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Thin layer chromatography analysis of antioxidant constituents from seagrasses of Gulf of Mannar Biosphere Reserve, South India

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Abstract: Aqueous Methanol extracts of four seagrass species- namely *Enhalus acoroides*, *Thalassia hemprichii*, *Halodule pinifolia* and *Syringodium isoetifolium* collected from Gulf of Mannar Biosphere Reserve, South India were analysed using thin layer chromatography (TLC) followed by DPPH (2, 2-Diphenyl-1-picrylhydrazyl) technique and total phenolic content (Folin-Ciocalteu method). 25-75% of major seagrasses resolved by means of TLC were active antioxidants. *Halodule pinifolia* showed a higher number of antioxidant constituents with strong activity (75%). The strength of antioxidant activity was shown to be stronger by the constituents of *H. pinifolia*, *T. hemprichii* and *E .acoroides*. The total phenolic content in these seagrass extracts was in the range of 0.2878-1.0807 mg tannic acid equivalent/g. The results suggested that seagrasses have strong antioxidant potential. Further studies are being carried out on the other species of seagrasses of different habitats in order to provide complete data of the antioxidant activity and characterization of the principle antioxidant agents, which can be used to treat various oxidative stress-related diseases in plants.

Keywords: Seagrasses,Antioxidant activity, TLC Bioautography, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), Total phenol.

Introduction

Antioxidants in biological systems have multiple functions, including depending against oxidative damage and in the major signaling pathways of cells. The major action of antioxidants in cells is to prevent damage caused by the action of reactive oxygen species (ROS). ROS such as superoxide radical ($O_2^{\cdot}$), hydroxyl radical ($OH^{\cdot}$), peroxide radical ($ROO^{\cdot}$) and nitric acid radical are generated in living organisms during excessive metabolism[1] and involved in extensive oxidative damage to the cells that leads to age related degenerative diseases, cancer and wide range of other human diseases[2]. Several synthetic antioxidants, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) are commercially available and are currently in use. Because of the carcinogenicity of synthetic antioxidants[3], there ispreference to develop effective antioxidants of natural origin[4]. The natural antioxidants (Phenolic compounds) play a key role in antioxidative defense mechanisms in biological systems and they act as free radical scavengers. So now-a-days attention has turned on to natural antioxidants because the use of synthetic antioxidants has been falling off due to their suspected action as cancer inducer[5]. There is considerable interest, in recent years, in finding out new natural antioxidants from living system for application in food, pharmaceutical and cosmetics. Moreover, searching for novel biologically active compounds sometimes leads to the discovery of new properties of known plant constituents.

It is therefore of interest to find new resource for this antioxidant constituents from Seagrasses. Seagrasses are a group of about 60 species of marine

flowering plants, which form the most widespread and productive coastal systems in the world[6]. In folk medicine, Seagrasses have been used for a variety of remedial purposes, e.g. for the treatment of fever and skin diseases , muscle pains, wounds and stomach problems, remedy against stings of different kinds of rays, tranquillizer for babies[7]. Marine and estuarine submerged aquatic angiosperms or seagrasses produce antimicrobial compounds that has antibacterial[8,9,10,11], antialgal[12], antifungal[13,14], antiviral[15,16], antiprotozoal[17], anti-inflammatory[18] and antidiabetic[19] activities. More recently, reports have revealed that seagrasses are rich source of antioxidant compounds[19,20,21].

A number of screening assays were developed and used to search potential antioxidants, which include, but are not limited to, high-throughput relative DPPH radical scavenging capacity (RDSC) assay[22], HO radical scavenging capacity (HOSC) assay[23] and thin layer chromatography (TLC) Bioautography assay[5,24]. Compared to other methods, the TLC Bioautography method can quickly detect and separate the active components in a complicated plant extract, and has additional advantage such as convenience, being simple to run, and requiring no specialized equipment. In this paper, we report the antioxidant constituents of four species of seagrasses from Gulf of Mannar (India) by using thin layer chromatography (TLC) followed by DPPH (2, 2-Diphenyl-1-picrylhydrazyl) technique and total phenolic content in aqueous methanolic extracts.

Materials and Methods

Chemicals and reagents

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich. Gallic acid, Tannic acid, Ascorbic acid, Folin-Ciocalteus's phenol reagent, Sodium carbonate was from Merck (Mumbai, India). All the chemicals used including the solvents were of analytical grade.

