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RESEARCH ARTICLE

Antioxidant Activity and Correlation of Three Different Seaweeds Along Gujarat Coastline, India

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ABSTRACT

The constant increase in the amount of free radicals in human body leads to the initiation of several diseases. Antioxidants quench free radicals and delay the oxidation process which ultimately raises defence mechanism against the pathogensis of molecular oxygen. As a treasure of bioactive compounds, seaweeds are attracting attention for the development of new healthy foods and drugs. The pharmacological activity of seaweeds can be predicted by their phyto-constituents. In the present study presence of phytoconstituents have been observed in *Caulerpa mycophysa, Iyengaria stellata,* and *Spatoglossum solieri* collected from Okha coast, Gujarat, India. Significant differences was observed in total phenolic and flavonoid contents, along with antioxidant activities through DPPH, ABTS and reducing power assays. A positive correlation between phenolic, flavonoid and scavenging activities of some extracts has been established. Results of this findings can be utilised in a sustainable manner in order to establish the use of these seaweeds as a potential source for antioxidants rich food which will help in utilization of these unexploited resources to combat diseases.

KEY WORDS: Seaweeds, Phytochemicals, Reactive oxygen species, Antioxidant assessment, Correlation

INTRODUCTION

Reactive oxygen species (ROS) are produced in the human body and are crucial for the proper course of many metabolic processes. For rhythmic body function, free radicals should be in an equilibrium state. The oxidative stress accelerates the aging process in body which eventually leads to an initiation of many diseases, such as cardiovascular, obesity, diabetes, atherosclerosis, Alzheimer's disease, cancer and other (Salachna *et al.,* 2021). Human body should always have the aptness to combat effects of antioxidants in order to prevent cell degradation from ROS. Many pharmaceutical, food and personal care products contain synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). However, due to their carcinogenicity and detrimental health effects, prohibitions on the use of synthetic antioxidants are being implemented (Khairul *et al.*, 2021).

The trend for natural and healthy food represents a great demand for food products worldwide with an additional approach of treating several diseases (Fernandes *et al.*, 2016). Use of food that possess natural antioxidants are gaining lime light in the field of research. Large number of medicinal plants has been investigated for their antioxidant activities until now (Chaudhari & Mahajan, 2015). The therapeutic role of specific plants can justifiably be attributed to having the phytochemicals especially the phenolic acids, flavonoids, terpenoids, alkaloids, lignans, tannins, saponins, etc. Phytochemicals are biologically active chemical compounds that protect cells from hazards and stress. By acting as a synergistic agents, these secondary metabolites provide health benefits through helping the body to utilise nutrients more efficiently (Nyamai *et al.,* 2016). Natural products of marine algae have made major gains in recent years, and have been proven to create a range of chemicals, some of which

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Table 1: Preliminary phytochemical screening of aqueous extracted seaweeds collected from Okha coast, Gujarat, India

