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RESEARCH ARTICLE

Isolation, characterization and bioactive properties of polysaccharides extracted from the peel of *Cucumis melo* Mill. melon (Gurvak variety)

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Abstract

Melon peel is a by-product that contains significant amounts of bioactive substances. It has potential for developing functional foods. In this work, we studied the chemical composition of melon peel from the Gurvak variety, including various fractions of polysaccharides and their monosaccharides, free amino acids, water-soluble (WS) vitamins and flavonoids. The fractions of WS, acidic and basic polysaccharides were obtained with yields of 14.5 %, 4.60 % and 5.63 %, respectively. Their viscosity results were linked with the carboxyl group-related parameters and molecular masses. The dominant molar mass of free amino acids was attributed to nonessential amino acids, with glycine and glutamine being the most abundant ones. In the peel pulp, flavonoids and vitamins were determined in nanomolar and micromolar concentration, respectively. After oral administration at a dose of 5000 mg/kg, the WS polysaccharides showed no toxicity in mice. Furthermore, the fraction exhibited wound-healing properties that accelerated skin recovery in mice. The polysaccharides extracted from the melon peel of the local Gurvak variety may be used as a wound-healing source. In terms of free amino acids, both the quantity and quality may not be appropriate for functional foods. A similar conclusion can be drawn made for quantities of flavonoids and vitamins. The results obtained can serve to expand knowledge and support further analysis in this discipline, which may lead to technological improvements.

Keywords: *C. melo* Mill.; melon peel; monosaccharides; polysaccharides; wound-healing

Introduction

Cucumis melo L., commonly known also as melon, includes many varieties. Naudin developed an analytical system for *C. melo* and divided this species into ten varieties. Munger and co-authors simplified the taxonomy of this species (1). Several varieties of this plant are grown in Uzbekistan and the local melon is famous in the Central Asian region for its taste. Melon varieties have been cultivated for decades and several species are included in the State Register. A total of 150000 to 155000 hectares of land are allocated for melon crops in Uzbekistan.

Gurvaks (*var. gurvak* Fil.) are distributed in Turkmenistan and northern Uzbekistan. Unlike other melons, their fruit has a smooth or scaly surface and their shape is round or oval. Approximately 10.2-12.6 % of the fruit mass consists of moisture and the sugar content ranges from 7.4-10 % to 16.0 %. These varieties are medium-sized; the growing period is 75-105 days. They are used for local consumption (2).

In the food processing industry, a significant amount of waste is generated, particularly during the processing of fruits and vegetables (3). In this regard, *C. melo* was one of the most widely produced fruits globally, with an annual production of 8-20 million tons, which generated a considerable number of by-products during processing (4). Melon processing residues were rich in proteins, dietary fibers, macro- and microelements, vitamins and phenolic compounds with high biological activity (5). Physicochemical studies demonstrated that such wastes contained antioxidants, antimicrobials, antidiabetics and other biologically active substances (6). Thus, the utilization of fruit processing waste not only helped in reducing environmental burden but also provided a sustainable source of valuable compounds for food, pharmaceutical and nutraceutical applications (7).

Melon peel was one of the raw materials that could be used as a source of carbohydrates. Besides, it contained proteins, fiber, fat, minerals, polyphenols, etc (8). Melon peel was reported as a rich source of cellulose, hemicellulose and lignin, with over 60 % of the mass belonging to these classes of

substances (9). The quantity of total carbohydrates in melon peels could make up more than 50 % of the dry mass. However, there was a high ratio of insoluble fiber, the main mass of which belonged to carbohydrates (10).

Cellulose, hemicellulose and pectin were distributed in the melon peel as cell wall polysaccharides. Pectin was located in the middle lamella and linked with some parts of cellulose, interacting with hemicellulose. Therefore, various approaches were explored to achieve higher extraction yields of different fractions of polysaccharides (11). Yield and degree of esterification of polysaccharides from melon peel were found to vary highly depending on extraction time, temperature, peel-to-solvent ratio and pH of medium. For example, 35-95 °C, 40-200 min, pH 1-3, 10-50 v/w resulted in ~2.9 - 29 % yield and 1.33 - 29.33 % esterification degree of pectin isolated from melon peel (12). Microwave-assisted extraction appeared to result in higher pectin yield and improved the sustainability of the raw material (13). Compared to citric acid or tartaric acid, the treatment of melon peel with hydrochloric acid was reported to be more beneficial in terms of achieving higher yields of microcrystalline cellulose. The authors claimed melon peel could be used as a biosource for obtaining microcrystalline cellulose (14).

