



## Extraction and correlation of total phenolic and flavonoid contents in seaweeds collected from Rameshwaram during pre- and post- monsoon period using different solvent systems with their antioxidant activity

K M Smita<sup>a</sup>, L Stanley Abraham<sup>\*b</sup>, S Sunitha<sup>c</sup>, R Vasantharaja<sup>b</sup> & R Thirugnanasambandam<sup>b</sup>

<sup>a</sup>Sathyabama Institute of Science and Technology, Rajiv Gandhi salai, Chennai – 600 119, India

<sup>b</sup>Centre for Ocean Research, Sathyabama Institute of Science and Technology, Rajiv Gandhi salai, Chennai – 600 119, India

<sup>c</sup>Department of Chemistry, Sathyabama Institute of Science and Technology, Rajiv Gandhi salai, Chennai – 600 119, India

\*[E-mail: stanleyabraham.cor@gmail.com]

Received 29 January 2022; revised 27 April 2022

The present study was carried out to compare and correlate the phenolic and flavonoid contents of *Turbinaria* sp., *Sargassum* sp. and *Gracilaria* sp. extracted using different solvents including methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate and hexane, and elucidated for their anti-oxidant activity. The total phenolic and flavonoid contents of the solvent extracts were determined using the Folin-Ciocalteu assay and aluminium chloride colorimetric assay with gallic acid and quercetin as standards, respectively. The anti-oxidant activity in phenolic and flavonoid content was also estimated by phosphomolybdenum method and was compared with gallic acid and quercetin standard. The quantitative analysis of flavonoid content reveals that methanolic extract of *Sargassum* sp. ( $9.56 \pm 0.38$  mg QE/g during pre-monsoon and  $9.44 \pm 0.48$  mg QE/g during post-monsoon season); acetone extract of *Gracilaria* sp. ( $2.16 \pm 0.11$  mg QE/g during pre-monsoon and  $2.12 \pm 0.07$  mg QE/g during post-monsoon season) and methanolic extract of *Turbinaria* sp. ( $4.11 \pm 0.12$  mg QE/g during pre-monsoon and  $4.22 \pm 0.15$  mg QE/g during post-monsoon season) had higher concentration of flavonoid content. However, the quantitative analysis of phenolic content was found to be lower in all the seaweed extracts as compared to flavonoids. The anti-oxidant activity of a phenolic content and flavonoid content were also found to be correlated. The findings of the current study conclusively demonstrate the content of phenolic and flavonoid compounds significantly correlate with anti-oxidant activity.

[**Keywords:** Anti-oxidant activity, Flavonoids, Phenolic compounds, Seaweeds]

### Introduction

Ocean is the source of ninety percent of the living organisms in the universe, which comprise approximately 50 % of the world's biodiversity<sup>1-2</sup>. Seaweeds or marine macroalgae have been identified as an underutilized resource among the marine organisms<sup>3</sup>. Potential natural resources known as seaweeds are categorized into three classes: Red algae (Rhodophyceae), Brown algae (Phaeophyceae), and green algae (Chlorophyceae)<sup>4-6</sup>. Several research efforts have proved that compounds present in the seaweeds have the property of anti-cancer<sup>7</sup>, anti-allergic<sup>8</sup>, anti-oxidant<sup>9-11</sup>, anti-inflammatory<sup>12</sup>, anti-obesity<sup>13-15</sup>, and neuro-protective activity<sup>16</sup> with excellent biomedical and pharmaceutical potential. Moreover, marine algae have been used as food and traditional remedies in Eastern countries, Europe, and America<sup>16</sup>.

Butylated Hydroxyanisole (BHA) and Propyl Gallate (PG), two synthetic antioxidants, have

negative side effects like liver damage and carcinogenesis<sup>17</sup>. Consequently, there is a need to find natural antioxidants with minimal or no adverse effects, can be used as food or medicine to replace the synthetic anti-oxidants. In recent years, seaweed extracts have been identified to have antioxidant and antimicrobial properties<sup>18-23</sup>. Phenolic and flavonoid compounds present in the seaweed extract have increasing attention due to its anti-oxidative property and additionally, it has immense potential for reducing the risk of numerous oxidative stress-related diseases<sup>24-25</sup>. Catechins, Flavonols and Flavonol glycosides are poly phenolic compounds identified in red and brown algae<sup>26-27</sup>. Flavonoids found in seaweeds have a strong correlation with increased life expectancy, a decline in the risk of chronic illness, and the prevention of various cancers<sup>28-30</sup>. In phenolic compounds, the radical scavenging capability was determined due to the possession of phenolic

hydroxyl groups (donates hydrogen atom to an oxidants) and extended covalent aromatic system (Unpair the electron and delocalize it)<sup>31-32</sup>.

