



ORIGINAL ARTICLE

Biochemical and Anti-proliferative activities of seven abundant tropical red seaweeds confirm nutraceutical potential of *Grateloupia indica*



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Abstract Seaweeds are being used as food items in Asian countries from ancient times. Seaweeds mainly grow in intertidal zone and survive extreme environmental conditions and thus have developed metabolites, which help them survive in such conditions. Red seaweeds have proven to be rich in compounds, which are antioxidant and having many other health benefits. In this study, seven most abundantly grown red seaweeds, *Gracilaria corticata* (GC), *Grateloupia indica* (GI), *Kappaphycus alvarezii* (KA), *Solieria robusta* (SR), *Amphiroa anceps* (AA), *Halymenia porphyrifomis* (HP), and *Sarconema scinaoides* (SS) were collected from Saurashtra coast of Arabian Sea. Harvested seaweeds were subjected to metabolite profiling, flavonoid and phenolic content analysis,

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Red seaweeds

different biological activities including radical scavenging, antioxidant, reducing and proliferation inhibition. Overall, GI was found to contain high contents (flavonoid and phenolic), biological activities and proliferation inhibition. Study confirms nutraceutical potential of red seaweed *Grateloupia indica* to be explored further for the bioactive compound or to be used as functional food.

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1. Introduction

Seaweeds, especially red seaweeds have proven to be rich source of dietary supplements (Tanna et al., 2019; Francavilla et al., 2013; Ragaza et al. 2015). Most of the seaweeds showed high antioxidant property, which can help decode their use as nutraceuticals application (Tanna and Mishra, 2018a; Tanna and Mishra, 2019; Choudhary et al. 2021). Total aquaculture production was 120 million tonnes in the year 2019, and algae-production contributed about 36 million tonnes (FAO, 2021). Out of total 36 million tonnes algal-production, about 50% algae were produced by cultivation. There is a large number of seaweeds available which includes 125 species of Rhodophyta (red algae), 64 Phaeophyceae (brown algae) and 32 Chlorophyta (green algae). Amongst available 221 species, 145 species are being used for foods while 101 species for the production of phycocolloid. There are many types of seaweeds, which are extensively cultivated that includes *Kappaphycus alvarezii* and *Gracilaria* spp. of red seaweeds. Maximum cultivation of seaweed has been observed in Chile and then in China (FAO, 2021). In East Asia countries specifically in China, Korea and Japan seaweeds are mainly used for food purpose (Nayar and Bott, 2014).

Red seaweeds from Rhodophyta phylum are widely used as seasonings, noodles, condiments, sushi wrappings and vegetables, also being use as food additive, functional-food, and in phycocolloid industry for agar and carrageenan due to their gelling, emulsifying, water retention and other physical properties (Arulkumar et al., 2018). Since the Viking age, in Norway red algae *Palmaria palmata* has been traditionally consumed (Roleda et al., 2019). The protein from red seaweed *Palmaria palmata* has shown hypotensive activity on rats by Renin Inhibitory mechanism (Fitzgerald et al., 2014). There is also edible film developed with antioxidant activity from *Pyropia columbina* (formerly *Porphyra columbina*) with their phycocolloids and phycobiliproteins enriched fractions (Cian et al., 2014). There have been many studies to check antioxidant activities of different seaweeds and have obtained good results like ethanolic extracts of two *Gracilaria* spp. from Brazil have shown high phenolic and antioxidant activities (Souza et al., 2011).

Metabolomics is newly emerged form of 'omics' study, which is biochemical phenotyping study to increase our knowledge about primary as well as secondary metabolites of given organism at a given time (Tanna and Mishra, 2018b). Tropical red seaweeds were reported to be rich in metabolites, flavonoid, and phenolic compounds (Tanna et al., 2019). Further seaweeds are considered natural antioxidants and possess high bioactivity (Choudhary et al., 2021; Tanna et al., 2018).

There are no reports, so far, on tropical red seaweeds from the Arabian Sea coast. Therefore, we studied the different

biological activities of the selected red seaweeds. Seven abundantly growing red seaweeds (*Gracilaria corticata*, *Grateloupia indica*, *Kappaphycus alvarezii*, *Solieria robusta*, *Amphiroa anceps*, *Halymenia porphyroides*, and *Sarconema scinaoides*) were analyzed for the different phytochemical composition (total flavonoid and phenolic contents) and biological activities including total antioxidant, reducing and scavenging activities, and proliferation inhibition on human cancer cell lines (HeLa and Huh7).

2. Materials and methods

2.1. Collection of seaweeds samples and preparation of extracts

Tropical seven red seaweeds; *Gracilaria corticata* (GC; family Gracilariaceae), *Grateloupia indica* (GI; family Halymeniaceae), *Kappaphycus alvarezii* (KA; family Solieriaceae), *Solieria robusta* (SR; family Solieriaceae), *Amphiroa anceps* (AA; family Lithophyllaceae), *Halymenia porphyroides* (HP; family Halymeniaceae), and *Sarconema scinaoides* (SS; family Solieriaceae) were collected from Saurashtra coast (N. 22° 28' 8.19"; E. 69° 04' 8.24") of Arabian Sea (Tanna et al., 2019). All collected samples were processed as described previously and stored for further analysis. Dried seaweed powder (10 g) was extracted overnight with aqueous methanol (70%, v/v) and supernatant was collected by centrifugation at 7000 g for 10 min. Supernatant was concentrated using rotary evaporator (100–150 mbar at 37 °C), lyophilized and stored at –20 °C until further use.

2.2. Estimation of total antioxidant activity

Total antioxidant of the red seaweed extracts were analyzed by measuring scavenging capacity of ABTS (2,2'-azino-bis(3-ethyl benzothiazoline-6-sulphonic acid)) free radicals and compared with standard trolox (Hazra et al., 2008; Re et al., 1999). Briefly, ABTS free radicals were generated by mixing ABTS diammonium salt (7 mM) with potassium persulphate (2.45 mM) in dark followed by incubation for 12–16 hrs at room temperature. ABTS free radicals (1 ml; OD₇₃₄ = 0.70 ± 0.02) was mixed with various concentration (200, 400, 600, 800 and 1000 µg ml⁻¹) of seaweed extracts or standard (1–5 µg ml⁻¹ trolox), incubated for 90 min and absorbance was read at 734 nm. ABTS scavenging activity was compared with standard trolox and percentage inhibition was calculated.