Polyphenols were another class of compounds in melon peel that deserved attention. Among total polyphenols, total flavonoids were expected to make up 25 - 30 % of this class of substances (10). The main subclass of flavonoids was reported to be flavones, including luteolin, apigenin, naringenin and their derivatives (9). Because of the polyphenols and flavonoids, melon peel was claimed to be a functional if fortified (2).

Melon peel is also a source of minerals that can be used to develop functional foods. Among minerals, potassium (K), calcium (Ca), magnesium (Mg), sodium (Na) and phosphorus (P) have been identified as the macroelements (15). A review of melon peel composition reported highly varying amounts of metals in the peel composition. Several-fold differences were observed in the quantities of zinc, copper, iron, potassium and magnesium that could be attributed to higher or lower amounts of macro or microelements in the soil in which the plants were grown (16). These results imply the need to study the concentrations of metals to ensure they are within permissible limits.

In this work, we aimed to study the contents and composition of polysaccharides as well as amino acids, vitamins and flavonoids in the *Cucumis melo* Mill. Gurvak variety with a view to their practical utilization.

Materials and Methods

Object of study

The object of the study was the dried rind of the *C. melo* Mill. melon variety, collected in the Khodjeyli region of Karakalpakstan. In this work, we used the melon peel isolated from the seeds, which was further dried at room temperature and ground to a powder. The raw material was then extracted step by step using the following methods.

Polysaccharides

Defatting melon peel for the extraction of polysaccharides

For defatting, 100 g of raw material was extracted in chloroform in a water bath at 61.5 °C using a reflux condenser. The process was repeated three times (pulp to chloroform ratio was 1:5 and then 1:3). The defatted melon pulp was air-dried at room temperature. The raw material (95.261 g) was extracted in 82 % ethyl alcohol (1:5) to remove low molecular weight impurities of amphiphilic nature. The raw materials were dried at room temperature until the odour of the solvent disappeared (17).

Obtaining water-soluble polysaccharides

The water-soluble (WS) polysaccharides were extracted from the defatted melon pulp in a water bath at 95 °C using a reflux condenser three times (defatted pulp to water ratios were 1:20, 1:20, 1:15, 1:15). Each extraction lasted 2hr, the extracts were combined and concentrated in a rotary evaporator at 50 °C until one-fifth volume of the solution remained. The resulting concentrate was precipitated with 96 % ethyl acetate four times and filtered. The precipitate was washed with ethyl alcohol and dried at room temperature (18).

Deproteinization of polysaccharides

The deproteinization of the isolated WS polysaccharides was carried out by using the Sevag method (17). A 5-fold volume of CHCl3-nBuOH at a 4:1 mass ratio was prepared and added to the solution of WS polysaccharides and mixed for one hour. The mixture was centrifuged (10,000 rpm) for 10 min to avoid proteins. The same procedure was repeated two times. Then, the supernatant was dialyzed (MwCO 3500D, MD44 mm) for a few days and further freeze-dried.

Extraction of acidic polysaccharides

Following the extraction of WS polysaccharides, the residue was extracted twice with a mixture of 0.5 % solutions of oxalic acid and ammonium oxalate (1:1) at 75 °C for 90 min (pulp to solvent ratios were 1:4 and 1:2). The filtrates were combined, concentrated and precipitated with 96% alcohol. The precipitate was dried at room temperature (19). Acidic polysaccharides were separated from the residue. Re-extraction was carried out twice at a 1:5 mass ratio of pulp and solvent. The combined extracts were concentrated, filtered, washed with ethyl alcohol and dried at room temperature.

Extraction of basic polysaccharides

Hemicellulose was usually obtained using an alkaline solution. The residue after pectin isolation was extracted with a fivefold volume of 5 % alkaline solution and left at room temperature for 12 hr. Then it was filtered using four layers of gauze. The resulting filtrate was titrated with acetic acid (pH 4 - 4.5). As a result, a precipitate (hemicellulose A) was formed. The precipitate was separated by centrifugation and dried at room temperature. The supernatant remaining after centrifugation was evaporated in a rotary evaporator, concentrated and precipitated in 96 % ethyl alcohol. The resulting precipitate (hemicellulose B) was filtered, washed in ethyl alcohol and dried at room temperature (20).

