Antioxidant potential and GC-MS profiling of *Turbinaria conoides* (J. Agardh) Kutzing

Sivaraj C.[1*], Uma Maheswari M.[1], Reena A.[2], Arumugam P.[1] and Arunagirinathan N.[3]
1. Armats Biotek Training and Research Institute, Chennai, Tamil Nadu, INDIA
2. PG and Research Department of Microbiology, Mohamed Sathak College of Arts and Science, Chennai, Tamil Nadu, INDIA
3. Department of Microbiology and Biotechnology, Presidency College (Autonomous); Central Research Laboratory, Chennai, Tamil Nadu, INDIA
*shivaraj27@gmail.com

Abstract
Seaweeds are one of the most abundant resources of the nature. They are generally known to possess a large variety of bioactive components that can be used in medicine, food and various other industrial applications. In the present study, the brown seaweed Turbinaria conoides was investigated for its phytochemical composition and antioxidant potential. To determine the antioxidant potential, DPPH radical, $ABTS^{\cdot+}$ radical cation scavenging assays, phosphomolybdenum reduction and Fe^{3+} reducing power assays were carried out.

The maximum DPPH radical scavenging activity was found to be 61.74% at 600 µg/mL concentration. The GC-MS analysis revealed the phytochemical composition of Turbinaria conoides, especially the presence of flavone and coumarine promising bioactive potential.

Keywords: Seaweeds, *Turbinaria conoides*, DPPH, GC-MS, antioxidant.

Introduction

Marine organisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents[1]. Especially, seaweeds are a resolution to the growing crisis of antibiotic resistance and their side effects. Marine algae have been used in a wide array of traditional remedies and serve as a good source for antimicrobial agents. Also, they have shown evidence of being a potential source of antioxidant and active volatile compounds for human welfare [2, 3]. Thus, in the present study, a marine algae *Turbinaria conoides* was investigated for its bioactive potential.

Turbinaria conoides is a type of brown algae (Phaeophyceae) seen growing on rocky substrates in the tropical marine waters. It belongs to the family Sargassaceae. The thallus is erect and dark brown in color. It possesses many polysaccharides, polyunsaturated fatty and proteins along with mineral salts like potassium, calcium and iron. It also has a wealthy source of dietary fibers with iodine which plays an immense role in enhancing the food quality and biochemical homeostasis. It is traditionally used as a fertilizer, insect repellent, antibacterial and pesticide and also for curing children's fever. Hence, further analysis of its phytochemical composition by GC-MS can reveal its rich constituents and the antioxidant analysis can be promising in the search for natural antioxidant agents.

Material and Methods

Collection of seaweed: The seaweed *Turbinaria conoides* was collected from Rameswaram, Tamil Nadu, India. It was then washed thoroughly with water and allowed to dry in shade. The ground material was finely ground in a mechanical blender. The powdered material was stored in a container for further use.

Preparation of crude extract: The finely ground algal material was extracted with methanol in the ratio of 1:10 (w/v) in a conical flask for 72 h. The extract was then filtered using filter paper in a separate container. The above process was repeated two times with the same residue but using fresh solvent. All the supernatants were collected together and then the solvent was removed by rotor evaporator. The resultant extract was stored for further analysis[4].

***In vitro* antioxidant assays**

1. DPPH radical scavenging assay: The antioxidant activity of the methanol extract of *Turbinaria conoides* was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical[5]. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 ml of various concentrations (100-600 µg/mL) of methanol extract of *Turbinaria conoides*. The mixture was then allowed to stand for 30 min incubation in dark. Ascorbic acid was used as the reference standard. One mL methanol and 1 mL DPPH solution were used as the control. The decrease in absorbance was measured using UV-Vis Spectrophotometer at 517 nm. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of DPPH radical inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

2. ABTS radical cation scavenging assay: The antioxidant capacity was estimated in terms of the ABTS radical cation scavenging activity following the procedure described by Delgado-Andrade et al[6]. ABTS was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 12-16 h before use. The ABTS solution (stable for 2 days) was diluted with 5 mM phosphate-buffered saline (pH 7.4) to an absorbance of 0.70±0.02 at 730 nm. After the addition of methanol extract

of varying concentrations (10-60 µg/mL) to 1 mL of diluted ABTS solution, the absorbance was measured after 10 min. The ABTS radical-scavenging activity of the samples was expressed as:

$$\% \text{ of ABTS radical cation inhibition} = \frac{\text{Control} - \text{Sample X 100}}{\text{Control}}$$

3. Phosphomolybdenum reduction assay: The antioxidant capacity of the extract was assessed as described by Prieto et al [7]. The methanol extract with concentrations ranging from 10 to 60 µg/mL was combined with reagent solution containing ammonium molybdate (4 mM), sc phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in water bath at 90°C for 90 min. The absorbance of the coloured complex was measured at 695 nm. Ascorbic acid was used as the standard reference.

