Evaluation of physicochemical characteristics and antioxidant property of Prunus avium gum exudates

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A B S T R A C T
In this study some physicochemical properties and elemental analysis of Prunus avium gum exudates were investigated. The gum studied had, on average, 75.14% carbohydrate, 11.3% uronic acids, 1.11% protein, 7.53% moisture content (w.b.) and 3.12% ash. Measured values for the angle of repose, Carr’s index and Hausner ratio showed the good flow ability for the gum powder. The viscosity of 1% aqueous solution of the gum exhibited a Newtonian type of flow and with pH reduction the swelling index was increased. The average molecular weight of the main polysaccharide fraction was about 1.46 × 10^5 Da (146 kDa). GC analysis showed that the main polysaccharide was composed of four kinds of neutral monosaccharides, namely mannose (Man), arabinose (Ara), galactose (Gal) and xylose (Xyl) with a relative molar ratio of 1.0:14.7:7.1:2.4. FTIR analysis showed the presence of carboxyl and hydroxyl groups and glycosidic linkage. The antioxidant activity of the gum was evaluated by determining DPPH scavenging and total phenolic contents which showed poor antioxidant property.

1. Introduction

Natural gums are obtained as exudates from different tree species. With wide multiplicity of uses, they have various and exclusive physicochemical properties [1]. For many years, gum exudates have been used in food applications, including, emulsification, thickening and stabilization of processed foods [2].

The exudate gums have a different component profile. They are composed mainly of polysaccharides with diverse structures. The composition of gum polysaccharides depends on species and cultivar of the plant [3]. The composition of monosaccharides, types and patterns of linkage, chain shapes, side branches and degree of polymerization govern the structural features of polysaccharides. These features determine the physical properties of gums including solubility, flow behavior, gelling potential and interfacial properties [4].

The Biological activity of this compounds for example anticancer, immunomodulation and antioxidant properties have been reported in several investigations. [5–10]. The presence of galactose, arabinose, rhamnose, uronic acids, galacturonic acid, protein, Ca and Mg as the main structural constituents of plant gum exudates, have been reported [11].

Species of the genus Prunus L. are mostly found in the northern hemisphere. Most of the species occur in semiarid climates [12]. Prunus is economically important because they are sources of fruits, oil, timber, and ornamentals [13]. The gum exudates of cherries (Prunus cerasus L.), ornamental Japanese cherries (Prunus yedoensis), plums (Prunus domestica L.), apricots (Prunus armeniaca L.), Japanese apricots (Prunus mume Sieb. et Zucc.), peaches (Prunus persica Batsch) and almonds (Prunus amygdalus Batsch.) have been studied [3].

Ščerbučin, Rosík and Kubala [14] showed that acidic polysaccharide of Prunus avium L. tree gum (var. duracina L.), composed of d-glucuronic acid, 4-O-methyl-d-glucuronic acid, d-galactose, d-mannose, ×-arabinose, and ×-xylose. It has been reported that the exudates gum polysaccharide of P. avium and P. cerasus, have the compact internally cross linked structure [15].

The aims of this work were to investigate the physicochemical, functional and antioxidant properties of Prunus avium exudate gums.

2. Materials and methods

The gum exudates of sweet cherry trees (Prunus avium) were collected from Marand, East Azerbaijan Province (Iran) during the
month of July–September (2015). The gum was dried in oven at 40°C, grounded in to powder and passed through sieve no. 50. DEAE-Cellulose A52 and Sephadex G–100 were purchased from the Pharmacia Co. (Uppsala, Sweden). Dextran of different molecular weights, bovine serum albumin (BSA) and pure monosaccharide standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All the used chemical materials were of analytical grade.

2.1. Polysaccharide purification

The polysaccharides of gum were purified according to the method described by Pachau, Lalhenmawia and Mazumder [16] with slight modification. Briefly, crude gum powder was boiled with 80% ethanol with a ratio of 1:4 (w/v) to enzymes deactivation and remove low molecular weight carbohydrates and coloring substances. It was dispensed in deionized water and gently stirred overnight in a magnetic stirrer. The gum solution was then centrifuged at 12000g to separate any undissolved matters. The gum solution was then filtered through Whatman No.1 filter paper and precipitated with 3 volumes of 2-propanol, collected and air dried. They were passed through sieve no. 85, stored in airtight container for elemental analysis.