Test		Observations (Indicating Positive Test)	*C. mycophysa*	*I. stellata*	*S. solieri*
		Detection of alkaloids			
2)	Hager's test	A creamy white precipitate	+	+	+
3)	Mayer's/ Bertrand's/Valser's test Valser's test	A creamy white/yellow precipitate	+++	-	++
4)	Wagner's test	A brown/reddish precipitate	+++	+++	-
5)	Picric acid test	An orange colour	-	-	+
6)	Iodine Test	A blue colour, which disappears on boiling and reappears on cooling	-	-	-
8)	Tannic acid test	A buff colour precipitate	-	-	-
		Detection of Carbohydrates			
1)	Barfoed's test	A red precipitate {mono-saccharides}	-	++	++
2)	Molish's test	A violet ring	+	++	+++
3)	Seliwanoff's Test	A rose red colour {ketoses}	-	-	-
4)	Resorcinol test	A rose colour {ketones}	-	-	-
5)	Test for pentoses	A red colour	-	-	-
6)	Test for starch	A canary colouration	-	+++	-
		Detection of Reducing sugars			
1)	Benedict's test	Green/yellow/red colour	-	++	+
2)	Fehling's test	A red precipitate	-	-	-
		Detection of Glycosides			
1)	Borntrager's test	A pink coloured solution	-	-	-
2)	Modified Borntrager's test	A rose-pink to blood red coloured solution	+	+	+
3)	10% NaOH test	A brick red precipitate	-	-	-
5)	Aqueous NaOH test	A yellow colour	-	+	+
6)	Concentrate H_2SO_4 test	A brown ring	-	-	-
		Detection of Cardiac Glycosides			
1)	Keller-Killani test	A blue coloured solution (in acetic acid layer)	-	-	-
2)	Test for Cardenolides	A red colour, fades to brownish yellow	+++	+++	+++
3)	Bromine water test	A yellow precipitate	-	-	-
4)	Baljet test	A yellow-orange colour	+	+	+
		Detection of Proteins and Amino acids			
1)	Biuret test	A pink coloured solution (in ethanolic layer)	-	+	-
3)	Ninhydrin test	A purple coloured sol. {Amino acids}	+	-	-
4)	Xanthoproteic test	A yellow coloured solution	-	-	+
		Detection of Flavonoids			
1)	Alkaline reagent test	An intense yellow colour, becomes colourless on addition of diluted acid	+	++	+++
2)	Lead acetate test	A yellow precipitate	++	+	++
3)	Shinoda's test/ Mg-hydrochloride reduction test	A pink to crimson coloured solution {flavonal glycosides}	-	-	+
4)	Shibata's reaction/Cyanidin test	A red colour {flavonols}	+	-	-
5)	Ferric chloride test	A green precipitate	+	-	-
6)	Pew's test	A red colour {flavonols}	-	-	-
7)	Zinc-hydrochloride reduction test	Magenta colour	-	-	-
8)	Ammonia test	A yellow colour	-	-	-
9)	Conc. H_2SO_4 test	An orange colour	+	-	++
		Detection of Phenolic compounds			
1)	Iodine test	A transient red colour	+	+	+
2)	Ferric chloride test	Dark green/bluish black colour	+	+++	-
3)	Gelatin test	A white precipitate	-	+	-
4)	Lead acetate test	A white precipitate	+++	+	-
5)	Ellagic Acid Test	Solution turns muddy /Niger brown precipitate	-	+	++
6)	Potassium dichromate test	A dark colour	-	-	-
8)	Test for Cartenoids	A blue colour at the interface	-	-	-

		Detection of Tannins			
1)	Gelatin test	A white precipitate	-	+	-
2)	Braymer's test	Blue-green colour	-	-	-
3)	10% NaOH test	Formation of emulsion {Hydrolysable tannins}	+	+	-
4)	Bromine water test	Decoloration of bromine	-	+	+
		Detection of Phlobatannins			
1)	HCl test	A red precipitate	-	-	+
		Detection of Saponins			
1)	Foam Test	Persistent foam for 10 min.	+	++	+
2)	$NaHCO_3$ test	Stable honeycomb like froth	+	+	+
3)	Olive oil test	Appearance of foam	+	+	+
		Detection of Phytosterols			
1)	Salkowski's test	Red colour (in lower layer)	-	-	+
2)	Libermann-Burchard'stest	An array of colour change	+	-	+
3)	Acetic anhydride test	Change in colour from violet to blue/green	-	-	-
4)	Hesse's response	Pink ring / Red colour (in lower chloroform layer)	+++	-	-
		Detection of Cholesterol			
1)		A red-rose colour	-	-	-
		Detection of Terpinoides			
1)		A grey coloured solution	-	-	-
		Detection of Triterpinoides			
1)	Salkowski's test	Golden yellow layer (at the bottom)	-	-	+
		Detection of Diterpenes			
1)	Copper acetate test	Emerald green colour	+	++	-
		Detection of Lignins			
1)	Labat test	A olive green colour	-	-	-
2)	Furfuraldehyde test	A red colour	+	-	+
		Detection of Carotenoids			
1)	Carr-Price reaction	A blue-green colour eventually changing to red	+	-	-
		Detection of Quinones			
1)	Alcoholic KOH test	Red to blue colour	-	+	+
2)	Concentrated HCl test	A green colour	-	-	+++
3)	Sulphuric acid test	A red colour	-	+++	+++
		Detection of Anthraquinones			
1)	Borntrager's test	A pink, violet, or red coloured solution	-	-	-
2)	Ammonium hydroxide test	Formation of red colour after 2 min.	-	-	-
		Detection of Anthocyanins			
1)	HCl test	Pink-red solution which turns blue-violet after addition of ammonia	-	-	-
		Detection of Leuconthocyanins			
1)	Isoamyl alcohol test	Upper layer appears red	-	-	-
		Detection of Coumarins			
1)	NaOH test	A yellow colour	-	+	+
		Detection of Emodins			
1)		A red colour	-	-	-
		Detection of Resins			
1)	Acetic anhydride test	Orange to yellow	-	+	+

