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Pectin from Three Vietnamese Seagrasses: Isolation, Characterization and Antioxidant Activity

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Abstract: This study focused on the isolation and structural characterization of pectin from three distinct species of Vietnamese seagrass including *Enhalus acoroides*, *Thalassia hemprichii*, and *Halophila ovalis*. The pectin yield obtained from *Enhalus acoroides* was the highest, corresponding to 24.15%, followed by those from *Thalassia hemprichii* (20.04%) and *Halophila ovalis* (19.14%). The physicochemical properties of pectin including total carbohydrate content, anhydrouronic acid (AUA) content, equivalent weight (EW), methoxyl content (MeO), and degree of esterification (DE) were determined using various analysis techniques. The pectin obtained from all three species were found to be low-methyl-esterified pectin, with the MeO content and DE for *E. acoroides*, *T. hemprichii*, and *H. ovalis* being 6.15% and 27.18%, 3.26% and 43.31%, and 4.65% and 33.25%, respectively. The average molecular weight (MW) of pectin was analyzed by size-exclusion chromatography. Pectin from *T. hemprichii* had the highest MW of 173.01 kDa, followed by pectin from *E. acoroides*, with a MW of 127.32 kDa, and that from *H. ovalis*, with a MW of 56.06 kDa. Furthermore, the pectins from all three seagrass species exhibited high antioxidant activity and might be promising as antioxidants.

Keywords: Vietnamese seagrass; *Enhalus acoroides*; *Thalassia hemprichii*; *Halophila ovalis*; pectin; isolation; characterization; antioxidant activity

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1. Introduction

Seagrasses are aquatic plants that thrive in marine and brackish water. Similar to terrestrial plants, seagrasses have leaves, stems, roots, flowers, fruits, and seeds and can synthesize carbohydrate through photosynthesis [1]. On the other hand, seagrasses can adapt to wave and flow activity. The seagrass ecosystem is one of the typical tropical marine ecosystems and serves as organic matter, food source, habitat and spawning grounds for aquatic species. Seagrasses also play an important role in maintaining and regulating the economy of coastal water [2]. They have been used as folk remedies to treat a variety of diseases such as fever, skin conditions, muscle aches, burns, stomach issues and as pain relievers for kids [3]. Seagrasses have a therapeutic impact because they contain a variety of biologically active compounds including polyphenols, flavonoids, terpenoids, pectins, and others [4].

Pectins are a group of glycanogalacturonans composed mainly of α-D-galactopyranosiluronic acid (α-D-GalpA) residues linked together in a 1,4-configuration. They are classified into three categories depending on the polysaccharide they contain: (1) a linear homopolymer known as homogalacturonan (HG), (2) a branched polymer called rhamnogalacturonan I (RGI), and (3) substituted galacturonans, with rhamnogalacturonan II (RGII) being the

most widely distributed. HG accounts for 57–69% of pectin and is composed of 1,4-linked α-D-GalpA chains, where 8 to 74% of the carboxyl groups can be methyl-etherified [5].

The physicochemical characteristics of pectin are correlated with its degree of methylation (DM) [6]. Two common types of pectin can be distinguished based on their DM values. Pectin with a DM value above 50% is known as high-methoxylation pectin, while pectin with a DM below 50% is referred to as low-methoxylation pectin. The DM value has a notable influence on the gelation properties of pectin. When heated in a sugar solution at a concentration higher than 55% and a pH lower than 3.5, high-methoxylation pectin quickly gelates [7]. In the presence of calcium ions, low-methoxylation pectin can be formed with a tiny amount of sugar [8]. Pectins with diversity in structure and complexity are widely used in the food, drug, and cosmetic sectors due to their gelation properties, and employed in jellies, confections, medical treatments as a gelling agent and stabilizer. Based on their beneficial biological properties such as antioxidant, antibacterial, antiviral, anticancer, anti-inflammatory, and metal-binding activities, pectins can be used as medicine and functional foods [9].

Commercial pectins are mostly produced from citrus wastes (pulp and peel), apple pomace, and sugar-beet pulp [7,10]. Natural pectin is also extracted from various sources such as terrestrial plants and seagrasses [11]. Similar to other plant polysaccharides, the chemical structure and composition of seagrass pectin varies depending on seagrass species, extraction conditions, and other environmental factors. Pectin recovered from seagrasses possesses a low DM value, with methylation in less than 10% of the carboxyl groups in galacturonan, compared to pectin that extracted from terrestrial plants [12].

