https://doi.org/10.15255/KUI.2021.076
KUI-35/2022
Original scientific paper
Received December 2, 2021
Accepted February 9, 2022

Functional Properties and Physicochemical Characterization of Mucilage Extracted from *Punica granatum* L. Peels and Their Application in Pharmaceutical Suspension Preparation

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Abstract

The aim of this study was the extraction, characterization, and evaluation of functional properties of mucilage extracted from *Punica granatum* L. peels. The isolated mucilage showed good swelling properties and emulsion capacity. The aqueous dispersion of mucilage showed pseudoplastic flow behaviour. In addition, the mucilage had good flow properties, which may be suitable for direct compression formulation. Structural analysis by FTIR indicated the presence of the characteristic binding of mucilage. Isolated mucilage was found to have good properties and could be exploited in food and pharmaceutical sector.

Keywords

Mucilage, Punica granatum L.*, functional properties, physicochemical properties*

1 Introduction

Use of natural polymers has increased in recent times, and has found applications in the food and pharmaceutical industries.[1] Natural polymers are materials of large molecular weight from natural origins, such as plants, animals, and microorganisms. Compared to synthetic polymers, natural polymers are easily available, biodegradable, they are non-toxic and low cost; these are typical environmentally friendly materials.[2] They are classified into three groups: polysaccharides, polypeptides, and polynucleotides.[3] Polysaccharide-base polymers reveal high biocompatibility, biodegradability, accessibility, stability, low cost, and low toxicity.[3,4] Mucilage is typically polysaccharide complex, naturally occurring viscous colloidal dispersions and high molecular weight,[5] offering an ideal product for the development/improvement of health products with beneficial properties to human consumption.[6] Recently, mucilage has evoked enormous interest and the demand for natural mucilage is increasing. Therefore, new sources of mucilage need to be explored in order to meet the demands.[3]

Mucilage has a wide range of applications in food, pharmaceutical, and cosmetic preparations for several applications such as thickeners, gelling agents, binding, suspending and emulsifying agents.[7] Several studies have shown the medicinal uses of mucilage due to its cholesterol lowering, anti-inflammatory, and antiulcer activities.[8] Also, mucilage has been used by phytotherapists to create reflex demulcensy, especially to ease irritable and ticklish dry cough.[9]

In a previous study, mucilage has been cited in hydroalcoholic extract of the entire fruit of *Punica granatum* L., a fruit widely grown in all Mediterranean countries. It is known for its therapeutic virtues, while its peels have been used for a long time in traditional medicine.[10,11]

Punica granatum L. (pomegranate) is a dicotyledonous plant belonging to the *Rosidae* subclass, *Myrtales* order, and *Punicaceae* family. The name pomegranate comes from the Latin word *pomum*, meaning apple, and *granatus,* meaning full of seeds.[12]

Therefore, the aim of this study was to investigate the physicochemical, functional properties of mucilage extracted from pomegranate peels, and evaluate its properties as suspending agent.

2 Materials and methods

2.1 Materials

Pomegranate was purchased from the local market of Blida in north of Algeria. Peels were dried at room temperature, and stored in clean bags for extraction. All the chemical reagents were of analytical grade.

2.2 Extraction and isolation of mucilage

Punica granatum L. peels mucilage was extracted as previously described,[13–16] with minor modification. Barks (50 g) were ground and macerated in distilled water with a ratio

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of (1 : 7) for 24 h, then boiled for 1 h under reflux with stirring, and kept aside for 2 h for release of mucilage into water. The material was pressed in muslin cloth in order to separate marc from the filtrate. For isolation of mucilage, a volume of ethanol was added to the filtrate (1 : 3) to precipitate the mucilage. The sample was stored in the refrigerator for 24 h. After filtration, the mucilage was dried for 48 h at a temperature of 37 °C. The extraction yield was calculated based on the amount of dried mucilage powder obtained and the amount of dried *Punica granatum* L. peels (Eq. (1)).[7,17]

$$\text{yield}(\%) = \frac{\text{weight of dried mucilage obtained}}{\text{weight of pomegranate peels sample taken}} \cdot 100 \quad (1)$$

The extracted mucilage was confirmed by Molish test[18] and characterized for organoleptic properties such as colour, odour and texture.

