sustainability

MDPI

Article

How Extraction and Purification Affect MALDI-TOF MS Characterization of Mangrove Condensed Tannins, An Ecologically Important Secondary Metabolites in Coastal Wetland Ecosystem

Tao Lang [1,2,3], Ping Xiang [4], Mingdang Li [1,3], Zhiteng Chen [1,3], Fenglan Li [3,5], Mingguo Jiang [5] and Haichao Zhou [1,*]

1 MNR Key Laboratory for Geo-Environmental Monitoring of Great Bay Area & Shenzhen Key Laboratory of Marine Bio-Resource and Eco-Environmental Science, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, China
2 College of Physics and Optoelectronic Engineering, Shenzhen University, Shenzhen 518060, China
3 Greater Bay Area Coastal Mangrove Wetland Research & Development Centre, Guangdong Neilingding Futian National Nature Reserve, Shenzhen 518040, China
4 Key Laboratory of Ministry of Education for Coastal and Wetland Ecosystems, Xiamen University, Xiamen 361005, China
5 School of Science and Technology, The Hong Kong Metropolitan University, Ho Man Tin, Kowloon, Hong Kong 999077, China
* Correspondence: zhouhc@szu.edu.cn

check for updates

Citation: Lang, T.; Xiang, P.; Li, M.; Chen, Z.; Li, F.; Jiang, M.; Zhou, H. How Extraction and Purification Affect MALDI-TOF MS Characterization of Mangrove Condensed Tannins, An Ecologically Important Secondary Metabolites in Coastal Wetland Ecosystem. *Sustainability* **2022**, *14*, 14960. https://doi.org/10.3390/su142214960

Academic Editors: Long Sun, Wenzhao Guo and Tianjiao Feng

Received: 12 October 2022
Accepted: 10 November 2022
Published: 11 November 2022

Abstract: Mangrove plants are rich in tannins, especially condensed tannins (CTs), which play an important role in biogeochemistry in coastal wetland ecosystem due to their functions of binding nutrients and heavy metal chelation. This study aims to obtain authentic chemical structures of mangrove CTs by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Four organic solvents (n-hexane, ether, chloroform, and EtOAc (ethyl acetate)) were used for extraction tests and three purification methods (Method 1, Sephadex LH-20 absorbed tannins, and nontannins fraction were washed by 50% methanol (mp) solution; Method 2, Sephadex LH-20 absorbed tannins and nontannins fraction were washed by 100% ethanol (ep), and released in 70% acetone solution; and Method 3, Yb^{3+} selectivity precipitated tannins) were conducted to investigate their influences on the characterization of CTs from two mangrove species, *Bruguiera gymnorhiza* and *Kandelia obovata*. The results showed that (1) EtOAc was used as an extraction solvent, leading to unauthentic structural properties of CTs; (2) the distribution patterns of the polymers in mangrove CTs purified with 50% methanol elicited the least different trends with those of CTs in the two mangrove crude extracts, and the lower oligomers (dimmer-hexamer) and higher polymers were lost during purification of CTs by 100% ep. Therefore, based on the toxicity and price of solvents, the crude CTs from mangrove plants can be extracted with n-hexane or ether to remove lipid and pigment impurities and then purified with 50% methanol, which is a complete set of methods to obtain completely authentic structural information of mangrove CTs. This study can offer more accurate structural information of mangrove CTs and new insights for the conservation of mangrove living environments for follow-up research.

Keywords: mangroves; condensed tannins; extraction; purification; MALDI-TOF MS

1. Introduction

Mangrove forests are unique tidal saline wetland ecosystems that are found along the upper intertidal regions of tropical and subtropical coasts and estuaries [1]. Mangrove plants are extremely abundant in large numbers of tannins, which are vital secondary metabolites and are the largest subgroup of macromolecular phenolic compounds in nature [2]. Interestingly, mangroves in China are collectively named "red trees" since

their internal tannins are peculiarly prone to being oxidized by air exposure and then turning red [3]. Previously, several studies discovered that the classes of tannins from common mangrove species in China fell into two major categories: condensed tannins (CTs, mainly from *B. gymnorhiza*, *K. obovata*, *Aegiceras corniculatum*, etc.) and hydrolysable tannins (HTs, mainly from *Sonneratia apetala*, *Sonneratia caseolaris*, *Avicennia marina*, etc.) [4–7]. Currently, considerable evidence has revealed that mangrove tannins, especially CTs, are involved in multiple ecological processes in mangrove wetland ecosystems, such as allelopathy [8], heavy metal chelation [9], nitrogen (N) resorption [6], and microbial diversity alterations [10,11]. In terms of the conservation of mangrove surroundings, two previous studies showed that (1) CTs from *K. obovata* could chelate massive quantities of Cr, Zn, Cu, Pb, Ni, and Cd in sediments of the Zhangjiang River Estuary [12]; (2) bound CTs (BCT), such as protein-bound CTs, in leaves of *K. obovata* visibly increased during the decomposition stage, suggesting that CTs played a key part in humification during N immobilization, and then they could avoid the loss of N nutrient in sediments [6]. Based on the various crucial functions of CTs, their accurate determination is the overwhelming foundation for research on mangrove wetland ecosystems; hence, it is indispensable and valuable to draw out a professional specification for CT extraction and purification prior to their determination.

The basic formula of plant CTs is uniformly composed of polymers of three-ring flavanols linked with C–C and/or C–O–C bonds [13]. However, the variations in monomer units, degree of polymerization (DP), pattern of hydroxylation, glycosylation, substitutions, and intermonomer linkages make the structures of CTs generally diverse and complex [14] and contribute to the chemical properties [13,15,16]. Indeed, this is precisely because the reactive chemical characteristic of CTs is the cornerstone of their matrices, physiological activities, effects on animal nutrition metabolism, and remarkable ecological functions [13,17,18].