One distinctive aspect of the seagrass cell wall is the presence of low-methyl-esterified pectins rich in apiose [5]. Pectin found in seagrasses contains apiogalacturonan fragments, in which *D*-apiose residues are linked via 1,2- and/or 1,3- bonds to *D*-galacturonic acid residues; it has been isolated from *Zostera marina* [13], *Zostera caespitosa* [14], and *Phyllospadix iwatensis* [15]. This unique polysaccharide is referred to as zosterin in *Z. marina* and consists of a GalAp backbone with 1,2-linked apiose oligosaccharides or single apiose residues attached as substituents [14,16]. The biological activities of seagrass pectin revealed several pharmacological effects including antivirus, antibacterial, antitumor, antioxidant, and hypocholesterolemic properties [5,17,18]. The antioxidant property of pectin has recently garnered a lot of attention. It is well accepted that the structural characteristics, including monosaccharide composition, molecular weight distribution, and chemical structure, are connected to the antioxidant activity [19–21]. However, pectin structure–activity correlations are still unknown.

Fourteen distinct species of seagrasses are currently known to exist in Vietnam, with numerous and wide dispersion throughout the sea, but the most common species around the coast of Nha Trang bay were found to be *Enhalus acoroides*, *Thalassia hemprichii*, and *Halophila ovalis* [22]. Research on Vietnamese seagrasses has mostly focused on their ecology, distribution, and taxonomy [22–25], while their valuable biologically active components such as pectin, have not yet been systematically studied. In this study, valuable pectic polysaccharides from these three seagrass species were extracted, and their physiochemical characters and antioxidant activity were investigated.

2. Materials and Methods

2.1. Materials

Fresh Vietnamese seagrasses were collected in the Thuy Trieu lagoon, Khanh Hoa province, in March 2020. The fresh seagrasses were cleaned by sea water and ground into 2–3 mm pieces, and then the samples were immersed in ethanol 96% for 10 days to remove pigments and lipids. To extract pectin, the defatted seagrass samples were air-dried at room temperature and stored in plastic bags.

2.2. Pectin Isolation

Pectin was extracted by a previously described method [15]. Briefly, 200 g of dry seagrass sample was pretreated with a hydrochloric acid solution (1.0 M) at pH 2–3 (w/v = 1:20, weight of seagrass to acid solution ratio) for 3 h at 60 °C. After the hydrolysis, the seagrass biomass was rinsed with distilled water to remove the acidic solution. Pectin was extracted twice with a 0.5% ammonium oxalate solution (pH 6.0) at 85 °C for 3 h. The extracts were collected by centrifugation, then concentrated under vacuum, dialyzed against distilled water using a 10 kDa cut-off membrane for 48 h, and precipitated with four volumes of 98% ethanol. The precipitates were air-dried after rinsing with 70% ethanol and acetone. The yield of pectin was estimated by the ratio of the weight of the powdered pectin to the weight of the dried seagrass biomass (%, w/w).

2.3. Physicochemical Characterizations

2.3.1. Total Carbohydrate

The total carbohydrate content was determined using the phenol–sulfuric acid method [26]. Briefly, 1 mL of the polysaccharide solution was mixed with 1 mL of phenol solution (5%). After mixing, 5 mL of concentrated H_2SO_4 was rapidly added to the mixture, which was shaken. The mixture was set aside for 10 min at room temperature and then boiled in a water bath for 20 min. The absorption intensity was then measured at 490 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan).

2.3.2. Equivalent Weight

The determination of the equivalent weight of pectin was carried out according to a previous method [27]. Briefly, pectin (0.5 g), ethanol (5 mL), sodium chloride (1.0 g), and distilled water (100 mL) were added to a 250 mL conical flask. Then, six drops of phenol red were added, and the solution was titrated against NaOH (0.1 N). The titration point was indicated by a purple color. This neutralized solution was stored for the determination of the methoxyl content.

