Water-soluble non-starch polysaccharides of wild-simulated Dendrobium catenatum Lindley plantings on rocks and bark of pear trees

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ARTICLE INFO

Keywords:
Cultivation pattern
Dendrobium catenatum
Lithophytation
Epiphytation
Glucosamine
Non-starch polysaccharides

ABSTRACT

The total water-soluble polysaccharide (TP) of Dendrobium catenatum is composed of starch and active non-starch polysaccharides (NSPs) with glucosamine as the main structural type. Although the TP content has been used as a quality assessment indicator for many years, the NSPs content in samples from different environments and growth seasons has not been reported. In this study, we found that NSPs had stronger antioxidant activity than TP. The NSPs content was higher in wild-simulated environments including rocks and trees compared to plantings grown in greenhouse. The culture mode and growth period affected the ratio of NSPs and starch. Facility cultivation provided optimal growth conditions but produced more starch, whereas wild-simulated cultivation resulted in a higher ratio of NSPs, particularly in September. Therefore, cultivation by lithophytation and epiphytation may be preferable to facility plantings, which is expected to be enormously useful for the current production and quality control of D. catenatum.

1. Introduction

Dendrobium catenatum Lindley (synonym D. officinale Kimura et Migo) (Si, Zhang, Luo, Liu, & Liu, 2017), a lithophytic orchid found in subtropical and temperate regions, is one of the most valuable traditional Chinese medicinal plants, as well as an edible, flowering herb. Its fresh stem can be chewed directly and the dried stem, called “Tiepi Fengdou” in Chinese, can be stewed in porridge, soups, and other dishes; as such, it is widely used in China and Southeast Asia for its extraordinary tonic efficacy (Si et al., 2017). Because the D. catenatum stem has many medicinal attributes, such as immunomodulating, anti-tumor, anti-fatigue, anti-diabetic, and antioxidant properties, there is an increasing trend towards using it as a dietary supplement in functional beverages, health foods, and cosmetics (Chen et al., 2021a; Kanlayavattanakul, Lourith, & Chaikul, 2018).

Although wild D. catenatum has been listed as an endangered plant since the 1980s, tissue culture seeding and artificial cultivation techniques have been developed, allowing production to meet market needs over the past 20 years (Si, Wang, Liu, Liu, & Luo, 2017). Facility-aided cultivation in greenhouses is the most popular cultivation mode; cultivation in simulated natural environments, such as living tree epiphytic and rock lithophytic cultivation, has also advanced in recent years to further production efforts with ecological protection practices (Lin et al., 2017). However, little is known about the quality differences in D. catenatum under different cultivation conditions.

It is widely accepted that polysaccharides are the major bioactive component of the D. catenatum stem (Zhang et al., 2021). According to Chinese Pharmacopoeia (2020 Edition), total water-soluble polysaccharide (TP) has been commonly used as major quality assessment indicator of D. catenatum, and TP was acquired through hot water extraction and alcohol precipitation and quantified by phenol-sulfuric acid colorimetry (Liu, Liu, Zhang, & Si, 2021). The water-soluble carbohydrate polymers are ubiquitous in all known plants (Van Soest, Robertson, & Lewis, 1991). Starch, one of the most important water-soluble polysaccharides and major diet constituent, provides energy for human beings (Sumczynski, Bubelova, Sneyd, Erb-Weber, & Mlcek, 2016).
By contrast, water-soluble non-starch polysaccharides (NSPs) is considered an active ingredient of medicinal plants and functional foods known for their potential health-promoting effects, such as those related to alleviating oxidative stress and balancing intestinal microbiota and immune-system modulation (Chen et al., 2021b). Among NSPs, most of the research efforts have focused on mannan, glucan, and glucomannan (GM) (Korolenko, Bgatova, & Vetvicka, 2019). GMS have a main chain of β-(1,4)-linked β-glucopyranosyl and β-(1,4)-linked α-mannopyranosyl backbone sometimes with a α-(1,6)-linked α-galactose side chain (Korolenko et al., 2019). These NSPs have excellent immuno-enhancing, anti-tumor, hepatoprotective, cardioprotective, and antioxidant activity (Li et al., 2022; Wang, Zhou, Wang, & Cao, 2022). Particularly, mannose, one of the degradation products of GMS, is taken up by the same transporter(s) as glucose but slows growth in several tumor types in vitro and in vivo (González et al., 2018). Thus, there is an increasing demand for natural antioxidants and immunomodulators from polysaccharides of plants, due to their relatively low toxicity and relatively few side effects (Lin et al., 2019). D. catenatum is one of the most abundant natural plant resources with water-soluble polysaccharides, whose content of TP accounts for up to 50% of its dry matter content; its major structural type is GM in the stems (Yue, Zeng, & Ding, 2020). Recently, several studies e.g. (Deng et al., 2016) have assessed polysaccharides and their molecular weight distribution and monosaccharide composition in Dendrobium and Panax species, using saccharide mapping and chromatographic methods; however, the ability to apply such methods in the quantitative and qualitative determination of NSPs from different D. catenatum samples remains unknown.

