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A novel water-soluble polysaccharide named PLP1 was successfully isolated from root barks of *Periploca laevigata* by hot water extraction and further purified by DEAE-sepharose chromatography. PLP1 has a relative molar mass of 5.57 10^5 g/mol determined by Ultra-high performance liquid chromatography (UHPLC). The PLP1 structure was investigated by chemical and instrumental analysis including gas chromatography mass spectrometry (GC–MS), methylation analysis, Fourier transform infrared (FT-IR) spectroscopy and nuclear magnetic spectroscopy (^{1}H NMR and ^{13}C NMR). Results showed that PLP1 was mainly composed of glucose, galactose, mannose and glucuronic acid in a molar percent of 62.9, 19.4, 11.8 and 5.9, respectively. The backbone of PLP1 was composed of 1,3-β-D-Glc*p*, 1,3,4-α-D-Glc*p*, 1,4-α-D-Man*p*, 1,6-α-D-Man*p*, 1,3-α-D-Gal*p* and 1,6-β-D-Gal*p*. The thermogravimetry analysis (TGA) and the differential scanning calorimetry (DSC) were used and showed that PLP1 has good thermal stability under 229 °C. Moreover, the purified polysaccharide demonstrated an appreciable *in vitro* antioxidant potential and high antibacterial activity against several Gram (+) and Gram (−) strains. These finding suggested that PLP1 might be suitable for use as functional foods and as potential therapeutic agents.

1. Introduction

Polysaccharides are naturally occurring polymeric carbohydrate possessing repeating units linked together by glycosidic bonds. These molecules, widely distributed in plants and algae, play an important role in the development of new products including pharmaceuticals, foods and biodegradable packaging materials (Chang, 2002; Cui, 2005; Jiao, Yu, Zhang, & Ewart, 2011; Xie, Jin et al., 2016; Xie, Tang, Jin, Li, & Xie, 2016). In recent years, the water-soluble polysaccharides have attracted a great deal of scientific interest due to their potential as a source of natural antioxidants and biologically active compounds such as antibacterial, hypoglycemic, and hypolipidemic activities (Song et al., 2012; Zhao, Qian, Yin, & Zhou, 2014; Wang, Cheng et al., 2016; Wang, Liu et al., 2016; Xie, Jin et al., 2016; Xie, Tang et al., 2016). The functions of polysaccharides are closely related to their structural properties such as chemical composition, branch degree, linkage type, tertiary structure and molecular weight.

Oxidation of polyunsaturated fatty acids, which occurs during storage, processing, and heat treatment of raw materials, and further storage of final products, is one of the major factors resulting in losses in fatty food quality by formation of compounds with negative effects on the aroma and nutritional value of the foods (Shahidi & Wanasundara, 1992). Moreover, it has been shown that antioxidants and free radical scavengers are crucial in the prevention of pathologies such as cancer, heart diseases, biological damage in living tissues, and neurodegenerative diseases, in which Reactive Oxygen Species (ROS) or free radicals are implicated (Middleton, Kandaswamy, & Theoharides, 2000). The two most commonly synthetic antioxidants, used in stabilization of foods, are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) which are added to fatty and oily foods to prevent oxidative deterioration (Löliger, 1991). However, the use of these chemical compounds has begun to be restricted because of their induction of DNA damage and their toxicity (Ito et al., 1986). From this point of view, governmental authorities and consumers are concerned about the safety of their food and the potential effect of synthetic additives on their health (Reische, Lillard, & Eintenmiller, 1998). Hence, it is crucial to develop alternative natural antioxidant to overcome these issues. Recently, plant-derived polysaccharides have been recognized as

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safe, highly stable and effective natural antioxidants in pharmaceutical and food fields due to their nontoxicity and insignificant side-effects (Xu, Yao, Sun, & Wu, 2009).

On the other hand, food borne diseases caused by microorganisms are major dilemma in the third world and developing countries, and even in developed nations (Sokmen et al., 2004). The consumption of foods contaminated with some microorganisms represents a serious health risk to humans. The subsistence and growth of microorganisms in foods may lead to spoilage, formation of toxins and quality deterioration of food products (Celiktas et al., 2007). In this context, several researchers were challenged to discover new antimicrobials to prevent food spoilage and food poisoning, which are important issues faced by the food industry.

Periploca laevigata (Asclepiadaceae) is native to Mediterranean region and widely distributed in the Sahara area. In Tunisia, it is predominantly found in the south of the country, especially in the mountains. Different organs of the plant were used as a food ingredient such as in tea and as an herbal preparation because of its reputed medicinal properties including the treatment of headaches and diabetes (Askri, Mghiri, Bui, Das, & Hylands, 1989). The most studied *Periploca* species were *P. sepium*, *P. graeca* and *P. nigrescens*. It was reported that seeds, leaves and roots have various biological activities such as antiproliferative (Spera, Siciliano, De Tommasi, Braca, & Vessières, 2007), antitumor (Itokawa, Xu, & Takeya, 1988) and hypotensive (Askri et al., 1989). Previous studies described that the root barks of *P. laevigata* are a rich source of flavonoids, polyphenols and aromatic compounds mainly benzaldehyde (Hajji et al., 2009, 2010). To the best of our knowledge, there are no available reports on the purification and structural characterization of polysaccharides from *P. laevigata* root barks. Therefore, the aim of the present work was to purify, characterize and study the antioxidant and antibacterial activities of a novel polysaccharide from *P. laevigata*.

2. Materials and methods

2.1. Chemicals and reagent

1,1-diphenyl-2-picrylhydrazyl (DPPH), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), β-carotene, monosaccharide standards, trifluoroacetic acid (TFA) and linoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals, namely Vitamin C (Vc), hydrogen peroxide (H_2O_2), potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride ($FeCl_3$), sodium hydroxide (NaOH), Folin-Ciocalteu reagent, aluminium chloride, sodium nitrite, sodium carbonate, Tween 40 and other solvents, were of analytical grade. All solutions were freshly prepared in distilled water.

