ORIGINAL ARTICLE

Phytochemical screening and high-performance thin-layer chromatography profile of *Sargassum wightii* and its antioxidant activity

M. Sujana[1], P. P. N. Vijay Kumar[2]

[1]Department of Zoology, Andhra University, Visakhapatnam, Andhra Pradesh, India, [2]Advanced Analytical Laboratory, Andhra University, Visakhapatnam, Andhra Pradesh, India

Abstract

Objective: The present study was aimed to screening the phytochemical constituents present in various solvent extracts such as hexane, chloroform, acetone, ethyl acetate, and methanol of *Sargassum wightii*. Selected extract was subjected to high-performance thin-layer chromatography (HPTLC) profile and its antioxidant activity. **Materials and Methods:** All extracts were screening of phytochemicals using standard procedures as described by Harborne. The maximum constituents contained in extract were subjected to identify the functional groups using FT-IR and also subjected for HPTLC screening with suitable mobile phase as hexane:toluene:chloroform:ethyl acetate. The HPTLC fingerprint exhibited the bands of phytochemical constituents and was visualized under UV 254 nm and 366 nm. TLC spots were scanned by CAMAG TLC scanner. Anti-oxidant activity of ethyl acetate extract was determined by DPPH free-radical scavenging activity. **Results:** Ethyl acetate extract was contained maximum number of components such as alkaloids, phenols, steroids, saponins, flavonoids, and tannins. HPTLC chromatogram of Ethyl acetate extract recorded seven bands at 254 nm with different Rf values whereas at 366 nm, 12 bands were recorded with maximum Rf values. The DPPH assay indicates *S. wightii* as a potential antioxidant. IC_{50} value of *S. wightii* is 320.05 µg/ml. **Conclusion:** The results suggest that *S. wightii* has an edible source for many nutrients show potential antioxidant activity.

Key words: *Sargassum wightii*, High-performance thin-layer chromatography, Phytochemicals, DPPH, Antioxidant activity

INTRODUCTION

Macro algae are very important marine resources of the world. Macroalgae are distributed widely in all oceans around the world, but was exploited in limited areas only. It flourishes, wherever rocks, coral, or suitable substrata are available for their attachment. Now the biodiversity and density of macroalgae in Southern Coastal region of Tamil Nadu have come down gradually over period of years.[1,2] Seaweeds are potential renewable resources in the marine environment. Seaweeds have been used by human as medicine and food for at least 13,000 years. Over the past several decades, seaweeds and their extracts have generated an enormous amount of interest in the pharmaceutical industry as the fresh source of bioactive compounds with immense medicinal potential. Compared to terrestrial plants, seaweeds are an untapped resource offering substantial potential for the isolation of original natural ingredients of interest for food and health purposes. Of the diverse classes of seaweeds, edible brown seaweed is considered to be the most nutritious and possesses a range of compounds with biological properties.[3] Seaweeds are rich in antioxidant such as carotenoids, pigments, poly phenols, enzymes, and diverse functional polysaccharides.[4] Sargassum, one of the marine macroalgal genera belonging to the class Phaeophyceae, is widely distributed in tropical and temperate oceans. It belongs to the family Sargassaceae

Address for correspondence:
Dr. P. P. N. Vijay Kumar, Advanced Analytical Laboratory, Andhra University, Visakhapatnam, Andhra Pradesh, India. Phone: +91-9948028048.
E-mail: vijaykumarppn@gmail.com

Received: 24-01-2019
Revised: 05-09-2020
Accepted: 03-10-2020

and order Fucales. It is a large, economically important, and ecologically dominant brown algae present genus among Phaeophyta in India and is represented by 38 species. *Sargassum wightii* is one of the important species belonging to the genus *Sargassum* and a wide range of bioactive properties have been reported from this species.[5]

The objective of this study was to screening of *S. wightii* for phytochemical analysis of five different solvents such as hexane, chloroform, acetone, ethyl acetate, and methanol extracts, tested for various chemical compounds such as alkaloids, phenols, steroids, saponins, flavonoids, and tannins. The maximum compounds contained extract which was used for high-performance thin-layer chromatography (HPTLC) profiling and also antioxidant analysis of *S. wightii*.

MATERIALS AND METHODS

Materials

S. wightii was purchased from Tamil Nadu, India. The obtained seaweeds were washed with tap water followed by distilled water. Seaweeds were kept on the blotting paper and spread out at room temperature in shade in a week. The shade dried seaweeds were grounded to fine powder using tissue blender.