It seems beyond dispute that the synthesis and accumulation of plant active ingredients largely depend on genes and the environment. Typically, cultivation conditions have a significant effect on the content and properties of polysaccharides from natural materials, including the growth stages (Détrar et al., 2021), genetics (Pavloveus & Kumita, 2011), and environmental conditions, such as light (Horrer et al., 2016), temperature (Wu et al., 2016), drought (Ahl et al., 2019), and precipitation (Walla et al., 2021). In previous studies, we examined the morphological and agronomic characteristics of wild-simulated D. catenatum plantings on rocks and the bark of pear trees and compared our findings to those obtained from facility-aided cultivation; our results revealed the complexity of the phytochemical contents and composition in wild simulated environments (Lin et al., 2017). In addition, the harvest time affects the polysaccharide content in the stems and leaves of D. catenatum; the TP content is highest before flowering, and the amount of glucose that constitutes TP decreases significantly after the flowering period (Liu et al., 2015). However, the content changes in NSPs, the core bioactive ingredients of D. catenatum, have not been clarified with respect to the cultivation conditions and growth stages. In this study, we investigated the differences in starch and bioactive polysaccharide content in the stems of D. catenatum obtained from three cultivation modes (lithophyte on rocks, epiphyte on the bark of pear trees and facility cultivated on pots) and harvest times (May and September) by comparative analyses of the activity, structure, and contents. To the best of our knowledge, this is the first study to analyze the NSPs of D. catenatum in different cultivation modes. Consequently, our results are expected to provide useful information for current production and quality control practices related to D. catenatum.

2. Materials and methods

2.1. Plant materials

Wild D. catenatum is a scarce resource in nature. It requires a bit of luck for D. catenatum hunters to seek them. In recent years, it has been occasionally found in moderately damp mountains at an altitude of 1600 m in some provinces of China, such as Zhejiang, Guangdong, Fujian, Hunan, and Yunnan. Typically, the native environment where D. catenatum is living is the vertical rock wall of Danxia landform or the position close to the top of a tree trunk. Because of the scarcity of wild D. catenatum, its production is insufficient to meet market demand. As a result, the raw products of D. catenatum currently sold on the market are all come from artificial cultivation.

D. catenatum ‘Jingpin Tianmushan’, numbered Zhejiang SVVDC0112018, was cultivated in a standard planting base in Lin’an, Zhejiang Province, China. The precise location was 119°26′11″ E, 30°20′30″ N, at 280 m above sea level. Fig. 1 shows the three cultivation modes: lithophyte (plant epiphytes on vertical rock walls with no obstructions above, under natural light), pear tree epiphyte (shading of trees and leaves of 70–80%, with a light intensity of 3000–5000 lx), and greenhouse cultivation (with 70% net shade and an irrigation spray device). A common advantage of the first two modes is that the wild living of Dendrobium is restored as much as possible.