Compared to other softer electrospray ionizations, matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) can directly give the molecular/excimer ion peak of polymers with different molecular weights in CTs, which has an important potential value for the analysis and determination of the distribution mode of polymers, the degree of polymerization, and the hydroxyl mode in CTs [15,19] In addition, MALDI-TOF MS has a high resolution up to 1 Da due to the application of the reflection mode and delay extraction technique [20]. MALDI-TOF MS can avoid operations of cumbersome purification and separation, directly analyzing plant tannins in crude extract from samples, and even performing direct and rapid analysis of plant samples with a solid form [21]. However, in the actual analysis process for CTs, due to the content of tannins in the crude extract and the uncertain coexistence in the crude extract, not all the tannins in the crude extract of plant samples can be successfully analyzed by MALDI-TOF MS [5]. Therefore, prior to analyzing CTs through MALDI-TOF MS, some pretreatments are needed, including liquid-liquid extraction and purification, the method selection which would have a crucial impact on the result analysis of CTs.

In general, the extraction of CTs from the aqueous phase contains three optional solvents, diethyl ether, chloroform, and EtOAc, which are used to remove non-tannic components, for example, lipids, pigments, and polyphenols with small molecules [22]. Moreover, Dunford et al. (2010) found that n-hexane, petroleum ether, and chloroform, which possessed low dielectric constant solvents, were capable of efficiently extracting lipids [23]. Nevertheless, EtOAc is generally considered an extracting solvent which owns the greatest influence on extraction in terms of yield, phytochemical content, and antioxidant properties due to its polarity [24]. The purification methods for CTs prior to characterization include column chromatography via Sephadex LH 20 [25,26] and Yb^{3+} precipitation [27,28]. Additionally, pure ethanol is a potential purification solvent but the strong polarity depends on its easier soluble capacity for low molecular weight materials [29,30]. Butler et al. (1982) discovered that methanol was the best choice for purification since the reaction was much less sensitive to monomer units [31]. However, the best approaches of extraction and purification for mangrove CTs remain to be further investigated.

B. gymnorhiza and *K. obovata* are two true viviparous mangrove species that are widely distributed in the intertidal zone of southern China [32,33]. In the present study, the above-mentioned methods were used to extract and purify CTs from the calyx (mainly the part of the calyx tube) of *B. gymnorhiza* and the mature hypocotyl of *K. obovata*. Eventually, this study aimed to clarify the effects of extraction and purification methods for mangrove CTs on the distribution pattern of polymers, polymerization degree, and hydroxyl pattern using MALDI-TOF MS, which would offer more accurate structural information of mangrove CTs and new insights for the conservation of mangrove living environments for follow-up research.

2. Materials and Methods

2.1. Mangrove Material Collection and Pretreatment

B. gymnorhiza calyx and *K. obovata* hypocotyl without disease and mechanical damage were considered the present experimental materials, which were collected from the Jiulong River Estuary of Fujian Province, China (24°24′ N, 117°55′ E). The collected samples were placed in cold storage bags, immediately brought back to the laboratory, and extracted three times with 70% acetone solution. After that, the mangrove-extract solution was evaporated at 30 °C under rotary pressure to remove acetone. Then, the aqueous phase was freeze-dried to obtain the crude extract of CTs for later use.

2.2. Extraction and Purification of Mangrove Condensed Tannins

Both mangrove CT extraction and purification experiments needed to be performed prior to the MALDI-TOF MS test. In this study, *B. gymnorhiza* calyx as mangrove materials were used for selecting the potential method for extraction. First, 10 mg of crude extract from *B. gymnorhiza* calyx was weighed and then dissolved into 1 mL of 30% acetone solution (solid materials needed to be dissolved completely). After that, the CT crude extract solution was further extracted three times with n-hexane, ether, chloroform, and EtOAc. Subsequently, three replicates were mixed into one sample. The aqueous phase of the extracted crude extract was evaporated at 30 °C under rotary pressure to remove the organic solvent for backup use. After this extraction treatment, 5 types of mangrove samples were obtained, such as untreated Bg crude extract (B), Bg samples extracted with n-hexane (B-h), ether (B-e), chloroform (B-c), and EtOAc (B-a).

For the purification section, crude extract from mangrove CTs was dissolved with a small amount of 50% methanol solution or more than 80% ethanol solution, placed on a Sephadex LH-20 column, and then eluted with 50% methanol solution until no obvious color was found in the eluent [34,35] (or eluted with pure ethanol until the absorbance of the eluent approached 0 at 280 nm [36]). After that, the CT samples adsorbed on the Sephadex LH-20 column were eluted with 70% acetone solution. The eluent was then collected and evaporated at 30 °C under rotary pressure to remove acetone, and the aqueous phase was freeze-dried for later use. In addition, a detailed description of the CT samples prepared by Yb^{3+} precipitation was performed according to Krueger et al. (2000, 2003) [27,28]. Like the extraction section, 6 types of *B. gymnorhiza* and *K. obovata* samples were obtained from crude extract after the purification or precipitation treatments, such as Bg/Ko samples purified with 50% methanol (B-mp/K-mp), pure ethanol (B-ep/K-ep), and Yb^{3+} precipitation (B-ybp/K-ybp).

2.3. Characterization of Condensed Tannin Using MALDI-TOF MS

In this study, MALDI-TOF-MS was used to analyze the degree of polymerization (DP) and different segment distributions of mangrove CTs. The MALDI-TOF-MS analysis was performed using a Bruker Reflex III MALDI-TOF MS (Bruker, Bremen, Germany), and its parameters were set as follows according to previous study [37].