2.2. Physicochemical characterization

2.2.1. Determination of ash content

The ash content was determined by following the method of Yebeyen, Lemenih and Feke [17]. About 1 g of the gum powder was first heated on a burner in air to remove its smoke. Then, it was burned in a furnace at 550°C. The ash content was calculated as follow:

\[
\text{Ash content}(\%) = 100 \times \left( \frac{\text{weight of ash}}{\text{weight of gum powder}} \right)
\]

2.2.2. Moisture contents (percent loss on drying)

About 1 g of ground gum powder sample was weighed and oven dried at 105°C for 5 h. Oven dry weight was taken after allowing the sample to cool in a desiccator before reweighing. The Moisture content was expressed as a percentage of the weight loss from the original weight [17].

2.2.3. Determination of viscosity

Viscosity of 1% sample solution in distilled water was determined at 25°C using the Brookfield viscometer (Model DV-E, Spindle ULA). Effect of shear rate (rpm) on the viscosity was studied by varying the shear rate from 300 to 6000 rpm [9].

2.2.4. Determination of angle of repose

A fixed height funnel fitted at the height of 10 cm from the base was used to measure the angle of repose (the funnel is 60°, 4.8 cm in diameter, 0.5 cm internal stem diameter with 10 cm stem length). A pile was formed at the base by flowing about 20 g of the dried powder (sample) through the funnel into the base. The angle of repose was then calculated as follows:

\[
\text{Angle of repose} = \tan^{-1}(h/r)
\]

Where h and r are the height and radius of the pile, respectively [9].

2.2.5. Compressibility index

Bulk and tapped densities were used to determine the compressibility index of the sample. About 2 g of the gum powder was taken into a 10 ml graduated measuring cylinder and the initial volume (V₀) was recorded. The cylinder was then tapped 100 times using the bulk density apparatus to achieve a final volume (V₁). The experiment was performed in triplicate and is reported as means and standard deviations. The bulk density was calculated from the initial volume and tapped density from the final volume. Carr’s index and Hausner ratio were then determined by the following equations [9]:

\[
\text{Carr’s index} = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} \times 100
\]

\[
\text{Hausner ratio} = \frac{\text{bulk density}}{\text{tapped density}}
\]

2.2.6. True density and porosity

The true densities (ρ.true) of gum powders were determined by the liquid displacement method using xylene as the immersion fluid, and computed according to the following equation:

\[
\rho_{true} = \frac{w}{(a + w - b)} \times SG
\]

Where w is the weight of powder, SG is the specific gravity of solvent, a is the weight of bottle + solvent and b is a weight of bottle + solvent + powder [18]. The porosity of the test powders was derived from the values of the true and tapped densities fitted into the following equation:

\[
\text{Porosity} = \left( 1 - \frac{\text{tapped density}}{\text{true density}} \right) \times 100
\]

2.2.7. Swelling index

Swelling index of the gum powder was determined according to the WHO method [19] with a slight modification. Briefly, about 1 g of the fine sample, was introduced into the three 25 ml glass-stoppered measuring tubes. Then, 25 ml of distilled water, 0.1 N HCl and phosphate buffer (pH = 7.4) was added to each tube and the mixture was shaken every 10 min for 1 h and allowed to stand for 24 h at room temperature. The swelling index was calculated using the measured volume that occupied by the sample.

2.2.8. Foaming index

About 1 g of the sample was transferred into a 500 ml conical flask containing 100 ml of boiling water. The moderate boiling temperature was maintained for 30 min, then it was cooled and filtered. The decoction was poured into 10 glass-stoppered test tubes in successive portions of 1.2, 3 ml, up to 10 ml the volume of liquid in each tube was adjusted to 10 ml with water. The tubes were thoroughly shaken in for 15 s at two shakes per second. It was allowed to stand for 15 min the foam height was measured [19].

2.2.9. Total carbohydrates, uronic acid and protein contents

Phenol–sulphuric acid method was used to determine the total carbohydrate content of the gum. D-glucose was taken as standard [20]. The uronic acid content was also analyzed by carboxyl groups method, taking D-galacturonic acid as the standard [21]. The absorbance was measured by the UV–vis spectrophotometer (SP-UV 500DB) at 490 and 535 nm for total carbohydrates and uronic acids, respectively. Crude protein content was measured using the Kjeldahl method and considering 6.25 as the conversion rate of nitrogen to crude protein [22].