Polysaccharide content is higher during the growing season, with the highest content occurring before the flowering period in May and the lowest content coinciding with autumn and winter seasons (Yu, Guo, Si, Wu, & Wang, 2014). As such, we sampled the plantings at two time points for our analyses. The biennial stems from the three cultivation modes were collected on May 9, 2018, and September 6, 2018. Samples were freeze-dried to obtain a constant weight, crushed, passed through a 60-mesh sieve, and then stored for further use in a dryer. The botanical origin of plants was identified by Prof. Jinping Si. The voucher specimens were deposited in the School of Forestry and Biotechnology of Zhejiang A&F University.

2.2. Chemicals and reagents

Pullulan standards (molecular weight (Mw): 6.2, 10.0, 21.7, 48.8, 113.0, 200.0, 366.0, and 805.0 kDa) were purchased from American Polymer Standards Corporation (APSC) (Mentor, OH, USA). α-Amylase was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). α-mannose (Man), β-glucose (Glc), α-rhamnose (Rha), β-xylose (Xyl), β-galactose (Gal), β-galacturonic acid (GalA), and α-gluconic acid (GlcA) were obtained from the National Institutes for Food and Drug Control (Beijing, China). Iodine, potassium iodide, and ABTS are were acquired from Aladdin Biochemical Technology Co, Ltd (Shanghai, China). 1-phenyl-3-methyl-5-pyrazolone (PMP) was purchased from Macklin Biochemical Technology C., Ltd (Shanghai, China). Deionized water was prepared using a Millipore Milli-Q Plus system. Acetonitrile was chromatographic grade; all other reagents were of analytical grade.

An Agilent 1200 high performance liquid chromatography system (DAD Detector; RID Detector, Agilent Technologies, Santa Clara, CA, USA), a Nicolet Nexus FT-IR spectrometer (Thermo Nicolet Nexus, Waltham, MA, USA), a SpectraMax 190 light absorption microplate reader (Molecular Devices, San Jose, CA, USA), and an ALPHAlA 1–2 LD Plus lyophilizer (Christ, Germany) were used for the analyses.

2.3. Preparation of test sample solution

The reflux of powdered D. catenatum (0.15 g) was performed with distilled water (25 mL) for 2.5 h at 100 °C. After cooling, an aliquot of extracted solution was centrifuged at 6000 × g for 30 min. The supernatant (5 mL) was precipitated for 1 h below 4 °C via ethanol addition to a final concentration of 80% (v/v). The resulting precipitate was collected by centrifugation and washed twice with 80% ethanol (20 mL).
The residue was re-dissolved in hot water to 5 mL; then samples (2 mL) were freeze-dried. The crude polysaccharide solution was deemed TP.

In addition, 5 mL the supernatant was taken for α-amylase digestion (Xu et al., 2016). The supernatant was added to the same amount of 10 mM Tris-maleate buffer for 30 min. The sample was placed in a boiling water bath and boiled for 7 min to gelatinize the starch granules. After cooling and equilibrating the sample to 40 °C, α-amylase solution (15 U/mL) was added to the sample and incubated for 1.5 h at 40 °C. To test if the starch had degraded completely, an iodine reagent test (0.5% iodine in 5% potassium iodide) was conducted. Then, four volumes of cold absolute ethanol were added, and precipitate polysaccharides were allowed to precipitate overnight at −20 °C. Next, the sample was centrifuged at 6000 × g at room temperature for 1 h. The supernatant was removed and the samples were washed twice with cold 80% ethanol (20 mL). The residue was collected as NSPs and re-dissolved in hot water for high-performance gel permeation chromatography–refractive index detection (HPGPC-RID) analyses.

2.4. Starch–iodide reaction

The iodine-potassium iodide solution was prepared by dissolving 0.25 g iodine and 2.0 g potassium iodide in 25 mL distilled water. The starch–iodide reaction was carried out in a mixture of the chromogenic agent and 200 µL each sample (Juliano, 1971).