[(+++) = Strong presence, (++) = Moderate presence, (+) = Presence, and (–) = Absence)

have biological activity with potential medical utility (Farvin & Jacobsen, 2013). In comparison to other marine resources, algae, often known as seaweed, are exceptional candidates that may help to future global food security for the ever-growing population (Hossain *et al.*, 2021).

For this study *Caulerpa microphysa* (green), *Iyengaria stellata*(brown),and *Spatoglossum solieri* (brown) seaweeds were selected. In order to establish the biological potential of these seaweeds, a comparative co-relation was established by studying the preliminary phytochemical and antioxidant properties of these seaweeds.

MATERIALS AND METHODS

Seaweed sample collection, identification, and preparation

Three different seaweed species were collected during the low tide from Okha coast of Gujarat, India situated at 22°28' N and 69°05' E in the north of Gulf of Kutch by random sampling method. *Caulerpa microphysa* and *Iyengaria stellata* were primarily examined and identified morphologically with reference book on Seaweeds of India (Jha *et al.,* 2019). The collected samples were cleaned with filtered seawater to eliminate all the extraneous impurities and transported to the laboratory in refrigerating condition for further processing. From there on, the samples were thoroughly washed with tap water followed by distilled water and shade dried at ambient temperature for 10-15 days. By using pestle and mortar fine-coarse powder was prepared, and properly packed in zip lock bags then stored in the refrigerator at 4^0C for further use. The classifications of seaweed are shown in Supplementary Table 1.

Phytochemical screening

Qualitative phytochemical screening : Five gram of seaweed powder was macerated in 50 ml of distilled water in a conical flask and placed on incubator shaker. The extract was purified using the Whatman No. 1 filter paper and the Buchner funnel. The filtrate was concentrated and dried at 45°C. This dry extract was dissolved in known volume of distilled water to make 1000 µg/mL stock, and used for phytochemical screening as well as antioxidant activity and stored at 4°C for further use. Presence of different phyto-constituents were checked by the detailed protocol systemized in (Shaikh *et al.*, 2020).

Quantitative Phytochemical Analysis : Dried seaweed powder 5 g taken in a thimble and extracted in soxhlet apparatus using 150 ml of different organic solvents with increasing polarity, i.e.; n-hexane, chloroform, acetone, 70% ethanol, and methanol at 60-70°C. These dry extracts were dissolved in known volume of Dimethyl Sulfoxide (DMSO) to make 1000 µg/ml stock.

Table 2: Total phenolic compounds (TPC) expressed as µg Gallic acid equivalent (GAE)/gm and total flavonoid compounds (TFC) expressed as µg Quercetin equivalent QE/gm dry weight of powdered seaweeds.

	Total Phenolics		Total Flavonoids	
C. mycophysa	Concentration (µg GAE/gm)	Standard Deviation	Concentration (µg QE/gm)	Standard Deviation
Hexane	2298.83	0.31	27192.41	0.56
Chloroform	4207.53	0.18	26551.40	0.54
Acetone	3564.54	0.10	18652.13	0.06
70% Ethanol	2977.34	0.11	17537.75	0.07
Methanol	1943.72	0.06	8504.38	0.02
Water	1972.89	0.10	15506.23	0.30
I. stellata				
Hexane	1011.69	0.02	8031.02	0.07
Chloroform	1054.68	0.02	9184.84	0.12
Acetone	2118.74	0.06	13376.09	0.05
70% Ethanol	1419.94	0.07	10743.00	0.14
Methanol	1737.00	0.05	26294.99	0.51
Water	2475.11	0.06	13642.36	0.21
S. solieri				
Hexane	2097.18	0.06	22172.78	0.34
Chloroform	3958.96	0.06	36294.81	0.44
Acetone	7679.98	0.28	65692.71	0.38
70% Ethanol	3644.43	0.12	24697.39	0.10
Methanol	7318.53	0.72	40860.81	1.08
Water	2533.45	0.07	25979.42	0.83

Table 3: Correlation co-efficient of different extracts of seaweeds.