Gas chromatography analysis

Hydrolysis of polysaccharides was carried out under acidic conditions at 100 °C. The WS polysaccharides were hydrolyzed with a 1 N H_2SO_4 solution for 8 hr. Pectin substances and hemicelluloses were hydrolyzed with 2 N H_2SO_4 and hydrolysates were neutralized with $BaCO_3$ for 24 hr, deionized with KU-2 cation exchanger (H^+), evaporated and subjected to chromatography. Gas chromatography was run on a Shimadzu GC-2010 chromatographer with a flame ionization detector, a quartz

capillary column Shimadzu Rxi-624Sil MS (30 m × 0.25 mm × 1.40 m), a phase flow rate (N_2) of 1.5 mLmin, an injector temperature of 260 ℃, a detector temperature of 280 ℃ and a column temperature of 230 ℃. Samples were quantified as alditol acetates derivatives (21).

Titrimetric indicators of polysaccharides

In order to determine the free carboxyl groups of polysaccharide substances, 25mL of water was added to 0.25 g of polysaccharide, heated slightly, mixed, left for 2 hr and titrated with 0.1 M sodium hydroxide solution (indicator: phenolphthalein) until a pink colour was formed.

Molecular mass determination

Molecular masses of polysaccharides were calculated by Dublie's method and sedimentation constant (22).

Quantification of WS vitamins

For the quantification of WS vitamins, 10 g of the sample was weighed and extracted in 50 mL of deionized water by intensive stirring for 1 hr and then kept at room temperature for 2 hr. The process was repeated twice. The filtrates were combined and centrifuged (7000 rpm) for 10 min. The obtained extract was used for analysis. Quantification was carried out in an Agilent Technologies 1200 equipped with an autosampler, Eclipse XDB C 18 column (5 µm, 4.6 x 150mm) and a diode array detector (DAD).

Quantification of flavonoids

The flavonoids were quantified by using HPLC. For this purpose, 10 g of the sample was extracted in 50 mL of 70 % ethanol at 70-80 ℃ by stirring for 1 hr. Further, the process was continued at room temperature for 2 hr. After filtering, the remaining portion was re-extracted twice with 25 mL of 70 % ethanol. The filtrates were combined and centrifuged (5000 rpm) for 5 min. The resulting solution was used for analysis. Quantification was carried out in an Agilent Technologies 1200 system equipped with a column Eclipse XDB C 18, (5 µm, 4.6 x 250mm) and a DAD.

Quantification of free amino acids

For the quantification of free amino acids, 5 g of the ground sample was extracted with deionized water in a capped flask using an ultrasonic water bath for 3 hr. Further, extraction was carried out at room temperature for the next 3 hr. Then, 1 mL of extract was taken and centrifuged at 5000 rpm for 5 min. A 100 µL aliquot of the supernatant was taken and dried in a lyophilizer. 100 µL of a 1:7:1:1 mixture of H_2O, CH_3CN, trimethylamine and phenylisothiocyanate was added to the dried sample, incubated for 30 min at 37 ℃ and freeze-dried. This operation was repeated twice to neutralize the acidic condition. Phenylthiocarbamoyl derivatives of amino acids were synthesized by reacting with phenyl isothiocyanate. The prepared sample was treated with 0.5 mL of 4.3 M CH_3COONa solution with pH - 6.4 for 5 min in an ultrasonic water bath, then centrifuged at 5000 rpm. Further, the supernatant was taken for analysis in an HPLC - Agilent Technologies 1200 chromatographer equipped with a DAD detector, 75 x 4.6mm Discovery HS C18 column.

Identification and quantification of metals

The quantity of metals was determined by using an ICP-MS PlasmaQuant MS Elite equipped with discrete dynode electron multipliers.

Pharmacological analyses

Acute toxicity of polysaccharides

Acute toxicity was determined by oral administration of the test sample at a dose of 5000 mg/kg, in accordance with OECD (2002) guidelines (23). The study was conducted on male, outbred white laboratory mice weighing 22 ± 2.0g Five mice were used in each group. All pharmacological studies were conducted on healthy, sexually mature mice that had undergone a 10-14 day quarantine period. The experiments were carried out by introducing the studied sample into the stomach of mice at the above dose using a special probe. The control group animals were given purified water in an equal volume. The experiments were conducted in laboratory conditions. On the first day, the general condition of the animals in both the experimental and control groups was monitored every hour including observation for possible tremors and deaths. Over the following two weeks, the general condition, activity, coat and skin condition, respiratory rate and depth, body weight changes and other health indicators were checked daily under vivarium conditions. All experimental animals were maintained on a normal diet, with unlimited access to water and food. At the end of the experiment, the average lethal dose (LD_{50}) and toxicity class of the tested sample were determined.