2.5. Scavenging activity of polysaccharide on ABTS+ free radicals

Determination of antioxidant activity was carried out on the basis of previous reports, with some modifications (Zhang et al., 2017b). Briefly, ABTS radical cations were produced by reacting 25 mL ABTS+ solution (7 mM) with 0.44 mL potassium persulfate (2.45 mM); the mixture was allowed to stand in the dark at room temperature for 16 h. At the time of use, the ABTS+ solution was diluted with PBS (0.1 M, pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm. The mixture solution, containing 150 µL polysaccharide of different concentrations (1, 2, 3, and 4 mg/mL) and 100 µL ABTS, was allowed to undergo reaction in dark conditions for 30 min, followed immediately by absorbance measurements recorded at 734 nm using a Spectra Max 190 system (Molecular Devices). VC (4 mg/mL) was used as positive control set. The scavenging activity of polysaccharide on ABTS free radicals was calculated according to the following:

$$ABTS \text{ scavenging activity } (%) = \left[1 - \frac{(A_S - A_R)}{A_S}\right] \times 100$$

where $A_S$ represents the absorbance of the sample reaction, $A_R$ represents the absorbance of the sample reaction without adding the ABTS solution, and $A_T$ represents the absorbance of the sample reaction without adding the sample reaction.

2.6. Evaluation of polysaccharide content by phenol-sulfuric acid colorimetry

The phenol-sulfuric acid method was used for polysaccharide content analyses of TP and NSPs, according to the Chinese Pharmacopoeia (2020 Edition). Briefly, 1 mL 5% phenol was added to 1000 µL polysaccharide solution. Then the solution was mixed with 5 mL concentrated sulfuric acid via shaking. The reaction solution was placed in a boiling water bath for 20 min. After the reaction solution had cooled in an ice bath for 5 min, the absorbance was measured at 488 nm. Glucose standard solutions with a concentration range of 10 to 100 µg/mL were used to calculate a standard curve; the reaction solution to which 1000 µL distilled water was added was used as a blank control. Each sample was set as three replications.

2.7. Analyses of polysaccharides by high-performance gel permeation chromatography with a differential refractive index detector (HPGPC-RID)

The content and molecular weight of specific polysaccharides from D. catenatum were measured via HPGPC-RID according to a previous study (Balli et al., 2020), with minor modifications. Briefly, two columns (length: 300 mm; internal diameter: 7.8 mm; Welch, Shanghai, China) with particles 5 µm in diameter were used in XitmateTM SEC-1000 and XitmateTM SEC-300 analyses. An Agilent 1200 high-performance liquid chromatography (HPLC) system was coupled to a RID system for measurements conducted at 35 °C. The mobile phase used double-distilled water at a flow rate of 1.0 mL/min. The average weight-averaged molecular weight (Mw) values of the polysaccharides were determined based on a standard curve produced by Pullulan standards (Sigma-Aldrich). The polysaccharide solution was filtered through a 0.22 µm nylon membrane for analyses.

2.8. Monosaccharide composition analyses

The monosaccharide compositions of sample polysaccharides were determined using a PMP-labeling procedure. Polysaccharide solution (1 mL) was hydrolyzed with 500 µL 3 M hydrochloric acid at 110 °C for 1 h in a sealed test tube. Subsequently, excess hydrochloric acid was removed by neutralization with the addition of 500 µL 3 M NaOH. Next, the polysaccharide hydrolysis solution (400 µL) was labeled with PMP by adding 400 µL 0.3 M NaOH and 400 µL 0.5 M PMP methanol solution. The mixture was incubated at 70 °C for 100 min. The reaction product was neutralized with 400 µL 0.3 M HCl solution and extracted three times with 2 mL chloroform. The aqueous phase was filtered through a 0.22 µm nylon membrane for HPLC analyses. The HPLC analyses were coupled to a DAD detector and a Venusil MP C18 column (250 mm × 4.6 mm) maintained at 35 °C. The column (250 mm × 4.6 mm) was eluted with a mobile phase consisting of 0.02 M ammonium acetate solution and acetonitrile (83:17, v/v) at 1 mL/min. Standard monosaccharides

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solution mixed with 0.10 mg/mL Man, 0.10 mg/mL Glc, 0.10 mg/mL Rha, 0.12 mg/mL Xyl, 0.09 mg/mL Gal, 0.12 mg/mL GalA, and 0.10 mg/mL GlcA was determined using the same procedure. The monosaccharide content was calculated according to the calibration curve (peak area and concentration) of each monosaccharide standard.