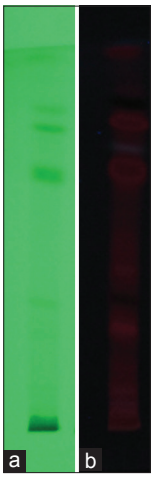

Figure 1: High-performance thin-layer chromatography profiles of *Sargassum wightii* at 254 nm (a) and 366 nm (b)

Preparation of the Extracts

50 g of *S. wightii* powder was extracted with 100 ml of different solvents such as hexane, chloroform, acetone, ethyl acetate, and methanol separately using Soxhelt apparatus. The obtained extracts were filtered through Whatman number-1 filter paper and the filtrate was stored for further experimental use.

Phytochemical Screening and HPTLC Profiling *S. wightii*

The different solvent extracts were subjected to the following chemical tests for screening of phytochemicals using standard procedures as described by Harborne.[6] Maximum constituents contained extract were subjected to HPTLC profile was performed on silica gel 60 F 254 where pre-coated aluminum sheets using CAMAG Linomat 5 sample applicator, CAMAG TLC Scanner, CAMAG UV Chamber, and WinCATS Software 4.03 were utilized during the processing. The plate was developed in mobile phase hexane:toluene:chloroform:ethyl acetate (3:3:2:2) the plate was dried and visualized under UV 254 nm and 366 nm. TLC spots were scanned by CAMAG TLC Scanner.

Antioxidant Assays of *S. wightii* by DPPH Method

0.1 mM DPPH in methanol solution was prepared and was protected from light by wrapping it with aluminum foil. Stock solutions of samples were prepared by dissolving 10 mg of dried extract in 10 ml of methanol. Different volume levels of test sample (100, 200, 300, 400, and 500 µg/ml) of each dose level were prepared by required dilution with methanol. The solution was made up to 3 ml by diluting the extract with desired quantity of methanol and then 150 µl of DPPH solution was added to each test sample. The solution without adding extract is used as the control. Absorbance was observed at 516 nm inUV-visible spectrophotometer (Shimadzu UV-VIS 2450) after 15 min using methanol as a blank. The % reduction and IC_{50} were calculated as follows: The free-radical scavenging activity (% antiradical activity) was calculated using the following equation: DPPH scavenging effect (%) or Percent inhibition = $A_0 – A_1/A_0 \times 100$.

Table 1: Preliminary phytochemical analysis of *Sargassum wightii* crude extracts

Solvent	Alkaloids	Phenols	Steroids	Saponins	Flavonoids	Tannins
Hexane	–	+	+	–	–	–
Chloroform	–	+	+	–	–	–
Acetone	+	+	+	–	–	–
E.acetate	+	+	+	+	+	+
Methanol	+	+	+	–	+	–

+: Present, –: Absent

Where A_0 was the absorbance of control reaction and A_1 was the absorbance in presence of test or standard sample.[7]

RESULTS AND DISCUSSION

S. wightii was screened for preliminary phytochemical constitutes of five different crude extracts (hexane, chloroform, acetone, ethyl acetate, and methanol) that were tested for various chemical compounds such as alkaloids, phenols, steroids, saponins, flavonoids, and tannins as showed in Table 1. Hexane and chloroform extracts have two metabolites, namely, phenols and steroids whereas acetone extract contains alkaloids, phenols, and steroids. From methanol extract, four constituents, namely, alkaloids, phenols, steroids, and flavonoids were observed, whereas from ethyl acetate extract and maximum number of components such as alkaloids, phenols, steroids, saponins, flavonoids, and tannins were observed. Hence, we have selected ethyl acetate extract for HPTLC profiling and antioxidant activity.

Ethyl acetate extract of HPTLC chromatogram was found to be varying with different Rf values and peak areas were observed at 254 nm and 366 nm [Figure 1]. At 254 nm 7 bands were detected with Rf values 0.19, 0.25, 0.35, 0.41, 0.45, 0.69, and 0.86 with peak areas 377.7, 221.6, 956.9, 248.4, 1278.0, 219.7, and 5907.8, respectively [Table 2 and Figure 2], whereas at 366 nm showed that 12 bands were observed in the chromatogram with maximum Rf values of 0.16, 0.19, 0.28, 0.32, 0.41, 0.45, 0.54, 0.68, 0.74,0.83, 0.96, and 1.03 with peak areas 350.2, 407.0, 212.6, 2615.6, 1605.1, 7777.8, 652.1, 340.5, 844.0, 14766.8, 1155.6, and 2923.1, respectively [Table 3 and Figure 3].