2.3 Physicochemical properties of mucilage

2.3.1 pH, solubility, and total ash value

The pH of the 1 % (w/v) aqueous mucilage solution was measured using a calibrated pH-meter (HANNA HI2210 pH instrument).[3,14]

Solubility of the extracted mucilage was evaluated using different solvents such as hot water, cold water, ethanol, acetone, methanol, and chloroform according to the following protocol:[19,20] 0.1 g of dry mucilage powder was added to 10 ml of solvent, the solution was magnetically stirred for 1 min, and then placed at a temperature equal to 25.0 ± 0.5 °C for 15 min. Solubility was determined by visual observation.

The mucilage was oven-dried, and then analysed for ash content by incinerating the sample in muffle four at 550 °C following the recommendations of the official method Analysis.[21] The total ash value was calculated according to the Eq. (2).

$$\text{Total ash value (\%)} = \frac{\text{weight of ash}}{\text{weight of mucilage}} \cdot 100 \quad (2)$$

2.3.2 Loss on drying

One gram of powder was weighed and dried at 105 °C using the procedure described in the following research.[18,22] The percentage of weight loss on drying by the powder was calculated according to the Eq. (3).

$$\text{Loss on drying (\%)} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \cdot 100 \quad (3)$$

2.3.3 Swelling Index

The swelling index is the volume (ml) taken up by swelling 1 g of the substance under specified conditions. The swelling index of mucilage was determined by taking 1 g of mucilage in 25 ml of distilled water and the mixture was agitated every 10 min for 1 h. The solution was then allowed to stand for 24 h at room temperature. Thereafter, the volume occupied by the mucilage was measured.[19,23]

2.3.4 Water holding capacity (WHC) and oil holding capacity (OHC)

Mucilage powder was used to determine its water holding capacity and oil absorption capacity according to the following procedure:[16] 25 ml of distilled water added to 0.25 g of mucilage, mixed by magnetic stirrer for 15 min, and centrifuged using a centrifuge SIGMA 3-30K at 10 000 rpm for 30 min. The supernatant was removed; the wet samples were weighed and then used to calculate the water holding capacity according to the Eq. (4).

$$\text{Water holding capacity} = \frac{\text{wet sample weight} - \text{dry sample weight}}{\text{dry sample weight}} \quad (4)$$

(g water/g dry sample weight)

For the oil absorption capacity, 0.5 g of sample was added to 10 ml of refined oil, mixed by vortex stirrer (WiseStir HS-30D stirrer) for 1 min, maintained at room temperature for 30 min, and then centrifuged at 10 000 rpm for 30 min, after which the supernatant was removed. The weight of the sample absorbed by the oil was weighed, and the oil absorption was calculated according to the Eq. (5).

$$\text{Oil absorption} = \frac{\text{oil absorbed sample weight} - \text{dry sample weight}}{\text{dry sample weight}} \quad (5)$$

(g oil/g dry sample weight)

2.3.5 Emulsion capacity

The emulsion capacity was determined using the mucilage powder according to the following procedure:[16] 1 g of sample dissolved in 50 ml of distilled water was added to 50 ml of refined oil. The emulsion was prepared using a homogenizer (IKA R104 homogenizer) for 1 min and then centrifuged at 4100 rpm for 5 min using a centrifuge SIGMA 3-30K brand. The emulsion capacity was determined according to the Eq. (6).

$$\text{Emulsion capacity (\%)} = \frac{\text{height of emulsified layer}}{\text{height of the whole layer}} \cdot 100 \quad (6)$$

2.3.6 Bulk and tapped density

Bulk density is a property of powder defined as the mass of particles of the material divided by total volume they occupy. It was measured by transferring a known amount of mucilage powder into a graduated measuring cylinder and the volume was noted (bulk volume). Tapped density was

measured by mechanically tapping the measuring cylinder containing mucilage powder until constant volume was observed:[14,18] 10 g of mucilage powder was introduced into a 100 ml graduated cylinder and placed on the tap density tester (ERWEKA brand densi-tape). The powders were then subjected to settlement until a constant volume was obtained.