2.9. Fourier transform infrared spectroscopy analyses

The IR spectra of D. catenatum polysaccharides were recorded using a Fourier transform infrared (FT-IR) spectrophotometer. The sample (2 mg) was ground into powder with spectroscopic-grade potassium bromide (KBr) powder and pressed into a disk for FT-IR measurement in the frequency range of 4000–400 cm⁻¹.

2.10. Data processing and statistical analyses

All experiments were repeated at least three times. The results were generated using EXCEL software (version 2007, Microsoft Corp., Redmond, WA, USA) and are expressed as the mean ± standard deviation. SPSS 19.0 software (IBM SPSS, Chicago, IL, USA) was used for the statistical analyses. Statistical significance was determined using one-way analysis of variance with GraphPad Prism 6.0 software (San Diego, CA, USA). A p-value < 0.05 was considered statistically significant. HPLC data were first processed by Agilent ChemStation and exported as AIA files to professional software, the Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (version 2004A), for calibration of the retention time of peaks. Principal component analyses were carried out using SIMCA 13.0 software (Umetrics, Malmö, Sweden).

3. Results and discussion

3.1. Extraction of NSPs from D. Catenatum

TP was extracted according to a classic method according to the Chinese Pharmacopoeia (2020 edition) as well as our previous study (Zhang et al., 2017a). After extraction with hot water and precipitation in 80% alcohol, the TP of D. catenatum was obtained. Fig. 2A shows the blue color of the starch–iodide reaction product indicating that TP from D. catenatum contained starch. Then the starch in the obtained TP was digested using α-amylase (final concentration: 15 U/mL) into small sugar molecules, and removed using alcohol (Li et al., 2019). As expected, after α-amylase digestion, the positive blue color response of polysaccharides on iodine and the potassium iodide solution had disappeared (Fig. 2A). Thus, the NSPs solution was obtained without starch. Since the main component of NSPs in D. catenatum is glucomannan, which contains β-(1,4)-linked α-glucopyranosyl and β-(1,4)-linked α-mannopyranosyl bonds and cannot be hydrolyzed by α-amylase, the loss of NSPs was only 1.3% in the previous study so the loss of this part could be neglected (Y. Li et al., 2019).

3.2. In vitro antioxidant activity of polysaccharides

As shown in Fig. 2B, the in vitro antioxidant activity of NSPs was significantly stronger than that of the TP. In contrast to the well-known antioxidant standard vitamin C (Vc), the 4 mg/mL NSPs solution showed similar antioxidant activity. The radical scavenging rate of the sample from lithophyte cultivation was significantly higher than those of other tested samples. The IC₅₀ of ABTS⁺ scavenging rate was calculated for the concentrations of 1, 2, 3, and 4 mg/mL each NSPs sample; the average IC₅₀ value was 1.89 mg/mL. Similar with the NSPs from D. devonianum, the D. catenatum NSPs also showed very strong scavenging abilities for ABTS⁺ radicals (Fan, Lin, & Luo, 2022). The increased antioxidant activity of the NSPs is likely due to starch removal, which is consistent with results showing that the starch content in wheat is negatively correlated with antioxidant activity (Sumczynski et al., 2015). However, it is not clear as to why there are differences in NSPs activity with respect to the cultivation method, which requires further study.