4. Ferric (Fe^{3+}) reducing power assay: The reducing power of the extract was determined by a slightly modified method of Yen and Chen[8]. One mL of extract of different concentrations (10 - 60 µg/mL) was mixed with phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1 mL, 1 %). The mixtures were then incubated at 50°C for 20 min. One mL of trichloroacetic acid (10 %) was added to each mixture. Then to the mixture, 1 ml of $FeCl_3$ (0.1 %) was added and the absorbance was measured at 700 nm using spectrophotometer. Ascorbic acid was used as the standard reference.

Gas chromatography–Mass Spectrometry (GC–MS): For GC-MS analysis, the sample was injected into a HP-5 column (30 m X 0.25 mm i.d. with 0.25 µm film thickness, Agilent technologies 6890 N JEOL GC Mate II GC-MS model). Following chromatographic conditions were used: Helium as carrier gas, flow rate of 1 mL/min and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV, ion source temperature of 250°C, interface temperature of 250°C and mass range of 50-600 mass units.

Identification of components: The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the interpretation of mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Statistical analysis: All the experiments were conducted in triplicate and data given in tables were average of the three replicates. All data were reported as mean ± standard deviation of three replicates.

Results and Discussion

DPPH radical scavenging assay: The ability of *Turbinaria conoides* to scavenge free radicals formed was assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The maximum DPPH radical scavenging activity was 61.74 % at 600µg/mL. The methanol extract of *Turbinaria conoides* demonstrated a high capacity for scavenging free radicals by reducing the stable DPPH (1,1-diphenyl-2- picrylhydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increased with increasing concentration of the extract. The IC_{50} value was found to be 443.16 µg/mL concentration and was compared with the standard (Ascorbic acid, IC_{50} = 11.98 µg/mL concentration). .

Table 1
DPPH assay of *Turbinaria conoides*

S.N.	Concentration (µg/ml)	% of inhibition
		DPPH˙
1	100	15.57±1.09
2	200	28.14±1.22
3	300	36.25±1.46
4	400	45.13±1.78
5	500	58.26±1.93
6	600	61.74±1.99

ABTS radical cation scavenging assay: The maximum ABTS radical cation scavenging activity was 61.26% at 60 µg/mL concentration. The experiment demonstrated high antioxidant activity the IC_{50} of 41.54 µg/mL concentration and was compared with the standard Ascorbic acid (IC_{50} = 4.21 µg/mL concentration).

Table 2
ABTS radical cation scavenging assay

S.N.	Concentration (µg/ml)	% of inhibition
		DPPH˙
1	10	19.56±1.11
2	20	28.96±1.46
3	30	35.64±1.85
4	40	48.15±2.06
5	50	53.56±2.33
6	60	61.26±2.50

Phosphomolybdenum reduction assay activity: The total antioxidant activity was measured spectrophotometrically by phophomolybdenum reduction method which is based on the reduction of Mo (VI) by the methanol extract of *Turbinaria conoides* and the subsequent formation of green phosphate/Mo (V) complex at acidic pH with a maximum absorption at 695 nm. The maximum absorbance was 0.173 at 60 µg/mL concentration. It was compared with the standard (0.359) Ascorbic acid.

Ferric (Fe^{3+}) reducing power activity: The reducing power of Fe^{3+} to Fe^{2+} by the methanol extract of *Turbinaria conoides* was studied and showed reduction ability in a dose-dependent manner. The maximum absorbance was 0.312 at

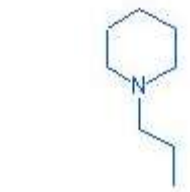
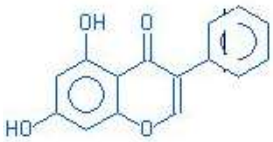
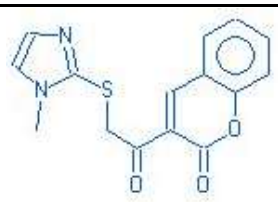
60 µg/mL and was compared with the standard (0.289) ascorbic acid.

GC-MS analysis: The GC-MS analysis was carried out for methanol extract of *Turbinaria conoides* and the eluted compounds are shown in table. The presence of flavone compounds (5,7-dihydroxy-2-phenyl-4H-1-Benzopyran-4-one and 3-[2-(1-methylimidazol-2-yl)sulfanylacetyl] chromen-2-one could be the reasons for the antioxidant property of the extract.