2.3. DPPH radical scavenging activity

DPPH activity test was Carried out according to the Blois method [23] with minor modifications. Butylated hydroxyl anisole was taken (BHA) as the reference standard for comparison. The series of sample solutions with different pre-determined concentrations gum powder was produced by dissolving the gum powder in water. In all cases 0.5 ml of DPPH solution in methanol (0.1 mM) was mixed
with 3 ml of the polysaccharide (and BHA) sample solution and incubated for 30 min at 37 °C. Absorbance was measured at 517 nm. The same procedure was also performed for gum powder and standard solutions of similar concentrations. The inhibition was then calculated by using the formula:

\[
\% \text{Inhibition} = \frac{\text{Abs}_{\text{cont}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{cont}}} \times 100
\]

Where \( \text{Abs}_{\text{cont}} \) is absorbance of control (DPPH in methanol at concentration of 0.1 mM) and \( \text{Abs}_{\text{sample}} \) is absorbance of sample solution (both gum powder and BHA solutions at the same concentration).

2.4. Determination of total phenolic contents

Folin-Ciocalteu method was used to determine the total phenolic content of the sample [24]. About 1 ml of the gum solution was mixed with 2.5 ml of reagent. After 1 min, 2 ml of sodium carbonate solution was added and the mixture was allowed to stand for 30 min at room temperature followed by measuring the absorbance at 765 nm. Gallic acid at different concentrations was used to prepare the calibration curve. The total phenolic compounds were determined and expressed as mg gallic acid equivalent (GAE) of dried gum exudate.

2.5. General analysis of gum polysaccharide

Concentrations were performed under diminished pressure at around 45 °C (Heidolph Laborota 4000 efficient rotary evaporator, Germany). The products were dried by vacuum freeze drying (Christ Alpha 1–2 freeze dryer, Germany). Protein in the polysaccharide was quantified according to the Bradford method [25], using bovine serum albumin (BSA) as the standard. Ultraviolet-visible absorption spectra were recorded with a Varian Cary100-Bio UV/visible spectrophotometer (USA). Infrared spectra were recorded using a Fourier transform infrared spectrophotometer (FT-IR, Nicolet 5700 Instrument, Thermo Company, Madison, USA) with KBr pellets in the frequency range 4000–400 cm\(^{-1}\). Gas chromatography (GC) was performed on a Varian 3400 instrument (Hewlett-Packard Component, USA) equipped with a DM-2330 capillary column (30 m × 0.32 mm × 0.20 µm) and flame-ionization detector (FID). The column temperature was kept at 120 °C for 2 min, then increased to 250 °C (maintained for 3 min) at a rate of 8 °C/min. N₂ was used as the carrier gas at a flow rate of 1.2 ml/min. The injector and detector heater temperature were 250 and 300 °C, respectively.

2.6. Purification of the polysaccharide

Polysaccharide was purified by the method of Jahanbin et al. [26] as described earlier. Crude \( P. Avium \) polysaccharides (CPAP) were redissolved in deionized water and forced through a filter (0.45 µm), then loaded onto a column (2.6 cm × 30 cm) of DEAE-Cellulose A52. After loading with the sample, the column was eluted with the gradient NaCl aqueous solution (0–1 M), and the procedure was monitored by the phenol-sulfuric acid method [27]. A major polysaccharide fraction (PAPS) was further purified on a Sephadex G-100 gel filtration column (1.6 cm × 70 cm), and eluted with deionized water, at a flow rate of 8 ml/h. The main polysaccharide fraction was collected and lyophilized to obtain white purified polysaccharide named, as PAPS-1 and used for further study.

2.7. Molecular weight determination

The molecular weight of PAPS-1 was evaluated and determined by high performance gel permeation chromatography (HPGPC) with a Shimadzu HPLC system equipped with a TSK-GEL PWXL (Tosoh Biosep, Japan) column (7.8 mm × 300 mm), eluted with 0.1 M Na₂SO₄ solution at a flow rate of 0.5 ml/min and detected by a RID-10A refractive index detector (Shimadzu, Japan) at 40 °C. The molecular weight of PAPS-1 was estimated by reference to a calibration curve made from a set of Dextran T-series standards of known molecular weights (200,000, 70,000, 40,000, 10,000 and 5000 Da).