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR analysis showed different peaks at 3363 due to N-H Stretching, at 2930 due to C-H Stretching, at 2360 due to C-N stretching, whereas 1620 due to N-H Bending, at 1421 due to C-O-H bending, at 1251 due to O-C bending occur, and at 1027 due to C-O stretching vibrations occurred,

Table 2: Peak list and Rf values of the chromatogram of *Sargassum wightii* ethyl acetate extract at 254 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.19	0.6	0.20	24.2	7.29	0.24	0.2	377.7	4.10	Unknown*
2	0.25	0.2	0.28	10.7	3.21	0.31	2.1	221.6	2.41	Unknown*
3	0.35	1.1	0.38	38.2	11.48	0.41	13.8	956.9	10.39	Unknown*
4	0.41	14.2	0.42	16.4	4.95	0.45	0.0	248.4	2.70	Unknown*
5	0.45	0.3	0.48	61.9	18.65	0.52	1.2	1278.0	13.88	Unknown*
6	0.69	5.9	0.71	11.1	3.34	0.75	1.6	219.7	2.39	Unknown*
7	0.86	2.7	0.90	169.7	51.08	0.97	4.7	5907.8	64.14	Unknown*

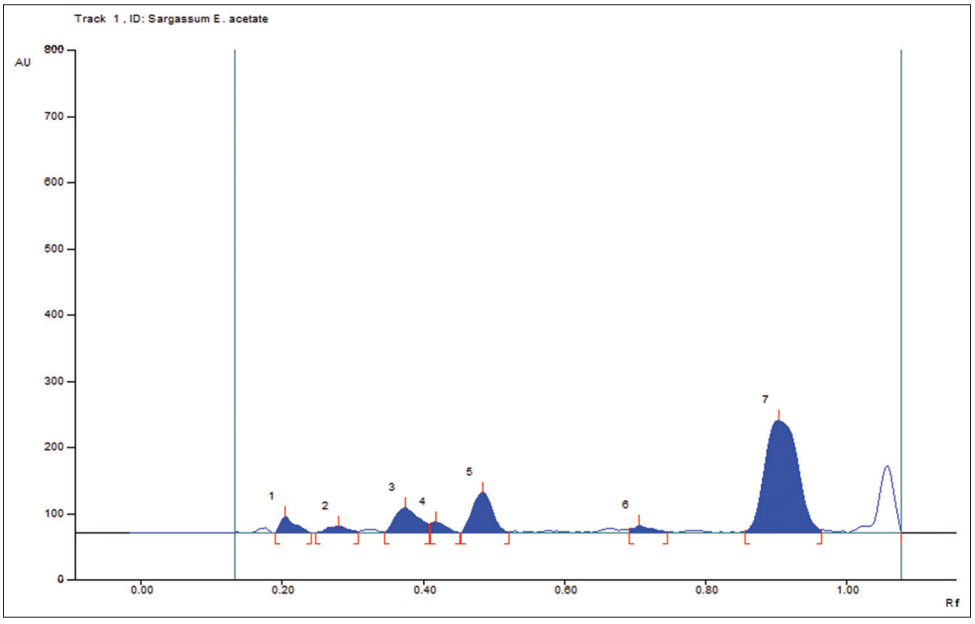

Figure 2: High-performance thin-layer chromatography chromatogram of *Sargassum wightii* at 254 nm

Table 3: Peak list and Rf values of the chromatogram of *Sargassum wightii* ethyl acetate extract at 366 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.16	0.9	0.18	29.5	2.28	0.19	8.6	350.2	1.04	Unknown*
2	0.19	9.7	0.20	37.7	2.91	0.23	0.1	407.0	1.21	Unknown*
3	0.28	0.1	0.30	16.3	1.26	0.32	0.4	212.6	0.63	Unknown*
4	0.32	0.4	0.39	90.5	6.98	0.41	67.4	2615.6	7.77	Unknown*
5	0.41	67.6	0.42	76.9	5.94	0.45	47.5	1605.1	4.77	Unknown*
6	0.45	47.8	0.48	306.1	23.62	0.53	9.8	7777.8	23.11	Unknown*
7	0.54	8.8	0.58	20.9	1.61	0.61	2.0	652.1	1.94	Unknown*
8	0.68	0.2	0.71	14.9	1.15	0.74	5.6	340.5	1.01	Unknown*
9	0.74	5.7	0.77	26.4	2.04	0.82	8.0	844.0	2.51	Unknown*
10	0.83	6.7	0.90	418.4	32.29	0.95	0.3	14766.8	43.88	Unknown*
11	0.96	0.5	1.00	49.8	3.84	1.02	1.2	1155.6	3.43	Unknown*
12	1.03	0.1	1.06	208.3	16.08	1.07	68.1	2923.1	8.69	Unknown*