This study was intended to the quantitative determination of phenolic and flavonoid content present in *Turbinaria* sp., *Sargassum* sp. and *Gracilaria* sp. which was collected during pre and post monsoon period. The antioxidant activity of total phenolic and flavonoid content was also intended to estimate during pre- and post-monsoon period. In order to extract bioactive compounds from seaweeds, various solvents of different polarity have been used, including methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate, and hexane. To compare the extraction yield, samples have been collected during two different seasons (pre- and post-monsoon period). In addition, this study was focused to determine the relationship between the flavonoid and phenolic content and its correlation with antioxidant activity.

## Materials and Methods

### Collection and extraction of seaweed

Freshly collected seaweeds, including *Turbinaria* species, *Sargassum* species, and *Gracilaria* species, were taken from the intertidal area of Rameswaram and Mandapam in the Ramnad district of Tamilnadu, India (9°16'59" N and 79°11'32" E). The seaweeds were brought to the laboratory in a poly bag and thoroughly washed with tap water to remove all the debris and then finally washed in distilled water, shade dried and pulverized. The mesh with a diameter of 0.5 mm was used to sieve the seaweed powder. The pulverized seaweed powders were sealed in a polypropylene sampling bag and kept under refrigeration until use<sup>33</sup>. 10 g of ground seaweeds, *Turbinaria* sp., *Sargassum* sp. and *Gracilaria* sp. were weighed individually in a 250 ml screw capped bottle and labelled. Each sample was mixed with 20 ml of 6N Hydrochloric acid<sup>34</sup>, 80 ml of solvents including methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate and hexane. Then the extraction was carried out in shaking condition for 24 h at RT<sup>35</sup>. Each extract was filtered after 24 h using Whatman filter paper No. 42, and the filtrate was then dried in a water bath at 100 °C until completely dried. Finally, 100 ml of deionized water was added to each dried extract and then sonicated until dissolved completely. The chlorophyll content was then removed from each extract by mixing it with

petroleum ether at a 5:2 (v/v) ratio in a separating funnel and extracting it at ambient conditions for 10 minutes<sup>36</sup>. Then the ether layer was discarded. The extraction with petroleum ether was repeated thrice for the complete removal of chlorophyll content. Finally, the resulting extracts were kept under refrigeration for further experiments.

### Analysis of Total Phenolic Content (TPC)

The Folin-Ciocalteu assay had been performed to estimate the total amount of phenolic concentration as the gallic acid equivalent<sup>37-38</sup>. An aliquot of seaweed extract (1 ml) was added to 25 ml of volumetric flask, containing 9 ml of deionized water. Folin-Ciocalteu phenol reagent (2N) volume of 0.5 ml was added to the mixture, which was then shaken for 3 min. 3 ml of 2 % Na<sub>2</sub>CO<sub>3</sub> solution was then added to the mixture after 3 min. Then the reaction mixer was vortexed and incubated for 15 min at RT. After that the reaction mixture was made up to the mark using deionized water. The absorbance against the reagent blank was measured at 550 nm after 15 min of incubation. The standard curve of gallic acid (0.1, 0.2, 0.3, 0.4, and 0.5 mg/l) was prepared to calculate the overall amount of phenolic content present in this extract, which was then denoted as mg Gallic Acid Equivalents (GAE)<sup>39</sup>.

### Analysis of Total Flavonoid Content (TFC)

The total flavonoid concentration estimated as quercetin equivalent by using aluminum chloride colorimetric assay<sup>34</sup>. An aliquot of seaweed extract (1 ml) was transferred to 10 ml of volumetric flask, containing 4 ml of d.H<sub>2</sub>O. 0.3 ml of 5 % of sodium nitrite was added to the solution and shaken well. After 5 min, 0.3 ml of 10 % Aluminum chloride was added to the solution. Following the addition of 2 ml of 1M NaOH, D.H<sub>2</sub>O was used to make the volume up to the required level. After thoroughly mixing the reaction mixer, the absorbance read at 415 nm in comparison to the reagent blank. The amount flavonoid content in the seaweed extract had been estimated using the standard curve of quercetin (20, 40, 60, 80 and 100 mg/ml) and denoted as mg Quercetin Equivalents (QE)<sup>39</sup>.

### Analysis of Total Anti-oxidant Activity (TAA)

The total anti-oxidant activity was estimated by phosphomolybdenum method<sup>40</sup>. 1 ml of seaweed extract was transferred to 25 ml of volumetric flask and mixed with 1 ml of 0.6 M Sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate.

The volume was then made up to the mark using deionized water. Then reaction solution kept at 95 °C for 90 min and then mixed well. The absorbance was read against the reagent blank at 550 nm. The total Antioxidant activity in the seaweed extract was estimated using the standard curve of Gallic acid (1, 2, 3, 4, and 5 mg/l) and denoted as mg Gallic Acid Equivalents (GAE).

Similarly, seaweed extract of 1 ml was transferred to 10 ml of volumetric flask and mixed with 1 ml of standard reagent and then the volume was adjusted to the mark using deionized water. The mixture was then kept for 90 min at 95 °C. After mixing, the absorbance was measured at 415 nm against a blank. The standard curve of quercetin (20, 40, 60, 80, and 100 mg/ml) was used to calculate the overall antioxidant activity in the seaweed extract, which was denotes as mg Quercetin Equivalents (QE).