	ABTS-TPC		DPPH-TPC		Reducing Power-TPC	
	r	r^2	r	r^2	r	r^2
C. mycophysa	0.242	0.058	-0.032	0.001	0.499	0.249
I. stellata	-0.387	0.149	0.148	0.022	-0.636	0.405
S. solieri	0.406	0.165	0.374	0.140	-0.104	0.010
	ABTS-TFC		DPPH-TFC		Reducing Power-TFC	
	r	r^2	r	r^2	r	r^2
C. mycophysa	0.774	0.599	-0.396	0.156	0.556	0.310
I. stellata	-0.316	0.099	0.230	0.053	-0.562	0.316
S. solieri	0.338	0.114	0.076	0.005	-0.278	0.077

Estimation of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) : TPC was estimated according to the Folin-Ciocalteu method described in (Muflihah *et al.*, 2021) with minor change. Folin-Ciocalteu reagent 1 ml was incubated with 1 ml seaweed extract for 5 minutes in dark at room temperature. After addition of sodium bicarbonate (10%), incubation was done for 30 min. at room temperature. Absorbance was measured at 725 nm using spectrophotometer (Shimadzu UV-1800). A calibration curve was prepared using different aliquots of gallic acid (5-50 µg/ml) and the amount of TPC was calculated as gallic acid equivalents in mg/g of dried seaweed.

TFC was determined according to the aluminium chloride colorimetric method with slight modification (Muflihah *et al.*, 2021). Seaweed extract 1 ml was mixed with 0.1 ml of 1 M potassium acetate. After 5 minutes, 0.1 ml of 10% aluminium trichloride was added to the mixture and allowed to stand for 5 minutes then 1 ml of 1M NaOH was added followed by addition of 1.8 ml of distilled water and incubated at room temperature for 30 minutes. Absorbance was measured at 422. A standard curve was prepared using different aliquots of concentration of Quercetin (30-300 µg/ml) and the amount of TFC was calculated as quercetin equivalents in mg/g of dried seaweed.

Antioxidant Assays

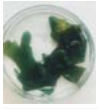
DPPH radical scavenging assay : The DPPH radical scavenging assay was performed based on the protocol described by Khairul *et al.* (2021). In brief, 1 mL of seaweed extract at a concentration of 125, 250, 500, and 1000 µg/mL was reacted with 2 mL of DPPH reagent followed by 2 mL of methanol. After incubation in the dark for 30 minutes at room temperature, the absorbance was measured at 517 nm against the blank (methanol only), and DPPH reagent served as a control. Ascorbic acid at different concentrations (6.25-800 µg/mL) was taken as the standard. The capability to scavenge the DPPH radical was calculated as follows:

$$\%DPPH\ scavenging\ activity = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A_0 =Absorbance of control, A_1 =Absorbance of sample/ standard

ABTS radical scavenging assay : ABTS assay was performed according to the protocol of Khairul *et al.* (2021). ABTS solution (7 mM) was mixed with a potassium persulfate (140 mM) solution and incubated overnight in the dark. Prior to use, the ABTS was diluted with 50% methanol to obtain an initial absorbance of about 0.70±0.02 at 734 nm. Free radical scavenging activity was assessed by mixing 50 µl of different concentrations (125, 250, 500, 1000 µg/ml) of seaweed extract with 1.0 ml of ABTS working standard. After incubation at room temperature for 30 minutes in dark and absorbance was measured at 734 nm against blank (only methanol), while ABTS solution served

Supplementary Table: Classification of seaweeds with their geographic location

Seaweed	Distribution	Class	Order	Family	Genus	Species
	N 22º 28.43'; E 69º 4.17'	Ulvophycea	Bryopsidales	Caulerpaceae	Caulerpa	*Caulerpa microphysa*
	N 22º 28.52'; E 69º 04.32'	Phaeophyceae	Extocarpales	Scytosiphonaceae	Iyengaria	*Iyengaria stellata*
	N 22º 28.52'; E 69º 04.32'	Phaeophyceae	Dictyotales	Dictyotaceae	Spatoglossum	*Spatoglossum solieri*

as control. Different concentrations of ascorbic acid (at a concentration range of 6.25-800 µg/ml) were taken as a standard. The capability to scavenge the ABTS* cation was calculated as follows:

$$\%ABTS\ scavenging\ activity = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A_0 =Absorbance of control, A_1 =Absorbance of sample/standard