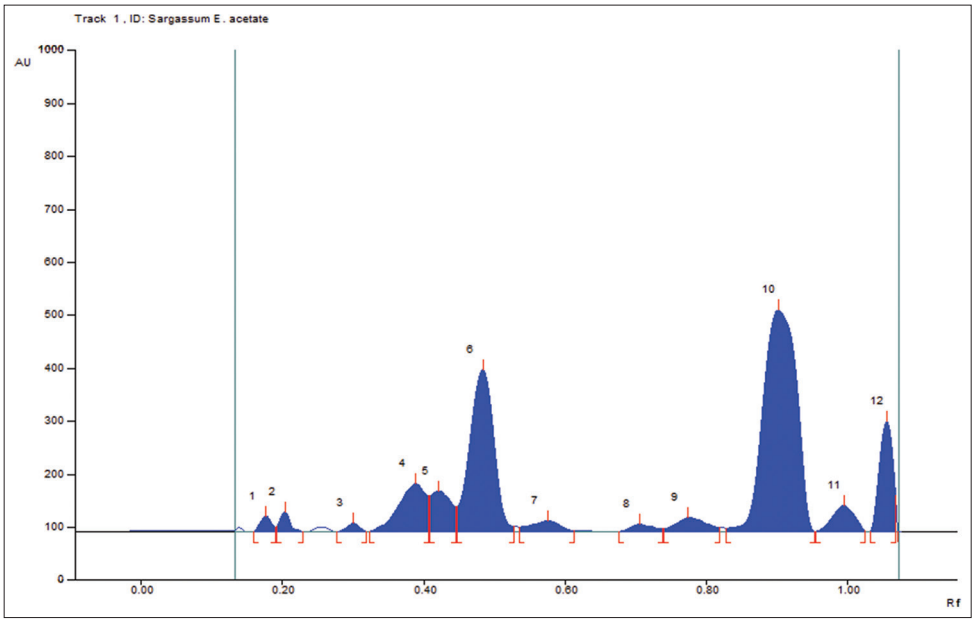

Figure 3: High-performance thin-layer chromatography chromatogram of *Sargassum wightii* at 366 nm

respectively [Figure 4]. The crude extract of *S. wightii* showed different peaks which confirmed the presence of functional groups such as amides, alcohols, and phenolic compounds.

Antioxidant or free radical scavenging, activities of the extract of *S. wightii* were determined using DPPH radical scavenging assay. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule at room temperature.[8] The reduction capacity of the DPPH radical is determined by the decrease in its absorbance at 516 nm induced by antioxidants present in the extract. The maximum absorption of a stable DPPH radical was at 516 nm. The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and radical progresses, which result in the scavenging of the radical by hydrogen donation.[9] Increase in scavenging activity of DPPH radicals in dose-dependent manner is due to the scavenging ability of the *S. wightii* ethyl acetate extract. IC_{50} value of *S. wightii* is 320.05 µg/ml [Figure 5]. High intake of antioxidant-rich foods is inversely related to the onset or progression of cancer as revealed by a number of epidemiological studies.[10-12] Indeed, a number of phytochemical antioxidants are known to confer protection against carcinogenic assault, cytotoxic damage to normal cells wrought during cancer therapy and acute and long-term effects of free radicals produced.[13,14]