## Results and Discussion

Three different species of seaweeds, *Turbinaria* sp., *Sargassum* sp. and *Gracilaria* sp. were collected from Rameshwaram during pre- and post-monsoon period and extracted using different solvents such as methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate and hexane to find out the extraction efficacy of the phenolic and flavonoid content. The variation in distribution of total phenolic content in three species was shown in Table 1. The variation in distribution of total flavonoid concentration in three species was shown in Table 2. A higher quantity of flavonoid content was observed in methanolic extract

of *Sargassum* sp. (9.56±0.38 mg QE/g during pre-monsoon and 9.44±0.48 mg QE/g during post-monsoon season), acetone extract of *Gracilaria* sp. (2.16±0.11 mg QE/g during pre-monsoon and 2.12±0.07 mg QE/g during post-monsoon season) and methanolic extract of *Turbinaria* sp. (4.11±0.12 mg QE/g during pre-monsoon and 4.22±0.15 mg QE/g during post-monsoon season). The amount of total phenolic content was observed lower concentration when compared to flavonoids. Thus, the present study clearly infers that the extraction efficacy of phenolic and flavonoid content was greatly depending on the type of solvent.

Similarly, the total anti-oxidant activity was determined by phosphomolybdenum method for the three species extracted using different solvent system. The total anti-oxidant activity was estimated using the standard calibration curve of gallic acid concentration range (1, 2, 3, 4, and 5 mg/l) as well as quercetin concentration (20, 40, 60, 80 and 100 mg/ml) denotes as mg GAE (Gallic Acid Equivalents) and mg QE (Quercetin Equivalents), respectively. The variation in anti-oxidant activity of three species with respect to gallic acid standard as well as quercetin standard is tabulated in Tables 3 and 4.

The current study performed the linear regression analysis to determine the relationship between phenolic and flavonoid concentration with antioxidant activity. Linear relationship between the concentration of entire phenolic compound and anti-oxidant activity of *Gracilaria* sp. (collected during pre- and post-monsoon period), extracted using

Table 1 — Total phenolic content in seaweed, extracted using different solvent systems during pre- and post-monsoon period

Name of the seaweed	Total phenolic content during pre-monsoon period (mg GAE/g)					
	Methanol	Acetone	Acetonitrile	Isopropyl alcohol	Hexane	Ethyl acetate
<i>Gracilaria</i> sp.	0.03±0.001	0.04±0.001	0.06±0.002	0.05±0.002	0.001±0.0001	0.04±0.001
<i>Sargassum</i> sp.	0.04±0.002	0.03±0.001	0.04±0.002	0.03±0.001	0.002±0.0001	0.03±0.001
<i>Turbinaria</i> sp.	0.03±0.001	0.02±0.001	0.06±0.002	0.05±0.002	0.001±0.0001	0.02±0.001
Name of the seaweed	Total phenolic content during post-monsoon period (mg GAE/g)					
	Methanol	Acetone	Acetonitrile	Isopropyl alcohol	Hexane	Ethyl acetate
<i>Gracilaria</i> sp.	0.03±0.001	0.04±0.001	0.06±0.003	0.05±0.001	0.001±0.0001	0.04±0.001
<i>Sargassum</i> sp.	0.04±0.002	0.03±0.001	0.04±0.002	0.02±0.001	0.002±0.0001	0.03±0.002
<i>Turbinaria</i> sp.	0.03±0.001	0.02±0.001	0.06±0.003	0.05±0.002	0.001±0.0001	0.02±0.001

Table 2 — Total flavonoid content in seaweeds, extracted using different solvent systems, during pre- and post-monsoon period

Name of the seaweed	Total flavonoid content during pre-monsoon period (mg QE/g)					
	Methanol	Acetone	Acetonitrile	Isopropyl alcohol	Hexane	Ethyl acetate
<i>Gracilaria</i> sp.	1.56±0.07	2.16±0.11	1.21±0.04	1.72±0.06	0.09±0.004	1.78±0.05
<i>Sargassum</i> sp.	9.56±0.38	2.64±0.13	1.82±0.06	7.62±0.32	0.15±0.01	2.76±0.09
<i>Turbinaria</i> sp.	4.11±0.12	2.05±0.10	1.79±0.09	2.14±0.08	0.23±0.01	1.60±0.07
Name of the seaweed	Total flavonoid content during post-monsoon period (mg QE/g)					
	Methanol	Acetone	Acetonitrile	Isopropyl alcohol	Hexane	Ethyl acetate
<i>Gracilaria</i> sp.	1.51±0.04	2.12±0.07	1.17±0.06	1.69±0.09	0.08±0.004	0.72±0.06
<i>Sargassum</i> sp.	9.44±0.48	2.59±0.13	1.79±0.06	7.51±0.37	0.15±0.004	2.71±0.10
<i>Turbinaria</i> sp.	4.22±0.15	2.10±0.11	1.89±0.09	2.19±0.10	0.28±0.01	1.65±0.05