Specifically, the irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm, and the duration of the laser pulse was 3 ns. Under the reflection mode, the MALDI-TOF MS of fractions FE, FW, and F1–F4 were recorded with a reflection voltage

of 23.0 kV and an accelerating voltage of 20.0 KV in the process of deionization with Amberlite IRP–64 cation exchange resin and 1.52 mg/mL CsCl (Cs^+) as the cationization reagent. Angiotensin ii (1046.5 MW), Bombesin (1619.8 MW), ACTHclip 18–39 (2465.2 MW), and somatostatin 28 (3147.47 MW) were used as external standards. The MALDI-TOF mass spectrograms of CT samples were acquired by the accumulation of 100–150 laser bombardments of CT crystals.

The matrix (DHB, 10 mg/mL of 30% acetone solution) and samples, such as 10 types of extracted CT crude extract and 6 types of purified CT materials, which were dissolved in 10 mg/mL of 30% acetone solution, were fully deionized with a strong acid cation exchange resin, Dowex 50 × 8–400. After that, the deionized sample solutions were mixed with NaCl (0.52 mg/mL aqueous solution) and CsCl (1.52 mg/mL aqueous solution) in a volume ratio of 1:1, and the mixture was immediately mixed with the deionized matrix solution in a volume ratio of 1:3. Then, the samples were spotted on the sample target.

2.4. Statistical Analysis

Since CTs are mixtures of polydisperse polymers with similar properties, the mean degree of polymerization (mDP) and the distribution patterns of different polymers have become the most used indexes to measure their structures and activities [32,35]. The mDP was calculated by comparing the peak areas based on the equation as follows:

$$\mathrm{mDP} = \frac{\sum_i m_i \times Pmax_i \times CCm_i}{\sum_i Pmax_i \times CCm_i}$$

where m is the DP of each polymer, $Pmax$ is the absolute or relative intensity of the most intense molecular ion peak or isotope peak in each polymer isotope cluster in the mass spectrogram, CCm is the correction coefficient corresponding to the respective DP.

In the present study, all the comparisons between different treatments were conducted by paired sample t test using SPSS software (version 17.0, IBM, NY, USA).

3. Results and Discussion

3.1. Effects of Different Extraction Methods on the Results of CT Structure Analysis

In the present study, four organic solvents, i.e., n-hexane, ether, chloroform, and EtOAc, were used to remove the lipids, pigments, and micromolecular polyphenols in the crude extracts of *B. gymnorhiza* calyx and *K. obovata* hypocotyl. It was found that the distribution patterns of polymers in CTs and the results of their corresponding mDP were affected by the extraction with different solvents (Figure 1). Specifically, EtOAc had the most obvious effects on the analysis results, which resulted in a decrease in the composition proportion of flavan-3-ol oligomers with small DPs in CTs of *B. gymnorhiza*, indicating that the greater polarity and strong extraction ability of EtOAc might play a key role in the interesting results (Figure 1). Indeed, Nawaz et al. (2020) reported that extraction yield, phytochemical content, and antioxidant properties could be notably influenced by the polarity of extracting solvents, such as EtOAc [24]. The better extraction capacity of EtOAc mainly depended on its stronger biocompatibility and selectivity [24]. In addition, EtOAc had a low boiling point of 77.0 °C at 1 atm, which was subsequently conducive for extraction from the solvent with less energy input [38]. Similarly, the results from the crude extract of *K. obovata* hypocotyl were in keeping with those from *B. gymnorhiza* calyx. Dunford et al. (2009) considered that low dielectric constant solvents, such as n-hexane, petroleum ether, and chloroform, were capable of efficiently extracting lipids, which was in accordance with resent results [23]. Nevertheless, based on the security, price, and all the extraction results, n-hexane or ether should be selected as the potential extraction solvents to obtain the true structural information of mangrove CTs in the process of removing non-tannin fractions.

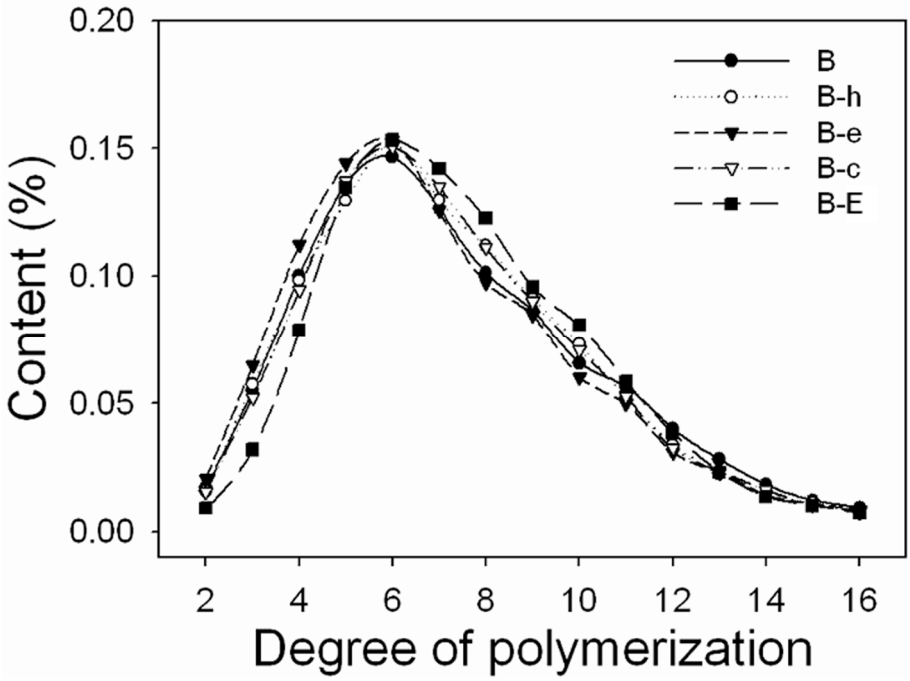

Figure 1. Distribution patterns of flavan-3-ol oligomers and polymers in the crude extract from *B. gymnorrhiza* calyx which was further extracted by different solvents, Cs^+ as the ionization reagent. Note: B-h, B-e, B-c, B-E, and B, mean CTs from *B. gymnorrhiza* calyx were extracted by n-hexane, ether, chloroform, and EtOAc or not, respectively.

