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RP-HPLC Profile of Major Phenolics from Brown Marine Macro Algae

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Abstract

To exemplify quantitative analysis of major phenolics from a brown marine macro algae *Sargassum cinereum*, *Sargassum ilicifolium*, *Sargassum tenerrimum* and *Sargassum wightii* by RP-HPLC chromatographic profile. Polyphenol content was determined and quantified by reversed phase high performance liquid chromatography (RP-HPLC) methods.

Keywords: Microalgae; Phenolics; RP-HPLC; *Sargassum* spp.

Introduction

Phaeophyceae is the largest and most complex type of algae. There are about 1800 species of brown seaweed, broadly distributed from tropical to polar zones of ocean in the world; some species are highly exploited for industries, such as *Laminaria japonica*, *Undaria pinnatifida*, *Ascophyllum nodosum*, and *Hizikia fusiformis*. To date, several well traded products are alginates, foods, animal feeds, fertilizers and there is also a little number of some emerging nutraceutical products [1-3].

Seaweeds are known as functional food because of their richness in lipids, minerals and certain vitamins, and also several bioactive substances like polysaccharides, proteins and polyphenols, with potential medicinal uses against cancer, oxidative stress [4] inflammation [4] allergy [5] diabetes [6] thrombosis [7] obesity [8] lipidemia [9] hypertensive [10] and other degenerative diseases.

Sargassum is a large genus, they are economically and medicinally important. In India, nearly 38 species are showed their existence [11]. *Sargassum* can be used as fertilizers, food additives, and animal feed [12,13]. *Sargassum* showed promising antibacterial, antipyretic, analgesic and anti-inflammatory, cytotoxicity, and antitumor activity [14-17]. *Sargassum* is a good source for the phytochemical investigation to identify the occurrence of biomolecules.

With this knowledge, the present study was indented to determine the phytochemical profiles of *Sargassum* spp by using Reverse phase-high performance liquid chromatography (RP-HPLC).

Materials and Methods

Seaweed collection and identification

Sargassum spp was collected from the west coast of Maharashtra. The samples were washed with seawater and then transfer to laboratory in a polythene bags. After that it was washed with double distill water. Algal authentication was done by Dr. B. B. Chaugule Ex-Head, Department of Botany, Shavitribai Phule, Pune University. Seaweed material was shade dried and grinded in a mechanical grinder to obtain fine powder and stored at -20°C refrigerator for further analysis. The reference standards for phenolics viz., gallic acid, 3,4-dihydroxybenzoic acid, vanillic acid, *p*-coumaric acid and ferulic acid were purchased from Hi-media (Mumbai, India) [18].

Quantitative RP-HPLC analysis of major phenolic compounds

Sample preparation was done by continuous shaking with constant stirring for (110 ± 2) rpm at room temperature (25 ± 2) °C); for the quantification of phenolics. Methanol was used as a solvent for the extraction. The extracted samples was filtered, final volume was adjusted with methanol and stored in the vials at 4°C until RP-HPLC analysis. Each sample was run in triplicates on a Reverse phase high-performance liquid chromatography (RP-HPLC).

Analysis was performed by Shimadzu prominence HPLC system equipped with degasser DGU-20A 5R, low- pressure quaternary pump LC 20 AD and photo diode array detector SPD-M20 A. Separation was achieved by using reverse phase Nova-Pak C-18 column (4 µm × 4.6 mm, 250 mm) from water (Milford, USA) was used for the chromatographic separation. All the solvents were used for the analysis was purchased from Sigma Aldrich HPLC grade purity 99.9%. Water, methanol and acetonitrile (5:3:2) containing 0.2% triethylamine used as mobile phase having pH=3.3. A total of 20 µL volume was used for the injection (Table 1).