The bulk density was obtained by dividing the weight of the sample by the volume of the sample contained in the graduated cylinder, as shown in the Eq. (7).

$$\text{Bulk density } \rho_{\text{bulk}}\ (\text{g ml}^{-1}) = \frac{\text{weight of dry powder}}{\text{bulk volume}} \tag{7}$$

The tapped density was calculated by dividing the ratio of the weight of the dry powder to its packed volume (Eq. (8)):

$$\text{Tapped density } \rho_{\text{tapped}}\ (\text{g ml}^{-1}) = \frac{\text{weight of dry powder}}{\text{tapped volume}} \tag{8}$$

2.3.7 Compressibility index and Hausner ratio

Compressibility or Carr index and Hausner ratio measure the flow properties of powder, and were calculated using estimated value of bulk and tapped density (Eqs. (9) and (10)):

$$\text{Compressibility index (\%)} = \frac{\text{tapped density} - \text{bulk density}}{\text{trapped density}} \cdot 100 \tag{9}$$

$$\text{Hausner ratio} = \frac{\text{tapped density}}{\text{bulk density}} \tag{10}$$

2.4 Fourier transforms infrared spectral analysis (FT-IR)

The mucilage was analysed using FT-IR (Jasco FT/IR-4100 FTIR) with spectral range of 400 to 4000 cm^{-1}. The transmission of the samples within 7 mm diameter KBr pellets was measured.

2.5 Scanning electron microscopy (SEM)

The mucilage morphology was inspected using JEOL JSM 60-63 LV scanning electron microscope at an electrical voltage of 10 kV.

2.6 Determination of zeta potential

Zeta potential is a representative of particle charge. Mucilage solution using Nanotrac wave instrument (Microtrac Inc., PA, USA). Briefly, the sample was dissolved in purified water at 1/100 (w/v). All measurements were done in triplicate at 25 °C.

2.7 Suspending properties of *Punica granatum* L. mucilage

Suspension properties are usually measured in terms of sedimentation volume, flow rate, pH, viscosity, and ability to redisperse in suspension.[2]

2.7.1 Preparation of mucilage suspension with paracetamol

Dried mucilage (0.5, 1.0, 1.5, and 2.0 g) and 5 g of paracetamol (Paracetamol tablets (PARALGAN 500 mg) were triturated with 50 ml of distilled water to form a smooth paste. The mixture was transferred to a 100-ml measuring cylinder, made up to the required volume with distilled water, and then vigorously mixed under magnetic stirrer for 2 min. To the suspension, 0.1 % (w/v) benzoic acid was added as a preservative.[2,4,24,25]

The procedure was repeated using suspending agent: sodium carboxymethyl cellulose powder (sodium CMC) (0.5, 1.0, 1.5, and 2.0 g).

2.7.2 Suspending activity evaluation

pH measurement

1 % (w/v) dispersion of the sample was mixed by magnetic stirrer in distilled water for 5 min and the pH was measured.

Measurement of flow rate

The flow rate, η_a, was measured by determining the ratio of the volume of the suspension in the 10 ml pipette to the time required for flow of the suspension (Eq. (11)).

$$\eta_a\ (\text{ml s}^{-1}) = \frac{\text{volume of solution without pipette (ml)}}{\text{flow time (s)}} \tag{11}$$

Determination of sedimentation volume

Each suspension (50 ml) was stored in a 50-ml tube for 10 days at 35 °C and protected from light. Observations were made every 24 h for 10 days.