Reducing power assay : The reducing power of the seaweed extract was determined using a modified method described by Jayanthi & Lalitha (2011). Firstly, 1 ml of the extract (at a concentration range of 125, 250, 500, 1000 µg/ml) was mixed with 2.5 mL of 1% potassium ferricyanide and phosphate buffer (pH = 6.6) and then incubated at 50°C for 20 minutes. Then, 2.5 ml of 10% trichloroacetic acid was added to the mixture, and the resulting solution was centrifuged at 3000 rpm for 10 minutes. After centrifugation, the upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of freshly prepared 0.1% ferric chloride solution. A calibration curve was prepared using different aliquots of ascorbic acid. The absorbance was measured at 700 nm using a blank solution without ferric chloride.

Statistical Analysis

All assays were performed in triplicate, and the results were presented as mean ± SD (standard deviation). The Pearson coefficient correlation for total phenolic and flavonoid content with antioxidant activity was analysed by Graph Pad Prism for windows (Version 9). A p-value < 0.05 was considered to be significant.

RESULTS

Qualitative phytochemical analysis

This study brings out adequatedata on the presence of phytochemical constituents in aqueous extracts of three different seaweed species. Most important secondary metabolites such as alkaloid, flavonoids, phenols, and saponins were detected in the higher or moderate extent in every sample in at least one of the listed tests. Among all these tests, test for Cardenolides (specific category of cardiac glycosides) gave strong positive result for all the species, while other phytochemicals such as Anthocyanins, Resins, Anthraquinones, Cholesterol, Leuconthocyanins, and Emodins were absent. Interestingly, *I. stellata* and *S. solieri* gave positive results for quinones. Detailed results with individual category of phytoconstituents are given in the Table 1.

TPC-TFC

Total phenolicand flavonoid content was determined using standards of Gallic acid and Quercetin respectively. The amount of total phenolics was measured by Folin Ciocalteau method at a concentration of 1000 µg/ml of each extract, detected from minimum concentration of 1011.69±0.02 to maximum of 7679.98±0.28 µg GAE/gm of dry weight (Table 2). The highest level of phenolics was found in acetonic extract of *S. solieri*. Only hexane, chloroform, and 70% ethanolic extracts of *I. stellata* had relatively very low levels (<1500 µg GAE/gm) of phenolics compare to others.

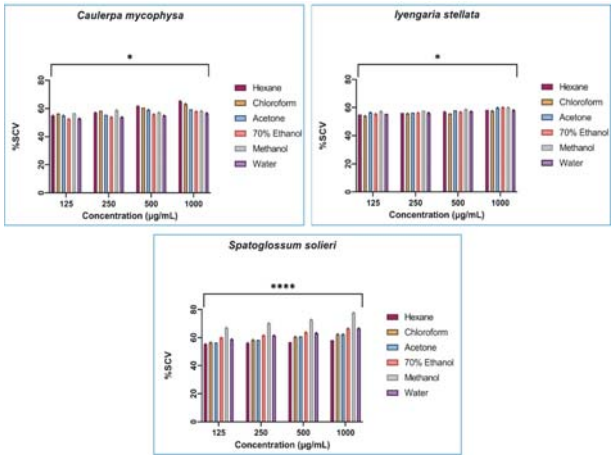


Figure 1. DPPH scavenging activity of different seaweed extracts prepared in different solvents are shown as mean±SD. Error bars indicate the value of standard deviation. *p< 0.05, ****p< 0.0001

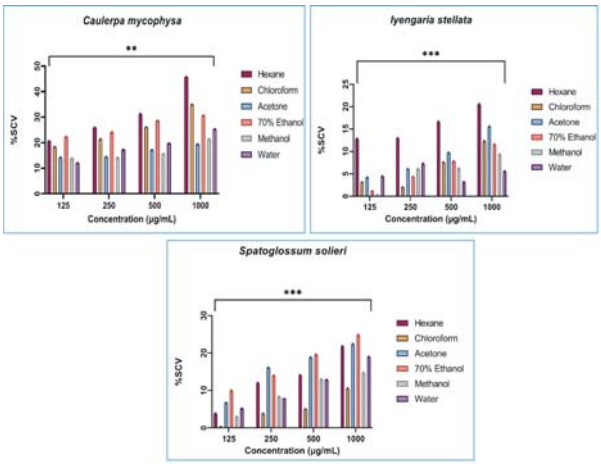


Figure 2: ABTS activity of different seaweed extracts are shown as mean±SD. Error bars indicate the value of standard deviation. **p< 0.005, ***p< 0.0005