3.2. Effects of Different Purification Methods on the Results of CT Structure Analysis

After further purification of these mangrove samples, the structural characteristics of the CTs purified by the three purification methods were notably changed compared with those in the crude extract. For instance, *B. gymnorhiza* calyx samples were analyzed using MALDI-TOF MS prior to purification with pure ethanol (B-ep), 50% methanol (B-mp), and Yb^{3+} precipitation (B-ybp) or not (B), and the results are shown in Figures 2 and 3 (quantitative information summarized from Figure 2). Specifically, in comparison with control group (B), B-mp, and B-ybp, the structural composition of B-ep was strikingly different using MALDI-TOF MS analysis, namely, some low polymers (trimer to pentamer) were obviously lost by purification with pure ethanol (Figure 2). This phenomenon was mainly because oligomers in mangrove CTs were low molecular weight phenolic compounds [5,6], which were easily soluble in a solvent with strong polarity, such as pure ethanol [29,30]. Furthermore, compared with B, the distribution patterns of polymers in CTs of B-ep were the most different, followed by CTs of B-ybp, while the distribution patterns of the polymers in CTs of B-mp purified with 50% methanol elicited the least different trend (Figure 3), indicating that 50% methanol might be the best method for mangrove CT purification. Indeed, Butler et al. (1982) found that an organic solvent, glacial acetic acid, could be used for the estimation of DP during the quantitative determination of proanthocyanins (CTs); however, methanol seemed to be the best choice for DP estimation since the reaction was much less sensitive to monomer units such as catechin than it was to polymeric tannins [31].

DP is an important factor which is dramatically affected by the purification process [39]. In Figure 3A, the mDP values of B-ep, B-mp, and B-ybp were different from that of the control group. Specifically, the mDP value of B-ep obtained by purification with pure ethanol was higher (8.5), further suggesting that some polymers with low DP in CTs might be easily dissolved in pure alcohol due to their strong polarity [29,30]. Nevertheless, the mDP values of B-mp and B-ybp were 6.9 and 6.4, respectively, which were lower than that of CTs in crude extract (7.4, Figure 3A). Moreover, the composition ratios of polymers with low DP in CTs from B-mp and B-ybp were higher than those in CTs from crude extract (Figure 3A); however, the trimer, tetramer, and pentamer in B-ep were

eluted from a Sephadex LH20 column by pure ethanol along with impurities during purification (Figure 2D). In terms of the CT purification methods with 50% methanol and Yb^{3+} precipitation, the composition ratio of polymers with low DP in CTs increased, indicating that a certain proportion of polymers with high DP in CTs might be lost in the purification process during these two types of purification processes. In Figure 3B, the relative abundance of nonamer was defined as unit 1 for benchmark to clearly illustrate the alterations and distinctions among different purification treatments and control in terms of DPs. Compared to the control group, the purification of CTs via all three of the abovementioned purification methods led to the loss of polymers with high DPs, especially those ranging from nonamer to hexadecamer (Figures 2 and 3B). Indeed, Belone et al. (2021) reported that there was a reduction in the tensile strength of polymers of approximately 50–60% after purification, suggesting that polymers with high DP could be prone to fragment into smaller pieces during the process, which was broadly in line with the resent results [40]. In summary, the selection of the purification method could induce alterations in DP and true structural information of mangrove CTs.

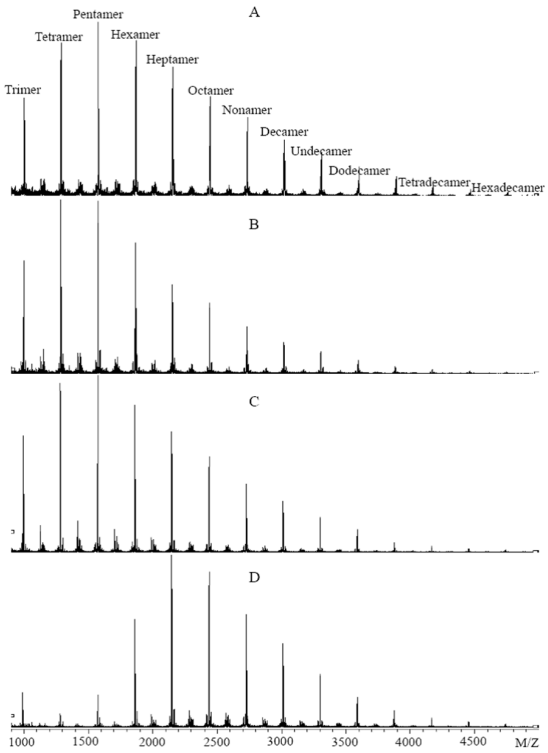

Figure 2. MALDI-TOF spectra of different purification methods of CTs from *B. gymnorhiza* calyx under linear mode. Note: (**A**), crude extract; (**B**), B-ybp; (**C**), B-mp; (**D**), B-ep.

