxicity of polyphenol on Hela cells viability

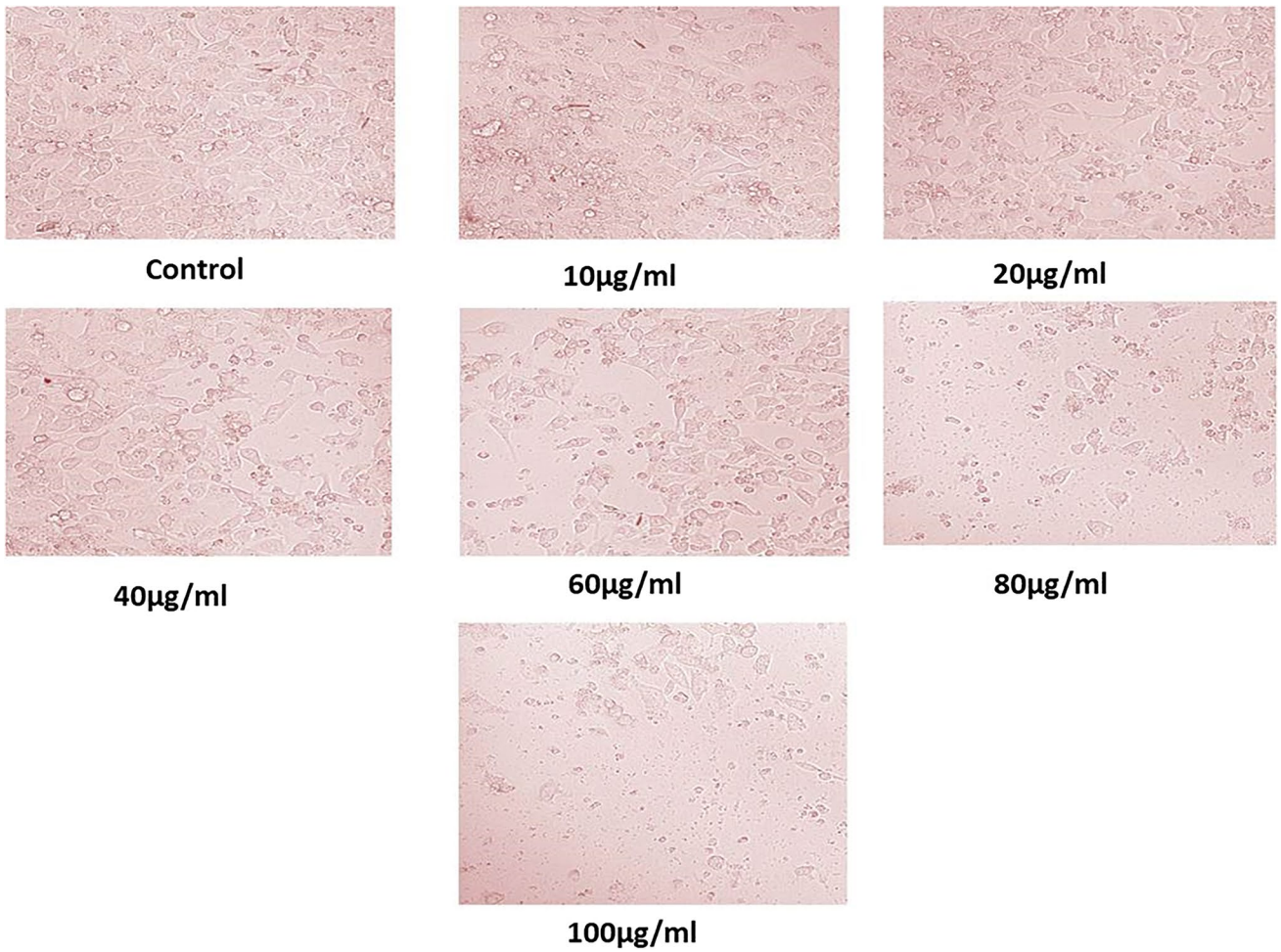

Fig. 3 Morphological examination of Hela cells for cell proliferation inhibitory of polyphenol