The equivalent weight (EW) was calculated by Formula (1).

$$\mathrm{EW} = \frac{\text{Weight of pectin sample (g)}}{\text{mL of alkali} \times \text{Molarity of alkali}} \times 100 \quad (1)$$

2.3.3. Methoxyl Content (MeO)

The MeO content of pectin was determined according to a previous method [27]. Sodium hydroxide (25 mL, 0.25 N) was added to the above-described neutral solution prepared for the determination of the equivalent weight. After carefully stirring, the mixture was kept at room temperature for 30 min. Then 25 mL of hydrochloric acid (0.25 N) was added, and the resulting solution was titrated against NaOH (0.1 N). The volume of alkali (mL) required for the neutralization reaction was measured, and the MeO content was calculated by the following Formula (2).

$$\mathrm{MeO\%} = \frac{\text{volume of alkali (mL)} \times 0.1 \times 31 \times 100}{\text{Weight of pectin sample (g)}} \quad (2)$$

2.3.4. Anhydrouronic Acid Content (AUA)

The AUA content of pectin was determined using the equivalent weight and the methoxyl content values that were obtained by the procedures described above [28]. The AUA value was calculated by Equation (3) [29].

$$\mathrm{AUA(\%)} = \frac{176 \times 0.1z \times 100}{W \times 1000} + \frac{176 \times 0.1y \times 100}{W \times 1000} \quad (3)$$

where y and z are the titled volume of NaOH from the AUA and equivalent weight determinations, respectively, and W is the weight of the pectin sample.

2.3.5. Degree of Esterification (DE)

The DE values were evaluated by the titrimetric method of the Food Chemical Codex [15] and calculated using Equation (4).

$$DE(\%) = \frac{176 \times MeO\ (\%)}{31 \times AUA\ (\%)} \times 100 \quad (4)$$

2.3.6. Molecular Mass Distribution

The HPLC system used was equipped with the following instruments: a CBM-20A prominence communications bus module, a DGU-20A5R degasser, an LC-20AD system controller, a degasser, a CTO-20A column oven, and an RID-20A refractive index detector (Shimadzu, Kyoto, Japan). Data acquisition and analysis were performed with the LC Solution Version 1.25 with GPC option.

The analysis of pectins was performed by high-performance size-exclusion chromatography (SEC) on the GPC column PSS SUPREMA combination ultrahigh (3 columns, dimensions 8 mm × 300 mm, particle size 10 µm, PSS, Mainz, Germany). Different dextran standards (Sigma, Cibolo, TX, USA) were used for the calibration: Dex1 (MW = 1.27 KDa), Dex5 (MW = 5.22 kDa), Dex12 (MW = 11.6 kDa), Dex25 (MW = 23.8 kDa), Dex50 (MW = 48.6 kDa), Dex150 (MW = 147.6 kDa), Dex270 (MW = 273 kDa), Dex670 (MW = 667.8 kDa). All reagents and mobile phases were prepared with ultrapure water. Each standard in triplicate at a concentration of 1.0 mg/mL was separately injected into the chromatographic column. Lithium nitrate (0.1 M) at a flow rate of 1.0 mL/min was used as the mobile phase. The column oven was set at 40 °C.

2.3.7. FTIR Spectroscopy

The infrared spectral analysis of pectin was performed on a Fourier transform infrared (FT-IR) spectrophotometer (Bruker Tensor 27, Bruker Corporation, Billerica, MA, USA) in the wavenumber range of 4.000–400 cm^{-1} with a resolution of 2 cm^{-1}. Powder samples of pectin were mixed with KBr powder and pressed into pellets for FT-IR spectra measurement in the frequency range of 400 to 4.000 cm^{-1}.

2.4. Antioxidant Assay

2.4.1. Total Antioxidant Activity

The total antioxidant activity was determined as previously reported [30]. A sample (1 mL) was added to 3 mL of a mixture containing 0.6 M H_2SO_4, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The solution was vortexed and kept for 90 min at 95 °C, and then the absorbance was measured at the wavelength of 695 nm. Ascorbic acid (AA) was used as a standard. All the results are expressed as mg of AA per gram of pectin sample.

2.4.2. DPPH Free-Radical Scavenging Activity

The free-radical scavenging activity was measured as described previously [31]. To this aim, 1,1-diphenyl-2-picrylhydrazyl (DPPH) was dissolved in ethanol to 0.2 mM, and a pectin sample was dissolved in distilled water to obtain a solution (1000 µg/mL) for testing the antioxidant activity. A sample (5 µL) was mixed with the DPPH solution in a ratio of 1:1. The mixture was kept at room temperature for 30 min in the dark. A UV–Vis spectrophotometer (UV-1800, Shimadzu, Japan) was used to measure the absorbance at 517 nm. The scavenging activity was calculated using Equation (5):

$$\text{DPPH scavenging activity}\ (\%) = \frac{A_0 - A_1}{A_0} \times 100 \quad (5)$$

where A_0 and A_1 are the absorbance of the control and of the sample, respectively.