The sedimentation volume, F (%), was then calculated using the Eq. (12).

$$F\ (\%) = \frac{\text{ultimate volume of the test sample (mucilage + paracetamol)}}{\text{original volume of the suspension}} \cdot 100 \tag{12}$$

Measurement of viscosity

The viscosity was measured using Brookfield viscometer. The viscosity of the sample was determined at 25 °C. All

determinations were made in triplicate, and the results obtained expressed as the mean values.

Redispersion

Fixed volume of each suspension (50 ml) was kept in calibrated tubes stored at room temperature for 20 days. At regular intervals of 5 days, a tube was shaken to observe the redispersibility of the sediment and the presence of deposits.[2]

3 Results and discussion

3.1 Mucilage extraction

The presence of the mucilage in the extracted material was confirmed by carrying out the Molisch test. The test was positive by the appearance of a purplish red colour.

The yield of mucilage extract from *Punica granatum* L. peels was 12 % w/w, brownish, irregular with a characteristic odour. Mucilage was completely soluble in hot water, formed a colloidal solution in cold water, and insoluble in organic solvents such as ethanol, acetone, methanol, and chloroform. According to *Alpizar-Reyes et al.*,[6] solubility is an important factor of analysis in food systems because it affects other functional properties and serves as a useful indication of the performance of hydrocolloids in dispersion systems. The pH measurement was 5.6, which is slightly acidic. This value is comparable to those reported for gum arabic[26] and *O. ficus-indica*.[26]

The pH value has a great influence on the viscosity of the solutions of the polymers and consequently on the stability of the pharmaceutical suspensions.[24] The pH is also a critical factor in coagulation/flocculation processes, where the pH should be between 5 and 7.5.[26] Loss on drying of the extracted mucilage was 16 %. This moisture content appeared to be high compared to the results obtained by *Bindu* and *Fahsa*.[2] This difference can be justified on the one hand by the type of drying used, and on the other hand by the type of grinding, i.e., the particle size of the mucilage. The ash content varies according to the origin of the raw material used and reflects the content of mucilage organic matter.[2] The ash content of the extracted mucilage was 5.4 %, which allows us to say that the peels of *Punica granatum* L. are rich in mineral matter. Swelling index (63 %) was similar to that observed with *Abdelmochus* seeds mucilage (66 %).[2] Swelling index denotes the degree of granule hydration. Thus, high swelling capacity is an indication of weaker binding forces in the granules.[6]

3.2 Water and oil holding capacity

Mucilage presents a better WHC (5.08 g water/g dry mucilage) than OHC (1.3 g oil/g dry mucilage). The WHC influences the formation of viscous solutions that can facilitate industrial processes. Mucilage forms a three-dimensional network in contact with water, trapping it and resulting in highly viscous solutions. Because of this large water absorption capacity, mucilage may find applications in the food, cosmetics, and pharmaceutical industries, in which it can dissolve, be dispersed, and form colloids.[13] The OHC would allow its use in combination with other products to collect petroleum and various oils in case of spills on water. In addition, the good OHC value suggests that mucilage could improve the texture of food products.[26]

3.3 Emulsion capacity

Emulsion capacity (75 %) of extracted *Punica granatum* L. mucilage was higher than that observed for *Ziziphus mauritiana* lam (52.22 %)[16] and similar to that of *Ocimum canum* Sims (74.41 %).[16] Emulsion is related to the stability of the product because it can improve the formation of small droplets and reduce their melting rate.[8]

3.4 Flow properties of the powder

The flow properties of mucilage are shown in Table 1. Similar values were observed for that of *Hibiscus esculentus*[19] and *Opuntia dillenii cladode*.[8]