The Aluminium Chloride procedure was used to calculate the concentration of total flavonoid content in different crude extracts at a concentration of 1000 µg/ml. In this study, significant differences were found in the level of total flavonoid content (TFC) for most of the extracts. The differences ranged from 8031.02±0.07to 65692.71±0.38 µg QE/gm dry extract (Table 2). Acetonic extract of *S. solieri* was found to have the highest TFC, while hexane extract of *I. stellata* had the lowest.

Antioxidant Activity

DPPH radical scavenging assay : A stable free radical 1-diphenyl-2-picrylhydrazyl (DPPH) at room temperature, possesses a nitrogen free radical that readily destroyed by a free radical scavenger. The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517 nm, which is induced by different antioxidants as proton radical scavengers or hydrogen donors (Bhalodia *et al.,* 2013).

Crude extract of seaweeds, *C. mycophysa, I. stellata,* and *S. solieri* prepared using Hexane, Chloroform, Acetone, 70% Ethanol, Methanol, and Water solvents showed concentration-dependent DPPH scavenging activity. These tests allowed assessing the variation of % SCV versus solvents at different concentration of sample (Fig. 1). In general, all the extracts showed >50% radical scavenging activity. Among three different seaweed species tested, methanolic extract of *S. Solieri* showed high mean antioxidant capacity, while 70% ethanolic extract of *C. mycophysa* showed lowest mean antioxidant capacity. In overall comparative results of three seaweed species indicated that their DPPH antioxidant capacity decreased in the following order: *S. solieri > I. stellata > C. mycophysa.*

ABTS radical scavenging assay : *In vitro* antioxidant activity by 2, 2-Azinobiz-3-Ethylbenthiazoline-6-Sulfonic Acid radical (ABTS) scavenging assay comprises the reaction that results in the formation of a blue-green ABTS chromophore between ABTS and hydrogen donating oxidizing agent, potassium per-sulphate here. Intensity of the colour increases as the concentration of sample increases indicating that the samples have antioxidant property (Khairul *et al.,* 2021).

As the result obtained in DPPH activity, the scavenging activity also increased with the increase in concentration of extract, being highest in *C. mycophysa* (45.74±0.04 at 1000 µg/ml concentration) and lowest in *S. solieri* (6.73±0.01 at 125 µg/ml concentration) as shown in Fig. 2. In all species of seaweed, ABTS scavenging activity was lower as compared to DPPH scavenging activity. The decreased order of ABTS activity among all seaweed species: *C. mycophysa > I. stellata > S. solieri.*

Reducing Power Ability : The antioxidant activity of seaweed crude extracts was also evaluated using the reducing power assay. This method is dependent on the hydrogen ion present in antioxidants reducing potassium ferricyanide (Fe^{+3}) to potassium ferrocynide (Fe^{+2}) product, changing the color of the substance to different shades of green to blue depending on the antioxidant function (Bhalodia *et al.,* 2013).

The reducing power of the extract increases with the increase in amount of sample concentrations. *S. solieri* exhibited highest reducing power showing 0.53 absorbance on 700 nm at 1000 µg/mL concentration while *I. stellata* showed the lowest absorbance 0.01 at 125 µg/mL concentration (Fig. 3). In our overall comparative results, the decreased order of ABTS activity among all seaweed species: *S. solieri >C. mycophysa > I. stellata.*

The Correlation Results between the Total Phenolics and Flavonoid Content, and Antioxidant Activity

Pearson's correlation analysis was performed to investigate the relationship between total phenolics and total flavonoids content with antioxidant capacity for all the extracts. The Pearson correlation coefficient (PCC), also referred to as Pearson's r, was taken in consideration to express the strength and direction of the linear relationship of correlation. The correlations can be said as in dependent manner wherein, extract which have a high level of TPC would eventually possess high antioxidant