2.3. Estimation of radical scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) is used for the study of scavenging activity (Saeed et al., 2012). About 1 ml of DPPH

solution (0.024%, w/v in methanol; $OD_{517} = 0.98 \pm 0.02$) was mixed with different concentration of seaweed extracts (200, 400, 600, 800 and 1000 $\mu\text{g ml}^{-1}$) or trolox, incubated overnight and absorbance was read at 517 nm. The scavenging activity was determined by comparing the absorbance of extracts with standard trolox (Mishra et al., 2015; Patel et al., 2016).

2.4. Estimation of reducing power

The reducing power was determined using ascorbic acid as standard (Tanna et al., 2018; Tanna et al., 2021). Different concentration of seaweeds extracts (100, 200, 300, 400 and 500 $\mu\text{g ml}^{-1}$) was mixed with 1 ml of phosphate buffer (0.2 M, pH 6.6) and 1 ml of $\text{K}_3\text{Fe}(\text{CN})_6$ (10 mg ml^{-1}). The reaction mixture was incubated at 50 °C for 20 min, 1 ml TCA (100 mg l^{-1}) was added to terminate the reaction. Supernatant was collected by centrifugation at 7000 g for 10 min at room temperature. Freshly prepared FeCl_3 (0.2 ml; 0.1%, w/v) was added to 1 ml of supernatant, incubated for 10 min at room temperature, absorbance was read at 700 nm, and reducing power was calculated.

2.5. Estimation of total phenolic and flavonoid contents

Total phenolic content (TPC) and total flavonoid content (TFC) were estimated with the help of gallic acid and quercetin as a standard, respectively (Tanna et al., 2019; Saeed et al., 2012; Zhishen et al., 1999). Different concentration of seaweed extracts (200, 400, 600, 800 and 1000 $\mu\text{g ml}^{-1}$) was mixed with Folin-Coicalteu reagents (2.5 ml; 0.2 M), incubated for 90 min, 2 ml sodium carbonate (Na_2CO_3 ; 75 g l^{-1}) was added, further incubated for 90 min in dark, and the absorbance was read at 760 nm. Total phenolic content was calculated using a standard curve of gallic acid and represented as mg ml^{-1} gallic acid per 100 mg of extract. Similarly, different concentration of seaweed extracts (200, 400, 600, 800 and 1000 $\mu\text{g ml}^{-1}$) was mixed with 0.3 ml NaNO_2 (5%, v/v), incubated at room temperature for 5 min, 0.3 ml AlCl_3 (10%, v/v) and 2 ml NaOH (1 M) were added, and absorbance of the reaction mixture was read at 510 nm. TFC was determined as mg ml^{-1} quercetin per 100 mg of extract from a standard curve.

2.6. Cell culture and anti-proliferative activity

The human hepatoma cancer cell line (Huh-7) and human cervical cancer cell line (HeLa) were seeded in 96-well plate with a concentration of 10^4 cells in each well and incubated at 37 °C under 5% CO_2 in the air for 24 h. Seaweed extracts were added and incubated further for 4–6 h. MTT based assay was performed for the study of anti-proliferative activity following the previously optimized protocol (Patel et al., 2018; Patel et al., 2019; Tanna et al., 2020). Absorbance was taken at 570 nm and following equations were used to calculate the percent anti-proliferative activity:

Anti – proliferative activity(%)

$$= 100 \times \left[1 - \frac{(abs_{570}(\text{sample}) - abs_{690}(\text{blank}))}{(abs_{570}(\text{control}) - abs_{690}(\text{blank}))} \right]$$

2.7. Extraction and analysis of metabolites

Previously optimized method was used for the extraction and quantification of metabolites (Pandey et al., 2015; Patel et al., 2020; Yadav et al., 2021). Briefly, dried seaweed samples were extracted with methanol (ice-cold) for 10 min at 70 °C. Supernatant was collected by centrifugation 11000 g for 10 min at 4° C, dried and proceed for derivatization (Mishra and Jha, 2009). Metabolites adonitol/ribitol was used as an internal standard for quantification, which was performed by GC–MS. Mass spectra peaks were compared with the library (NIST– National Institute of Standards and Technology) for the identification, whereas quantification was done using internal standard. Metabolite data were analyzed using partial least squares-discriminant analysis (PLS-DA) (Yadav et al., 2021).

2.8. Statistical analysis

All experiments were done in triplicates and three biological replicas were there in each experiment. Analysis of variance (ANOVA) was applied to all data and threshold for statistical significance was considered $p < 0.05$, all values are represented as mean \pm SD (standard deviation of the mean), and significant difference is shown by different letters. Correlation studies and multivariate analysis were performed using principal component analysis and Pearson's correlation matrix.

3. Results

3.1. Biological activities of red seaweed–extracts

All red seaweed–extracts showed dose dependent biological activities including total antioxidant, scavenging, and reducing activities (Fig. 1). Seaweeds, GI (70 ± 0.5 %) and AA (62 ± 0.3 %) have high total antioxidant activity at 400 μg concentration. Whereas, GC and HP showed 70 ± 0.5 % and 65 ± 0.5 % total antioxidant activity at 1000 μg concentration. Seaweeds, SS and KA showed high antioxidant activity of 64 ± 0.7 % and 56 ± 0.3 % with 400 μg and 1000 μg concentration of extract, respectively (Fig. 1A). High scavenging activity, about 68 ± 0.3 % was observed with GI extract at 1000 μg concentration. Though $> 50\%$ scavenging activity was noticed with AA (55 ± 1 %) and SS (52 ± 0.6 %) at 600 and 800 μg concentration, respectively. But, both (AA and SS) have shown similar about 58 ± 0.6 % scavenging activity at 1000 μg concentration. Other seaweeds, HP, SR and GC have shown comparatively less activity, about 51 ± 1 , 49 ± 0.6 and 46 ± 0.4 %, respectively at 1000 μg concentration (Fig. 1B). Seaweed GI showed the highest reducing activity 40 ± 1 % at 100 μg concentration. Overall, SS has been observed to have the highest reducing activity of 68 ± 0.1 % with 500 μg concentration. The reducing activity was followed by AA, GI, GC and HP with 63 ± 0.2 , 57 ± 0.5 , 45 ± 0.4 and $43 \pm 1\%$, respectively at 500 μg concentration (Fig. 1C).