2.2. Plant material

Fresh roots of *P. laevigata* were collected from Matlegg Mountain (Regueb, Tunisia, LAT: 34.8792/LON: 9.7284) on September 2017. The raw material was washed with distilled water and then dried at room temperature for at least 5 days. The dried root barks were further crushed to obtain a fine powder, and then stored in glass bottles at room temperature.

2.3. Isolation and purification of PLP

P. laevigata root barks powder (100 g) was treated with 500 mL of 96% aqueous ethanol for 2 h to remove interfering components, polyphenols, pigments and lipids, and then dried at room temperature. The residues were mixed with 500 mL of hot water at 70 °C for three times (3 × 3 h). After vacuum filtration, the supernatants were collected and condensed to about 200 mL, and then mixed with 5 volumes of anhydrous ethanol at 4 °C overnight. Precipitates were dissolved in deionized water and deproteinized two times by Sevag solution (chloroform: butyl-alcohol, 4:1). The deproteinized solution was re-precipitated by addition of 500 mL anhydrous ethanol. The crude polysaccharide was dissolved in deionized water and centrifuged at 5000 rpm for 20 min. The supernatant, containing soluble polysaccharide, was applied to a DEAE-Sepharose Fast Flow column (25 mm × 30 cm) and eluted with 200 mL water, followed stepwise elution with, 0.2, 0.4 and 0.6 mol/L NaCl at a rate of 3 mL/min, the volume of each aliquot collected was 6 mL. Two peaks named PLP1 and PLP2 were obtained. Fractions of each peak were collected, concentrated and lyophilized for further use.

2.4. Carbohydrate, uronic acid and protein contents

The content of carbohydrate was determined *via* phenol–sulfuric acid method with D-Glucose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The content of uronic acid was determined through the m-hydroxybiphenyl colorimetric procedure by using D-glucuronic acid as the standard (Blumenkrantz & Asboe-Hansen, 1973). Bradford method was used to determine the content of protein using BSA (bovine serum albumin) as the standard (Bradford, 1976).

2.5. Molar mass determination

The homogeneity and molar mass of PLP1 was studied by UHPLC-SEC system equipped with a Waters Acquity UPLC pump, a differential refractometer, a multiangle laser light scattering detector and a TSK Gel GMPWXL type column. After filtration through 0.22 μm syringe filters (Whatman, England), 50 μL of the polysaccharide sample, at a concentration of 2.0 mg/mL, was injected. The mobile phase was 10 mmol/L sodium nitrate with 0.02% sodium azide (NaN_3) at a rate of 0.4 mL/min. Astra version 6.1.2 Wyatt software (Wyatt Technology Co., USA) was applied for the data acquisition and analysis.

2.6. Thermal properties

TGA analysis was performed on Q500 high resolution (TA Instruments) as previously described (Paramakrishnan, Jha, & Jayaram Kumar, 2015). PLP1 sample (~1.5 mg) was placed in an aluminum oxide pan and heated within a temperature range of 30–700 °C at a heating rate of 10 °C/min under nitrogen atmosphere. Regarding the DSC (Q20 Modulated, TA Instruments) analysis, 5 mg of purified PLP1 sample was used.

2.7. Monosaccharide composition analysis

The monosaccharide composition of PLP1 was identified by a gas chromatography using an Agilent 7890A instrument equipped with an HP-5 capillary column (30 m × 0.25 mm × 0.25 μm) and a flame-ionization detector (FID) and was carried out according to Xie et al. (2010). Briefly, PLP1 sample (5 mg) was hydrolyzed with 3 mL of 2 mol/L TFA, at 110 °C for 3 h. The excess acid was removed by vacuum evaporation with methanol after hydrolysis. Then, the hydrolyzed product was reduced with 50 mg sodium tetrahydruroborate ($NaBH_4$) and acetylated by pyridine (0.5 mL) and acetic anhydride (0.5 mL) at 40 °C for 2 h. The resulted alditol acetates were then filtred *via* 0.45 μm filter membrane and then analyzed by GC. The temperature program was as follows: increasing from 120 °C (1 min) to 240 °C at 10 °C/min and holding at 240 °C for 5 min. The temperatures of both injector and detector were set at 250 °C. Nitrogen was used as carrier gas. The standard sugars used were D-galacturonic acid, D-glucuronic acid, D-galactose, D-mannose, D-glucose, L-arabinose and L-rhamnose.

2.8. Methylation analysis

To determine glycosyl linkages, methylation analysis was carried

out according to Ciucanu and Kerek (1984). Briefly, a sample of PLP1 (10 mg) was dissolved in 5 mL of anhydrous dimethylsulfoxide at room temperature. Then, 10 mg of NaOH were added and the sample was well mixed and stirred for 1 h. The mixture was methylated by adding 3 mL of methyl iodine and the reaction was maintained 1 h at room temperature. The reaction mixture was treated extracted with three volumes of chloroform and the organic layer was washed with double-distilled water. The chloroform was then dried by vacuum rotary evaporator. Complete methylation was confirmed by the disappearance of the −OH band by infrared examination. The methylated sample was dissolved in 3 mL of 90% formic acid for 3 h at 100 °C. The residues were further hydrolyzed using 3 mL of 2 mol/L TFA for 3 h at 110 °C. After hydrolysis, excess TFA was removed by evaporation under reduced pressure. The methylated PLP1 was converted into alditol acetates by reduction with 50 mg $NaBH_4$ at room temperature. The acetylation process was the same as for the monosaccharide composition. After dissolution of the acetate derivatives in chloroform, the reaction mixture was analyzed with GC–MS.

2.9. Infrared spectroscopy

The PLP1 powder was applied to the FT-IR (Nexus of ThermoFisher) equipped with an attenuated reflection accessory (ATR) containing a diamond/ZnSe crystal, at room temperature (25 °C) in the spectral range frequencies of 650–4000 cm^{-1}. For each spectrum, 32 scans of interferograms were averaged and the spectral resolution was 4 cm^{-1}. Data analysis and treatment was carried out by using the OMNIC Spectra software (ThermoFisher Scientific).