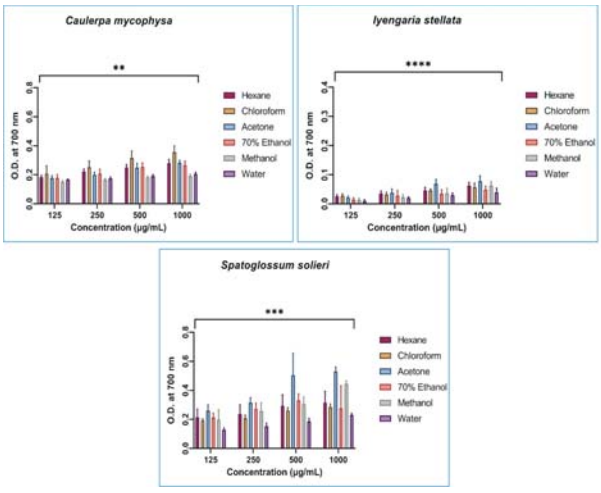


Figure 3. Reducing power ability of the different seaweeds extracts are shown as mean of absorbance at 700 nm with standard deviation. Error bars indicate the value of standard deviation. **p< 0.005, ***p< 0.0005, ****p< 0.0001

capacity as demonstrated in Table 3. A strong (0.7–0.89), moderate (0.4-0.69), weak (0.10-0.39) and negligible (0.00-0.10) correlations were observed between TPC-TFC and antioxidant activities for species under study. In contrast, negative correlation coefficient was also obtained in some of the samples.

Extracts of *C. mycophysa* gave strong positive correlation ($r = 0.774$, $r^2 = 0.599$) between ABTS activity and total flavonoid content at a 95%confidence level. As expected, not all the extracts showed a positive correlation between all the combinations. *S. solieri* extracts gave contradictory results; they possessed the weak or negligible correlation even though it contained comparatively good level of TPC and TFC. However, no correlation is found between the phenolics content and ABTS activity followed with radical scavenging activity of the *I. stellata* extracts in the present study, wherein negligible correlation was found between the phenolic content and DPPH activity. The same trend was found with total flavonoid content and DPPH activity in case of *I. stellata* extracts.

DISCUSSION

The profile of phytochemical is immensely important to determine the active principles responsible for the biological activities exhibited by any plant. Phytonutrients screening provides new ideas for the development of new drugs against diseases (Shaikh *et al.,* 2020). From the phytochemical profiling results, it is confirmed that the extracts of *C. mycophysa, I. stellata,* and *S. solieri* can act as a precursor for isolation of selective secondary metabolites having clinical applications related with pharmaceutical industries in future. In the field of medicine and nutraceuticals, seaweed contributes its efficacy based on the presence of their chemical profiles. The presence of essential phytoconstituents basically influenced by the physicochemical properties of the seaweed, and polarity of solvents used for extraction. Different extracts have different concentrations of phenolics and flavonoids contents (Abdullahi & Abubakar, 2022). The difference in polarities of the seaweed components can affect the efficiency of the extraction (Patricia *et al.*, 2017). Sea-algal polyphenols, and flavonoids are known to having low toxicity with biological properties such as antimicrobial effects, antioxidant activities, modulation of de-toxification enzymes, decrease of platelet aggregation, modulation of hormone metabolism and stimulation of the immune system (Nyamai *et al.,* 2016; Gómez-Guzmán *et al.,* 2018). During normal cellular function, production of free radicals miss an electron and give them an electric charge. For neutralizing it, free radicals try to withdraw an electron from a neighbouring molecule. The high content of phytochemicals like phenols, tannins, flavonoids, alkaloids, and quinones could explain its high radical scavenging activity, mainly due to their unusual and unique redox properties (Bhalodia *et al.,* 2013).

The antioxidant potency of any extract cannot be estimated with a single test. Therefore, to strengthen our investigation, three different assays (DPPH scavenging, ABTS, reducing power assay) have been done for verification. DPPH assay is mainly depending on the hydrogen donating capacity to scavenge radicals. Response of antioxidants to the DPPH is dependent of the equivalent number of DPPH molecules turns to hydroxyl ions prevailing (Singh *et al.,* 2016). Same principle is behind the ABTS assay, but DPPH assay is appeared to be more adhesive to OH groups. Furthermore, the radicals of DPPH and ABTS model are structurally distinct. Reducing power assay is a rapid screening method and may serve as a significant reflection of the antioxidant activity. Compounds having reducing power activity indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary or secondary antioxidants (Jayanthi & Lalitha, 2011). All the extracts appeared to have almost similar trend in outcome and we can correlate them. The results of green seaweed species under study have been verified with previously reported results (Nazarudinet *et al.,* 2022). Supported study revealed the DPPH reduction activity of *Halimeda opuntia*seaweed, collected from Port Dickson, Negeri Sembilan, Malaysia ranged from 56.29 to 63.91 % at a concentration of 200-1000 ìg/ml (Nazarudin *et al.*. 2022).