Compared to the structural characteristics of CTs from *B. gymnorhiza* calyx, the effects of various purification methods on those from the *K. obovata* hypocotyl sample were obviously inconsistent (Figure 4A,B). In terms of DP, the trend of the crude extract from *K. obovata* hypocotyl was like that from *B. gymnorhiza* calyx; the mDP in K-mp was smaller, that in K-ep was larger, and that in K-ybp was like that in the crude extract (Figure 4A). However, the CTs contained in the *B. gymnorhiza* calyx were simpler in structure than those contained in the *K. obovata* hypocotyl. The polymers with different DPs in *B. gymnorhiza* calyx were mainly homopolymers composed of catechin/epicatechin (C/EC) structural units, while the latter contained large numbers of heteropolymers, which were composed of C/EC and gallocatechin/epigallocatechin (GC/EGC) structural units [5]. Like CTs from *B. gymnorhiza* calyx, some high polymers ranging from nonamers to hexadecamers of CTs from *K. obovata* hypocotyl were lost as a result of sample purification, regardless of the purification method used (K-ybp, K-mp, and K-ep, Figure 4C). However, since the number of heteropolymers

in each polymer was more than that of homopolymers in terms of the hydroxyl groups, a larger proportion of these polymers was retained in the process of Yb^{3+} precipitation and column chromatography purification with Sephadex LH 20 as a chromatograph filler, resulting in partial concentration effects. Typically, Zhou et al. (2012) in one of the previous studies considered a higher DP should correspond to a lower solubility and a greater resistance to degradation [6]. Hence, the distribution patterns of K-ybp, K-mp, and K-ep, especially K-ybp, in the high molecular weight region were different from those of the homopolymers from nonamers to hexadecamers (Figure 4B,C). Compared to CTs in the crude extract of *K. obovata* hypocotyl, some of the polymers with high DP in K-mp and K-ep were still lost in terms of the polymer distribution patterns from nonamer to hexadecamer, especially those between dodecamer and hexadecamer (Figure 4B,C). Furthermore, using pure ethanol to remove impurities from CTs would still lead to the loss of polymers with low DP. Compared with the CTs in the crude extract, the CTs obtained after purification with pure ethanol still had the highest distortion degree in structural features.

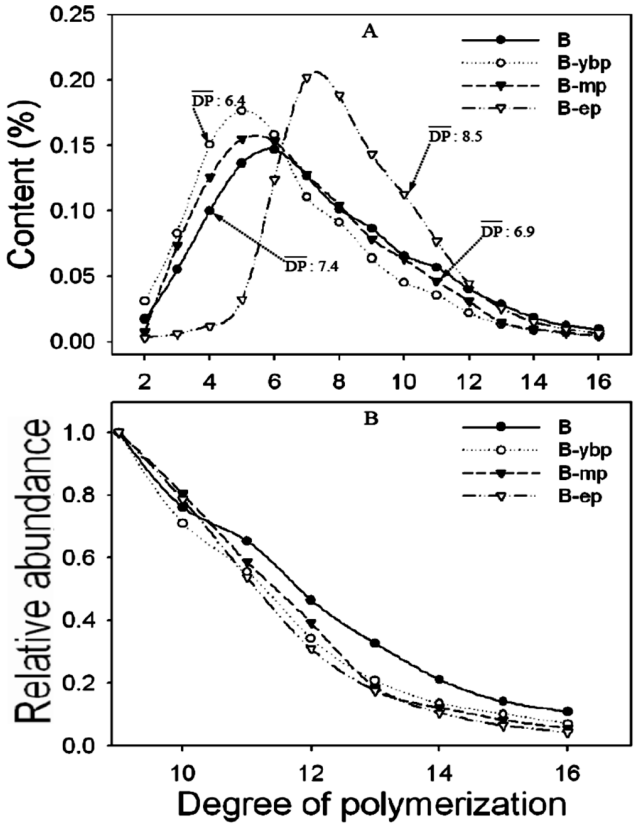

Figure 3. The mDP and distribution patterns of a full range of polymers (**A**) and specific polymers with DP from nonamer to hexadecamer in the high molecular weight region (**B**) after purification with 50% methanol (B-mp), pure ethanol (B-ep), and Yb^{3+} precipitation (B-ybp) or not (B). In Figure 3B, the relative abundance of nonamer was defined as unit 1 for benchmark.

The changes in the hydroxyl mode of CTs obtained by the three different purification methods were more complicated. The hydroxyl distribution patterns of the hexamer in K-ybp obtained by purification with Yb^{3+} precipitation was consistent with those in the crude extract of *K. obovata* hypocotyl (Figures 5 and 6). However, in the purified K-ep obtained by purification with pure ethanol, the composition ratio of hexamers with more substituted hydroxyl groups in the C5′ position of the B ring of the flavan-3-ol structure unit was significantly higher than that in K from the crude extract (Figures 5 and 6), which was mainly because the strong polarity of pure alcohol. Although K-mp had the same trend with K-ep, the degree of increase was far less than that of K-ep (Figures 5 and 6). Similarly, the effects of different purification methods on the distribution patterns of hydroxyl in a

single polymer also existed in the CTs from *B. gymnorhiza* calyx. In conclusion, given the present results and realistic issues, such as the price of Yb^{3+}, the 50% methanol was the optimum selection for purification of mangrove CTs.

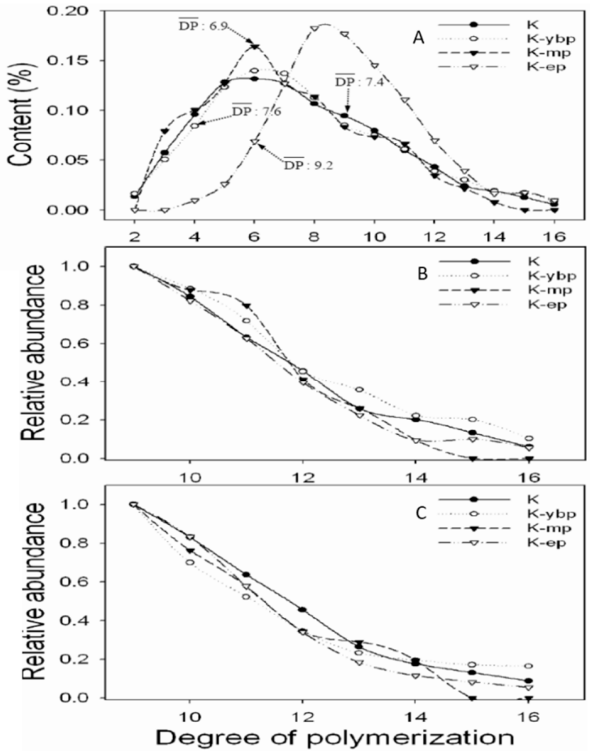

Figure 4. The mDP and distribution patterns of a full range of polymers (**A**), specific polymers with DP from nonamer to hexadecamer in the high molecular weight region (**B**), and homopolymers made up of C/EC from nonamer to hexadecamer (**C**) after purification with 50% methanol (B-mp), pure ethanol (B-ep), and Yb^{3+} precipitation (B-ybp) or not (B). In (**B**,**C**), nonamer was defined as unit 1 for benchmark.