2.4.3. Ferric-Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out according to a method described previously [31]. In this assay, 1 volume of a TPTZ (tripyridyl triazine) solution (10 mM) in HCl (40 mM) and 1 volume of $FeCl_3$ (20 mM) were mixed with 10 volumes of sodium acetate buffer (300 mM, pH 3.6) to create the FRAP agent before performing testing process. A sample solution (1 mL) was added to 3 mL of the FRAP reagent, and then the mixture was incubated at 37 °C for 30 min. The absorbance at 593 nm was recorded using deionized water as the blank sample. A fresh working solution of $FeSO_4$ was used for the calibration. From the linear calibration curve, the antioxidant activity, based on the ability to reduce ferric ions, of the sample was calculated and expressed as milligram of Fe^{2+} equivalents per gram of sample [32].

3. Results and Discussion

3.1. Pectin Isolation

Pectin from the three distinct species of Vietnamese seagrass *E. accoroides*, *H. ovalis*, and *T. hemprichii*, was extracted using a two-steps process. The dried samples were pretreated by hydrolysis with hydrochloric acid as the hydrolyzing agent, which was followed by pectin extraction using ammonium oxalate. In the first step, hydrochloric acid was used to degrade the bonds between pectin and hemicellulose and release the pectin chains from the seagrass samples. In the next step, pectin could be completely collected from the ammonium oxalate solution.

The yields of pectin and the total carbohydrate contents of various seagrass species are reported in Figure 1. The results showed that *E. accoroides* seagrass provided the highest pectin yield of 24.15%, followed by *T. hemprichii* (20.04%), and *H. ovalis* (19.14%), while *H. ovalis* had the highest carbohydrate content of 29.43%, followed by *E. accoroides* (27.28%), and *T. hemprichii* (24.68%). When compared to the other seagrass species which were also collected in temperate water areas, the pectin concentration and total carbohydrate content in the Vietnamese seagrass species appeared significantly different. In fact, the pectin and total carbohydrate contents found in the seagrass species *Zostera marina* were 11% and 39.1%, respectively [13,33] and those in *Phyllospadix iwatansis* were 6.91% and 39.45%, respectively [15,34]. The variation in the content of pectic polysaccharides and total carbohydrate content depends on the source of the seagrass biomass, the harvest time, the habitat of the seagrass species, and the extraction method [15,16,27,35]. For example, Neelakandan et al. extracted pectin from Indian *H. ovalis* seagrass (6.5%) using a mixture of sodium acetate, papain, ethylenediamine tetraacetic acid, and cystein, heating the samples at 60 °C for 24 h [36]. In the present work, the acidic hydrolysis method [15] was used with slight modifications such as changes in the extraction time and temperature and using ammonium oxalate after the hydrolysis. In general, the pectic polysaccharides in each species of seagrasses vary in total carbohydrate content, which leads to a great diversity of biological activities [13,15,34,37].

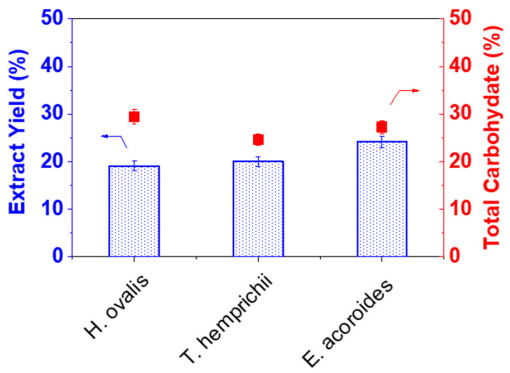

Figure 1. Yield (bar, left axis) and total carbohydrate content (red solid square, right axis) of pectin isolated from Vietnamese seagrass species.