Table 3 — Total anti-oxidant activity in seaweeds, extracted using different solvent systems, during pre- and post-monsoon period (Expressed as mg gallic acid equivalents)

Name of the seaweed	Total anti-oxidant activity during pre-monsoon period (mg GAE/g)					
	Methanol	Acetone	Acetonitrile	Isopropyl alcohol	Hexane	Ethyl acetate
<i>Gracilaria</i> sp.	0.36±0.01	0.41±0.01	0.34±0.02	0.41±0.01	0.04±0.002	0.44±0.02
<i>Sargassum</i> sp.	0.46±0.02	0.43±0.02	0.30±0.01	0.30±0.01	0.0001±0.00	0.31±0.01
<i>Turbinaria</i> sp.	0.33±0.02	0.16±0.01	0.58±0.03	0.26±0.01	0.03±0.001	0.30±0.01
Name of the seaweed	Total anti-oxidant activity during post-monsoon period (mg GAE/g)					
	Methanol	Acetone	Acetonitrile	Isopropyl alcohol	Hexane	Ethyl acetate
<i>Gracilaria</i> sp.	0.34±0.02	0.27±0.01	0.32±0.01	0.38±0.01	0.03±0.001	0.41±0.02
<i>Sargassum</i> sp.	0.44±0.01	0.48±0.02	0.27±0.01	0.28±0.01	0.0001±0.00	0.29±0.01
<i>Turbinaria</i> sp.	0.38±0.02	0.18±0.01	0.61±0.02	0.27±0.01	0.03±0.001	0.33±0.01

Table 4 — Total anti-oxidant activity in seaweeds, extracted using different solvent systems, during pre- and post-monsoon period (Expressed as mg quercetin equivalents)

Name of the seaweed	Total anti-oxidant activity during pre-monsoon period (mg QE/g)					
	Methanol	Acetone	Acetonitrile	Isopropyl alcohol	Hexane	Ethyl acetate
<i>Gracilaria</i> sp.	3.05±0.10	4.23±0.19	3.50±0.13	3.13±0.16	0.40±0.02	3.43±0.10
<i>Sargassum</i> sp.	7.07±0.37	3.25±0.13	2.96±0.13	4.21±0.15	0.28±0.01	3.38±0.14
<i>Turbinaria</i> sp.	3.29±0.11	2.51±0.07	2.23±0.07	2.43±0.10	0.43±0.02	2.71±0.09
Name of the seaweed	Total anti-oxidant activity during post-monsoon period (mg QE/g)					
	Methanol	Acetone	Acetonitrile	Isopropyl alcohol	Hexane	Ethyl acetate
<i>Gracilaria</i> sp.	2.94±0.09	4.09±0.19	3.41±0.19	2.99±0.10	0.28±0.01	3.35±0.16
<i>Sargassum</i> sp.	6.95±0.36	3.03±0.10	2.53±0.13	4.13±0.17	0.20±0.01	3.19±0.14
<i>Turbinaria</i> sp.	3.44±0.17	2.71±0.08	2.38±0.08	2.58±0.11	0.50±0.02	2.82±0.11

different solvents including methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate and hexane have shown in Figure 1 with correlation coefficient of 0.8518, and coefficient of determination ( $R^2$ ) of 0.7255. Linear relationship between the concentration of total flavonoid content and anti-oxidant activity of *Gracilaria* sp., (collected during pre- and post-monsoon period), extracted using different solvents including methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate and hexane is shown in Figure 2 with correlation coefficient of 0.8633 and coefficient of determination ( $R^2$ ) of 0.7453. Based on this study, *Gracilaria* sp. was found to have significant correlation between flavonoid concentration and its antioxidant activity as well as phenolic substance and its antioxidant activity. Hence the phytoconstituents such as phenolic compound and flavonoid present in *Gracilaria* sp. were identified as natural source of anti-oxidant substances of high importance.

The interrelation between the concentration of total phenolic compound and antioxidant activity of *Sargassum* sp. (collected during pre- and post-monsoon period), extracted using different solvents including methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate and hexane have shown in Figure 3 with correlation coefficient of 0.8451 and coefficient of determination ( $R^2$ ) of 0.7142. Similarly, the linear relationship between the concentration of total flavonoid content and antioxidant activity of