2.10. ^{1}H NMR and ^{13}C NMR analysis

^{1}H NMR and ^{13}C NMR spectra of PLP1 were recorded on a Bruker 400 spectrometer (Bruker Biospin AG, Fallanden, Switzerland) at 25 °C. PLP1 was dried in a vacuum over P_2O_5 for 48 h, and then 20 mg of sample was dissolved in 1 mL of 99% deuterium oxide (D_2O). Analysis of data was carried out using MestRe Nova 5.3.0 (Mestrelab Research S.L.) software. Chemical shifts were given in ppm.

2.11. Antioxidant activity

2.11.1. $DPPH^{\cdot}$ radical scavenging capacity

The DPPH radical-scavenging ability of PLP1 was assessed as described by Bersuder, Hole, and Smith (1998). The PLP1 at different concentrations (0.25–2 mg/mL) was incubated with $DPPH^{\cdot}$ solution (0.2 mmol/L in ethanol) at room temperature for 1 h in the dark. Absorbance was recorded at 517 nm UV–vis spectrophotometer. The $DPPH^{\cdot}$ radical scavenging capacity was calculated as follows:

$$DPPH^{\cdot} \text{ radical scavenging activity (\%)} = (A_0 - A_1)/A_0 \times 100$$

where A_0 is the absorbance of the control reaction (without addition of the sample), A_1 was the absorbance of PLP1 solution in the reaction mixture.

IC_{50} value (μg/mL) is the inhibitory concentration of PLP1 at which $DPPH^{\cdot}$ radicals were scavenged by 50% and was determined by the linear regression analysis of the concentration-response curve of $DPPH^{\cdot}$ radical scavenging activity. The used standard was Vc. Three replicates were performed for each test sample.

2.11.2. $ABTS^{\cdot+}$ radical-scavenging activity

The reduction of $ABTS^{\cdot+}$ by PLP1 was determined as described by Re et al. (1999). The $ABTS^{\cdot+}$ was prepared by mixing equal volumes of 7 mmol/L ABTS solution and 2.45 mmol/L potassium persulphate. The resulting mixture was incubated for 16 h at room temperature in the dark to obtain a dark-colored solution containing $ABTS^{\cdot+}$ radicals and then diluted with ethanol to an absorbance of 0.7 ± 0.05 at 734 nm. This solution was added to the PLP1 sample at different concentrations (0.25–2 mg/mL). The decrease of absorbance at 734 nm was measured by spectrophotometer against ethanol used as a blank. The $ABTS^{\cdot+}$ radical scavenging ability was calculated using the following equation:

$$ABTS^{\cdot+} \text{ radical scavenging activity (\%)} = (A_0 - A_1)/A_0 \times 100$$

where A_0 was the absorbance of the mixture without sample and A_1 was the absorbance of the mixture with sample. Vc was used as positive standard. Data for each assay was recorded in triplicate.

2.11.3. Reducing power assay

The capacity of PLP1 to reduce iron (III) was evaluated according to the method of Yildirim, Mavi, and Kara (2001). A volume of 0.5 mL of sample at different concentrations (100–700 μg/mL) was mixed with 1.25 mL of 0.2 mol/L potassium phosphate buffer at pH 6.6 and 1.25 mL of potassium ferricyanide solution (1% v/v). After their incubation at 50 °C for 30 min, a volume of 0.5 mL of 10% (w/v) trichloroacetic acid (TCA) was added to the reaction mixtures. Then, 1.25 mL of each sample mixture was mixed with 1.25 mL of distilled water and 0.25 mL of 0.1% (w/v) ferric solution. The absorbance of the reaction mixtures was recorded at 700 nm after 10 min of incubation and a concentration-response curve was determined. Higher absorbance of the resulting solution indicated higher reducing power capacity. The commercial antioxidant Vc was used as positive control. Results were presented as means of experiments performed in triplicate.

2.11.4. DNA nicking assay

DNA nicking assay was performed according to the method of Lee, Kim, Kim, and Jang (2002). A volume of 8 μL of PLP1 sample at the concentrations of 0.5 and 1.0 mg/mL was added to 2 μL of pGAP plasmid DNA (0.5 μg/well). Then, the mixtures were kept for 10 min at room temperature followed by the addition of 10 μL of Fenton's reagent (80 μmol/L $FeCl_3$, 50 μmol/L ascorbic acid and 30 mmol/L H_2O_2). The mixture solutions were then incubated for 5 min at 37 °C. Finally, the DNA was analyzed on 0.8% (w/v) agarose gel electrophoresis and visualized under ultraviolet light.

2.12. Antibacterial activity

2.12.1. Bacterial strains

The antibacterial activity was studied against seven bacterial species from the American Type Culture Collection: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 19430), *Listeria monocytogenes* (ATCC 19117), *Staphylococcus aureus* (ATCC 25923), *Micrococcus luteus* (ATCC 4698) and *Bacillus cereus* (ATCC 11778).

2.12.2. Agar diffusion method

The antibacterial activity of PLP1 was evaluated according to the method described by Berghe and Vlietinck (2011). Culture suspensions (200 μL) of the microorganisms (10^6 cfu/mL of bacterial cells estimated by absorbance at 600 nm) were spread on Miller Hinton agar. Then, 60 μL of PLP1 in sterilized water were loaded into wells (6 mm in diameter) punched in the agar layer. The Petri dishes were kept at 4 °C for 1 h before incubation at 37 °C for 24 h. Antibacterial activity was estimated by determining the zone of growth inhibition (diameter expressed in millimeters) around the wells. Gentamicin at 20 μg/mL was used as positive standard to determine the sensitivity of bacterial strains and sterilized water as negative control. Tests were carried out in triplicate.