3.2. Physicochemical Characterizations

Anhydrouronic acid (AUA) is a principal parameter used to determine the purity of pectin [35]. The AUA content is also crucial to determine the DE values, as well as to evaluate physical properties of pectin. The percentage of uronic acid in Vietnamese seagrass pectin is presented in Figure 2A. The results showed that the highest uronic acid content was determined for pectin isolated from *H. ovalis* (79.4%), followed by those from *E. acoroides* pectin (68.1%) and from *T. hemprichii* (65.5%). The uronic acid content of *H. ovalis* pectin was higher than those from *Z. marina* (71.5%), *P. iwatenis* (74.9%), and *T. ciliatum* (55%) [26,37], while the uronic acid content of pectin from *T. hemprichii* and *E. acoroides* was lower than the values obtained for *Z. marina* and *P. iwatenis* pectins. Another study found that *P. iwatensi* pectin contained 57.2% of uronic acid [15]. The difference in uronic acid content between the two studies could be explained by the fact that different extraction method as well as raw material sources were used. The uronic acid content of pectins obtained from the Zosteraceae species *Z. marina*, *Z. pacifica* and *P. iwatenis* [33] was 38%, 36%, and 40%, respectively, and was significantly lower than those of pectin extracted from Vietnamese seagrass and other sources [15]. Therefore, as for pectin obtained from vegetables and fruits, the uronic acid content of seagrass pectin depends on the source of pectin and the method used to extract it [27,28].

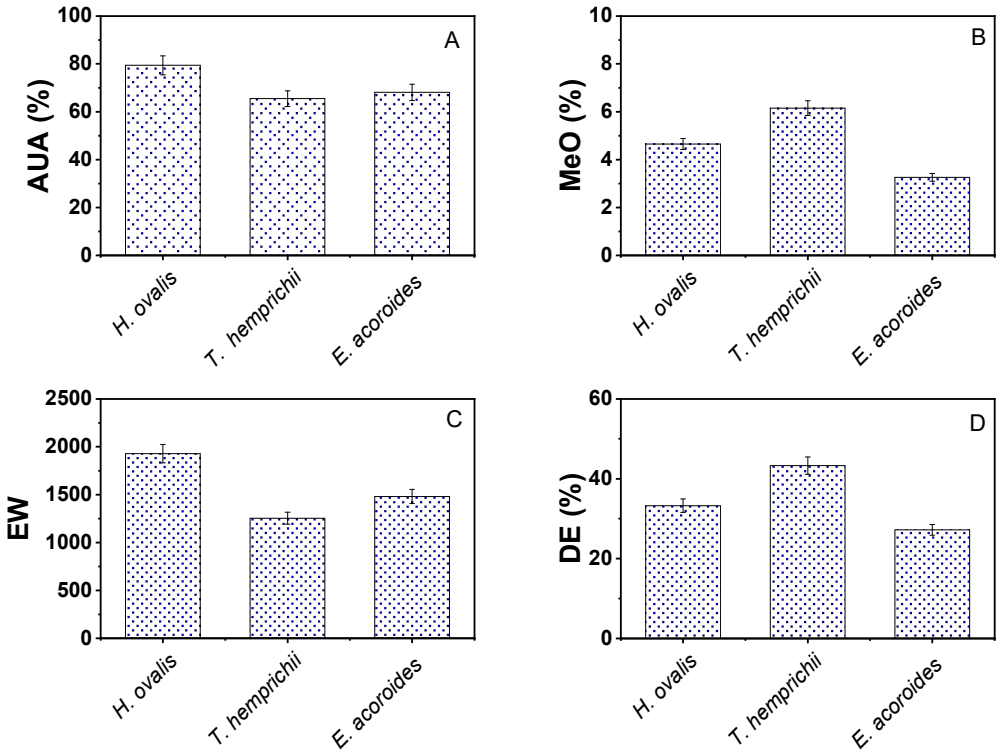

Figure 2. Anhydrouronic acid content (**A**), methoxyl content (**B**), equivalent weight (**C**), degree of esterification of pectin from three Vietnamese seagrass species (**D**).

The MeO value is an important factor in controlling pectin setting time and gelation ability. The MeO analysis is shown in Figure 2B. The results showed that the MeO content of *T. hemprichii* pectin (6.15%) was higher than that of *E. acoroides* (4.65%) and *H. ovalis* (3.26%). These values were higher than the MO content of pectin from other seagrass species. Ovodova et al. found that pectin from *Z. marina, Z. pacifica* and *Phyllospadix* had a MeO value of 0.97%, 0.80%, and 0.78%, respectively [33]. The MeO value of pectin is affected by the source of pectin as well as by the MO determination method [35]. The MO content of pectin derived from Vietnamese seagrasses indicated a low-methoxyl pectin with a MO value of less than 7%.