For all above biological activities, half maximal concentration (EC_{50}) was calculated for each red seaweed–extract (Fig. 2). Seaweed GI showed the highest antioxidant activity with the lowest EC_{50} values of 120 ± 4 $\mu\text{g ml}^{-1}$ followed by SS (190 ± 10 $\mu\text{g ml}^{-1}$), whereas the lowest activity was observed with SR as it showed the highest EC_{50}

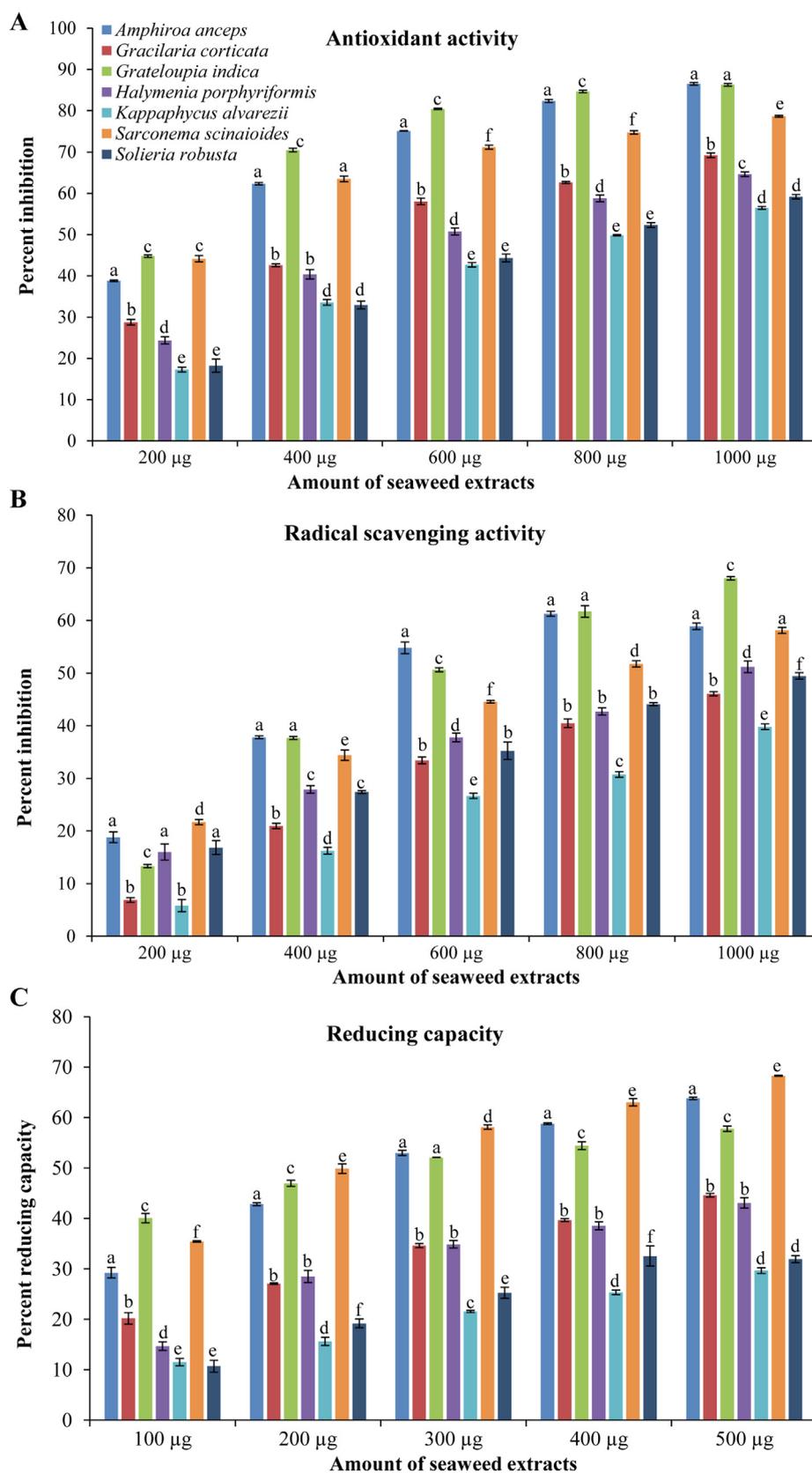


Fig. 1 Different biological activities of selected tropical red seaweeds. (A) Total antioxidant, (B) scavenging, and (C) reducing activity. All activities are shown as mean \pm SD (n = 3) and different small letters show a statistically significant difference at $p < 0.05$ (Tukey test).

($770 \pm 6 \mu\text{g ml}^{-1}$). Seaweed, GI also showed the highest scavenging activity with EC_{50} $656 \pm 1 \mu\text{g ml}^{-1}$, followed by AA ($672 \pm 4 \mu\text{g ml}^{-1}$). The lowest scavenging activity was found with KA extract (EC_{50} $1235 \pm 20 \mu\text{g ml}^{-1}$). In case of reducing power capacity, the lowest EC_{50} was observed with SS ($237 \pm 3 \mu\text{g ml}^{-1}$), followed by GI ($294 \pm 4 \mu\text{g ml}^{-1}$) and AA ($306 \pm 2 \mu\text{g ml}^{-1}$). Seaweed KA also showed the lowest reducing activity with the highest EC_{50} ($938 \pm 18 \mu\text{g ml}^{-1}$).

3.2. Total phenolic and flavonoids contents

The maximum amount of total phenolic content was found with seaweed GI ($80 \pm 10 \text{ mg ml}^{-1}$ per g extract), followed by AA and SS which contained 20 ± 2 and $15 \pm 2 \text{ mg TPC per ml per g of extract}$, respectively (Fig. 3A). Similarly, the highest flavonoid content is also detected with GI ($617 \pm 72 \text{ mg ml}^{-1}$ per g extract) followed by AA and SS, in which 472 ± 113 and $408 \pm 103 \text{ mg TFC per ml per g of extract}$ were determined (Fig. 3B). The lowest TPC ($8\text{--}10 \text{ mg ml}^{-1}$ per g extract) was found in SR, HP and KA; whereas KA also have the lowest TFC ($352 \pm 110 \text{ mg ml}^{-1}$ per g extract).