Table 3
Phosphomolybdenum reduction and Fe^{3+} reducing power assays of methanol extract of *Turbinaria conoides*

S.N.	Concentration (µg/ml)	Fe^{3+} Reducing Power Assay Absorbance at 700 nm	Phosphomolybdenum reduction assay (695nm)
1	10	0.089±0.017	0.024±0.022
2	20	0.185±0.021	0.046±0.029
3	30	0.219±0.021	0.058±0.039
4	40	0.273±0.074	0.128±0.044
5	50	0.297±0.083	0.166±0.023
6	60	0.312±0.095	0.173±0.053

Table 4
Active compounds identified in methanol extract of *Turbinaria conoides* by GC-MS analysis

S.N.	RT	Name	Structure	Mol. Wt g/mol	Mol. formula	IUPAC Name
1.	16.10	1-Propylpiperidine		127.227	$C_8H_{17}N$	N-Propylpiperidine
2.	17.05	Dodecanoic acid, 10-methyl-, methyl ester		228.370	$C_{14}H_{28}O_2$	Methyl 10-methyldodecanoate
3.	17.75	Chrysin		254.238	$C_{15}H_{10}O_4$	5,7-dihydroxy-2-phenyl-4H-1-Benzopyran-4-one
4.	18.83	Methyl linolenate		292.463	$C_{19}H_{32}O_2$	Methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate
5.	19.63	7-Methyl-Z-tetradecen-1-ol acetate		268.441	$C_{17}H_{32}O_2$	[(Z)-7-methyltetradec-8-enyl] acetate
6.	20.28	3-[2-(1-methyl-2-imidazolylthio)-1-oxoethyl]-coumarine,		300.332	$C_{15}H_{12}N_2O_3S$	3-[2-(1-methylimidazol-2-yl)sulfanylacetyl]chromen-2-one
7.	20.57	Tricosane		324.637	$C_{23}H_{48}$	n-Tricosane
8.	22.45	Pentacosane		352.691	$C_{25}H_{52}$	n-Pentacosane