2.12.3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

MIC, defined as the lowest concentration that prevents visible growth, was determined according to Farag, Daw, Hawedi, and El-

Batory (1989) in sterile 96-well microplates with a final volume of 200 µL in each well. The PLP1 was dissolved in Tween 80 (1% w/v) then two-fold serial dilution was performed in *Luria-Bertani* (LB) medium. Each well of the microplate included 100 µL of diluted PLP1, 80 µL of the growth medium (LB) and 20 µL of cell suspension (10 ^{6}cfu/ml). Bacteria only in LB medium and 1% Tween 80 were used as positive and negative controls, respectively. After incubation of the plates for 24 h at 37 °C, 25 µL MTT (0.5 mg/mL) were added to the wells. The reaction of viable cells with MTT showed violet color. To estimate the minimum bactericidal concentration (MBC), an aliquot of 20 µL from each well presented no visible growth were spread on LB dishes (Sfeir, Lefrançois, Baudoux, Derbré, & Licznar, 2013). The MBC was the lowest concentration that gave no culture on the Petri dishes after incubation for 24 h at 37 °C. MIC and MBC were expressed in mg/mL. The determination of MIC and MBC values was assessed in triplicate.

2.13. Statistical analysis

Statistical analyses were performed with SPSS ver.17.0, professional edition. The mean differences between tests were examined by Duncan test and compared by one-way analysis of variance (ANOVA). Differences were considered significant at *p-value* < 0.05. All tests were carried out in triplicate.

3. Results and discussion

3.1. Isolation and purification of polysaccharides

The crude polysaccharides designed as PLPs were obtained from *P. laevigata* root barks with a yield of 7.75% by hot water extraction and ethanol precipitation. The chemical composition of PLPs was determined and data were summarized in Table 1. The content of proteins was less than 5% and the amount of total sugars and uronic acid were 86.3% and 10.7%, respectively. After deproteinization, PLPs solution was loaded into DEAE-Sepharose column and was then eluted with NaCl gradient from 0 to 0.6 mol/L. As shown in Fig. 1, two fractions named PLP1 and PLP2 were obtained with 0.92% and 0.14% yield, respectively. The PLP1 was eluted with 0.2 mol/L NaCl solution and the PLP2 was eluted with 0.4 mol/L NaCl. As shown in Table 1, the total sugar of PLP1 and PLP2 was estimated by phenol-sulfuric acid method at 93.3% and 62.8%, respectively. The protein content, determined according to Bradford method, was not detected for PLP1 and was approximately 6.7% for PLP2. Knowing that free proteins in the crude polysaccharides were removed by Sevag method, the above results confirmed that PLP2 was a protein conjugate polysaccharide. The uronic acid content showed that PLP2 was richer than PLP1 with 26.2%. Jiang, Qiu, Li, Li, and Wang (2016) reported a lower yield (1.96%) of polysaccharides extraction by ethanol precipitation from *Marsdenia tenacissima* leaves, belonging to the Asclepiadaceae family. In addition, the chemical characterization of this polysaccharide extract revealed the average values of 5.6% proteins and 93.8% carbohydrates, among them 21.3% are uronic acids, which were higher than those obtained for PLP1. Based on extraction yield and carbohydrate richness, the PLP1 was selected to be further characterized.

Table 1
The content of carbohydrate, protein, uronic acid of PLPs, PLP1 and PLP2.

	PLPs	PLP1	PLP2
Carbohydrate (%)	86.3 ± 2.6a	93.3 ± 2.3b	62.8 ± 1.7c
Proteins (%)	4.8 ± 0.7c	ND	6.7 ± 0.8a
Uronic Acid (%)	10.7 ± 1.1c	4.1 ± 0.5a	26.2 ± 1.1c

ND: not detected.
Different letters (a, b and c) for each row showed significant difference ($p < 0.05$).

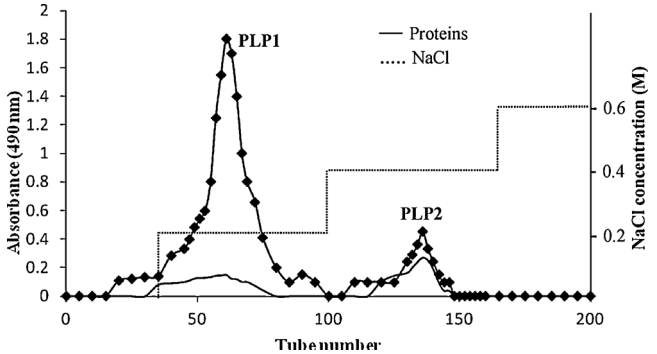

Fig. 1. The elution profile of crude polysaccharides PLP isolated from *Periploca laevigata* root barks on a DEAE-Sepharose Fast Flow column (25 mm × 30 cm) eluted with 200 mL distilled water and NaCl solution (0 to 0.6 mol/L) step gradient at a flow rate of 3 mL/min, the volume of each aliquot collected was 6 mL.

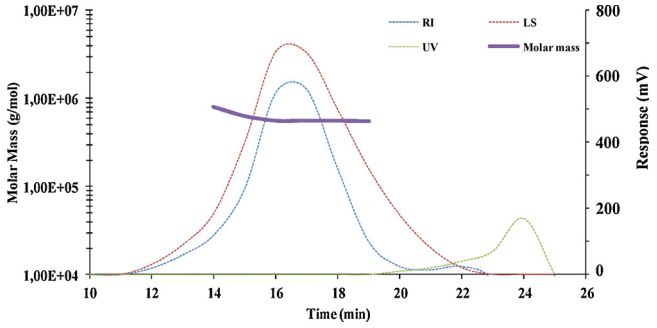

Fig. 2. Molar mass determination of PLP1 by size exclusion chromatography/ multiangle laser light scattering/ refractive index system. The sodium nitrate (10 mmol/L) with 0.02% NaN_3 used as mobile phase at a flow rate of 0.4 mL/min.
LS: Laser light Scattering; UV: Ultra Violet; RI: Refraction Index.

3.2. Molar mass and monosaccharide composition

The molar mass and homogeneity of the PLP1 were evaluated by UHPLC-SEC. As shown in Fig. 2, the profile revealed that the previously eluted fraction corresponding to PLP1 is a single, indicating that PLP1 is a homogeneous component with a high purity. The molar mass was estimated to be 5.57 10^{5} g/mol. PLP1 had negative response to Bradford test and had no absorption in UV spectrum, demonstrating the absence of nucleic acid and protein.