Table 1 – Flow properties of mucilage powder

	Mucilage of *Punica granatum*	Mucilage of *Hibiscus esculentus*	Mucilage of *Opuntia dillenii cladode*
Bulk density / g ml^{-1}	0.59	0.57	0.67
Tapped density / g ml^{-1}	0.71	0.69	0.80
Compressibility index / %	16.90	17.39	16.20
Hausner ratio	1.20	1.21	1.19

The values for bulk density, tapped density, compressibility index, and Hausner ratio were 0.59 g ml^{-1}, 0.71 g ml^{-1}, 16.9 % and 1.2, respectively (Table 1). According to *P. Kalegowda et al.*[8] compressibility index of 12–18 % and a Hausner ratio of < 1.25 indicate the desirable packing characteristics (less cohesiveness, good compressibility and flow ability) of biomaterial in pharmaceutics. Polymers with lower bulk density would be useful in better disintegration due to increased water absorption of particles by capillary action through the pores.[8]

3.5 Fourier transform infrared spectroscopy (FT-IR)

The functional group bands found in the *Punica granatum* L. mucilage (Fig. 1) are characteristic for proteins and polysaccharides. The spectrum showed characteristic peaks of −OH between 3412.79 and 3372.51 cm^{-1}, $-CH_2$ at 2925 cm^{-1}, C=C at 1618.78 cm^{-1}, acid at 1234.64 cm^{-1} and an ether group between 1101.67 and 1028.36 cm^{-1}.[9,10,25]

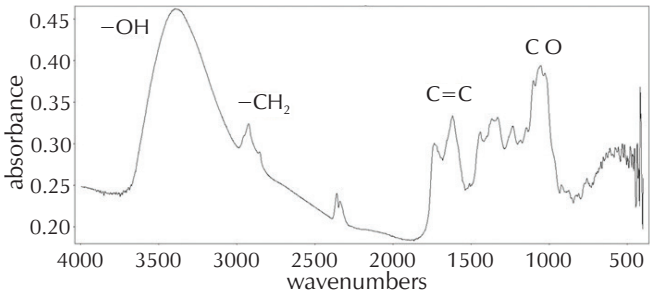

Fig. 1 – FTIR spectrum of *Punica granatum* L. mucilage

3.6 Scanning electron microscopy (SEM)

Surface morphology images analysed by SEM are shown in Figs. 2(a), 2(b), 2(c), and 2(d), respectively. The particles were aggregates of different shapes (dendrite and spheric) and dimensions. Previous studies have shown that the surface topography, structure and properties of polysaccharides can be influenced by extraction, purification, and preparation conditions.[11,18]

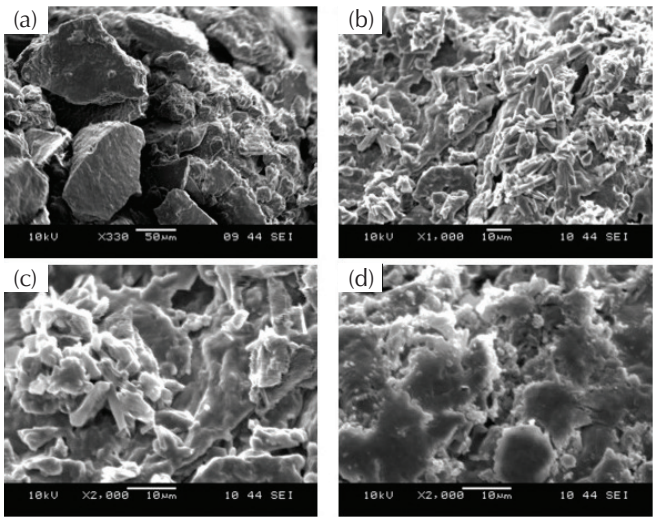

Fig. 2 – Surface morphology of mucilage extracted from *Punica granatum* L. mucilage observed under a scanning electron microscope (SEM)