3.3. Phenol-sulfuric acid colorimetry analyses

As shown in Fig. 3, in the flowering period of May, the TP contents of lithophytic, tree epiphytic, and facility cultivation of D. catenatum were 512.2, 473.3, and 462.8 mg/g, respectively; after pretreatment with α-amylase, the contents decreased by 14.0%, 20.9%, and 24.3% respectively, which means that the proportion of starch in TP can be as high as one-fifth, particularly in facility cultivation. Consistently, in September, corresponding to the period after flowering, the TP contents were 418.2, 385.9, and 431.4 mg/g accordingly for lithophytic, tree epiphytic, and facility cultivation, respectively, and α-amylase pretreatment reduced polysaccharide content by 6.0%, 7.7%, and 19.5%. Therefore, TP and NSPs content and the ratio between NSPs and TP were influenced by the cultivation environment and the development stage.

The cultivation conditions significantly affected polysaccharide content. The stems of D. catenatum collected from the lithophytic mode contained the highest NSPs content, whereas facility cultivation produced the most starch. It is interesting to note that D. catenatum cultivated in the greenhouse had the highest TP content in September, but contained the lowest active polysaccharide NSPs as a result of the highest content of starch among the three cultivation modes. As the NSPs content was greater in lithophytic samples than epiphytic ones, and even higher than facility-cultivated samples from both May and September, it can be deduced that the simulated natural cultivation
pattern is conducive to the synthesis and accumulation of active polysaccharide components, whereas \textit{D. catenatum} grown in a greenhouse, the best growth mode for maximum yield, showed relatively poor quality due to starch (inactive polysaccharides) accumulation.

The harvest time also had a significant effect on polysaccharide content. In the same environment, both TP and NSPs contents were higher in May than in September. May is the time during which \textit{D. catenatum} begins to bloom in Zhejiang Province, China. Blooming consumes a significant amount of nutrient starch (Liu et al., 2014). Thus, the stems of \textit{D. catenatum} synthesize and accumulate more starch before blooming in May. As a result, there was a significant decrease in TP content. The content of NSPs, accounting for 75.5% to 94.0% of the TP, decreased after flowering to a varying degree, thus suggesting that GM and other NSPs may also act as energy storage substances and may be consumed during the flowering period.

3.4. Content of specific polysaccharides in \textit{D. Catenatum}

Although it is difficult to discriminate the starch and NSPs using a direct one-step on-line HPGPC-RID method due to the complexity of the components, the method combining two-step HPGPC-RID analyses with \(\alpha\)-amylase digestion has proven to be very efficient for quantitative determination of polysaccharide content including TP and NSPs. The polysaccharide contents of the three cultivation modes were analyzed;

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{NSPs and starch content (mg/g) of \textit{D. catenatum} in different cultivation patterns and harvest times. The unit of mg/g was calculated as milligrams of polysaccharides per gram of \textit{D. catenatum} powder dry weight. The data are presented as means ± SD from three independent experiments. Different lowercases above each columns show significant difference in the TP content \((P < 0.05)\). The relative percentages of each NSPs and Starch in TP are also marked on the figure.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{HPSEC chromatograms of total polysaccharides (red line) and non-starch polysaccharides (blue line) from \textit{D. catenatum} samples. (A, B, C) samples collected from rock (L), pear tree (E) and greenhouse (F) in May; (D, E, F) samples collected from rock (L), pear tree (E) and greenhouse (F) in September. Elution time for three different molecular weight fractions are shown (vertical dash line). 1, 2, 3, means the different HPLC fractions of polysaccharides.}
\end{figure}
the results are shown in Fig. 4. The standard curve and regression equation were established as follows: \( \log M_w = 9.6971 - 0.3076t \), with \( R = 0.9969 \) (in which \( M_w \) is the weight-average molecular weight and \( t \) is the retention time). The polysaccharides from \( D. \) catenatum were divided into three fractions according to the chromatogram peak shapes and their relative molecular mass: fraction 1 \((4.3 \times 10^5 - 6.6 \times 10^6) \text{ Da}\), fraction 2 \((1.5 \times 10^6 - 4.3 \times 10^6) \text{ Da}\), and fraction 3 \((0.0085 \times 10^6 - 1.5 \times 10^6) \text{ Da}\). The standard curve for calculating the polysaccharide content of each fraction was \( Y = 79949X + 1596.4 \), with \( R^2 = 0.9991 \) (where \( Y \) is the peak area of each polysaccharide fraction and \( X \) is the content of polysaccharide fractions calculated using the Pullulan standard with \( M_w \) of \( 8.05 \times 10^5 \) Da). Our results revealed a linear relationship from 0.01 to 2.00 mg/mL polysaccharide concentration. The established HPGPC-RID method was applied to quantify TP (including starch and specific polysaccharides), NSPs (amylase-treated polysaccharides), and their three fractions in \( D. \) catenatum samples. Table S1 summarizes the contents of the investigated polysaccharides.