3.3. Correlation analysis

A correlation analysis was performed individually for all seven red seaweeds (Table S1). A very strong ($0.9\text{--}1.0$; $R^2 > 0.9$) significant ($p < 0.05$) correlation was observed among all biological activities (total antioxidant, scavenging and reducing) and contents (TPC and TFC) for red seaweeds AA, GC, GI, HP and SR. Seaweed SS also showed strong significant correlations ($0.9\text{--}1.0$; $R^2 > 0.9$; $p < 0.05$) among biological activities and content, except TFC, which showed moderate ($0.5\text{--}0.7$; $R^2 = 0.3\text{--}0.6$) insignificant ($p > 0.05$) correlation with other biological activities including TPC. Seaweed KA showed

strong significant correlation ($0.9\text{--}1.0$; $R^2 > 0.9$; $p < 0.05$) amongst TPC, total antioxidant and scavenging activities. However, reducing activity showed weak ($0.35\text{--}0.45$) while TFC showed strong ($0.6\text{--}0.7$) correlations with other biological activities.

3.4. Anti-proliferative activity

Seaweeds, HP and GI were found to have the highest proliferation-inhibition activities (about $40 \pm 0.5\%$) on HeLa cells. Similarly, GI and KA showed the maximum $44 \pm 2\%$ proliferation-inhibition on Huh-7 cells (Fig. 4). Other red seaweeds showed low anti-proliferation activities; GC and AA showed $32\text{--}35\%$, and SR and KA showed $26\text{--}28\%$ proliferation-inhibition on HeLa cells. Similarly, seaweeds, AA, GC, HP, SR and SS showed $<40\%$ proliferation-inhibition on Huh-7 cells.

3.5. Multivariate correlation analysis

A cumulative multivariate correlation analysis was performed between biological activities and phytochemical composition (Table S2). Overall total antioxidant of tropical red seaweeds showed a significant ($p < 0.5$) and very strong ($0.9\text{--}1.0$) correlation with scavenging activity (0.940 ; $R^2 = 0.833$), TFC (0.870 ; $R^2 = 0.757$) and proliferation-inhibition of Huh-7 cells (0.708 ; $R^2 = 0.501$). Overall total antioxidant activity showed a significant strong ($0.7\text{--}0.9$) correlation with reducing activities (0.792 ; $R^2 = 0.627$), and a moderate ($0.4\text{--}0.7$) correlation with TPC (0.628 ; $R^2 = 0.395$) and proliferation-inhibition of HeLa cells (0.538 ; $R^2 = 0.290$). Similarly, overall scavenging activity was strongly correlated with reducing activity (0.763 ; $R^2 = 0.582$), TFC (0.897 ; $R^2 = 0.804$) and proliferation-inhibition of Huh-7 cells (0.801 ; $R^2 = 0.642$). Within phytochemical composition, TPC showed a strong correlation with TFC;

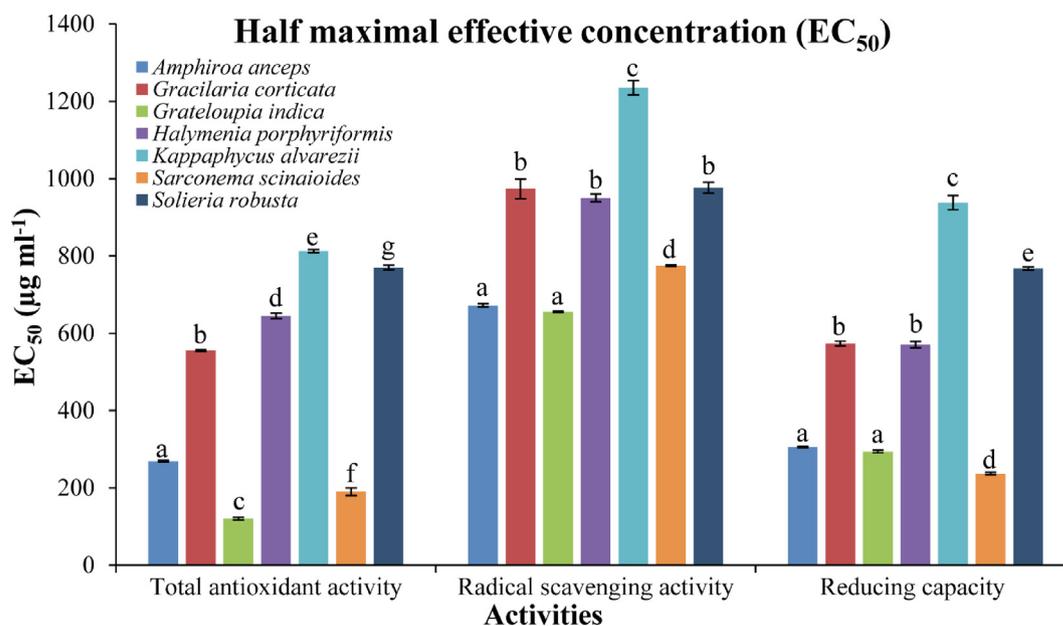


Fig. 2 Half maximal effective concentration (EC_{50} ; $\mu\text{g ml}^{-1}$) of red seaweeds for different biological activities. All activities are shown as mean \pm SD ($n = 3$) and different small letters show a statistically significant difference at $p < 0.05$ (Tukey test).