3.7 Zeta potential measurement

Zeta potential represents an indicator for investigating the type and extent of electrostatic interactions where charged biopolymers exist at the same time.[17] Its measurements provide an understanding of the causes of dispersion, aggregation or flocculation, and a solution for improving the formulation of dispersions, emulsions or suspensions. Zeta potential value of extracted mucilage was equal to -34.67 ± 0.68 mV, corresponding to a zone of stability. To obtain a stable suspension, the zeta potential must be the highest (in absolute value). A suspension is usually considered stable when the zeta potential is greater than 30 mV in absolute value.[5]

3.8 Suspension properties of mucilage extract

Paracetamol suspensions were prepared using isolated mucilage at different concentrations of 0.5 %, 1 %, 1.5 %, and 2 % (w/v) and similar suspensions were prepared with sodium CMC. The properties of the suspension were evaluated using various parameters such as pH, sedimentation volume, flow rate, redispersion values, and viscosity of the suspension. Suspension properties are summarised in Table 2. The sedimentation volume was directly proportional to the concentration of the suspending agent (mucilage and sodium CMC), while the flow rate was inversely proportional. The pH values were slightly acidic for the mucilage suspensions and neutral for the sodium CMC suspensions.

These suspensions were shaken at 5-day intervals for 20 days to evaluate the redispersibility of paracetamol. Since the suspensions produce sediment during storage, they must be easily dispersible so as to ensure more uniform dosage administration of the drug after agitation.

Redispersibility of suspensions at low concentrations was found to be faster than at higher concentrations. This observation can be attributed to the higher viscosity of these formulations with a higher concentration of suspending agents.

4 Conclusion

Mucilage yield isolated from *Punica granatum* L. peels was 12 % w/w. It exhibited good swelling properties and

Table 2 – Suspension properties of the extracted mucilage

Suspending agent	Concentration / %	Sedimentation volume / %	Flow rate / ml s^{-1}	pH	Redispersion / ml	Viscosity / poise
Mucilage	0.5	0.15	0.42	5.7	2.0	5.9
	1.0	0.18	0.36	5.4	2.4	6.2
	1.5	0.28	0.25	5.3	2.8	7.1
	2.0	0.40	0.18	5.2	3.0	7.9
Sodium CMC	0.5	0.16	0.62	7.4	2.0	0.57
	1.0	0.20	0.52	7.5	2.3	0.85
	1.5	0.26	0.49	7.6	2.6	1.04
	2.0	0.30	0.47	7.7	2.9	1.20

good emulsifying capacity. Structural analysis by FTIR indicated the presence of the characteristic mucilage bonds. The value of the zeta potential is negative and equal to 34.67 ± 1.68 in absolute value, corresponding to a zone of stability. Mucilage properties as well as suspension agent have shown that mucilage has good properties, and has the potential to be used in the formulation of pharmaceutical products.

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SAŽETAK

Funkcionalna svojstva i fizikalno-kemijska karakterizacija sluzi ekstrahirane iz kore nara (*Punica granatum* L.) i njezina primjena u pripravi farmaceutskih suspenzija

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Cilj ovog istraživanja bio je ekstrakcija, karakterizacija i procjena funkcionalnih svojstava sluzi ekstrahirane iz kore nara (*Punica granatum* L.). Izolirana sluz pokazala je dobro bubrenje i dobar kapacitet emulzije. Vodena disperzija sluzi pokazala je pseudoplastično ponašanje. Osim toga, sluz je imala dobra protočna svojstva, što bi moglo biti prikladno kod izrade pripravaka metodom izravnog prešanja. Strukturna analiza FTIR-om pokazala je prisutnost karakterističnog vezivanja sluzi. Utvrđeno je da izolirana sluz ima dobra svojstva i može se upotrebljavati u prehrambenom i farmaceutskom sektoru.

Ključne riječi
Sluz, Punica granatum L., *funkcionalna svojstva, fizikalno-kemijska svojstva*

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Izvorni znanstveni rad
Prispjelo 2. prosinca 2021.
Prihvaćeno 9. veljače 2022.