The monosaccharide composition of PLP1 was analyzed by comparing the retention time against standards by GC. PLP1was composed of glucose, galactose, mannose and glucuronic acid in a molar percent of 62.9, 19.4, 11.8 and 5.9, respectively. Arabinose, rhamnose and galacturonic acid were not detected in PLP1. These results indicated that PLP1 was a heteropolysaccharide with glucose as the major sugar ingredient. Differently, the purified polysaccharide from *M. tenacissima* exhibited higher contents of galactose (30%), mannose (17.7%) and glucuronic acid (20.6%) with absence of glucose and presence of arabinose (9.1%) and xylose (22.4%) (Jiang et al., 2016).

3.3. Thermal properties

The thermostability is an important parameter for bioactive molecules by considering sterilization process. As shown in Fig. 3, the PLP1 TGA profile displayed two different stages. The first one, covered from 33 °C to 154 °C, was mainly associated with the loss of the bound water (Castaño, Rodríguez-Llamazares, Carrasco, & Bouza, 2012) and the mass loss was about 19.81%. The second stage, occurred between 154 °C and 537 °C, was attributed to the change of functional groups,

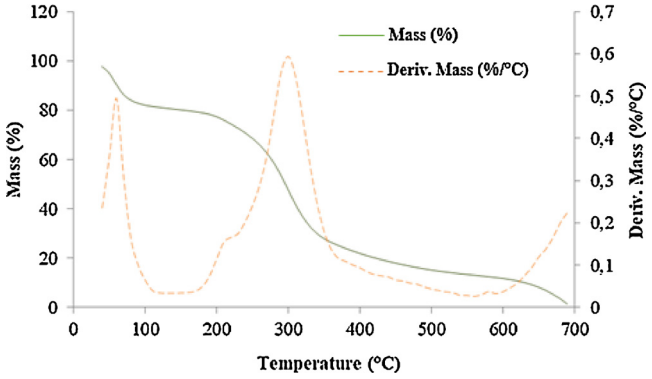

Fig. 3. Thermogravimetry and differential thermogravimetry curves of PLP1. The sample (1.395 mg) was heated within a temperature range of 30–700 °C at a heating rate of 10 °C/min under N_2 atmosphere.

depolymerization and decomposition structure fractions (Zhen, Hong, Yong, & Tan, 2015), with a loss rate of 86.07%.

DSC was further used to determinate the occurrence of exothermal or endothermal changes with an increased temperature. The T_0 and T_p were approximately 60 and 300 °C, respectively. The enthalpy (ΔH) value of PLP1 was 183.4 J/g. The thermal behavior is associated to the ratio of sugars constituting PLP1. The results from TGA and DSC showed that PLP1 may structurally be stable and has good thermal stability under 229 °C.

3.4. FT-IR spectroscopy

The FT-IR spectrum of the purified PLP1 from 650 to 4000 cm^{-1} was illustrated in Fig. 4. The characteristic intense band of hydroxyl groups stretching vibration was detected at around 3359 cm^{-1}. The weak peaks at 2934 cm^{-1} and 1243 cm^{-1} were attributed to C—H stretching band (Xie et al., 2013). Uronic acid was evidenced by one absorbance band at 1415 cm^{-1} (O—C═O binding), with the band located at 1621 cm^{-1} consistent with the asymmetric stretch vibration of COO— as was found for uronic acids (Jia et al., 2013). The three strong absorption peaks in the range of 1000 cm^{-1} and 1200 cm^{-1} were attributed to the presence of C—O—C and C—O—H stretching vibration, which were ascribed to pyranose ring (Ding, Hou, & Hou, 2012). PLP1 showed strong absorption peak at 1038 cm^{-1} and 1086 cm^{-1} suggesting the presence of glucose and galactose, respectively (Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000). The weak peaks in the range of 900 cm^{-1} and 800 cm^{-1} suggested that both

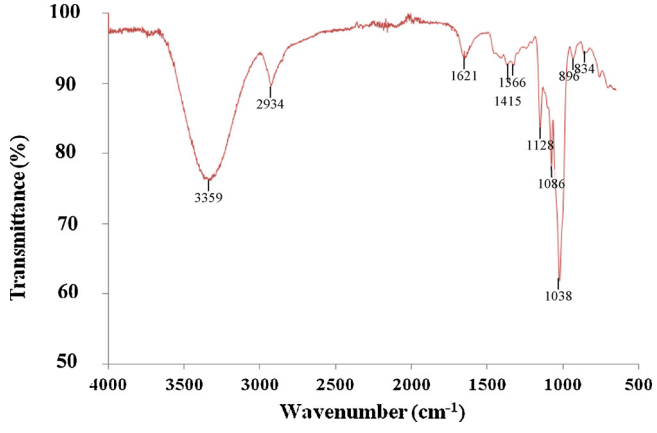

Fig. 4. FT-IR spectrum of PLP1. The PLP1 powder was applied to the FT-IR at room temperature (25 °C) in the spectral range frequencies of 650–4000 cm^{-1}. Data analysis and treatment was carried out by using the OMNIC Spectra software.

Table 2
Methylation analysis results of PLP1.

Methylated sugar	Linkage types	Molar percentage (%)
2,4,6-Me_3-Glc	1,3-Glc*p*	44.2
2,6-Me_2-Glc	1,3,4-Glc*p*	20.7
2,3,4,6-Me_4-Glc	1-Glc*p*	6.8
2,3,6-Me_3-Man	1,4-Man*p*	4.6
2,3,4-Me_3-Man	1,6-Man*p*	6.0
2,3,4,6-Me_4-Gal	1-Gal*p*	3.9
2,4,6-Me_3-Gal	1,3-Gal*p*	6.5
2,3,4-Me_3-Gal	1,6-Gal*p*	6.4

α and β-configuration simultaneously exist in PLP1 (Yu et al., 2015).