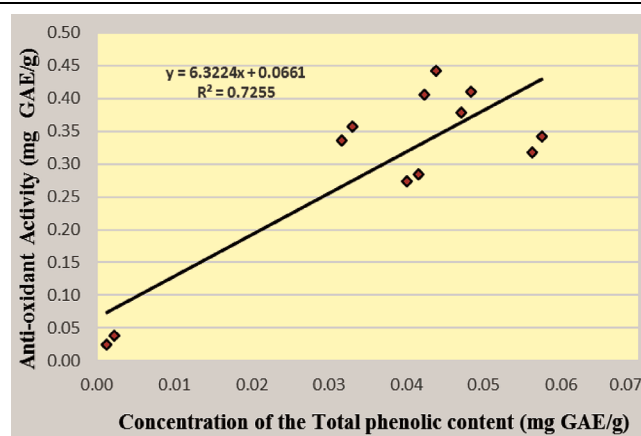


Fig. 1 — Linear correlation between the concentration of total phenolic content and anti-oxidant activity of *Gracilaria* sp. collected during pre- and post-monsoon period and extracted using different solvents (methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate and hexane). Correlation coefficient = 0.8518, coefficient of determination ( $R^2$ ) = 0.7255

*Sargassum* sp. (collected during pre- and post-monsoon period), extracted using different solvents including methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate and hexane have shown in Figure 4 with correlation coefficient of 0.9136 and coefficient of determination ( $R^2$ ) of 0.8347. Based on this study, *Sargassum* sp. was observed to exhibit significant relationship between flavonoid concentration and the activity against to the antioxidant as well as phenolic concentration and its antioxidant activity. Hence the phenolic compound

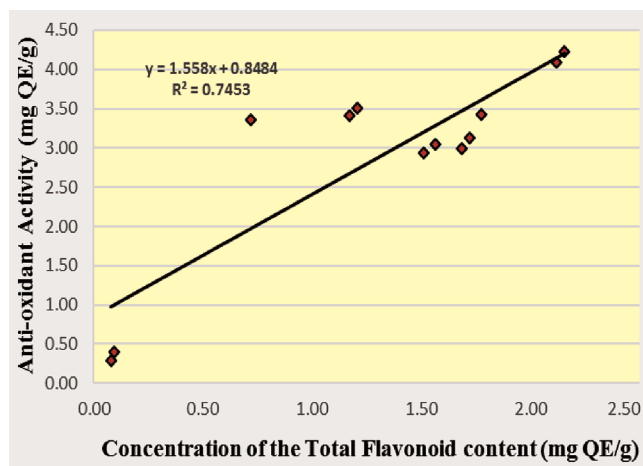


Fig. 2 — Linear correlation between the concentration of total flavonoid content and anti-oxidant activity of *Gracilaria* sp. collected during pre- and post-monsoon period and extracted using different solvents (methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate and hexane). Correlation coefficient = 0.8633, coefficient of determination ( $R^2$ ) = 0.7453

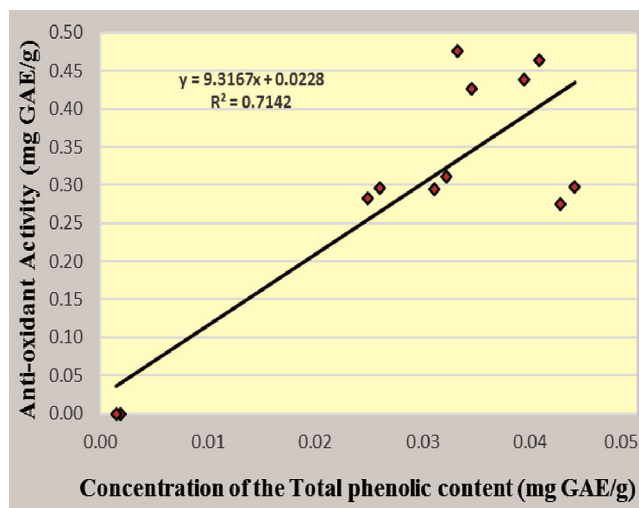


Fig. 3 — Linear correlation between the concentration of total phenolic content and anti-oxidant activity of *Sargassum* sp. collected during pre- and post-monsoon period and extracted using different solvents (methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate and hexane). Correlation coefficient = 0.8451, coefficient of determination ( $R^2$ ) = 0.7142

and flavonoid found in *Sargassum* sp. were identified as natural source of anti-oxidant substance of high importance.

Significant relation between the concentration of total phenolic content and anti-oxidant activity of *Turbinaria* sp. (collected during pre- and post-monsoon period), extracted using different solvents (methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate and hexane) is shown in Figure 5 with

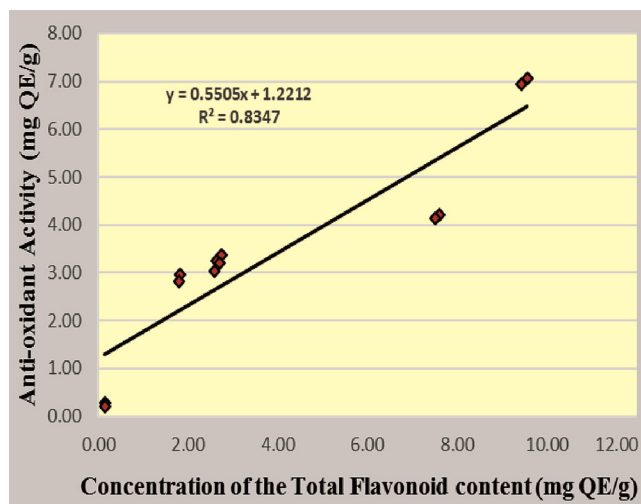


Fig. 4 — Linear correlation between the concentration of total flavonoid content and anti-oxidant activity of *Sargassum* sp. collected during pre- and post-monsoon period and extracted using different solvents (methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate and hexane). Correlation coefficient = 0.9136, coefficient of determination ( $R^2$ ) = 0.8347