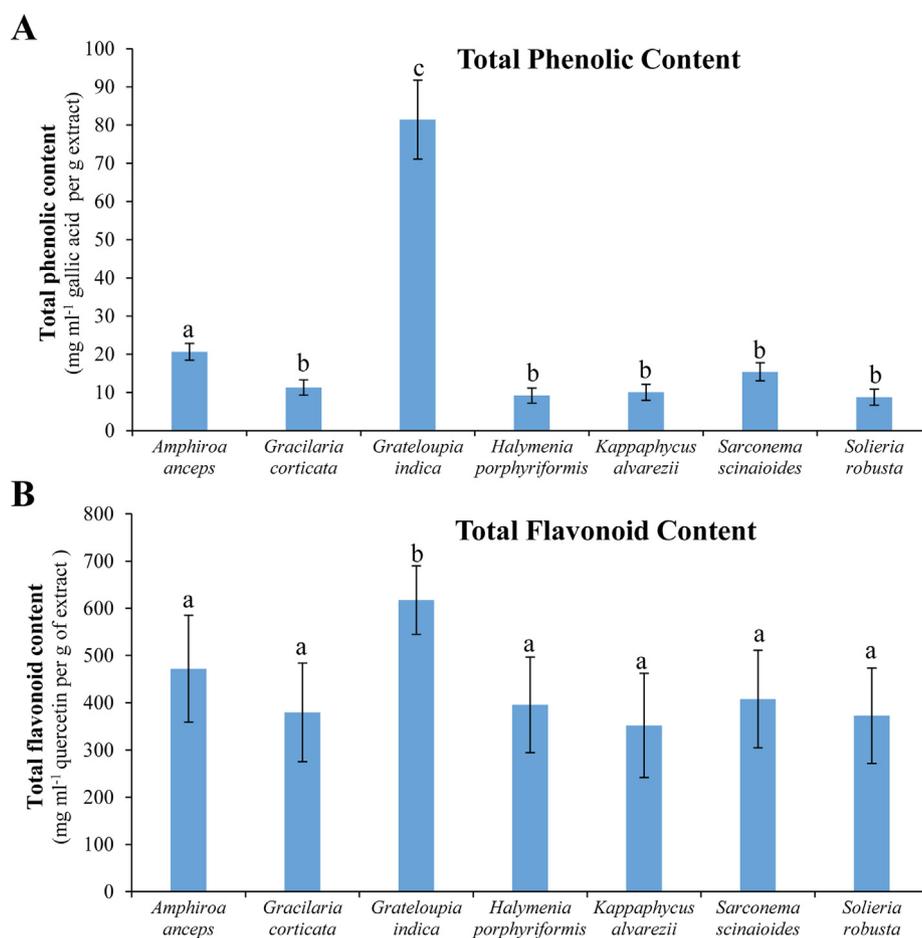


Fig. 3 TPC and TFC of tropical red seaweeds. (A) TPC is shown as GAE (mg ml⁻¹) per gram of extract. (B) TFC is shown as quercetin equivalent (mg ml⁻¹) per gram of extract. All data are shown as mean \pm SD (n = 3) and different small letters show a statistically significant difference at p < 0.05 (Tukey test).

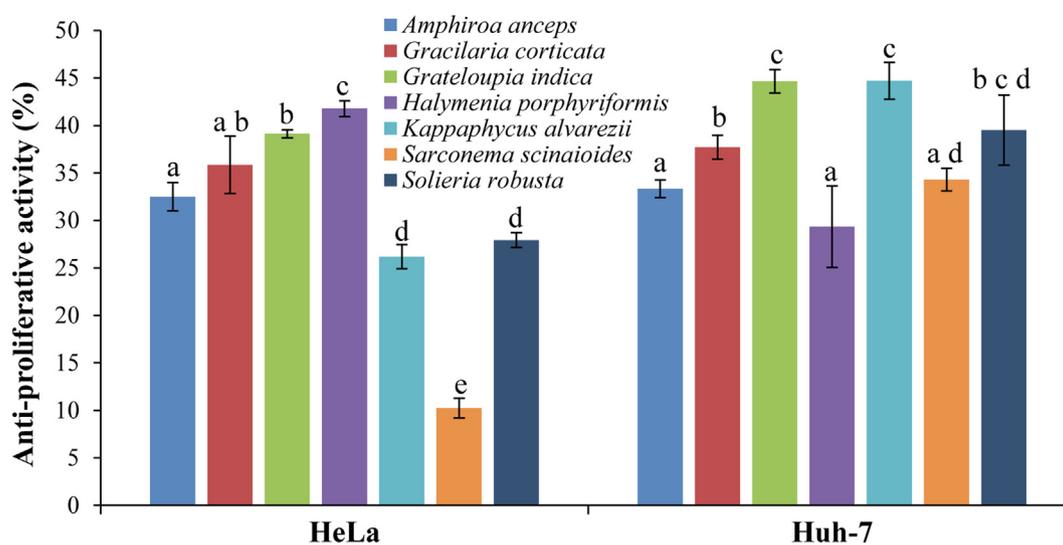


Fig. 4 Proliferation-inhibition of tropical red seaweed extracts on carcinoma cell (HeLa and Huh-7) lines using MTT assay. All data are shown as mean \pm SD (n = 3) percent inhibition and different small letters show a statistically significant difference at p < 0.05 (Tukey test).

whereas TFC not only showed strong correlation with TPC but also with antioxidant and scavenging activities.

A cumulative principal component analysis of different biological activities and contents were performed for red seaweeds (Fig. 5). The biplot inferred from PCA showed 82.42% variation (PC1), and categorized red seaweeds based on their biological activities. The analysis revealed that GI has nutraceutical potential as it showed high biological activities including total antioxidant, total scavenging, anti-proliferative activity of HeLa cells and also have maximum TPC.

3.6. Metabolite profiling

A total of 57 different metabolites were detected in red seaweeds (Table 1). These metabolites were grouped in the different categories; about 25 types of sugars, 15 fatty acids, 9 sugar derivatives, 7 different amino acids and one polyol were quantified. Maximum 39 different metabolites were detected in SS followed by 23 in GI, 22 in HP, 21 in GC and AA, 19 in KA, and lowest 13 was found in SR. It was also observed that seaweed SS have maximum 12 exclusive metabolites, followed by 4 unique metabolites owned by GI, HP contained 2 while GC have 1 specific metabolites. No exclusive metabolites were detected in other seaweeds AA and SR. Sugar mannobiose was abundantly detected in seaweeds GI ($5600 \pm 500 \mu\text{g g}^{-1}$ DW) followed by AA ($4000 \pm 250 \mu\text{g g}^{-1}$ DW) and SS ($2500 \pm 150 \mu\text{g g}^{-1}$ DW). Seaweed GC contained maximum glucose ($11160 \pm 185 \mu\text{g g}^{-1}$ DW), and KA contained maximum galactose ($1275 \pm 260 \mu\text{g g}^{-1}$ DW), while no abundance of sugars were found in HP and SR seaweeds. Amino acid valine was detected in all seaweeds in the range of $0.5\text{--}7 \mu\text{g g}^{-1}$ DW. Among fatty acids, palmitic acids were detected in all sea-

weeds with maximum amount $83 \pm 7 \mu\text{g g}^{-1}$ DW in GI, followed by SS ($40 \pm 5 \mu\text{g g}^{-1}$ DW). About $20 \mu\text{g g}^{-1}$ DW palmitic acids were estimated in seaweeds HP and KA, $17 \pm 6 \mu\text{g g}^{-1}$ DW palmitic acids was found in AA. Comparatively low amount of palmitic acid was detected in seaweeds SR ($10 \pm 2 \mu\text{g g}^{-1}$ DW) and GC ($5 \pm 2 \mu\text{g g}^{-1}$ DW). Interestingly, GC contained high amount of heptanoic acid ($252 \pm 43 \mu\text{g g}^{-1}$ DW) and octanoic acid ($123 \pm 7 \mu\text{g g}^{-1}$ DW). Metabolites allose, galactonic acid, glucuronic acid and pinitol were exclusively detected in GI. Similarly, glucose and tagatofuranose were found exclusively in GC and KA, respectively. Two sugar-metabolites lyxose and ribofuranose were detected exclusively in HP. About 12 different metabolites, decanoic acid, dodecanoic acid, lignoceric acid, octadecenoic acid, allopyranose, mannitol, sucrose, tagatose, talose, trehalose, xylose and arabinitol were observed exclusively in seaweed SS.