The proportions of the three fractions in polysaccharides (TP and NSPs) were similar among the samples with respect to the different harvest times and cultivation modes. Fraction 1 accounted for 1.73–3.21% of polysaccharides, fraction 2 accounted for 26.88–34.31%, and fraction 3 accounted for 63.68–70.80%. Although the method of HPGPC-RID method using the Pullulan standard with \( M_w \) of \( 8.05 \times 10^5 \) Da was different from the polysaccharide content measured by phenol-sulfuric acid method, the sum of the contents of the three fractions was consistent with the results of TP and NSPs content determined using glucose as the standard of the phenol-sulfuric acid method. With respect to the TP content for the three cultivation modes, the order from high to

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**Fig. 5.** HPLC chromatograms of monosaccharides. (A) Mixed standards, including 1. mannose (man), 2. rhamnose (rha), 3. glucuronic acid (glcA), 4. galacturonic acid (galA), 5. glucose (glc), 6. galactose (gal), 7. xylose (xyl), 8. arabinose (ara). (B) The composition of polysaccharides from \( D. \) catenatum cultivated in different environment and collected in May. (C) Samples collected in September.
low was as follows: lithophytic > epiphytic > facility-aided cultivation. The harvest time affected the TP and NSPs content, in which the total content was higher in May than in September.

Comparing the curves of TP and NSPs, the peak height was considerably lower after starch removal in NSPs (Fig. 3). The starch was relatively more distributed in fraction 3 when samples were collected in May than in September. For example, the D. catenatum collected from pear trees in May contained 2.19, 10.88, and 44.49 mg/g starch in the three fractions (1–3, respectively), compared to the results from September showing amounts of 0.64, 17.21, and 7.69 mg/g. These results indicate that starch in D. catenatum had no fixed molecular weight, consistent with studies on rice and buckwheat (Deng, Yang, Li, Zeng, Li, Zhong, & Ren, 2021; Wang, Wang, Wang, Qiu, & Li, 2021). In addition, flowering consumed more starch with related lower molecular weight fractions. By contrast, from May to September, D. catenatum of lithophyte consumed 49.95 mg/g fraction 3 of NSPs, the samples from pear tree epiphyte consumed 12.48 mg/g, whereas the greenhouse cultured ones increased 5.56 mg/g. This may be because facility-aided cultivated D. catenatum has sufficient starch to provide energy for flowering, as opposed to simulated naturally cultivated ones, particularly cliff-cultured plants, that cannot provide enough starch and consequently consume NSPs to support flowering.

3.5. Monosaccharide composition

HPLC with pre-column derivatization has been widely employed for qualitative and quantitative analyses of compositional monosaccharides in polysaccharides from plants (Li et al., 2013). The results in Fig. 5 and Table S2 show that the HPLC profiles of TP and NSPs from D. catenatum were similar; the main compositional monosaccharides were Man and Glc with the concentration from 20.05 mg/g to 125.54 mg/g, as well as minor Glca, Gal, Xyl and Ara with the concentration less than 5.23 mg/g. However, the monosaccharide content fluctuated with a change in harvest time, cultivation mode, and enzyme pretreatment. The glucose content showed a remarkable decrease after α-amylase treatment (up to 58.11 mg/g in the samples collected from greenhouse in May), which confirmed that the polysaccharides of D. catenatum contained starch.