Sample collection and preparation

Fresh leaves of *Enhalus acoroides* (L.f.) Royle, *Thalassia hemprichii* (Ehrenb.) Asch., *Syringodium isoetifolium* (Asch.) Dandy and *Halodule pinifolia* (Miki) Hartog were collected in March 2009 from the intertidal region of the Mandapam coast, Gulf of Mannar Biosphere Reserve, India (Lat. 09˚ 17.417'N; Long. 079˚ 08.558'E) and immediately brought to the laboratory in plastic bags containing seawater to prevent evaporation. Then the plants were washed thoroughly with the tap water to remove all the sand particles and epiphytes. The samples were shade- dried at room temperature for five days until constant weight obtained and ground in an electric mixer. The powdered samples were then stored in refrigerator for future use.

Extraction

Dried finally crushed leaves (10g) were extracted for 24 h in 200 ml of aqueous methanol at room temperature. The extraction was twice repeated and filtered through glass funnel and Whatmann No. 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure using a rotary flash evaporator. Finally the dry extracts were lyophilized and then stored in refrigerator for further analysis.

Thin Layer chromatography analysis of antioxidant constituents

The antioxidant constituents were analysed using thin layer chromatography (TLC) followed by DPPH (2, 2-Diphenyl-1-picrylhydrazyl) technique[24]. About 100 µg of extract of seagrass species was loaded on TLC plates (Merck, 10 x 10 cm^2). The plates were developed in 10% chloroform in methanol and Methanol:Chloroform:Hexane(7:2:1) to separate the various constituents of the extracts. The developed plates were air dried and observed under visible and UV light (240 and 300 nm). Various separated spots were noted as their Rf values. After this examination, 0.05% of DPPH solution in methanol was sprayed on the surface of developed TLC plates and incubated for 10 min at room temperature. The active antioxidant seagrass constituents were detected as yellowish white spots produced by bleaching of DPPH by resolved bands on the TLC plates. After visual comparison with the intensity of bleached colour of the TLC band of positive standard, the antioxidant strengths of seagrass constituents were tentatively categorized as strong and week activities. All detected active antioxidant constituents were noted according to their Rf values. Ascorbic acid and Gallic acid were used as positive control, and blank TLC plate was taken as negative control.

Determination of total phenolic content

The total phenolic content of the extracts was measured using the modified Folin-Ciocalteu method[25]. An aliquot of the extract was mixed with 5 ml of Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/ l) of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40^oC for colour development. Absorbance was then measured at 765 nm using the PerkinElmer Lamda 25 UV-VIS Spectrophotometer. Samples from the extract were evaluated at a final concentration of 0.1 mg/ml. Total phenolic content was expressed as mg/g Tannic acid equivalent.

Results and Discussion

Seagrasses have been used as a biological source of several metabolites with various bioactivities. Seagrass

extracts have been shown to have various antioxidant activities[19,20,21]. Thus, the experimental data of these previous reports showed that seagrasses must have contained strong antioxidant constituents. The results of present investigation showed that 25-75% of major seagrasses resolved by means of TLC were active antioxidants (Table .1) in terms of DPPH free radical scavenging activity, supporting the previous studies of strong antioxidant potential of seagrasses. The extracts of *Halodule pinifolia* showed several resolved TLC bands with strong antioxidant activity of resolved bands (75%) followed by *Thalassia hemprichii* (60%) showed strong antioxidant activity and few spots with weak antioxidant activity of resolved bands. However, extracts of *Enhalus acoroides* and *Syringodium isoetifolium* showed faint spots, indicating relatively weak antioxidant activity of the resolved bands. The nature of the active antioxidant TLC bands of the crude extracts of all four seagrasses of two different solvent systems are presented in Table 1.