A score plot was inferred using PLS-DA (Partial Least Squares Discriminant Analysis) for metabolites which were commonly detected in all seaweeds (Fig. 6). PLS-DA plot showed two important key measures; one is variable importance in projection (VIP) and the second is the weighted sum of absolute regression coefficients. The colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each red seaweed group. A hierarchical cluster analysis was done and a heat map was inferred using Spearman's rank correlation coefficients (Supplementary Figure S1). The deduced heat map showed differential accumulation of metabolites in different red seaweeds.

4. Discussion

Recently the priority for consumers turned to the healthiness, lifestyle and comfort without neglecting environmental and sustainability concerns (Santos et al., 2020). The phrase "Good food, good health" means a lot itself. In the last few decades, seaweeds get attention of researchers because it offers the opportunity of discovering a broad range of both primary and secondary metabolites which have interesting properties and applications (Tanna and Mishra, 2018a; Tanna and Mishra, 2019; Choudhary et al., 2021). Seaweeds possess valuable metabolites like vitamins, polyphenols, minerals, as well as strong balance of n-3 to n-6 ratio of PUFA. Seaweeds can live and adapt in harsh and extreme conditions and in addition to that they are easily cultivated and thus economical (Ragaza et al., 2015). Researchers mainly focus on the seaweeds extracts and their chemical characterization which publicized their variety of components with very attractive biological activities such as antimicrobial, antiviral, anti-inflammatory, antitumor, fat-lowering, neuroprotective and antihypertensive activities (Hakim and Patel, 2020). In the present study, seven red seaweeds which are abundantly growing in their natural habitat were studied for their phytochemical composition and biological activities, keeping their further exploration in the future.

Primary metabolites contribute their role directly in physiological function of organism, under normal growth which includes photosynthesis, whereas secondary metabolites are produced under diverse environmental stress conditions which are temperature and salinity fluctuations, ultraviolet exposure and environmental pollutants (El-Beltagi et al., 2019). Primary

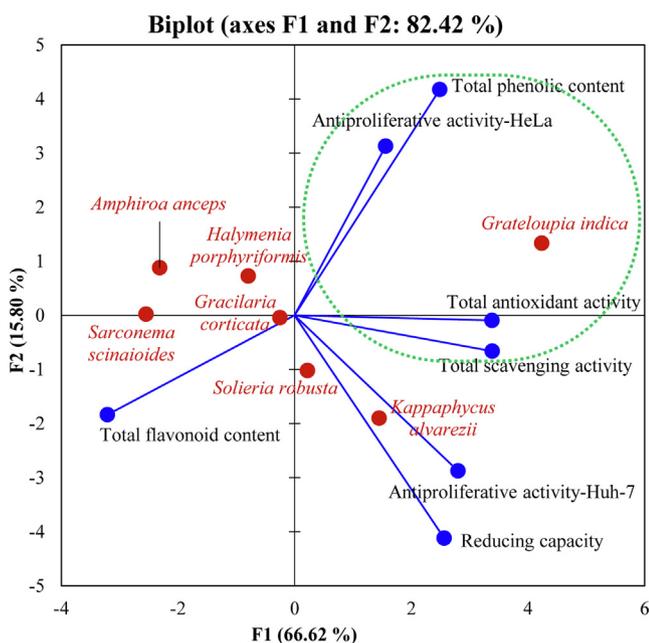


Fig. 5 A cumulative principal component analysis. A Bi-plot of different biological activities of red seaweeds deduced from the Pearson correlation matrix.

Table 1 Metabolite profiling of tropical red seaweeds. Metabolites were analyzed by GC–MS, identify using in build library and quantified using internal standard.