The EW was used to calculate the AUA content and the degree of esterification (DE). Figure 2C shows the EW values of pectin isolated from *H. ovalis*, *T. hemprichii*, and *E. acoroides*. The result showed that pectin from *H. ovalis* had the highest EW value (1928.6), followed by *E. acoroides* (1480.5) and *T. hemprichii* (1253.4). The EW of Vietnamese seagrass pectin is significantly higher than that of pectin isolated from other sources such as red and white grapefruit peel (624 and 749, respectively), *Azanza garckeama* fruit (813.64), and orange peel powder (381–485) [10,29,38]. The EW values of pectin are highly dependent on pectin material source and extraction method [10]. The gelation ability of Vietnamese seagrass pectin with a high equivalent weight is expected to be great.

The analysis of pectin DE is presented in Figure 2D. The result showed that pectin DE significantly depended on the seagrass species. *T. hemprichii* possessed the highest pectin DE value (43.31%), followed by *H. ovalis* and *E. acoroides*, with 33.25% and 27.18%, respectively. These findings revealed that Vietnamese seagrass pectin is a low-methyl-esterified pectin, with less than 50% DE [35]. However, the DE of these species is significantly higher than that of previously reported seagrass-derived pectin, such as pectin from *Z. marina* (7.9%) and *P. iwatensis* (10.2%) [16], whereas these values are significantly lower than those measured for terrestrial plant-derived pectins, which are estimated to be in the range of 50–76% [39–41]. These DE values suggest that this pectin may have more sophisticated physicochemical properties in comparison to other types of pectin. A high-DE pectin can hasten the gelation setting process, but its water solubility is low [29].

The MW distribution of Vietnamese seagrass pectin was evaluated by size-exclusion chromatography (SEC), as shown in Figure 3. The physicochemical characterization of the examined pectins is summarized in Table 1. The highest average MW was found for pectin isolated from *T. hemprichii* (173 kDa), followed by those from *E. acoroides* (127 kDa) and *H. ovalis* (56.6 kDa). In particular, for *E. acoroides* pectin, we observed a shoulder that corresponded to a high MW region (170 kDa), which indicates a broad polydispersity index (PDI) of this pectin. The results confirmed that the average MW of pectin strongly depends on the source of the crude materials. Indeed, the MW of pectin from these seagrass species was found to be similar to the average MW of pectin from apple, citrus, and dragon fruit (with MW of 144 kDa, 138 kDa and 88 kDa, respectively) [42], whereas it is significantly lower than those of pectin from fresh white cabbage (351 kDa), carrot (827 kDa), onion (900 kDa), sweet pepper (571 kDa), and the seagrass *Phyllospadix iwatensis* (325.45 kDa) [15,41]. Some species possess pectin with a MW lower than 100 kDa, such as *Zostera marina* (62 kDa) and *Zostera caespitosa* (77.2 kDa) [14,43].

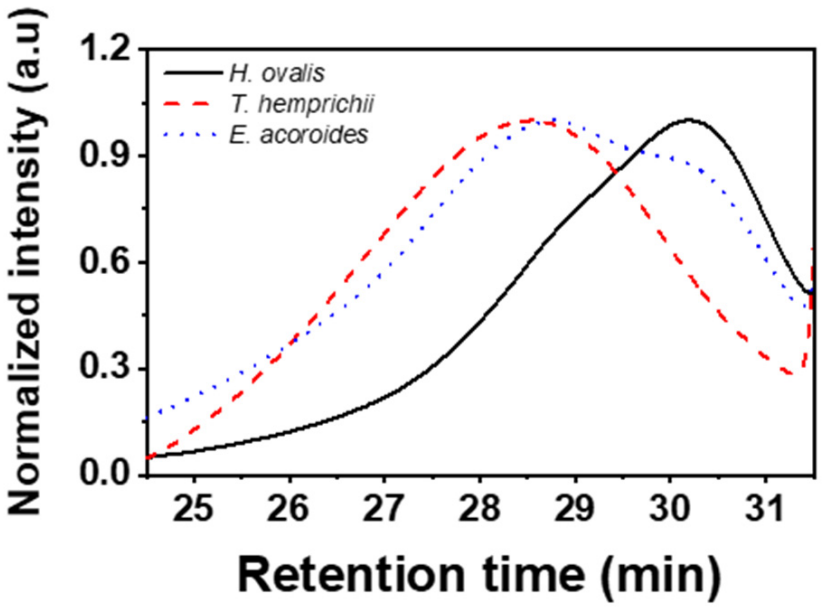

Figure 3. GPC curves of Vietnamese seagrass pectin.