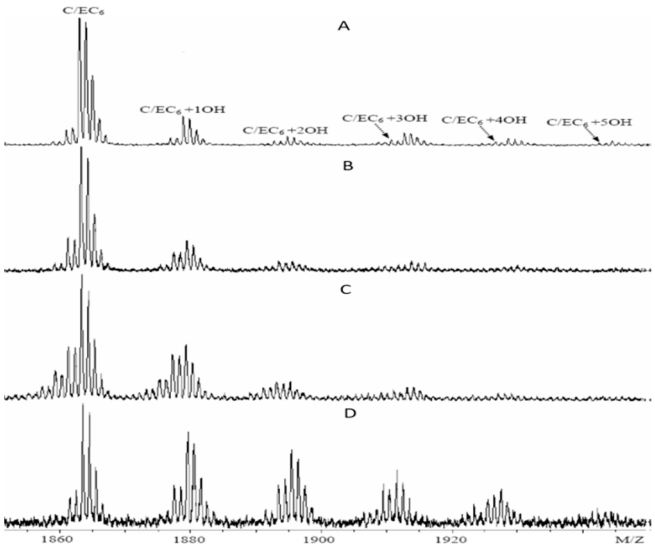

Figure 5. Enlarged MALDI-TOF spectra of hexamers in CTs from *K. obovata* hypocotyl purified by different methods. Note: (**A**), crude extract; (**B**), K-ybp; (**C**), K-mp; (**D**), K-ep; C/EC_6, homopolymer composes of six C/EC; n in C/EC_6 + nOH, the number of substituted hydroxyl groups in the C5′ position of the B ring of the flavan-3-ol structural unit in hexamers.

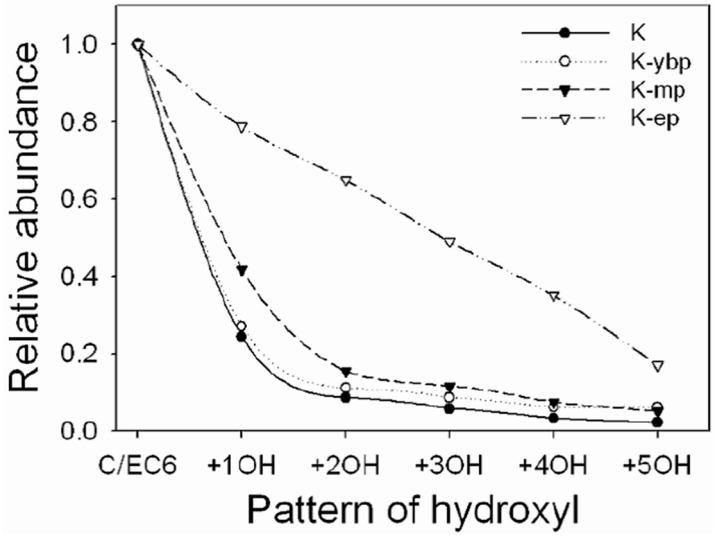

Figure 6. The patterns of hexameric hydroxyl mode in K, K-ybp, K-mp, and K-ep. The relative strength of each polymer was calculated as the relative strength of the homopolymer C/EC_6 in the MALDI-TOF MS in unit 1.

4. Conclusions

Extraction and purification are two necessary steps prior to the characterization of mangrove CTs using MALDI-TOF MS. These findings in this study indicated that serving as the extraction solvent, EtOAc rather than n-hexane, ether, and chloroform had the greatest influence on the true structural information of CTs in mangrove plant samples in the process of removing the non-tannic components of crude extract, suggesting that it could not be used for mangrove CT extraction prior to characterization. In addition, the pure ethanol purification was distinguished from 50% methanol purification and Yb^{3+} precipitation for mangrove CTs that the pure ethanol purification method also led to the loss of many polymers with low DP, indicating that the pure ethanol purification method had the greatest influence on the true structural information of CTs in mangrove plant samples. Additionally, all three purification methods increased the percentage of GC/EGC in the structural units of CTs, but the influence trend on the whole distribution patterns of hydroxyl groups was not completely consistent with those in each polymer. Considering all the present results for extraction and purification method selections and feasibility, such as toxicity and price of reagents, this study concluded that the potential options for mangrove CT extraction and purification were n-hexane/ether and 50% methanol, respectively.