3.5. Methylation analysis

Methylation analysis of PLP1 was performed to determine the linkage pattern of the monosaccharide residues. As shown in Table 2, the obtained data revealed the presence of eight components, namely, 2,4,6-Me_3-Glc, 2,6-Me_2-Glc, 2,3,4,6-Me_4-Glc, 2,3,6-Me_3-Man, 2,3,4-Me_3-Man, 2,3,4,6-Me_4-Gal, 2,4,6-Me_3-Gal and 2,3,4-Me_3-Gal. The molar percentages were 44.2%, 20.7%, 6.8%, 4.6%, 6.0%, 3.9%, 6.5% and 6.4%, respectively, indicating that PLP1 was mainly composed of (1→3), (1→3,4) connected glucose as the backbone.

3.6. ^1H and ^{13}C NMR analysis

The ^1H NMR and ^{13}C NMR spectra of PLP1 are shown in Fig. 5. Most chemical shifts were ranged from δ 3.0 to δ 5.4 ppm, which was the specific feature of polysaccharides in ^1H NMR spectrum (Hu, Liang, & Wu, 2015). As shown in Fig. 5A, PLP1 spectrum exhibited a set of wide and intense signals between δ 3.0 and δ 4.0 ppm corresponding to CH—O and CH_2—O sugars groups. The anomeric proton signals at δ 5.01, δ 5.17 and δ 5.32 ppm revealed that PLP1 might contain three kinds of monosaccharide residues. In addition to the anomeric region, shifts at δ 4.63, δ 4.83, indicated the existence of both α and β-configurations (Huang et al., 2016), consistent with the presence of weak bands between 800 cm^{-1} and 900 cm^{-1} in the FT-IR spectrum of PLP1.

The ^{13}C NMR spectrum of PLP1 was crowded in a narrow region ranging from δ 60.1 to 107.3 ppm (Fig. 5B). The signals in δ 99.6–107.3 ppm corresponded to anomeric carbons atoms of D-glucose, D-galactose and D-mannose. The resulted data revealed the presence of α and β anomeric configuration as obtained by ^1H NMR spectrum. The signal observed at δ 5.32 ppm (^1H NMR) corresponded to anomeric proton of α-glucopyranose unit in the main chain, which was confirmed by the signal at δ 99.6 ppm in ^{13}C NMR spectrum.

3.7. Antioxidant activity of PLP1

The antioxidant capacity of PLP1 was evaluated using four complementary tests, namely the scavenging ability of the DPPH˙ radicals, ABTS radical scavenging activity, reducing power and the oxidative damage DNA protective assay.

Free radical scavenging is thought to be one of the main mechanisms exhibited by antioxidants to delay oxidative processes. DPPH˙ is a stable free radical, which accepts a hydrogen radical to become a stable diamagnetic molecule (Kedare & Singh, 2011). As shown in Fig. 6A, the DPPH˙ radical scavenging activities increased in a PLP1 concentration-dependent manner. PLP1 exhibited a strong radical scavenging ability (IC_{50} = 0.82 mg/mL) and reached 95% at a concentration of 1.25 mg/mL which was higher than CPHP I, a polysaccharide extracted and purified from *Cissus ptereoclada Hayata*, showing radical scavenging activity of 87.69% at 1.4 mg/mL (Li et al., 2015). Wang, Cheng et al. (2016) and Wang, Liu et al. (2016) isolated a polysaccharide from *Cucurbita moschata* seeds with 40.5% scavenging rate at 1.0 mg/mL,