Quantitative analysis of major phenolics from *Sargassum* spp was carried out with a comparison of the standard RP-HPLC chromatographic profile. Figure 1 shows the profile of standard phenolics compounds while in Figure 2 *S. cinereum* contains gallic acid (0.1144 ± 0.0096 mg/g) and *p*-hydoxybenzoic acid (0.0474 ± 0.0005 mg/g). *S. ilicifolium* contains *p*-hydroxybenzoic acid (0.0094 ± 0.0005 mg/g) as shown in Figure 3. RP-HPLC profile of *S. tenerrimum* showed gallic (0.1165 ± 0.0010 mg/g) and *p*-hydroxybenzoic acid (0.0186 ± 0.0005 mg/g) in Figure 4 *S. wightii* contains gallic acid (0.0539 ± 0.0028 mg/g) presented in Figure 5.

Result and Discussion

Quantification of four *Sargassum* spp. was analyzed for phenolics from a brown marine macroalgae i.e. *S. cinereum*, *S. ilicifolium*, *S. tenerrimum* and *S. wightii*. Phenolics such as gallic acid, *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid and ferulic acid elution for brown algae; with reference to a pure standard is illustrated with a distinct peak. The validation parameters consisted at linearity range, precision, accuracy and limits of detection and quantification. The peaks were identified by their retention

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Sr. No	Phytochemical parameter	Name of the Test	Water extract	Chloroform	Acetone	Pet ether	Benzene
1.	Alkaloids	Hager's test	+	+	-	-	-
2.	Carbohydrates	Molish's test, Fehling test	+	+	+	-	-
3.	Glycosides	Legal's test	+	+	-	-	-
4.	Steroids	Salkowski test	--	-	+	-	-
5.	Flavonides	Shinodeis test	-	-	-		-
6.	Flavones		-	-	-	-	-
7.	Saponins		+	--	+	+	+
8.	Fixed oil and Fat	Spot test and saponification test		-	-	+	-
9.	Tannins		+	-	-	-	-
10.	Terpenoids		-	+	+	-	-
11.	Proteins	Xanthoproteic test	-	-	-	-	-
12.	Amino acids	Xanthoproteic test	-	-	-	-	-
13.	Phenols		-	-	-	-	-
14.	Coumarins		-	-	-	-	-
15.	Anthroquinone		-	-	-	-	-

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Table 1: Phytochemical constituents present in different extract of *Sargassum ilicifolium.*

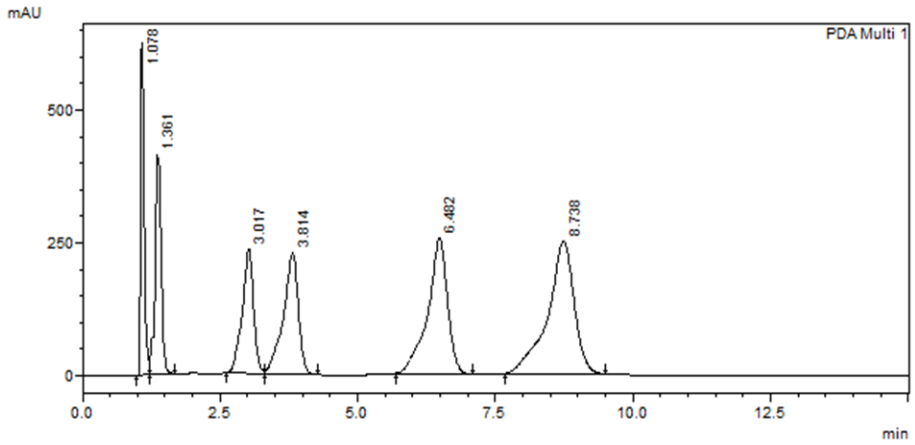

Figure 1: RP-HPLC profile of standard phenolics compounds P-1.078 (tannic acid), P-1.361 (gallic acid), P-3.017 (*p*-hydroxybenzoic acid), P-3.814 (vanillic acid), P-6.482 (*p*-coumaric acid) and P-8.738 (ferulic acid).