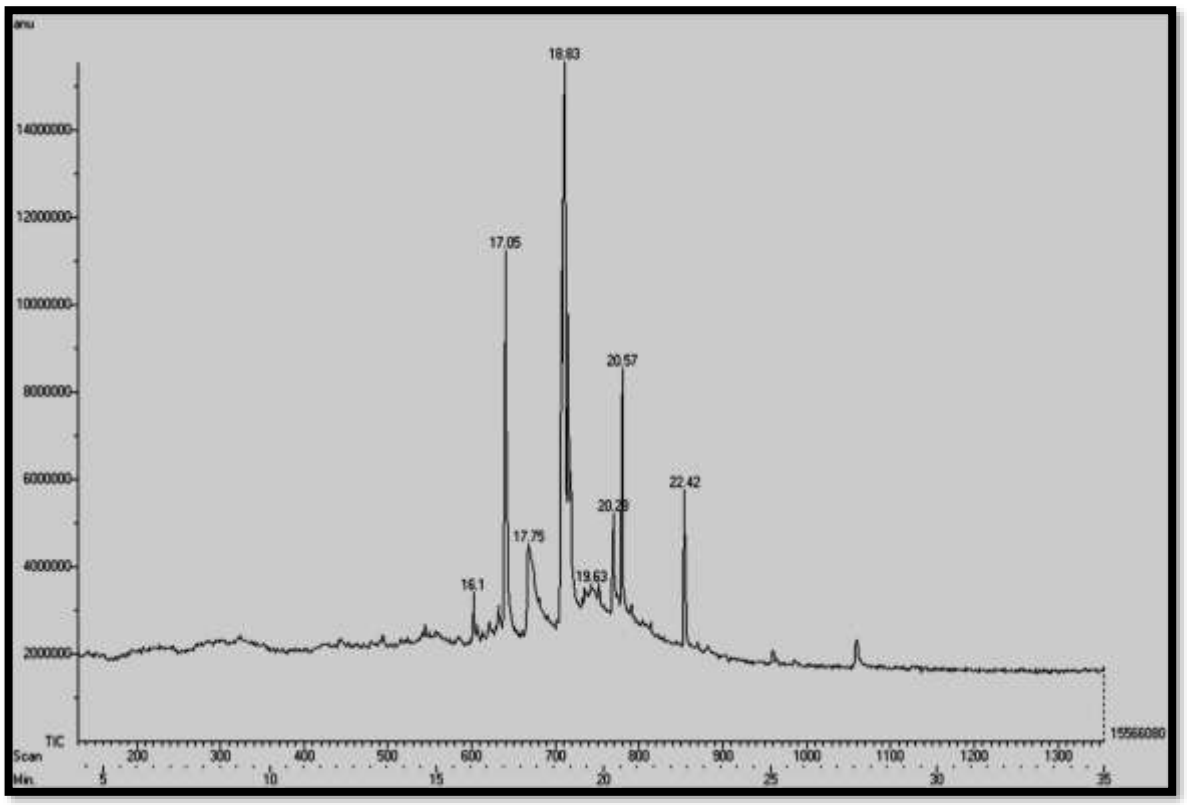


Figure 1: GC-MS Chromatogram

Conclusion

The antioxidant activity of *Turbinaria conoides* has been studied and the results indicate that this alga can be used as a natural antioxidant agent. The present study results are in agreement with the earlier studies by Kajal et al[9] Who reported the significant antioxidant activity of *Turbinaria conoides*. The results prove that this algal species is an excellent source of bioactive compounds with a wide variety of applications.

Reference

1. El Gamal A.A., Biological importance of marine algae, *Saudi Pharmaceutical Journal*, **18(1)**, 1-25 **(2010)**

2. Villarreal-Gomez L.J., Irma E.M., Graciela G.R. and Nahara E.S., Antibacterial and anticancer activity of seaweeds and bacteria associated with their surface, *Revista De Biologia Marina Oceanografia*, **45**, 267-275 **(2010)**

3. Matanjun P., Mohamed S., Mustapha N.M., Mohammad K. and Ming C.H., Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo, *J. Appl. Phycol.*, **20(4)**, 367-373 **(2008)**

4. Vimala M., Reginald M. and Irene W.J., Phytochemical analysis in different solvent extracts of *Padina tetrastromatica*, *International Journal of Development Research*, **5(3)**, 3761-3763 **(2015)**

5. Leelavathi M.S. and Prasad M.P., Evaluation of antioxidant properties of marine seaweed samples by DPPH method, *Int. J. Pure App. Biosci.*, **2(6)**, 132-137 **(2014)**

6. Delgado-Andrade C. and Morales F.J., Unraveling the contribution of melanoidins to the antioxidant activity of coffee brews, *J Agric Food Chem.*, **53**, 1403-1407 **(2005)**

7. Prieto P., Pineda M. and Anguilar M., Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E, *Anal. Biochem.*, **269**, 337-341 **(1999)**

8. Yen G.C. and Chen H.Y., Antioxidant activity of various tea extracts in relation to their antimutagenicity, *J. Agri. Food Chem.*, **43**, 27-32 **(1995)**

9. Kajal C., Praveen N.K., Vijayan K.K. and Roa G.S., Evaluation of phenolic contents and antioxidant activities of brown seaweeds belonging to *Turbinaria* spp. (Phaeophyta, Sargassaceae) collected from Gulf of Mannar, *Asian Pac. J. Trop. Biomed.*, **3(1)**, 8–16 **(2013)**

10. Indu H. and Seenivasan R., In vitro antioxidant activity of selected seaweeds from southeast coast of India, *Int. J. Pharm. Sci.*, **5(2)**, 474-484 **(2013)**

11. Palaksha M.N., Ravishankar K. and Girija S., Evaluation of in vitro antibacterial and anthelmintic activities of *Turbinaria conoides* plant extracts. *International Journal of Biological and Pharmaceutical Research*, **4(8)**, 577-581**(2013)**

12. Archana P., Mohit C.K. and Ajay K., Bioactive compounds and properties of seaweeds, *Review Open Access Library Journal*, **1**, e752 **(2013)**

13. Sradhasini R. and Anjan K., A review on the potentiality of marine seaweeds as a medicinal source, *World Journal of Pharmacy and Pharmaceutical Sciences*, **4(10),** 458-476 **(2015)**

14. Raja A., Vipin C. and Aiyappan A., Biological importance of marine algae- an overview, *Int. J. Curr. Microbiol. App. Sci.*, **2(5),** 222-227 **(2013)**

15. Susana M.C., Loic G.C., Paulo J.S., Mara S.R., Olivia R.P. and Leonel P., Bioproducts from seaweeds: a review with special focus on the Iberian peninsula, *Current Organic Chemistry*, **18(7),** 896-917 **(2014)**.

(Received 01st January 2020, accepted 12th March 2020)