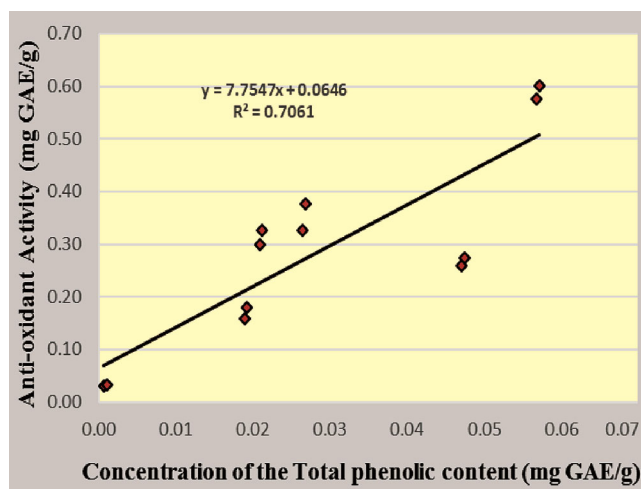


Fig. 5 — Linear correlation between the concentration of total phenolic content and anti-oxidant activity of *Turbinaria* sp. collected during pre- and post-monsoon period and extracted using different solvents (methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate and hexane). Correlation coefficient = 0.8403, coefficient of determination ( $R^2$ ) = 0.7061

correlation coefficient of 0.8403 and coefficient of determination ( $R^2$ ) of 0.7061. Likewise, total flavonoid content and antioxidant activity of *Turbinaria* sp. collected during pre- and post-monsoon period and extracted using different solvents (methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate and hexane) have a linear relationship with one another and is shown in Figure 6 with correlation coefficient of 0.8673 and coefficient of

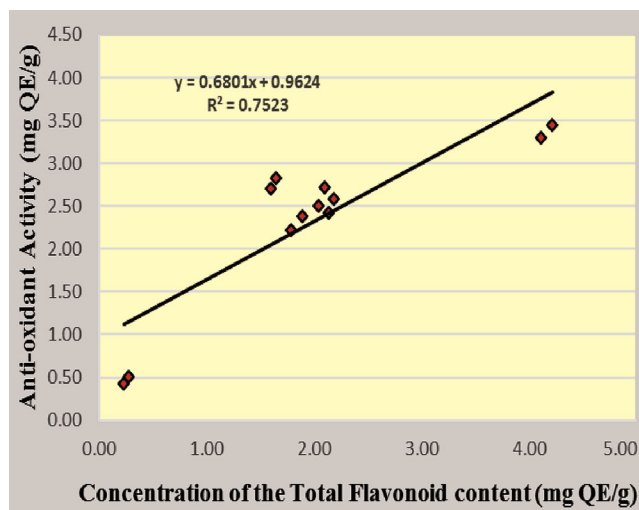


Fig. 6 — Linear correlation between the concentration of total flavonoid content and anti-oxidant activity of *Turbinaria* sp. collected during pre- and post-monsoon period and extracted using different solvents (methanol, isopropyl alcohol, acetone, acetonitrile, ethyl acetate and hexane). Correlation coefficient = 0.8673, coefficient of determination ( $R^2$ ) = 0.7523

determination ( $R^2$ ) of 0.7523. Based on this study, The anti-oxidant activity of *Turbinaria* sp. was found to significantly correlated with both its phenolic content and flavonoid content. Hence, the phenolic compound and flavonoid present in *Turbinaria* sp. were identified as natural source of anti-oxidant substance of high importance.

### Conclusion

In this study, it is observed that the extraction efficacy of phenolic and flavonoid content was greatly depending on the type of solvent. The results obtained in this study clearly infer that *Turbinaria* sp., *Sargassum* sp. and *Gracilaria* sp. were found to contain phenolic compound and flavonoid with potential anti-oxidant activity. Moreover, it is noticed that seasonal variation found to have less impact on extraction yield. As a result, neither the total phenolic content nor the anti-oxidant activity of flavonoids were significantly different. Based on this information, it could be used as a source of natural supplement as antioxidants. Further studies should be directed to carry out *in-vivo* studies to find out its therapeutic effect in order to prepare natural commercial pharmaceutical product of high value.

### Acknowledgements

Authors would like to thank the management of Sathyabama Institute of Science and Technology, Chennai for its staunch support in all research activities.