The approach of Pearson's correlation coefficient establishes a strong, moderate or mild positive linear correlation between total phenolic content (TPC) and total flavonoid content (TFC) of various seaweed extracts with DPPH radical scavenging activity, ABTS scavenging activity, and reducing power activity. Based on the present result, it can be said that *C. mycophysa* flavonoids are a potent radical scavenger among three species. The high correlations are giving lead to the role of flavonoid compounds may be contributing to the antioxidant activities of this seaweed extract. Flavonoids are also a group of phenolic compounds that exhibit important biological effects and its promising antioxidant activity owing to their capability to scavenge reactive oxygen species effectively (Muflihah *et al.,* 2021). Other two species showed relatively good antioxidant activity with moderate or mild correlation with total phenolics and flavonoid contents. In this case, non-phenolic compounds with aromatic ring such as polysaccharides, proteins, or

ascorbic acid or some other active components may have synergistic or antagonistic effects on the scavenging activity, which can give contradictory results. Farvin & Jacobsen (2013) have shown a positive correlation between TPC and antioxidant properties of the seaweed species collected from the coastal areas of Denmark. On the other hand, *Polysiphonia fucoides*was rich source of polyphenols, but it did not seem to contribute to their good radical scavenging properties due to negative correlation although it was collected from same coast (Farvin & Jacobsen, 2013). Hence, further characterization studies and more antioxidant assays are needed to characterize the active compounds that are responsible for the activity.

From the last decade, replacement of synthetic antioxidants is being focused in food industry. Seaweeds are potential reservoirs of novel biologically active components due to their extraordinary diversity (Admassu *et al.,* 2018). An ocean system is a tremendous competitive environment to survive; seaweeds have to make some discernibly diverse compounds to develop defence policies by altering their metabolic pathways. Primary and secondary metabolites present in seaweeds are progressively getting used in medical and biochemical research. Bioactivity of these metabolites can linked for the improvement of human health by alteration in genetic expression of cellular events (Choudhary *et al.*, 2021). The discovery of detailed chemical profile may enhance our understanding to recognize their true potential. Yet, more research will help to get insight in finding an untapped source of health-beneficial molecules from underutilized resource.

CONCLUSION

In order to infer a deduction on which species is best in the criterion of antioxidant properties among the three seaweed, several aspects are needed to be taken into account. Firstly, in the examination of variety of phytoconstituents, all three seaweed species *C. mycophysa, I. stellata,* and *S. Solieri* possesses phenol, flavonoid, carbohydrates, and alkaloids with clear manifestation. Secondly, the results of screening experiment demonstrated that extracts of the different seaweed species extracted with various solvents contained different levels of total phenolics and total flavonoid contents based on their composition, being maximum content in *S. solieri*. Thirdly, the three antioxidant parameters, namely: DPPH radical scavenging activity, ABTS activity, and reducing power activity showed a similar pattern in context to concentration, being highest scavenging capacity in *C. mycophysa*. Among all the species, *S. Solieri* species showed maximum DPPH activity, and reducing power activity. As there is a growing tendency of disease and an increased need for medicine, results of this preliminary study can be the basis for further development and recommends that macro algae being an underused bioresource. Thus, future research works being a novel candidate, should be made to identify and purify the definite bioactive compounds to promote the use of these seaweeds as natural sources of potential antioxidants having potential therapeutic efficiency.

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Competing interests

The authors declares that there is no competing interests.

Availability of data and material

All data generated or analysed during this study are included in this article.

Code availability

Not applicable

Authors' contributions

Nidhi Patel: Design and sample collection, Experimental work, Original draft preparation

Sejal Pal: Validation, Review and editing

Anjali Soni: Conceptualization, Methodology, Review and editing

Preeti Sharma: Supervision, Data Interpretation, Review and editing

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