Category	Metabolites↓ Seaweeds^ →	Amount ($\mu\text{g g}^{-1}$ DW)							
		AA	GC	GI	HP	KA	SS	SR	
Amino acids	Alanine	50 ± 10	44 ± 16	—	—	—	35 ± 7	22 ± 6	
	Glutamic acid	—	1 ± 0.5	—	—	—	20 ± 7	—	
	Glycine	3 ± 1	—	—	5 ± 1	1 ± 0.5	—	1.5 ± 0.5	
	Leucine	—	5.5 ± 2.0	—	2 ± 1	—	—	—	
	Threonine	1 ± 0.5	3.5 ± 1	—	—	—	2 ± 0.5	—	
	Tyramine	—	—	2.5 ± 2	—	—	1.5 ± 0.2	1.5 ± 0.5	
	Valine	2 ± 0.5	7 ± 2	—	4 ± 2	0.5 ± 0.1	3 ± 1	1.5 ± 0.5	
Fatty acids	Arachidic acid	—	—	1 ± 0.5	—	—	2.5 ± 1	—	
	Decanoic acid	—	—	—	—	—	0.2 ± 0.05	—	
	Dodecanoic acid	—	—	—	—	—	1 ± 0.1	—	
	Eicosanoic acid	—	—	1.5 ± 0.5	—	—	2 ± 0.2	—	
	Heptanoic acid	0.2 ± 0.1	2 ± 0.2	—	—	0.5 ± 0.1	1.5 ± 0.5	1.5 ± 0.1	
	Hexanoic acid	0.6 ± 0.02	—	2.5 ± 2	0.2 ± 0.03	1 ± 0.5	—	2 ± 0.05	
	Lignoceric acid	—	—	—	—	—	0.5 ± 0.1	—	
	Myristic acid	1 ± 0.05	—	4 ± 0.5	1.5 ± 0.5	1 ± 0.1	2 ± 0.5	1 ± 0.05	
	Octadecenoic acid	—	—	—	—	—	8.5 ± 5	—	
	Octanoic acid	1 ± 0.2	5 ± 1	1.5 ± 0.5	2 ± 1	2 ± 1	3 ± 0.1	—	
	Oleic Acid	1 ± 0.5	4.5 ± 2	—	—	—	—	—	
	Palmitic Acid	17 ± 6	125 ± 7	83 ± 7	20 ± 0.5	20 ± 1	40 ± 5	10 ± 2	
	Pentanoic acid	1 ± 0.5	—	1.3 ± 0.7	1 ± 0.5	0.5 ± 0.1	7 ± 1	—	
	Stearic acid	2.5 ± 0.5	20 ± 0.5	15 ± 2	3 ± 0.1	3 ± 0.1	7 ± 1	2 ± 0.5	
	Valeric acid	—	5 ± 1	—	—	—	8 ± 1	—	
	Sugars	Allopyranose	—	—	—	—	—	355 ± 15	—
		Allose	—	—	1 ± 0.1	—	—	—	—
Arabinofuranose		—	—	—	4 ± 0.2	1 ± 0.5	—	—	
Arabinose		1 ± 0.3	—	—	1 ± 0.05	—	—	—	
Cellobiose		—	—	—	—	30 ± 15	0.5 ± 0.1	3 ± 0.1	
Deoxyribose		—	6 ± 0.5	—	—	—	7 ± 0.5	—	
Fructose		—	2 ± 0.5	1 ± 0.1	1 ± 0.5	—	0.5 ± 0.05	—	
Galactofuranose		1.5 ± 1.0	4 ± 1	—	—	1.5 ± 0.5	2.5 ± 1	1 ± 0.1	
Galactose		1 ± 0.7	—	2 ± 1	—	1275 ± 260	1 ± 0.2	—	
Glucopyranose		—	—	15 ± 5	—	—	0.5 ± 0.1	2 ± 0.3	
Glucose		—	255 ± 45	—	—	—	—	—	
Glyceryl-glycoside		90 ± 10	58 ± 22	510 ± 80	10 ± 0.5	20 ± 2	70 ± 12	—	
Lyxose		—	—	—	0.5 ± 0.2	—	—	—	
Maltose		—	11160 ± 185	—	—	—	1 ± 0.1	—	
Mannitol		—	—	—	—	—	1 ± 0.5	—	
Mannobiose		4000 ± 250	—	5600 ± 500	—	—	2500 ± 150	—	
Myo-Inositol		1 ± 0.5	2 ± 0.5	3 ± 1.5	1 ± 0.1	1 ± 0.5	1 ± 0.1	—	
Ribofuranose		—	—	—	1 ± 0.1	—	—	—	
Scyllo-Inositol		—	—	—	4 ± 0.5	2 ± 0.2	10 ± 6	—	
Sucrose		—	—	—	—	—	6 ± 2	—	
Tagatofuranose		—	—	—	—	0.2 ± 0.1	—	—	
Tagatose		—	—	—	—	—	0.5 ± 0.1	—	
Talose		—	—	—	—	—	1 ± 0.1	—	
Trehalose		—	—	—	—	—	5 ± 1	—	
Xylose		—	—	—	—	—	2 ± 1	—	
Sugar derivatives		Arabinitol	—	—	—	—	—	2 ± 1	—
		Erythritol	—	—	2 ± 0.5	1.5 ± 0.5	—	2 ± 0.5	1 ± 0.5
	Galactonic acid	—	—	1 ± 0.1	—	—	—	—	
	Glucitol	0.5 ± 0.2	2 ± 0.1	—	2 ± 0.05	—	—	—	
	Glucuronic acid	—	—	2 ± 1	—	—	—	—	
	Threitol	—	0.5 ± 0.1	2 ± 0.1	—	—	—	—	
	Threonic acid	115 ± 55	35 ± 5	10 ± 3	5 ± 1	1 ± 0.2	3 ± 0.2	—	
	Xylitol	0.3 ± 0.1	—	0.5 ± 0.2	3 ± 0.2	0.5 ± 0.1	—	—	
	Xylonic acid	—	—	—	1 ± 0.1	—	—	—	
Polyol	Pinitol	—	—	1 ± 0.05	—	—	—		

^Seaweed codes used in the study; AA: *Amphiroa anceps*, GC: *Gracilaria corticata*, GI: *Grateloupia indica*, HP: *Halymenia porphyriformis*, KA: *Kappaphycus alvarezii*, SS: *Sarcomena scinaoides*, and SR: *Solieria robusta*.

‘—’ not detected or negligible amount detected.

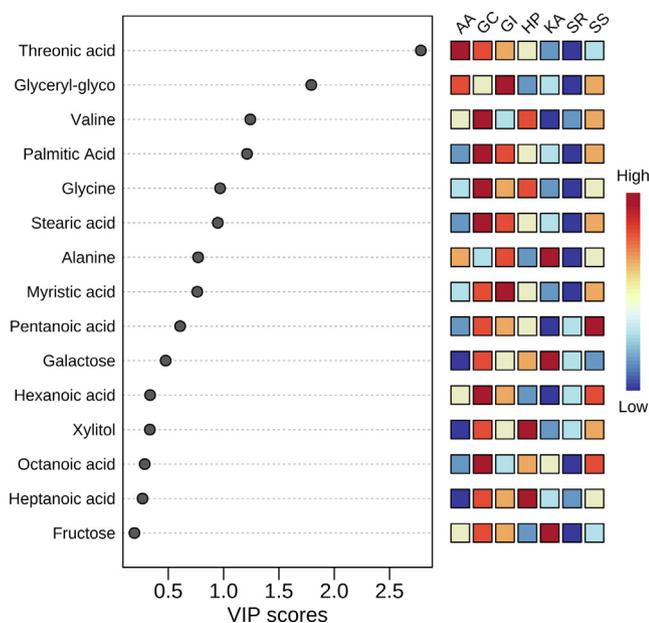


Fig. 6 A score plot inferred by partial least squares discriminant analysis for metabolites commonly detected in all red seaweeds. AA: *Amphiroa anceps*, GC: *Gracilaria corticata*, GI: *Grateloupia indica*, HP: *Halymenia porphyriiformis*, KA: *Kappaphycus alvarezii*, SS: *Sarconema scinaoides*, and SR: *Solieria robusta*.