### Conflict of Interest

Authors declare there are no conflict of Interest.

### Ethical Statement

The study does not involve any endangered or live organism.

### Authors Contribution

KMS – Experiment analysis, result interpretation and manuscript preparation; LSA – Design of experiment, guidance and interpretation of results; SS – Helping in analysis; RV – Review and editing; and RT – Experimental analysis and manuscript preparation.

### References

- Kim S & Wijesekara I, Development and biological activities of marine-derived bioactive peptides, A review, *J Funct Foods*, 2 (1) (2010) 1-9.
- Cavalcante L H A, Da Matta C B B, De Araújo M V, Barbosa-Filho J M, De Lira D P, *et al.*, Antinociceptive and anti-inflammatory activities of crude methanolic extract of red alga *Bryothamnion triquetrum*, *Mar Drugs*, 10 (9) (2012) 1977-1992.
- Heo S-J, Hwang J-Y, Choi J-I, Han J-S, Kim H-J, *et al.*, Diphlorethohydroxycarmalol isolated from *Ishige okamuraea* brown algae a potent  $\alpha$ -glucosidase  $\alpha$ -amylase inhibitor alleviates postprandial hyperglycemia in diabetic mice, *Eur J Pharmacol*, 615 (1-3) (2009) 252-256.
- Sen S K, Gupta M, Mazumder U K, Haldar P K, Saha P, *et al.*, Antitumor activity of *Citrus maxima* (Burm.) Merr. Leaves in Ehrlich's ascites carcinoma Cell-Treated Mice, *Int Scholar Res Netw Pharmacol*, 2011 (2011) 1-4.
- Sandeep R, Arun K, Jugran A B, Asutosh B, Indra D, *et al.*, Anti-oxidant and anti-microbial properties of some ethno-therapeutically important medicinal plants of Indian Himalayan Region, *3 Biotech*, 6 (2016) p. 154.
- Farghl A A, Al-Hasawi Z M & El-Sheekh M M, Assessment of Antioxidant Capacity and Phytochemical Composition of Brown and Red Seaweeds Sampled off Red Sea Coast, *Appl Sci*, 11 (23) (2021) p. 11079.
- Kong C S, Kim J A, Yoon N Y, Kim S-K, *et al.*, Induction of apoptosis by phloroglucinol derivative from *Ecklonia cavain* MCF-7 human breast cancer cells, *Food Chem Toxicol*, 47 (7) (2009) 1653-1658.
- Li Y, Lee S, Le Q, Kim M-M & Kim S-K, Anti-allergic effects of phlorotannins on histamine release via binding inhibition between IgE and Fc RI, *J Agr Food Chem*, 56 (24) (2008) 12073-12080.
- Heo S J, Park E J, Lee K W & Jeon Y-J, Antioxidant activities of enzymatic extracts from brown seaweeds, *Bio Resour Technol*, 96 (14) (2005) 1613-1623.
- Park P, Heo S, Park E, Kim S-K, Byun H-G, Jeon B-T, *et al.*, Reactive oxygen scavenging effect of enzymatic extracts from *Sargassum thunbergii*, *J Agr Food Chem*, 53 (17) (2005) 6666-6672.
- Zou Y, Qian Z, Li Y, Kim M M, Lee S H, *et al.*, Antioxidant effects of phlorotannins isolated from *Ishige okamuraea* free