Author Contributions: T.L.: investigation, data curation, methodology, writing—original draft, and funding acquisition. P.X.: investigation. M.L.: investigation. Z.C.: investigation. F.L.: investigation. M.J.: investigation. H.Z.: methodology, writing—review and editing, project administration, supervision, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding: This research was jointly supported by the National Natural Science Foundation of China (41876090; 32101367), the Guangdong Basic and Applied Basic Research Foundation (2022A1515010698), the Innovation of Science Technology Commission of Shenzhen Municipality (JCYJ20170818092901989; 20200827115203001), the Science and Technology Major Project of Guangxi (AA18242026), and the Specific Research Project of Guangxi for Research Bases and Talents (AD18281066).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Alongi, D.M. Carbon cycling and storage in mangrove forests. *Annu. Rev. Mar. Sci.* **2014**, *6*, 195–219. [CrossRef] [PubMed]
2. Salminen, J.P. Two-dimensional tannin fingerprints by liquid chromatography tandem mass spectrometry offer a new dimension to plant tannin analyses and help to visualize the tannin diversity in plants. *J. Agric. Food Chem.* **2018**, *66*, 9162–9171. [CrossRef] [PubMed]
3. Wang, Y.; Zhu, H.; Tam, N.F.Y. Polyphenols, tannins and antioxidant activities of eight true mangrove plant species in south China. *Plant Soil* **2014**, *374*, 549–563. [CrossRef]
4. Wei, S.D.; Lin, Y.M.; Liao, M.M.; Zhou, H.C.; Li, Y.Y. Characterization and antioxidative properties of condensed tannins from the mangrove plant *Aegiceras corniculatum*. *J. Appl. Polym. Sci.* **2012**, *124*, 2463–2472. [CrossRef]
5. Zhou, H.C. Biogeochemical Implications of Mangrove Plant Polyphenols and Their Antioxidant Activities. Ph.D. Thesis, Xiamen University, Xiamen, China, 2012.
6. Zhou, H.C.; Tam, N.F.Y.; Lin, Y.M.; Wei, S.D.; Li, Y.Y. Changes of condensed tannins during decomposition of leaves of *Kandelia obovata* in a subtropical mangrove swamp in China. *Soil Biol. Biochem.* **2012**, *44*, 113–121. [CrossRef]
7. Zhou, H.C.; Tam, N.F.Y.; Lin, Y.M.; Ding, Z.H.; Chai, W.M.; Wei, S.D. Relationships between degree of polymerization and antioxidant activities: A study on proanthocyanidins from the leaves of a medicinal mangrove plant *Ceriops tagal*. *PLoS ONE* **2014**, *9*, e107606. [CrossRef]
8. Lang, T.; Wei, P.; Chen, X.; Fu, Y.; Tam, N.F.Y.; Hu, Z.; Chen, Z.; Li, F.; Zhou, H. Microcosm study on allelopathic effects of leaf litter leachates and purified condensed tannins from *Kandelia obovata* on germination and growth of *Aegiceras corniculatum*. *Forests* **2021**, *12*, 1000. [CrossRef]
9. Jiang, S.; Weng, B.S.; Liu, T.; Su, Y.; Liu, J.C.; Lu, H.L.; Yan, C.L. Response of phenolic metabolism to cadmium and phenanthrene and its influence on pollutant translocations in the mangrove plant *Aegiceras corniculatum* (L.) Blanco (Ac). *Ecotoxicol. Environ. Saf.* **2017**, *141*, 290–297. [CrossRef]
10. Zhang, Q.F.; Laanbroek, H.J. The Effects of condensed tannins derived from senescing *rhizophora mangle* leaves on carbon, nitrogen and phosphorus mineralization in a *Distichlis spicata* salt marsh soil. *Plant. Soil* **2018**, *433*, 37–53. [CrossRef]
11. Zhang, Q.F.; Laanbroek, H.J. Tannins from senescent *Rhizophora mangle* mangrove leaves have a distinctive effect on prokaryotic and eukaryotic communities in a *Distichlis spicata* salt arsh soil. *FEMS Microbiol. Ecol.* **2020**, *96*, fiaa148. [CrossRef]
12. Lang, T.; Tam, N.F.Y.; Hussain, M.; Ke, X.R.; Wei, J.; Fu, Y.J.; Li, M.D.; Huang, X.Z.; Huang, S.Y.; Xiong, Z.J.; et al. Dynamics of heavy metals during the development and decomposition of leaves of *Avicennia marina* and *Kandelia obovata* in a subtropical mangrove swamp. *Sci. Total Environ.* **2022**, *855*, 158700. [CrossRef]
13. Kraus, W.; Ngoc, L.H.; Conrad, J.; Klaiber, I.; Reeb, S.; Vogler, B. Investigation of biologicallyactive natural products using online LC-bioassay, LC-NMR, and LC-MS Techniques. *Phytochem. Rev.* **2002**, *1*, 409–411. [CrossRef]
14. Monagas, M.; Quintanilla-Lopez, J.E.; Gomez-Cordoves, C.; Bartolome, B.; Lebron-Aguilar, R. MALDI-TOF MS analysis of plant proanthocyanidins. *J. Pharmaceut. Biomed. Anal.* **2010**, *51*, 358–372. [CrossRef]
15. Muchuweti, M.; Ndhlala, A.R.; Kasiyamhuru, A. Estimation of the degree of polymerization of condensed tannins of some wild fruits of zimbabwe (*Uapaca kirkiana* and *Ziziphus mauritiana*) using the modified vanillin-HCl method. *J. Sci. Food Agric.* **2005**, *85*, 1647–1650. [CrossRef]
16. Nierop, K.G.J.; Preston, C.M.; Verstraten, J.M. Linking the B ring hydroxylation pattern of condensed tannins to C, N and P mineralization. a case study using four tannins. *Soil Biol. Biochem.* **2006**, *38*, 2794–2802. [CrossRef]
17. Noferi, M.; Masson, E.; Merlin, A.; Pizzi, A.; Deglise, X. Antioxidant characteristics of hydrolysable and polyflavonoid tannins: An ESR kinetics study. *J. Appl. Polym. Sci.* **1997**, *63*, 475–482. [CrossRef]
18. Noble, A.C. Astringency and bitterness of flavonoid phenols. In *Chemistry of Taste: Mechanisms, Behaviorsand Mimics*; Given, P., Paredes, D., Eds.; ACS Symposium Series; American Chemical Society: Washington, DC, USA, 2002; Volume 825, pp. 192–201.
19. Reed, J.D.; Krueger, C.G.; Vestling, M.M. MALDI-TOF mass spectrometry of oligomeric food polyphenols. *Phytochemistry* **2005**, *66*, 2248–2263. [CrossRef]
20. Rejtar, T.; Hu, P.; Juhasz, P.; Campbell, J.M.; Vestal, M.L.; Preisler, J.; Karger, B. Off-line coupling of high-resolution capillary electrophoresis to MALDI-TOF and TOF/TOF MS. *J. Prote. Res.* **2002**, *1*, 171–179. [CrossRef]
21. Ishida, Y.; Kitagawa, K.; Goto, K.; Ohtani, H. Solid sampling technique for direct detection of condensed tannins in bark by matrix-assisted laser desorption/ionization mass spectrometry. *Rapid Commun. Mass Sp.* **2005**, *19*, 706–710. [CrossRef]
22. Wei, S.D.; Zhou, H.C.; Lin, Y.M. Antioxidant activities of extract and fractions from the hypocotyls of the mangrove plant *Kandelia candel*. *Int. J. Mol. Sci.* **2010**, *11*, 4080–4093. [CrossRef]
23. Dunford, N.; Imak, S.; Jonnala, R. Pressurized solvent extraction of policosanol from wheat straw, germ and bran. *Food Chem.* **2010**, *119*, 1246–1249. [CrossRef]
24. Nawaz, H.; Aslam Shad, M.; Rehman, N.; Andaleeb, H.; Ullah, N. Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds *Braz. J. Pharm. Sci.* **2020**, *56*, e17129.
25. Maie, N.; Behrens, A.; Knicker, H.; Kogel-Knabner, I. Changes in the structure and protein binding ability of condensed tannins during decomposition of fresh needles and leaves. *Soil Biol. Biochem.* **2003**, *35*, 577–589. [CrossRef]
26. Chen, Y.M.; Hagerman, A.E. Characterization of soluble non-covalent complexes between bovine serum albumin and beta-1,2,3,4,6-penta-O-galloyl-D-glucopyranose by MALDI-TOF MS. *J. Agric. Food Chem.* **2004**, *52*, 4008–4011. [CrossRef] [PubMed]