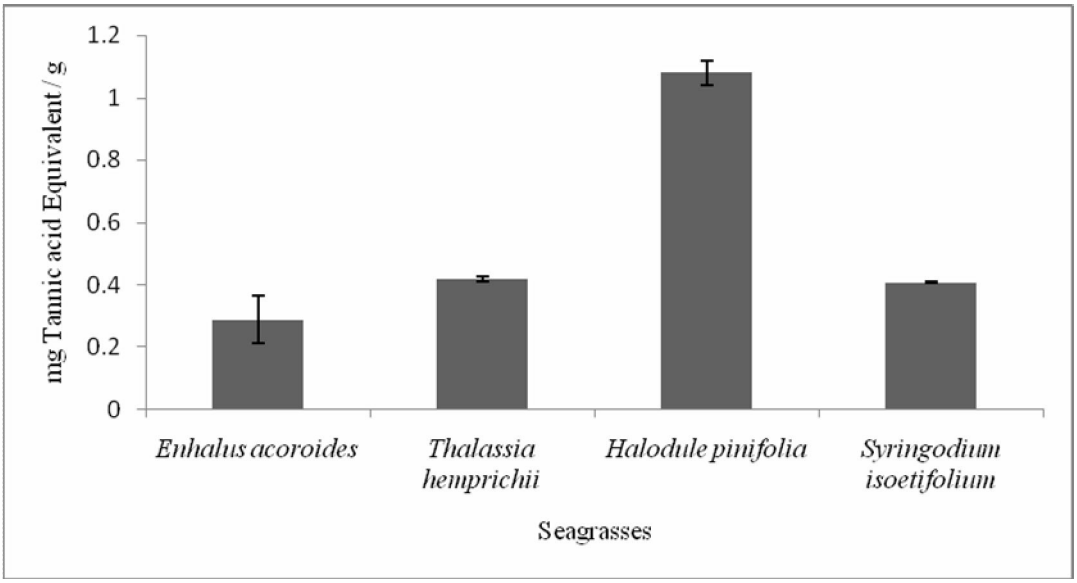
In the present experiment, the total phenolic content was determined and results are presented in Fig.1. The phenolic content in the test seagrass extracts was found to be higher in *Halodule pinifolia* (1.0807±0.039) followed by *T. hemprichii* (0.4187±0.007). In general, phenolic compounds were commonly found in plants and have reported several biological activities including potential antioxidants and free radical scavengers apart from primary defense role[26,27,28]. Earlier reports revealed that seagrasses especially their polyphenols have the antioxidant activity[19,20,21].

Table 1. Thin layer chromatography (TLC) profile of DPPH free radical active constituents of seagrass crude extracts in two different solvent systems

Extract	Total number of TLC spots [a]	Antioxidant active TLC spots	Characteristics of antioxidant active spots
Mobile phase – 10% chloroform in methanol			
Enhalus acoroides	8	0.11	*
		0.23	**
Thalassia hemprichii	8	0.05	**
		0.12	**
Halodule pinifolia	9	0.12	**
		0.32	**
		0.52	**
		0.63	**
		0.89	*
Syringodium isoetifolium	4	0.05	**
		0.24	*
Mobile phase – Methanol:Chloroform:Hexane(7:2:1)			
Enhalus acoroides	5	0.02	**
		0.23	*
Thalassia hemprichii	5	0.03	**
		0.20	*
		0.34	**
Halodule pinifolia	4	0.05	**
		0.25	**
		0.39	**
Syringodium isoetifolium	5	0.05	**
		0.25	*

[a] Indicates a sum of UV detected and no detected TLC bands before DPPH spray
*Comparatively weak in activity; **comparatively strong in activity

Fig. 1 Total phenolic content in the seagrass extracts



Conclusion

The present study concluded the presence of antioxidants in the seagrasses provides useful information of seagrasses on pharmacological activities and potential applications of such compounds as natural antioxidants in different food/pharmaceutical products. Further studies are being carried out on the other species of seagrasses of different habitats in order to provide complete data of the antioxidant activity and characterization of the principle antioxidant agents, which can be used to treat various oxidative stress-related diseases in plants.

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References

1. Aruoma I.O and Cuppette S.L., *Antioxidant methodology: In vivo and in vitro concepts*. IL: AOAS Press.1997.
2. Aruoma I.O.,. Antioxidant action of plant foods. Use of oxidative DNA damage, as a tool for studying antioxidant efficacy. *Free Radical Res.,* 1999, 30, 419-427.
3. Wichi H.P.,. Enhanced tumor development of bytylated hydroxyanisol (BHA) from the prospective of effect on fore stomach and oesophageal squamous epithelium. *Food Chem Toxicol.,* 1998, 26, 717-723.
4. Anandjiwala S., Srinavasa H., Kalola J and Rajani M., Free-radical scavenging activity of *Bergia suffruticosa* (Delie) Fenzl. *J Natl Med.,* 2007,61, 59-62.
5. Climpoiu C., Analysis of Some Natural Antioxidants by Thin-Layer Chromatography and High Performance Thin-Layer Chromatography. *J Liq Chrom Rel Technol.,* 2006, 29, 1125-1142.
6. Green E.P., Short F.T., *World atlas of Seagrasses*. Berkeley: University of California, 2003, 1-4.
7. de la Torre-Castro M. and Ro¨nnba¨ck P., Links between humans and seagrasses-an example from tropical East Africa. *Ocean & Coastal Management.*, 2004, 47, 361-387.
8. Harrison P.G. and Chan A.T., Inhibition of the growth of microalgae and bacteria by extracts of eelgrass (*Zostera marina*) leaves. *Mar Biol.,* 1980, 61, 21-26.
9. Bernard P. and Pesando D., Antimicrobial and antifungal activity of extracts from the rhizomes of the Mediterranean seagrass *Posidonia flavano* (L.) delile. *Bot Mar.,* 1989, 32, 85-88.
10. Devi P., Solimabi W., D'Souza L., Sonak S., Kamat Y. and Singbal S.Y.S., Screening of some marine plants for antiviral activity against marine fouling bacteria. *Bot Mar*., 1997, 40, 87-91.