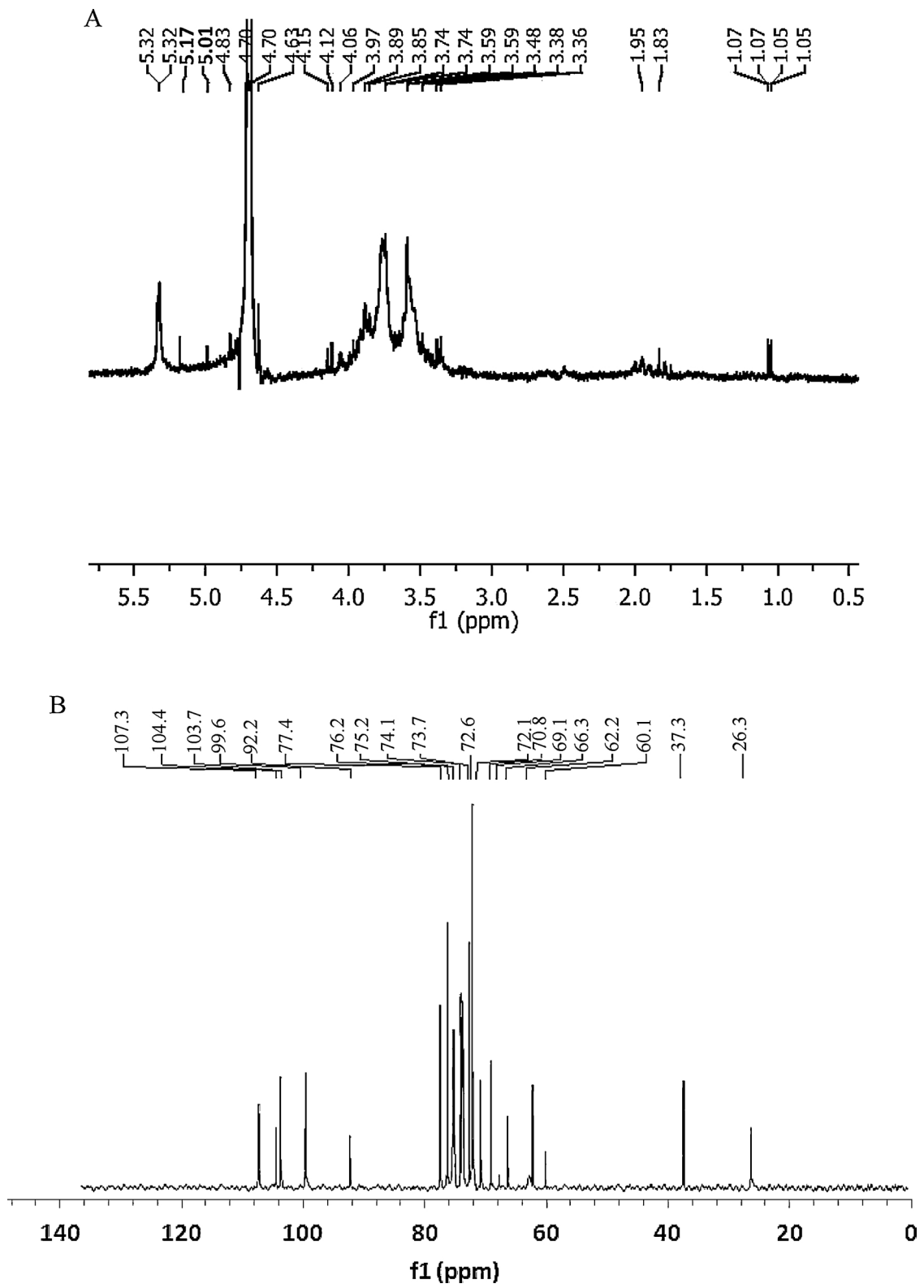

Fig. 5. ^{1}H (A) and ^{13}C NMR (B) spectra of the PLP1. NMR profiles were recorded at 25 °C on a Bruker 400 spectrometer using 20 mg PLP1 sample. The obtained data were analyzed using MestRe Nova 5.3.0 software.

which is lower than scavenging ability of PLP1. However, PLP1 exhibited lower activity compared with polysaccharides extracted from camellia seed cake (Shen et al., 2014) and from *Cyclocarya paliurus* (Batal.) Iljinskaja (Xie et al., 2010), whose inhibitory rates were 91.05% at a concentration of 0.6 mg/mL and around 90% at 0.27 mg/mL, respectively. Those differences could be due not only to the conformational characteristic and sugar composition of polysaccharides but also to the methodology of extraction and purification where possible modifications in the secondary structure could be occurred by some treatments such as deproteinization.

ABTS radical scavenging activity is used to determine the ability of antioxidants to donate a hydrogen atom by converting it to the non-

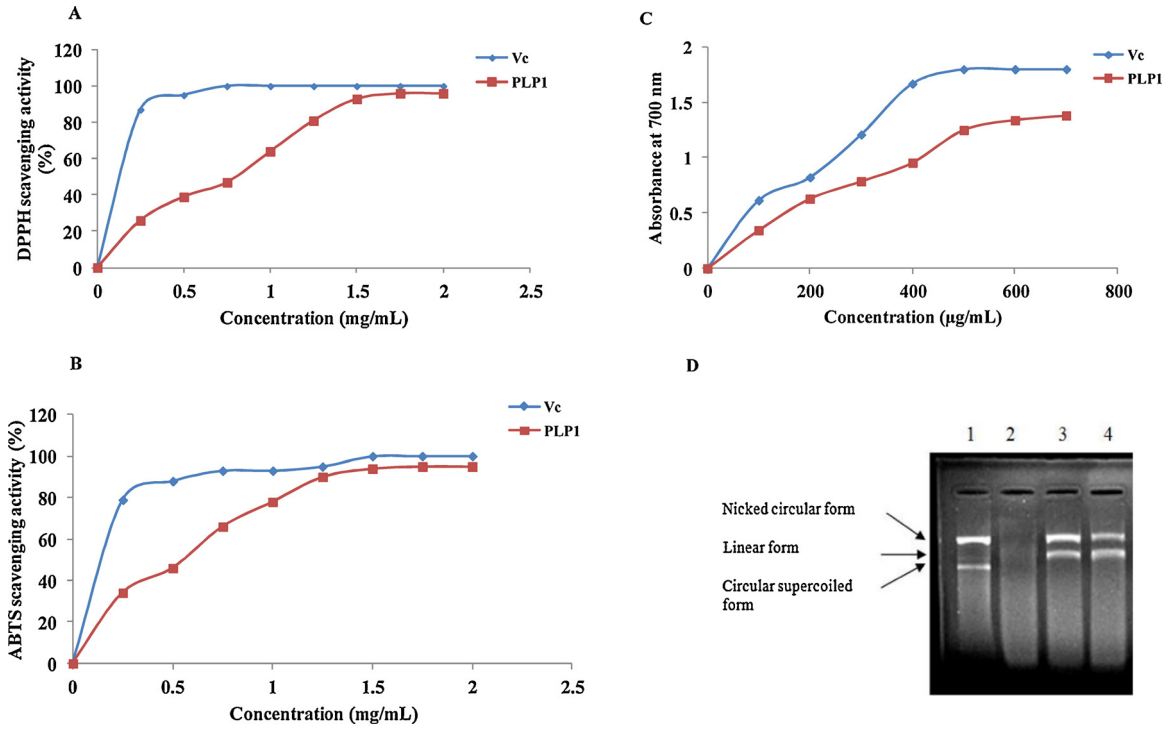

Fig. 6. Antioxidant activity of the polysaccharide purified from root barks of *P. laevigata*: (A) DPPH scavenging activity; (B) ABTS scavenging ability; (C) Ferric reducing power; (D) DNA protection assay: Lane 1: native pGap plasmid DNA, lane 2: plasmid DNA incubated with Fenton's reagent, lane 3 and 4: plasmid DNA incubated with Fenton's reagent and 0.5 and 1 mg/mL of PLP1, respectively.

radical species (Binsan et al., 2008). Specific absorbance at 734 nm wavelength can be used as an index reflecting the antioxidant activity of polysaccharide. As shown in Fig. 6B, PLP1 displayed obvious scavenging activity against ABTS radical in a concentration-dependent manner. The effective concentration (EC_{50}) was about 0.67 mg/mL. Compared to the scavenging power of polysaccharides from *Cistanche tubulosa* where the rate not exceed 86% at the same concentration (Zhang et al., 2016), PLP1 showed higher activity. These results indicated that PLP1 had strong scavenging power for ABTS radical and could be explored as novel potential antioxidant.