27. Krueger, C.G.; Dopke, N.C.; Treichel, P.M.; Folts, J.; Reed, J.D. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of polygalloyl polyflavan-3-ols in grape seed extract. *J. Agric. Food Chem.* **2000**, *48*, 1663–1667. [CrossRef]
28. Krueger, C.G.; Vestling, M.M.; Reed, J.D. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of heteropolyflavan-3-ols and glucosylated heteropolyflavans in sorghum [*Sorghum Bicolor* (L.) Moench]. *J. Agric. Food Chem.* **2003**, *51*, 538–543. [CrossRef]
29. Miller-Chou, B.A.; Koenig, J.L. A review of polymer dissolution. *Prog. Polym. Sci.* **2003**, *28*, 1223–1270. [CrossRef]
30. Tian, Y.; Liimatainen, J.; Alanne, A.L.; Lindstedt, A.; Liu, P.; Sinkhonen, J. Phenolic compounds extracted by acidic aqueous ethanol from berries and leaves of different berry plants. *Food Chem.* **2017**, *220*, 266–281. [CrossRef]
31. Butler, L.G.; Price, M.L.; Brotherton, J.E. Vanillin assay for proanthocyanidins (condensed tannins): Modification of the solvent for estimation of the degree of polymerization. *J. Agric. Food Chem.* **1982**, *30*, 1087–1089. [CrossRef]
32. Lin, Y.M.; Liu, J.W.; Xiang, P.; Lin, P.; Ye, G.F.; Sternberg, L.D.S.L. Tannin dynamics of propagules and leaves of *Kandelia candel* and *Bruguiera gymnorrhiza* in the Jiulong River Estuary, Fujian, China. *Biogeochemistry* **2006**, *78*, 343–359. [CrossRef]
33. Lang, T.; Sun, H.M.; Li, N.Y.; Lu, Y.J.; Shen, Z.D.; Jing, X.S.; Xiang, M.; Shen, X.; Chen, S.L. Multiple signaling networks of extracellular atp, hydrogen peroxide, calcium, and nitric oxide in the mediation of root ion fluxes in secretor and non-secretor mangroves under salt stress. *Aquat. Bot.* **2014**, *119*, 33–43. [CrossRef]
34. Hagerman, A.E.; Butler, L.G. Condensed tannin purification and characterization of tannin-associated proteins. *J. Agric. Food Chem.* **1980**, *28*, 947–952. [CrossRef]
35. Mane, C.; Sommerer, N.; Yalcin, T.; Cheynier, V.; Cole, R.B.; Fulcrand, H. Assessment of the molecular weight distribution of tannin fractions through MALDI-TOF MS analysis of protein-tannin complexes. *Anal. Chem.* **2007**, *79*, 2239–2248. [CrossRef]
36. Hagerman, A.E.; Zhao, Y.; Johnson, S. Methods for determination of condensed and hydrolyzable tannins. In *Antinutrients and Phytochemicals in Food*; Shahidi, F., Ed.; ACS Symposium Series; American Chemical Society: Washington, DC, USA, 1997; Volume 662, pp. 209–222.
37. Zhou, H.C.; Lin, Y.M.; Wei, S.D.; Tam, N.F.Y. Structural diversity and antioxidant activity of condensed tannins fractionated from mangosteen pericarp. *Food Chem.* **2011**, *129*, 1710–1720. [CrossRef]
38. Lu, W.; Wang, Z.; Yuan, Z. Characteristics of lipid extraction from *Chlorella* sp. Cultivated in outdoor raceway ponds with mixture of ethyl acetate and ethanol for biodiesel production. *Bioresour. Technol.* **2015**, *191*, 433–437. [CrossRef]
39. Hivechi, A.; Bahrami, S.H. A new cellulose purification approach for higher degree of polymerization: Modeling, optimization and characterization. *Carbohydr. Polym.* **2016**, *152*, 280–286. [CrossRef]
40. Lessa Belone, M.C.; Kokko, M.; Sarlin, E. Degradation of common polymers in sewage sludge purification process developed for microplastic analysis. *Environ. Pollut.* **2021**, *269*, 116235. [CrossRef]