metabolites include normal ones, polysaccharides, lipids and proteins while the secondary metabolites produced in seaweeds tissues which are halogenated compound, phenolic compounds, terpenes, phenolic compounds and small peptides along with their bioactivity (Rico et al., 2017). GC has shown the presence of essential amino acids such – leucine, threonine and valine. Amongst which valine and leucine are dietary branched chain amino acid that has shown to be useful for immune response (Calder, 2006) as well as injury induced cognitive repair system (Ibuki et al., 2011). Specifically, valine was found to be present in all selected seaweeds except for GI. Seaweeds AA, GI, SS showed very high levels of Mannobiose sugar, which has proven to activate innate immune system against *Salmonella enteritidis* (SE) infection which is food-borne illness in humans (Ibuki et al., 2011). Other sugars like glyceride-glycoside was present in all collected seaweeds except for *Solieria robusta*. Glucose was found to be present in very high amount in *Gracilaria corticata*. Other sugars such as arabinofuranose, fructose, galactofuranose were found to be present in multiple selected red seaweeds in significant quantity. Various sugar derivatives such as threonic acid was also found to be present in all selected seaweeds except for *Solieria robusta*. There has been study-showing magnesium salt of threonic acid to have beneficial effect on Alzheimer's patient condition (Wroolie et al., 2017). *Grateloupia indica* showed presence of important omega-6 polyunsaturated fatty acid-arachidic acid. In previous study also it has shown that tropical seaweeds are good source of PUFAs (Kumar et al., 2011).

Polyphenolics are secondary metabolites which are major antioxidants containing one or more hydroxyl group mainly found in plants, fruits, beverages such as wine, tea and coffee and also in vegetables (Machu et al., 2015). There have been many studies showing phenolic compounds are directly correlated to antioxidant activity in various red seaweeds. Marine

seaweeds are exposed to light and oxygen variations, which generate stress and radicals in them, but they are stable to these oxidation conditions, which indicate that they have well established defense mechanism (Matanjan et al., 2008). There have been studies involving direct correlation of brown (Tanna et al., 2021) and green seaweeds with their biological activities such as antioxidant activities with polyphenolic and flavonoid contents. In this study, we have shown the correlation of red seaweeds selected along the Gujarat coast.

Polyphenols from *Neopyropia tenera* (formerly *Porphyra tenera*), *Palmaria palmata* (Machu et al., 2015), *Kappaphycus alvarezii* (formerly *Eucheuma cottonii*), *Eucheuma denticulatum* (formerly *E. spinosum*) and *Halymenia durvillei* (Matanjan et al., 2008), *Gracilaria birdiae* and *Crassiphycus corneus* (*Gracilaria cornea*) from Brazil (Souza et al., 2011), *Gracilaria corticata* of Indian origin (Sachindra et al., 2010) have shown direct correlation between phenolic content and antioxidant activity of their extracts. Antioxidant activity and phenolic content of *Gracilaria gracilis* (collected in summer from Italy) has shown to be higher in comparison to commercial antioxidant products. In this study, red seaweeds, including *Amphiroa anceps*, *Gracilaria corticata*, *Grateloupia indica*, *Halymenia porphyriiformis* and *Solieria robusta* have shown significant correlation between phenolic content and antioxidant activity. *Grateloupia indica* has shown highest total phenolic ($80 \pm 10 \text{ mg ml}^{-1}$ per g extract) and flavonoid ($617 \pm 72 \text{ mg ml}^{-1}$ per g extract) content (Fig. 3) amongst all studied red seaweeds. The lowest EC_{50} of antioxidant ($120 \pm 4 \mu\text{g ml}^{-1}$) and scavenging ($656 \pm 1 \mu\text{g ml}^{-1}$) activity was detected for *Grateloupia indica*, while the lowest reducing power was observed in *Sarconema scinaoides* extract ($237 \pm 3 \mu\text{g ml}^{-1}$), followed by *Grateloupia indica* ($294 \pm 4 \mu\text{g ml}^{-1}$) (Fig. 2). *Grateloupia indica* has also shown the highest anti-proliferative activity on Huh-7 cell lines. Various species of *Grateloupia* have been shown to possess high antioxidant activities such as *Grateloupia livida* (Tang et al., 2017) due to presence of sulfated polysaccharides; *Grateloupia turuturu* with its UV-shielding capability due to antioxidant compounds (Félix et al., 2020).

Myristic acid has shown to possess antimicrobial activity in previous studies (Liu and Huang 2012). Red seaweed *Ceramium rubrum* possessed high content of myristic acid and also shown for high antimicrobial activity (Cortés et al., 2014). Similarly, red seaweeds *Polysiphonia virgata* and *Grateloupia turuturu* showed high antibacterial activities (Saravanakumar et al., 2008; García-Bueno et al., 2015). In our study, high content of myristic acid was detected in GI which suggests that this seaweed could explore further for antimicrobial activities. High anti-inflammatory activity was reported for *Kandelia candel* due to high content of glyceride glycosides (Dat et al., 2015). In this study, high glyceride glycoside content was found in GI, this could be responsible for the high antioxidant activity. Seaweed GI could also be explored for the anti-inflammatory activity.

Previous studies confirmed that *Grateloupia indica* possessed high antioxidant and flavonoids including ascorbic acid, catechins, gallic acid and luteolin (Tanna et al., 2019). Previously, *Amphiroa anceps* also reported to have high gallic acid and catechin along with low quantity of proanthocyanine and apigenin (Tanna et al., 2019). Overall study demonstrates that red seaweed, *Grateloupia indica* contains high TPC and TFC, also showed high proliferation inhibition.

5. Conclusions

Among all seven studied red seaweeds, high antioxidant activities were found in GI followed by SS. Similarly, GI showed high scavenging activity followed by AA. However, SS showed high reducing activity followed by GI and AA. Seaweed, GI was found to be rich in phenolics and flavonoid contents, followed by AA and SS. HP and GI showed high proliferation inhibition on Hela while GI and KA showed on Huh-7 cell lines. Overall, GI (*Grateloupia indica*) considered the potential candidate for nutraceutical applications followed by AA (*Amphiroa anceps*) and SS (*Sarconema scinaoides*). HP (*Halymenia porphyriiformis*) and KA (*Kappaphycus alvarezii*) also showed potential proliferation inhibition activities along with *Grateloupia indica*. The study confirms that flavonoid and phenolic compounds are major contributors for biological activities. Based on study, *Grateloupia indica* is recommended for nutraceutical application as it contain high nutritional values and antioxidant properties with anticancer activities.

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Data Availability Statement

Not applicable.

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Appendix A. Supplementary material

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