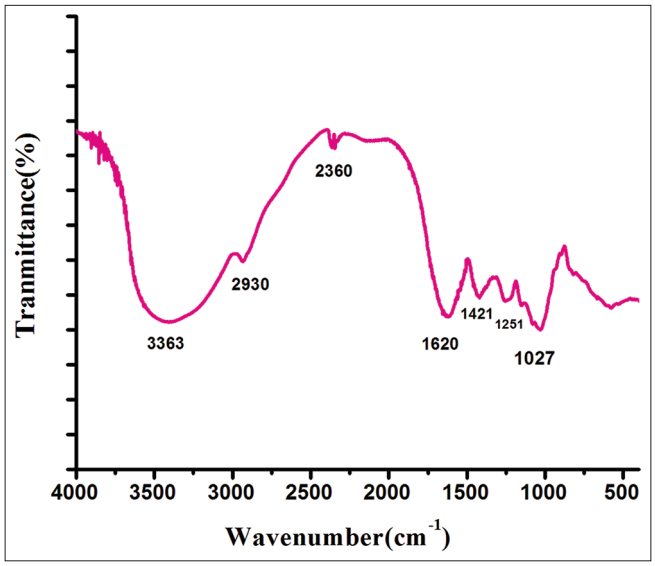

Figure 4: FT-IR spectrum for crude extract of *Sargassum wightii*

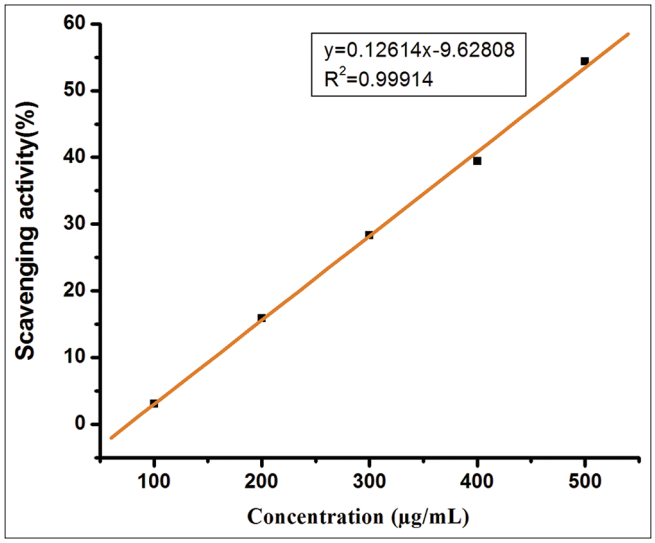

Figure 5: DPPH radical scavenging activity of *Sargassum wightii*

CONCLUSION

Finally, we conclude from this study that the extracts of seaweed *S. wightii* showed that maximum phytochemical constitutes were in ethyl acetate extract. The HPTLC finger print showed many peaks with Rf values, indicating different groups of phytochemicals present also concluded that the brown alga *S. wightii* is a potential source of bioactive compounds. These compounds can be utilized for the natural antioxidant activity.

ACKNOWLEDGMENTS

We are thankful for the financial assistance to the DST-PURSE Programme and Advanced Analytical Laboratory, Andhra University, for carrying out in this research work.

REFERENCES

1. Krishnamurthy V. Krusadi Island, Gulf of Mannar, A Paradise Lost; Can it be Regained? National Symposium on Algae, Man and Biosphere. Poondi: A.V.V.M. Sri Pushpam College; 2006. p. 20-4.
2. Kannan L, Thangaradjou T. Identification and assessment of biomass and productivity of seagrasses. In: National Training Workshop on Marine and Coastal Biodiversity Assessment for Conservation and Sustainable Utilization. Thoothukudi, Tamil Nadu: SDMRI Special Research Publication; 2006. p. 9-15.
3. Rodríguez-Bernaldo de Quirós A, Frecha-Ferreiro S, Vidal-Perez AM, López-Hernández J. Antioxidant compounds in edible brown seaweeds. Eur Food Res Technol 2010;231:495-8.
4. Vinayak CR, Sabu AS, Chatterji A. Bio-prospecting of a few brown seaweeds for their cytotoxic and antioxidant activities. Evid Based Complement Altern Med 2011;2011:673083.
5. Mizukoshi S, Matsuoka H, Kato H, Noda H. Search for bioactive substances from marine algae. Bull Fac Bioresour Mie Univ 1992;8:27-34.
6. Harbone J. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. New York: Chapman and Hall; 1998. p. 1-150.
7. Achola KJ, Munenge RW. Bronchodilating and uterine activities of *Ageratum conyzoides* extract. Pharm Biol 1998;36:93-6.
8. David JM, Barreiros AL, David JP. Antioxidant phenylpropanoid esters of triterpenes from *Dioclea lasiophylla*. Pharm Biol 2004;42:36-8.
9. Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yasuhara T, *et al*. Effects of the interaction of tannins with co-existing substances. VI: Effects of tannins and related polyphenols on superoxide anion radical, and on 1, 1-diphenyl-2-picrylhydrazyl radical. Chem Pharm Bull (Tokyo) 1989;37:2016-21.
10. Hunter DJ, Willett WC. Diet, body size, and breast cancer. Epidemiol Rev 1993;15:110-32.
11. Smith-Warner A, Giovannucci E. Fruit and vegetable intake and cancer. In: Heber D, Blackburn GL, Go VL, editors. Nutritional Oncology. San Diego, CA: Academic Press; 1999. p. 153-93.
12. Cohen JH, Kristal AR, Stanford JL. Fruit and vegetable intakes and prostate cancer risk. J Natl Cancer Inst 2000;92:61-8.
13. Borek C. Antioxidants and radiation therapy. J Nutr 2004;134:S3207-9.
14. Weiss JF, Landauer MR. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. Toxicology 2003;189:1-20..

Source of Support: Nil. **Conflicts of Interest:** None declared.