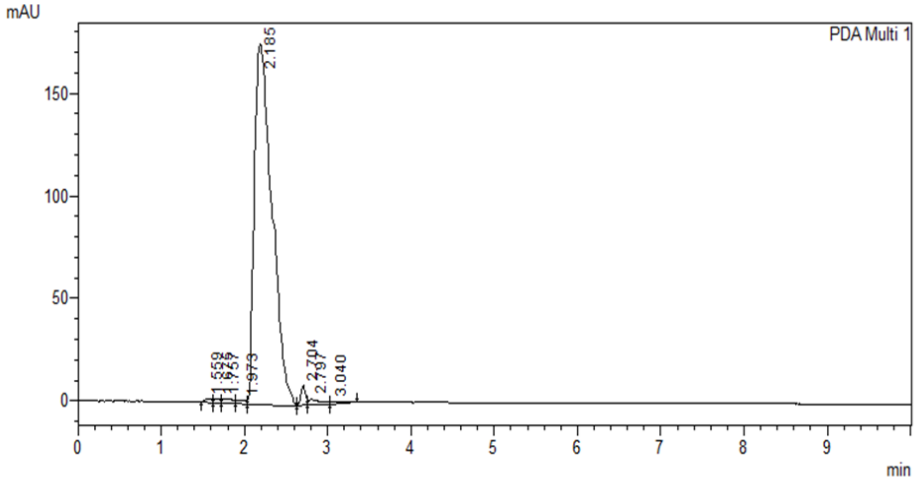

Figure 2: RP-HPLC profile of *S. cinereum* showing gallic acid 0.1144 ± 0.0096 mg/g and *p*-hydoxybenzoic acid 0.0474 ± 0.0005 mg/g (n=3).

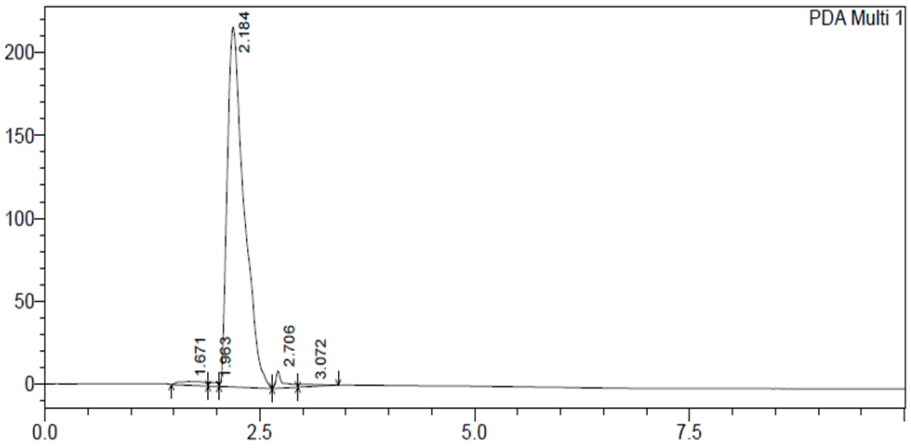

Figure 3: RP-HPLC profile of *S. ilicifolium* showing *p*-hydroxybenzoic acid 0.0094 ± 0.0005 mg/g (n=3).