11. Bhosale S.H., Nagle V.L. and Jagtab T.G., Antifouling potential of some marine organisms from India against species of *Bacillus* and *Pseudomonas*. *Marine Biotechnol.,* 2002, 4, 111-118.
12. Harrison P.G., Control of microbial growth and of amphipod grazing by water-soluble compounds from leaves of *Zostera marina*. *Mar Biol.,* 1982, 67, 225-230.
13. Ballesteros D., Martin D. Uriz M.J., Biological activity of extracts from Mediterranean macrophytes. *Bot Mar.,* 1992, 35, 481-485.
14. Jensen P.R., Kensin K.M., Porter D. and Fenical W., Evidence that a new antibiotic flavones glycoside chemically defends the seagrass *Thalassia testudinum* against zoosporic fungi. *Appl Environ Microbiol.,* 1998, 64, 1490-1496.
15. Premanathan M., Chandra K., Bajbai S.K. and Kathiresan K., A survey of some Indian medicinal plants for antiviral activity. *Bot Mar.,* 1992, 35, 321-324.
16. Rowley D.C., Hansen M.S.T., Rhodes D., Sotriffer C.A.. Ni H., McCammon J.A., Bushmann F.D. and Fenical W., Thalassiolins A-C: New Marine – Derived Inhibitors of HIV cDNA Integrase. *Bioorg Med Chem.,* 2002, 10, 3619-3625.
17. Orhan I., Sener B., Atici T., Brun R., Perozzo R. and Tasdemir D., Turkish freshwater and marine macrophytes extracts show in vitro antiprotozoal activity and inhibit Fabl, a key enzyme of *Plasmodium falciparum* fatty acid biosynthesis. *Phytomedicine.,* 2006, 13, 735-739.
18. Hua K.F., Hsu H.Y., Su Y.C., Lin I.F., Yang S.S., Chen Y.M. and Chao L.K., Study on the Antiinfalmmatory Activity of Methanol Extract from Seagrass *Zostera japonica*. *J Agr Food Chem.,* 2006, 54(2), 306-311.
19. Gokce G. and Haznedaroglu M.Z., Evaluation of antidiabetic, antioxidant and vasoprotective effects of *Posidonia oceanica* extract. *J Ethnopharmacol.,* 2008, 115, 122-130.
20. Hasina E.I., Kolenchenko E.A., Sgrebneva M.N., Kovalev V.V. and Khotimchenko Yu.S., Antioxidant Activities of a Low Etherified Pectin from the Seagrass *Zostera marina*. *Russian J Mar Biol.,* 2003, 29 (4), 259-261.
21. Sureda A., Box A., Terrados J., Deudero S. and Pons A., Antioxidant response of the seagrass *Posidonia oceanica* when epiphytized by the invasive macroalgae *Lophocladia lallemandii*. *Mar Environ Res.,* 2008, 66, 359-363.
22. Bhattarai H.D., Paudel B., Hong S.G., Lee H.K. and Yim J.H., Thin layer chromatography analysis of antioxidant constituents of lichens from Antarctica. *J Nat Med.,* 2008, 62,481-484.
23. Cheng Z., Moore J. and Yu L., High-throughput relative DPPH radical scavenging capacity assay. *J Agr Food Chem.,* 2006, 54(20), 7429-7436.
24. Moore J., Yin J. and Yu L., Novel fluorometric assay for hydroxyl radical scavenging capacity (HOSC) estimation. *J Agr Food Chem.,* 2006, 54(3), 617-626.
25. Wolfe K., Wu X. and Liu R.H., Antioxidant activity of apple peels. *J Agr Food Chem.,* 2003, 51, 609-614.
26. Kähkönen M.P., Hopia A.I., Vuorela H.J., Rauha J.P., Pihlaja K., Kujala T.S., et al. Antioxidant activity of plant extracts containing phenolic compounds. J Agr *Food Chem.,* 1999, 47, 3954-3962.
27. Rice-Evants C., Miller N.J., Bolwell G.P., Bramley P.M. and Pridham J.B., The relative antioxidants activities of plant-derived polyphenolic flavonoids. *Free Rad Res.,* 1995, 22, 375-383.
28. Sugihara A., Arakawa T., Ohnishi M. and Furuno K., Anti and pro-oxidative effects of flavonoids on metal induced lipid hydroperaxidase-dependant lipid peraxidation in cultured hepatocytes located with α-linolenic acid. *Free Rad Biol Med.,* 1999, 27, 1313-1323.

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