Table 1. Summary of the physicochemical characterization of pectins extracted from the examined Vietnamese seagrasses.

Pectin	Extraction Yields (%)	MW (kDa)	Total Carbohydrate (%)	AUA (%)	MeO (%)	EW	DE (%)
H. ovalis	19.14	56.6	29.43	79.4	3.26	1928.6	33.25
T. hemprichii	20.04	173	24.68	65.5	6.15	1253.4	43.31
E. acoroides	24.15	127	27.28	68.1	4.65	1480.5	27.18

The functional groups of Vietnamese seagrass pectin were determined using FTIR spectroscopy, as shown in Figure 4. The spectra revealed that the absorption bands of the three types of seagrass pectin were similar. The broad bands at 3127–3417 cm^{-1} were assigned to the asymmetric stretching of hydroxyl groups [44]. The stretching vibration of the double-bond region is responsible for the bands at 1500 to 1800 cm^{-1}. The intense bands at 1596–1662 cm^{-1} correspond to the carboxyl (COO^{-}) group [45]. The O-CH_3 groups in the polysaccharide chains are represented by the bands at 952–953 cm^{-1}. Differences in the FTIR data of the extracted pectins were observed in the bands at 1200–900 cm^{-1} [46], which were attributed to the C–O–C glycosidic linkage and the C–C vibrational modes. This finding confirmed the presence of saccharide molecules in the extracted samples.

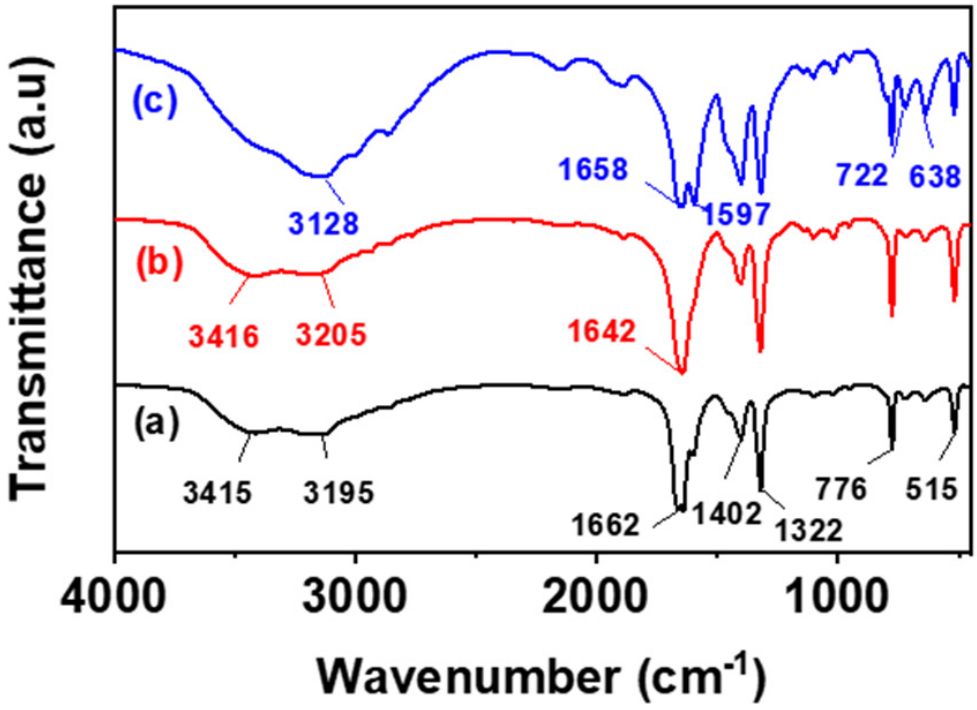

Figure 4. FTIR spectroscopy of pectin extracted from *H. ovalis* (**a**), *T. hemprichii* (**b**), and *E. acoroides* (**c**).

3.3. Antioxidant Activity

Figure 5 depicts the antioxidant activity of pectins extracted from the Vietnamese seagrass species, including total antioxidant capacity, ferric reducing antioxidant power (FRAP), and DPPH radical scavenging activity. The total antioxidant capacity and ferric reducing activity revealed that all seagrass pectins possessed different levels of activity (see Figure 5A,B). The strongest total antioxidant capacity was determined for *E. acoroides* pectin (32.72 mg/g), followed by *T. hemprichii* pectin (15.53 mg/g) and *H. ovalis* pectin (12.67 mg/g), while *H. ovalis* pectin had the greatest FRAP activity, corresponding to 1151.56 mg/g, followed by *E. acoroides* and *T. hemprichii* pectins, with values of 726.89 mg/g and 599.14 mg/g, respectively.