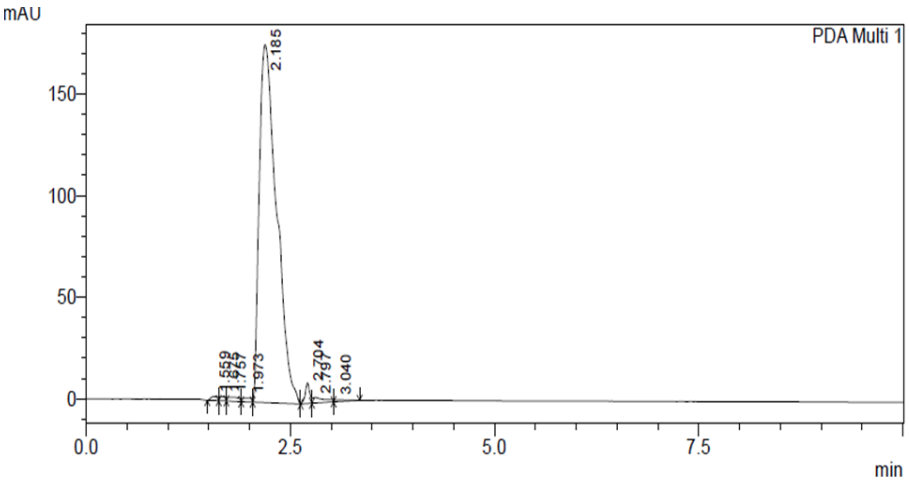

Figure 4: RP-HPLC profile of *S. tenerrimum* showing gallic 0.1165 ± 0.0010 mg/g and *p*-hydroxybenzoic acid 0.0186 ± 0.0005 mg/g (n=3).

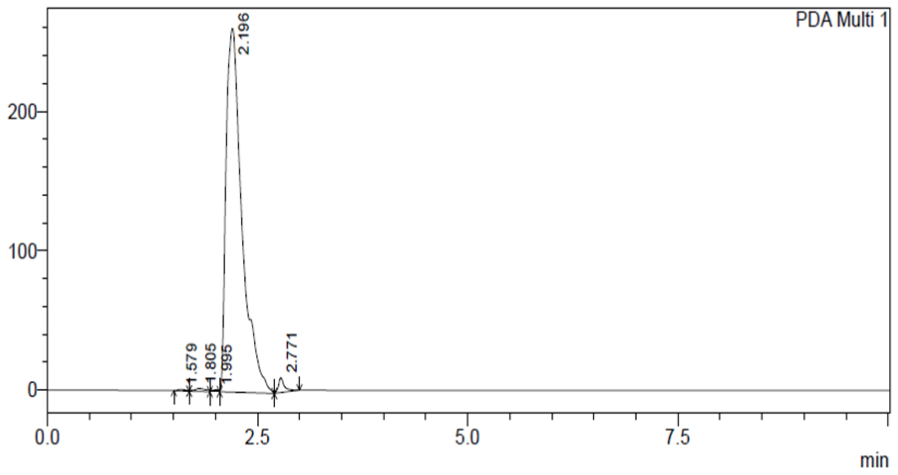

Figure 5: RP-HPLC profile of *S. wightii* showing gallic acid 0.0539 ± 0.0028 mg/g (n=3).

time comparing the UV-visible spectra and spiking with standards. Reverse phase high-performance liquid chromatography (RP-HPLC) analysis was performed by Shimadzu prominence unit with degasser DGU- 20A 5R, low pressure quaternary pump LC 20 AD and photo diode array detector SPD-M20 A. Water, methanol and acetonitrile (5:3:2) containing 0.2% triethylamine used as a mobile phase having pH=3.3. A total of 20 µL volume was used for the injection. Chromatographic separation was achieved by using reverse phase Nova-Pak C-18 column (4 µm × 4.6 mm, 250 mm) from water (Milford, USA) maintained at 24°C. Quantification was done by using an external standard curve with five points. The linearity range was evaluated by plotting the peak area corresponding to the analyte as a function of concentration introduced.

These data are useful for comparison with other phenolics producing species i.e. estimation of major phenolics content. It provides valuable phytocomponents such as polyphenols, flavonoids and terpenoids which have an antioxidant and anti-inflammatory potential.

Quantitative analysis of four species i.e. *S. cinereum*, *S. ilicifolium*, *S. tenerrimum* and *S. wightii* of phenolics were evaluated and analyzed using RP-HPLC. The flow rate of mobile phase was 1 mL/min with 15 min run time and 280 nm was used as absorbance channel. RP-HPLC quantification revealed that *S. cinereum* contains 0.1144 ± 0.0096 mg/g of gallic acid and 0.0474 ± 0.0005 mg/g (n=3) of *p*-hydroxybenzoic acid whereas *S. ilicifolium* eluted 0.0094 ± .0005 mg/g *p*-hydroxybenzoic acid. *S. tenerrimum* showed the presence of gallic acid 0.1165 ±.0010 mg/g and *p*- hydroxybenzoic acid 0.0186 ± 0.0005 mg/g in addition *S. wightii* observed 0.0539 ± 0.0028 mg/gm of gallic acid while vanillic acid, *p*-coumaric acid and ferulic acid were absent in above four species (Tables 1-4).