The glucose content of greenhouse samples was twice that of lithophyte and 2.4 times that of pear epiphyte (Table S2). But the contents sharply decreased in facility-grown samples after enzyme digestion, whereas lithophyte and epiphyte samples experienced a slight decrease. In the samples collected in May, the glucose content decreased 38.41 mg/g, 47.71 mg/g and 58.11 mg/g from rock lophyte, pear tree epiphyte, and facility cultivation patterns respectively. Meanwhile, the index of glucose content decreased by 0.88 mg/g, 7.56 mg/g and 56.21 mg/g in September. This result indicated that samples cultivated in the greenhouse accumulated much more starch than that in wild-simulated cultivation patterns in September, when the trend of the samples was similar to that in May. Moreover, there was no significant difference in the content of glucose contents for the three cultivation modes in May. But four months after, the glucose content for these three cultivation modes showed significant changes. Furthermore, glucose decreased predominantly from May to September, as a possible result of flowering that consumed glucose for energy.

In addition, there were no significant differences in the mannose or glucose content of NSPs. However, the molar ratio of mannose to glucose changed dramatically for the three cultivation modes and two growth stages. Particularly, in the three samples collected in May and the greenhouse samples in September, the ratio was less than 2, which was caused by the high content of starch composed of glucose. Turning to NSPs, the ratio of Man:Glc was ranged from 3.33 to 5.64. It suggested that the high proportion of mannose in NSPs is closely related to its enhanced biological activity (Huang et al., 2015).

3.6. Structure analyses by FT-IR spectroscopy

The main observed modes in the D. catenatum polysaccharides appeared at 3406, 2928, 2890, 1735, 1652, 1637, 1377, 1249, 1151, 1092, 1065, 1024, 958, 896, 878, and 809 cm⁻¹ (Fig. 6). The mode at 3406 cm⁻¹ is the characteristic stretching vibration of the –OH groups coupled with hydrogen bonding. In addition, the mode at 2928 and 2890 cm⁻¹ represents C–H stretching and arises from methyl units. Ester carbonyl groups (C=O), mainly sourced from the acetyl group of GM appeared at 1735 cm⁻¹. The modes at 1652 and 1637 cm⁻¹ are associated with the bending vibration of O–H. In addition, stretching vibration of the C=O fragment was observed at 1249, 1151, 1092, 1065, 1024, and 958 cm⁻¹. The existence of β-1,4 glycosidic and β-1,4 mannosidic linkages characteristic of GM were assigned to C=O–C stretching at 1024 and 1249 cm⁻¹. The peaks at 896 cm⁻¹ were of β-anomeric carbon, indicating that the two fractions mainly contained β-type glycosidic linkages (Y. Y. Deng et al., 2014). In addition, C=H bending appeared at 878 and 809 cm⁻¹ and is attributed to the β-pyranose form of glucose and mannose, respectively (Huang et al., 2016).

The difference between the TP and the NSPs is the obvious reduction in the intensity of the entire IR spectrum as a result of starch digestion. However, the absorption peak intensity at 958 cm⁻¹ and the uronic acid content in September were higher than in May, when the absorption intensity of the rock wall and pear tree at 1735 and 958 cm⁻¹ in different habitat samples became greater than that observed for greenhouse samples.

The authors declare the following financial interests/personal

Declaration of Competing Interest
relationships which may be considered as potential competing interests: Jinping Si reports administrative support was provided by Ministry of Science and Technology of the People’s Republic of China. Jingjing Liu reports financial support was provided by National Natural Science Foundation of China.

Funding

The authors are grateful for supported from the National Key Research and Development Program of China (No. 2017YFC1702201), the National Science Foundation of China (No. 81603228) and the projects supported by the State Key Laboratory of Subtropical Silviculture, ZAFU (ZY20180206 and KF201703).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100309.

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Fig. 6. The representative FT-IR spectra of polysaccharide solution in D. catenatum.
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