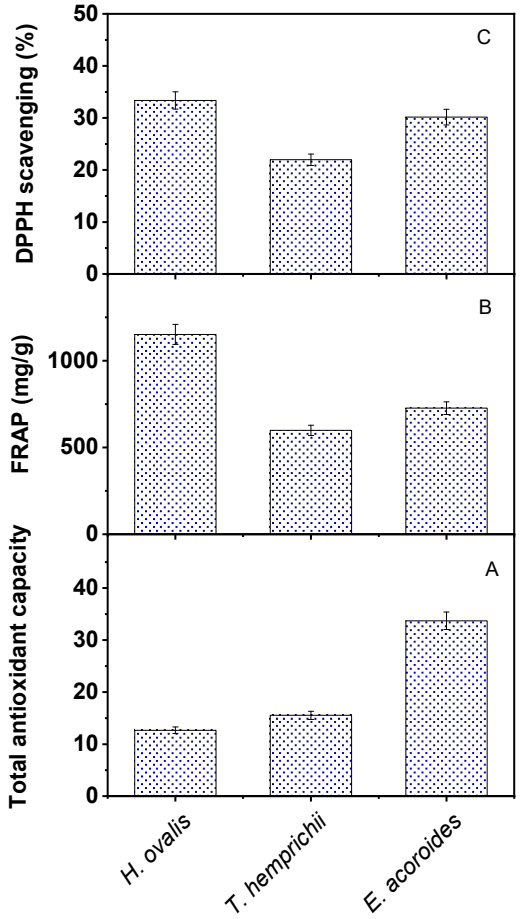

Figure 5. Total antioxidant capacity calculated in milligram of ascorbic acid per gram of pectin sample (**A**), ferric reducing antioxidant power (FRAP) activity (**B**), DPPH radical scavenging activity (**C**) of Vietnamese seagrass pectins.

A DPPH radical scavenging assay was also carried out to test the ability of the antioxidant compounds to function as proton radical scavengers or hydrogen donors [47]. Figure 5C shows the DPPH radical scavenging activity of the Vietnamese seagrass pectin. *H. ovalis* pectin had the highest DPPH radical scavenging activity (33.36%), followed by *E. acoroides* pectin (30.16%) and *T. hemprichii* pectin (21.98%). The result revealed a high antioxidant activity of Vietnamese seagrass pectin, which was attributed to the abundant presence of -OH and -COOH groups of galacturonic acid in the polysaccharide chains [48,49]. The result is agreement with previous reports [50,51]. The low-etherified pectin of the eelgrass *Z. marina* showed antioxidant activity superior to that of two antioxidant medicines, mildronat and empoxipin [51]. Another study found that the sulfated polysaccharide fractions extracted from *H. ovalis* seagrass had good antioxidant activity [36]. The Vietnamese seagrass pectin outperformed the DPPH radical scavenging activity of vegetable and apple pectins, which were found in the range of 10–30% [41,52]. The varying antioxidant activity of pectin could be attributed to differences in their molecular weight and galacturonic acid content. Although the mechanism is not fully understood, polysaccharides with a low molecular weight and a high uronic acid content have been shown to increase the antioxidant activity [31,53]. These findings suggest that pectin extracted from the three examined Vietnamese seagrasses could be used as potential antioxidants.

4. Conclusions

In this work, pectin from the three Vietnamese seagrass species *E. acoroides*, *T. hemprichii*, and *H. ovalis* were examined. The results revealed that the seagrasses have a high pectin content ranging from 19.14 to 24.15%. A physicochemical characterization including the

determination of the content of anhydrouronic acid, the equivalent weight, the methoxyl content, the degree of esterification, and the average molecular weight was carried out. The results showed that seagrass pectin is a low-methoxyl pectin. In the evaluation of the antioxidant activity, these pectins exhibited a high activity. These findings suggest that Vietnamese seagrasses have the potential to be developed as a biomaterial material for further applications in functional food. This could involve conducting clinical trials to evaluate the efficacy of seagrass pectin to help promote the sustainable utilization of marine resources.

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