Fe (III) reduction can be used as an indicator of electron-donating activity, which reflects an important mechanism of polysaccharide antioxidant action. As observed in Fig. 6C, the reducing power of PLP1 and Vc increased with their concentrations which is in agreement with previous reports of antioxidant potential of polysaccharides extracted from various plant sources (He, Zhao, Zhao, & Sun, 2015; Liang et al., 2011). PLP1 displayed strong reducing potential (EC_{50} = 0.175 mg/mL) as previously reported for polysaccharides extracted from *Edwardsia sipunculoides* (He et al., 2015). PLP1 could act as good reductant due to its electron donating capacity to transform Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}) leading to stabilize free radicals and inhibit the pursuit of the oxidative chain reactions.

In case of plasmid, damage of DNA results in a cleavage of one of the phosphodiester chains of the supercoiled DNA and produces a relaxed open circular form. Further, cleavage near the first breakage results in linear double-stranded DNA molecules. The formation of circular form of DNA is indicative of single-strand breaks and the formation of linear form of DNA is indicative of double-strand breaks (Burrows & Muller, 1998). In this study, oxidative DNA damage protective activity of PLP1 was evaluated against $OH^{\cdot}$ induced damage on pGAP plasmid DNA. As shown in lane 2 (Fig. 6D), incubation of DNA with Fenton's reagent resulted in a complete degradation of DNA, indicating that $OH^{\cdot}$ generated by Fenton's reagent produced multiple double-strand DNA breaks. Addition of PLP1 at 0.5 and 1 mg/mL (lane 3 and 4) showed partial retention of supercoiled DNA and mitigated the oxidative stress produced by Fenton's reagent. As previously depicted in the DPPH, ABTS and reducing activity assays, PLP1 exhibited great potential to quench reactive oxygen species by donating hydrogen atom or electron. This type of action might prevent the free radicals-mediated oxidation of DNA through directly scavenging $OH^{\cdot}$ and therefore protecting the supercoiled plasmid DNA from $OH^{\cdot}$ radicals. In this study, it was shown that PLP1 exhibited strong antioxidant activity through different mechanisms that could be used in various food and pharmaceutical applications to protect products from oxidation and enhance their health effects.

3.8. Antibacterial activity of PLP1

Since controlling bacterial infections has becoming a serious problem as a result of the developed resistance of bacteria against a large spectrum of commercial antibiotics, the research of new natural materials with antibacterial agents has recently expanded (Smith, 2004). In this study, antibacterial activity of PLP1 was assessed against three Gram- and four Gram + bacteria. As can be seen in Table 3, PLP1 showed varying degrees of antibacterial activity against all strains

Table 3
Antibacterial activities of PLP1 polysaccharide extracted from *P. laevigata* root barks.

Tested bacteria	Inhibition zone diameter (mm)		MIC (mg/mL)	MBC (mg/mL)
	PLP1	Gentamycin		
E. coli	15.5 ± 0.5	20.0 ± 1.0	0.5	0.8
P. aeruginosa	13.0 ± 1.0	18.0 ± 1.0	1.0	1.5
S. typhimurium	ND	16.0 ± 1.0	NT	NT
L. monocytogenes	9.0 ± 0.5	18.0 ± 1.0	0.8	0.8
S. aureus	11.0 ± 1.5	21.0 ± 1.0	0.3	0.8
M. luteus	12.0 ± 1.0	20.0 ± 1.0	0.5	0.6
B. cereus	14.0 ± 1.5	19.0 ± 1.0	0.3	0.5

ND: not detected, NT: not tested.

tested. The inhibition zones values ranged between 9 and 15.5 mm. Data showed that *E. coli*, a Gram-negative bacterium, was the most sensitive to PLP1, whereas, no antibacterial activity was detected in the case of *S. typhimurium*. These results are in agreement with the findings of Fakhfakh, Abdelhedi, Jdir, Nasri, and Zouari (2017) who reported that the polysaccharide extracted from *Malva aegyptiaca* exhibited the highest inhibitory effect against Gram-negative bacteria. Comparing MIC and MBC, bioactive components from natural products can be classified into bacteriostatic (MIC < MBC) and bactericide agent (MIC = MBC). According to these parameters, the purified polysaccharide from *P. laevigata* can be considered a bactericide agent against L. *monocytogenes*, however, a bacteriostatic agent against all other tested bacteria. Antibacterial activities have similarly been described for other polysaccharides isolated from plants such as *Olea europaea* (Khemakhem, Abdelhedi, Trigui, Ayadi, & Bouaziz, 2018), *Broussonetia papyrifera* (Han et al., 2016), *Diaphragma juglandis fructus* (Meng, Li, Xiao, Zhang, & Xu, 2017) and *Ilex paraguariensis* (Kungel, Correa, Correa, Peralta, & okovic, 2018). Previous studies have moreover shown that polysaccharides from *Cyclocarya paliurus* (Batal.) exhibited good antifungal activity against *Saccharomyces cerevisiae* and *Candida* sp. and moderate antibacterial activity against *E. coli*, *S. aureus* and *B. subtilis* at a concentration of 1 mg/mL with diameter zone inhibitions of 6.54, 6.57 and 6.93 mm, respectively (Xie et al., 2012). The mechanisms involved in the antibacterial activity of polysaccharides are worthy of further investigations but it suggested that they might act as barriers inhibiting bacterial growth by stopping the import of nutriments (Ren et al., 2014).

4. Conclusion

In this study, a novel polysaccharide PLP1 was extracted from *Periploca laevigata* by hot-water extraction and purified with DEAE-Sepharose chromatography. The average molecular weight of PLP1 was 5.57 10^5 g/mol. PLP1 was composed of glucose, galactose and mannose with molar percent of 62.9, 19.4, and 11.8, respectively. The main backbone of PLP1 was composed of 1,3-β-D-Glc*p*, 1,3,4-α-D-Glc*p*, 1,4-α-D-Man*p*, 1,6-α-D-Man*p*, 1,3-α-D-Gal*p* and 1,6-β-D-Gal*p*. PLP1 showed antioxidant activities in dose-dependent manner and performed a strong antibacterial activity. Overall, the present study suggests that the *P. laevigata*, a spontaneous and mountainous plant, could be considered as a promising source for polysaccharides with interesting bioactivities in the medical and food industries. Moreover, further investigation to elucidate the structure-activity relationship of PLP1 and the antibacterial possible molecular mechanism is necessary.

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