2.8. Monosaccharide composition analysis

The monosaccharide composition of PAPS-1 was analyzed by GC. PAPS-1 was hydrolyzed with 2 M TFA (2 ml) at 120 °C in a sealed tube for 2 h. The isolation of sugars from the TFA hydrolysate is relatively easy due to the high volatility of the acid and the excess acid was evaporated by co-distillation with distilled water. Then the hydrolyzed products were reduced with NaBH₄ (50 mg), followed by neutralization with dilute acetic acid and evaporated at 45 °C. They were then co-distilled with methanol to remove the excess boric acid. The reduced products (alditols) were acetylated with 1:1 pyridine-acetic anhydride in a water bath for 1 h at 90 °C to give the alditol acetates. Alditol acetates of authentic standards (d-glucose, d-xylene, d-galactose, d-mannose and l-arabinose) with myo-inositol (2 mg) as the internal standard were prepared and subjected to GC analysis separately in the same way [28]. Uronic acid contents were determined by measuring the absorbance at 525 nm using the m-hydroxybiphenyl colourimetric method with d-glucuronic acid as the standard [29].
2.9. Carboxy-reduction of PAPS-1 to give CR-PAPS-1

PAPS-1 was carboxy-reduced by the carbodiimide method [30], using NaBH₄ as the reducing agent, giving products (named as CR-PAPS-1) with the –COOH groups of its uronic acid residues reduced to –CH₂OH. CR-PAPS-1 was analyzed by GC as described above.

2.10. Statistical analyses

Analysis was performed using the SPSS software package (SPSS 23 for Windows, SPSS Inc., Chicago, IL, USA). Comparison of means was performed by Duncan’s test with confidence level as P ≤ 0.05.

3. Results and discussion

3.1. Physicochemical characteristics

Some laboratory analytical data for physicochemical characteristics of the gum is listed in Table 1. As the first physical characteristics can be noted Ash content and Moisture content. The ash content and moisture content of the crude gum powder were found to be 3.12 ± 0.06 and 7.53 ± 0.15, respectively. The moisture content of the gum is lower than limit that set by the most of the pharmacopeias (about 15%). Therefore, it is stable against activation of enzymes and proliferation of living organisms. It is reported that exudates are containing various metal ions that soil composition is the most important factor for determining the nature and amount of these constituents [16]. The reported value for total ash of acacia and gum tragacanth is 4% w/w which makes them suitable for food and pharmaceutical applications [31]. The obtained results showed that the total ash of sweet cherry gum is acceptable comparison with acacia and tragacanth gums. Physical features of gum solution including viscosity and gelling properties are affected by the ion content, especially Ca²⁺ and K⁺ [9].

The measured value of angle of repose for the powder was 37.69° ± 0.90. An angle of repose lower than 40° indicates good flowability, conversely an angle of repose higher that 40° is an indication of cohesiveness [32]. The amount of Hausner ratio and Carr index, were 0.88 ± 0.01 and 11.33 ± 1.15, respectively. In general, powders with poor flowability indicated by Hausner ratio greater than 1.25; Carr’s compressibility index below 16% indicates good flowability while values above 35% indicate cohesive powders [18,33].

3.1.1. Viscosity

Flow behavior of polysaccharide solutions is depended on their critical concentration. They exhibit Newtonian behavior at concentration below this critical concentration and show non-Newtonian behavior above this concentration [9,34]. Fig. 1 shows the viscosity–shear rate profile of the 1% aqueous solution of $P$. avium gum. Molecular size and structure are the main factors influencing viscosity- shear rate profile. The long chain structure creates high viscosity at low concentrations. However, the polysaccharides with long chain structure and high molecular weight create a high viscosity aqueous in low concentration. According to Fig. 1, Shear thinning region occurred between 10 and 40 rps where there is a significant reduction in viscosity. On the other hand, reduction in viscosity after 40 rps is not significant. The viscosity of 1% aqueous solution of the gum exhibited a Newtonian type of flow.

3.2. Antioxidant property

The antioxidant activity of many plant extracts measured with a free radical of DPPH. $IC_{50}$ is the concentration in g/ml of the sample to scavenge 50% of the DPPH radical. The higher $IC_{50}$ values indicate lower antioxidant activity. The $IC_{50}$ of sample and standard was calculated from the graph by plotting the% Inhibition against concentration of sample and standard (Fig. 2). The $IC_{50}$ of the $P$. avium gum solution and BHA solution was measured 429.46 μg/ml and 72.86 μg/ml respectively. The result showed that $P$. avium gum exudates exhibit low antiradical activity, which is significantly lower than the standard BHA (P < 0.05). The total phenolic compounds measured in the gum exudates of $P$. avium was 12.99 ± 0.8 mg/g weight of gum powder. The main reason for the low antioxidant activity of gum is poor phenolic compound.