Conclusion

To conclude, *Sargassum cinereum*, *Sargassum ilicifolium*, *Sargassum tenerrimum* and *Sargassum wightii* shows insignificant amount of polyphenols. The automated integration software LC Lab solution Shimadzu-Japan was used to acquire the area under curve. The standard curve and retention times i.e. (tannic acid) at 1.0 min, (gallic acid) at 1.3 min, (*p*-hydroxybenzoic) at 3.0 min, (vanillic acid) at 3.8 min, (*p*-coumaric acid) at 6.482 min and (ferulic) acid 8.7 min were calibrated using pure phenolics standard solubilized in methanol. Results were expressed as mg/g.

Sr. No	Phytochemical parameter	Water extract	Chloroform	Acetone	Pet ether	Benzene
1.	Alkaloids	+	+	-	-	-
2.	Carbohydrates	+		+	-	-
3.	Glycosides	+	+	-	-	-
4.	Steroids	-	-	+	-	-
5.	Flavonides	-	-	-		-
6.	Flavones	-	-	-	-	-
7.	Saponins	+	-	+	+	+
8.	Fixed oil and Fat		-	-	+	-
9.	Tannins	+	-	-	-	-
10.	Terpenoids	-	+	+	-	-
11.	Proteins	-	-	-	-	-
12.	Amino acids	-	-	-	-	-
13.	Phenols	-	-	-	-	-
14.	Coumarins	-	-	-	-	-
15.	Anthroquinone	-	-	-	-	-

Table 2: Phytochemical constituents present in different extract of *Sargassum cinereum*.

Sr. No	Phytochemical parameter	Water extract	Chloroform	Acetone	Pet ether	Benzene
1.	Alkaloids	+	+	-	-	-
2.	Carbohydrates	+		+	-	-
3.	Glycosides	+	+	-	-	-
4.	Steroids	-	-	+	-	-
5.	Flavonides	-	-	-		-
6.	Flavones	-	-	-	-	-
7.	Saponins	+	-	+	+	+
8.	Fixed oil and Fat		-	-	+	-
9.	Tannins	+	-	-	-	-
10.	Terpenoids	-	+	+	-	-
11.	Proteins	-	-	-	-	-
12.	Amino acids	-	-	-	-	-
13.	Phenols	-	-	-	-	-
14.	Coumarins	-	-	-	-	-
15.	Anthroquinone	-	-	-	-	-

Table 3: Phytochemical constituents present in different extract of *Sargassum tenerrimum*.

Sr. No	Phytochemical parameter	Water extract	Chloroform	Acetone	Pet ether	Benzene
1.	Alkaloids	+	+	-	-	-
2.	Carbohydrates	+		+	-	-
3.	Glycosides	+	+	-	-	-
4.	Steroids	-	-	+	-	-
5.	Flavonides	-	-	-		-
6.	Flavones	-	-	-	-	-
7.	Saponins	+	-	+	+	+
8.	Fixed oil and Fat		-	-	+	-
9.	Tannins	+	-	-	-	-
10.	Terpenoids	-	+	+	-	-
11.	Proteins	-	-	-	-	-
12.	Amino acids	-	-	-	-	-
13.	Phenols	-	-	-	-	-
14.	Coumarins	-	-	-	-	-
15.	Anthroquinone	-	-	-	-	-

Note: '+' active compound present
'-'active compound absent

Table 4: Phytochemical constituents present in different extract of *Sargassum wightii*.

Conflict of Interest

The authors declare that they have no conflicts of interest related to the contents of this article.

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