Table 6 IC_{50} Value of tested sample

log(inhibitor)	Normalized response	Variable slope
Best-fit values		
LogIC50	~3.382	1.362
HillSlope	~1.612	−1.123
IC50	~2412	23.02
95% CI (profile likelihood)		
LogIC50	(Very wide)	1.251 to 1.466
HillSlope	(Very wide)	−1.402 to −0.8924
IC50	(Very wide)	17.81 to 29.24
Goodness of fit		
Degrees of freedom	1	28
R squared	0.000	0.8811
Sum of squares	8.667e-006	3766
Sy.x	0.002944	11.60
Number of points		
# of X values	3	30
# Y values analysed	3	30

number of nonviable cells. Polyphenol treatment of Hela cells decreased cell proliferation in a dose-dependent manner. The IC_{50} value (inhibitory concentration) was 23.02 g/ml. The effect showed that as the concentration of polyphenols increased, the cell survival rate reduced. Table 6 shows that polyphenol was more effective against colon cancer cell line Hela cells as a result of the study's findings. Namvar et al. [29] determined the effect of *S. muticum* methanolic extract against MCF-7 and MDA-MB-231 breast cancer cell line proliferation. The MTT assay effects indicated that the extract was cytotoxic against breast cancer cell lines in a dose-dependent manner, with IC_{50} of 22 µg/ml for MCF-7 and 55 µg/ml for MDA-MB-231 cell lines. Apostolidis and Lee [30] reported that the polyphenols extracted from *A. nodosum* showed significant a-amylase and a-glucosidase inhibitory activity equal to acarbose, a commercially potent inhibitor drug.

3.7 HPLC analysis of polyphenol

HPLC analysis of polyphenol of *S. tenerrimum* is given in Fig. 4. The HPLC analysis of the polyphenol presence of various constituents as evidenced by the chromatogram obtained at various retention times (2.050, 2.157, 2.523, 3.023 and 4.377). The polyphenol like *p*-hydroxybenzoic acid was identified at Rt 3.023. Waghmode and Khilare [31] have been reported that the, measurable analysis of following brown seaweed such as *S. cinereum*, *S. ilicifolium*, *S. tenerrimum* and *S. wightii* of phenolics were evaluated and studied using RP-HPLC. Jayabarath and Jeyaprakash [32] reported that the high performance liquid chromatography was preferred analytical tool for fingerprints and quantification of marker compounds in seaweed extracts. HPLC analysis of *T. conoides* methanolic extract showed the presence of different constituents as evidence by the chromatogram obtained at various retention times (3.643, 3.819 and 6.463) at λmax 254 nm.

4 Conclusion

The present study concludes the opportunity to project the importance of brown seaweed *Sargassum tenerrimum* possessing medicinal. The polyphenol extracted from

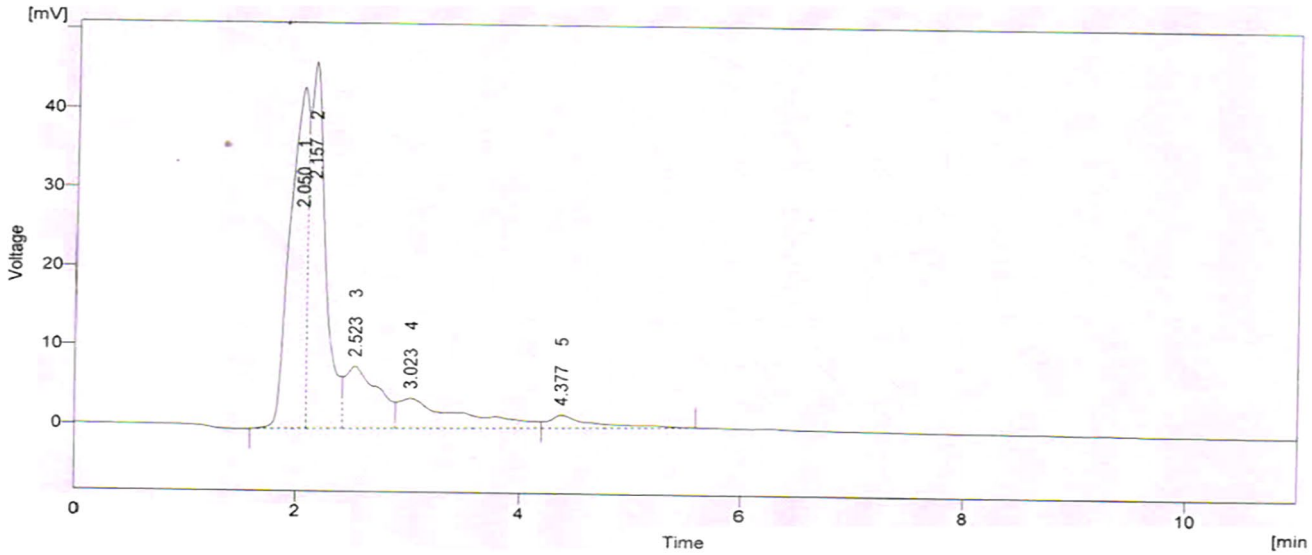

Fig. 4 HPLC analysis of polyphenol compound from *S. tenerrimum*

Sargassum tenerrimum showed higher antibacterial and antioxidant activity and cell line activity.

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Declarations

Conflict of interest The authors declare no competing interests.

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