3.3. Monosaccharide composition and molecular weight

CPAP was purified with DEAE-cellulose A52 and Sephadex G-100 gel-filtration columns. The main fraction (PAPS-1) was collected and lyophilized for further analysis, PAPS-1, which appeared as a white powder, showed a negative response to the Bradford method and had no absorption at 280 and 260 nm in the UV spectrum, indicating the absence of protein and nucleic acid. A single and symmetric narrow peak was observed in the HPGPC profile; it means that PAPS-1 is a homogeneous polysaccharide. (Fig. 3). The average molecular weight of PAPS-1 was about 1.46 × 10⁵ Da (146 kDa) according to the calibration curve with standard dextrans.

Gc analysis (Fig. 4A) showed PAPS-1 was composed of four kinds of neutral monosaccharides, namely mannose (Man), arabinose (Ara), galactose (Gal) and xylose (Xyl) with a relative molar ratio of 1.0:14.7:7.1:2.4:3. The content of uronic acids was determined spectrophotometrically and showed a value of 11%. The results indicated that PAPS-1 was an acidic polysaccharide and arabinose was the predominant monosaccharide in polysaccharide structure.

To characterize the uronic acid present in PAPS-, a part of the sample was carboxy-reduced (CR-PAPS-1) and submitted to monosaccharide analysis by GC (Fig. 4B). In this procedure, the uronic acid is converted to its corresponding neutral sugar. The result showed that CR-PAPS-1 was composed of Man, Ara, Gal, Xyl and glucose (Glc) with molar ratios of 1.0:14.67:2.5:3.2:3. A combination of monosaccharide composition analysis of native PAPS-1 and CR-PAPS-1 revealed that PAPS-1 contained 11.3% glucose, arising from glucuronic acid.

To study and identify main functional groups of plant polysaccharides, FT-IR spectroscopy was used. As shown in Fig. 5, the IR spectrum of PAPS-1 revealed a typical major broad stretching peak around 3430.7 cm⁻¹ for the hydroxyl group, and the small band at around 2923.5 cm⁻¹ was attributed to the C-H stretching of CH₂ groups. In addition, a peak at 1733.1 cm⁻¹ indicated
that there were carboxyl groups in PAPS-1. The result was in good agreement with that of chemical analysis, since PAPS-1 had uronic acid (11.3%) as mentioned above. The broad band at 1634.2 cm\(^{-1}\) was due to adsorbed water and absorption bands at 1459.5 and 1380.6 cm\(^{-1}\) could be assigned to deforming vibrations of C\(\text{H}\) bond. Polysaccharides have specific bands in the 1200–950 cm\(^{-1}\) region (i.e., the so-called fingerprint region). Each polysaccharide has the specific band with unique position and intensity, allowing its possible identification. The C–O ether bond showed stretching at about 1143.6 cm\(^{-1}\) while the C–O alcohol bond showed stretching at 1079.8 and 1031.3 cm\(^{-1}\). The absorptions at 925.1, 798.9 and 771.4 cm\(^{-1}\) confirmed the co-existence of furanose and pyranose
forms. Furthermore, the presence of β-type glycosidic linkage in PAPS-1, identified by an absorption peak at 890.7 cm⁻¹ [26]. On the basis of the aforementioned results, it can be concluded that PAPS-1 is an acidic polysaccharide containing β-configurations in both pyranose and furanose forms.

4. Conclusion

In this research physicochemical properties and monosaccharide composition of P. avium gum exudates were investigated. The carbohydrates and proteins were the major components of P. avium gums. GC analysis for monosaccharide composition indicates that mannose (Man), arabinose (Ara), galactose (Gal) and xylose (Xyl) with a relative molar ratio of 1.0:14.7:7.1:2.4 were the main monosaccharides of gum polysaccharides. The total phenolic compounds measured in the gum exudates of P. avium was 12.99 ± 0.8 mg/g weight of gum powder. Results from DPPH scavenging activity indicated that the gum possesses low antioxidant properties. The main reason for the low antioxidant properties attributed to low phenolic content.

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