- radical mediated oxidative systems, *J Agr Food Chem*, 56 (16) (2008) 7001-7009.
- 12 Kim M, Rajapakse N & Kim S, Anti-inflammatory effect of *Ishige okamurae* ethanolic extract via inhibition of NFB transcription factor in RAW 264.7 cells, *Phytother Res*, 23 (5) (2009) 628-634.
  - 13 Maeda H, Hosokawa M, Sashima T & Miyashita K, Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK-Ay Mice, *J Agr Food Chem*, 55 (19) (2007) 7701-7706.
  - 14 Tsukui T, Konno K, Hosokawa M, Maeda H, Sashima T, *et al.*, Fucoxanthin and fu-coxanthinol enhance the amount of docosahexaenoic acid in the liver of KKAY obese/diabetic mice, *J Agr Food Chem*, 55 (13) (2007) 5025-5029.
  - 15 Kong C, Kim J, Ahn B, Vo T S, Yoon N Y, *et al.*, 1-(3,5-Dihydroxyphenoxy)-7-(2,4,6-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin inhibits adipocyte differentiation of 3T3-L1 fibro-blasts, *Mar Biotechnol*, 12 (3) (2010) 299-307.
  - 16 Zarros A, In which cases is neuro protection useful? *Adv Altern Think Neuro Sci*, 1 (S1) (2009) 3-5.
  - 17 Wichi H P, Enhanced tumor development by butylated hydroxyanisole (BHA) from the properties of effect on fure stomach and oesophagel aquamous epithelium, *Food Chem Toxicol*, 26 (2007) 727-723.
  - 18 Ely R, Supriya T & Naik C G, Antimicrobial activity of marine organisms collected off the coast of South East India, *J Exper Mar Biol Ecol*, 309 (2004) 121-127.
  - 19 Duan X J, Zhang W W, Li X M & Wang B G, Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*, *Food Chem*, 95 (2006) 37-43.
  - 20 Kuda T T, Sunekawa M, Goto H & Araki Y, Antioxidant properties of four edible algae harvested in the Noto Peninsula, *Japan J Food Compos Anal*, 18 (2005) 625-633.
  - 21 Lim S N, Cheung P C K, Ooi V E C & Ang P O, Evaluation of tioxidative activity of extracts from brown seaweed, *Sargassum siliquastrum*, *J Agric Food Chem*, 50 (2002) 3862-3866.
  - 22 Park P J, Shahidi F & Jeon Y J, Antioxidant activities of enzymatic extracts from and edible seaweed *Sargassum horneri* using ESR spectroscopy, *J Food Lipids*, 11 (2004) 15-27.
  - 23 Chakraborty K, Lipton A P, Paulraj R & Vijayan K K, Antibacterial diterpernoids of *Ulva fasciata* Delile from South-western coast of Indian Peninsula, *Food Chem*, 119 (2010) 1399-1408.
  - 24 Yoshie Y, Wang W, Petillo D & Suzuki T, Distribution of catechins in Japanese seaweeds, *Fish Sci*, 66 (2000) 998-1000.
  - 25 Sandeep R, Arun K, Jugran A B, Asutosh B, Indra D, *et al.*, Anti-oxidant and anti-microbial properties of some ethno-therapeutically important medicinal plants of Indian Himalayan Region, *3 Biotech*, 6 (2016) p. 154.
  - 26 Santoso J, Yoshie Y & Suzuki T, The distribution and profile of nutrients and catechins of some Indonesian seaweeds, *Fish Sci*, 68 (2002) 1647-1648.
  - 27 Yoshie Stark Y, Hsieh Y P & Suzuki T, Distribution of flavonoids and related compounds from seaweeds in Japan, *J Tokyo Univ Fish*, 89 (2003) 1-6.
  - 28 Hodgson J M & Croft K D, Dietary flavonoids: effects on endothelial function and blood pressure, *J Sci Food Agric*, 86 (2006) 2492-2498.
  - 29 Halliwell B, Oxidative stress and cancer: have we moved forward? *Biochem J*, 401 (2007) 1-11.
  - 30 Yan S & Asmah R, Comparison of total phenolic contents and antioxidant activities of turmeric leaf, pandan leaf and torch ginger flower, *Int Food Res J*, 17 (2010) 417-423.
  - 31 Rice-Evans C A, Miller N J & Paganga G, Structure-antioxidant activity relationships of flavonoids and phenolic acids, *Free Radic Biol Med*, 20 (1996) 933-956.
  - 32 Sousa H G, Uchoa V T, Cavalcanti S M G, de Almeida P M, Chaves M H, *et al.*, Phytochemical screening, phenolic and flavonoid contents, antioxidant and cytogenotoxicity activities of *Combretum leprosum* Mart. (Combretaceae), *J Tox Envtl Health*, Part A, 84 (10) (2021) 399-417.
  - 33 Rattaya S, Benjakul S & Prodpran T, Extraction, antioxidative, and antimicrobial activities of brown seaweed extracts, *Turbinaria ornata* and *Sargassum polycystum*, grown in Thailand, *Int Aquatic Res*, 7 (1) (2015) 1-16.
  - 34 Hertog M G L, Hollman P C H & Katan M B, Content of potentially anticarcinogenic flavonoids of 28 vegetables and fruits commonly in the Netherlands, *J Agri Food Chem*, 40 (1992) 2379-2383.
  - 35 Lanfer-Marquez U M, Barros R M C & Sinnecker P, Antioxidant activity of chlorophylls and their derivatives, *Food Res Int*, 38 (2005) 885-891.
  - 36 Ragazzi E & Veronese G, Quantitative analysis of phenolic compounds after thin-layer chromatographic separation, *J Chromatography*, 77 (1973) 369-375.
  - 37 Xia L R, Wang Q, Zhuguo H, Li L, Shu B K, *et al.*, Simultaneous determination of 10 major flavonoids in *Dalbergi Oderifera* by high performance liquid chromatography, *J Pharm Biomed Anal*, 39 (3-4) (2005) 469-476.
  - 38 Morales L, Acero N, Galán A, Perez-Garcia C, Alguacil L F, *et al.*, Bioactive properties of *Tynanthus panurensis* (Bureau) Sanwith bark extract, the Amazonian "clavo huasca", *J Medicinal Food*, 14 (9) (2011) 939-943.
  - 39 Zhishen J, Mengcheng T & Jianming W, The determination of flavonoid contents in mulberry and their scavenging effects on Superoxide radicals, *Food Chem*, 64 (1999) 555-559.