Wound-healing effects of polysaccharides

For the experiment, healthy, white, non-breeding laboratory mice with a body weight of 25 ± 2.0 g, which had passed a 10-14 day quarantine period, were selected. Five mice were taken for each group. First, the mice were anesthetized, then the hair on the back of the body was shaved without damaging it using an electric razor intended for hair removal and cleaned with 70 % ethyl alcohol. The central area of the shoulder of the mice was incised with a sharp scalpel, thereby creating a simulated wound area with a diameter of 1 x 1 cm and a depth of 2 mm. The mice were then divided into control and treatment groups. After 3 hr, their initial wound area was measured and then the mice were treated with either purified water (control) or a 2.5 % polysaccharide solution (treatment), which was applied evenly to the injured skin to promote wound healing. In the following days, the surface of the open wound was cleaned with isotonic solution and the samples were applied once daily until the wound healed. The wound area was measured using a 150 mm (6'') digital caliper on days 1, 4, 8, 12 and 16 of the experiment.

Results and Discussion

The results obtained in this study showed that the highest ratio of polysaccharides belonged to the WS fraction. Approximately 14.5 % of the peel pulp was composed of the WS fraction. Pectins accounted for 4.6 % of the total mass of the peel pump. Hemicellulose A and B were presented at 2.99 % and 2.64 %, respectively (Table 1).

The average molecular mass of the WS fraction was ~ 73 kDa. The highest molecular mass was observed for pectin, at 140 kDa. Hemicellulose A and B had average molecular masses of average 78.5 and 132.7 kDa, respectively. A high degree of esterification was observed in the WS fraction, measured at 78 %. The degree of esterification of hemicellulose A and B were 89 % and 90 %, respectively (Table 1).

Table 1. Yield and titrimetric parameters of polysaccharides

Types of polysaccharides	Yield (%)	Free COOH groups (%)	COOH group esters (%)	Total COOH groups (%)	Degree of esterification (%)	Molecular mass (kDa)
Water soluble polysaccharides	14.5	1.62	5.76	7.38	78.0	72.7
Pectins	4.60	7.74	3.24	10.98	29.5	140.0
Hemicellulose A	2.99	0.54	4.5	5.04	89.3	78.5
Hemicellulose B	2.64	0.9	8.28	9.18	90.0	132.7

Note: Values are based on single determinations; no statistical analysis was applied.

We also investigated the viscosities of the obtained fractions (Fig. 1). The lowest and highest relative viscosities were found in the WS fraction and hemicellulose B, respectively, with hemicellulose B showing almost double the viscosity of the WS fraction (Fig. 1 A). The difference was likely attributed to the combined effect of three parameters: molecular weight, degree of esterification and total COOH group content, which together contributed to the high viscosity of the hemicellulose B fraction. The remaining fractions exhibited varying characteristics. For example, hemicellulose A had a similar degree of esterification to hemicellulose B, but displayed a significantly lower molecular mass and fewer carboxyl groups. In contrast, pectins had molecular mass and carboxyl group levels comparable to hemicellulose B but a much lower degree of esterification. WS polysaccharides showed both low molecular mass and low carboxyl groups (Table 1). The differences in the specific viscosity were more notable between WS polysaccharides and hemicellulose B (Fig. 1 B). The relative and specific viscosities of pectins and hemicellulose A were similar and their specific viscosities were several-fold higher levels than those of WS polysaccharides.

Our results on the chemical composition of various types of polysaccharides isolated from melon peel indicated varying monosaccharide units in their compositions. Glucose, arabinose and galactose were found in similar mass ratios in the WS polysaccharides fraction; while rhamnose and mannose were not detected in this fraction. In pectins, arabinose, xylose, galactose and rhamnose were present in comparable quantities, each measured at two to three times higher levels than glucose. Glucose, arabinose and xylose were identified as the main monosaccharides in hemicellulose A. Xylose and glucose were present in higher amounts in hemicellulose B compared to arabinose, galactose and rhamnose. Mannose was not determined in any of the fractions (Table 2).

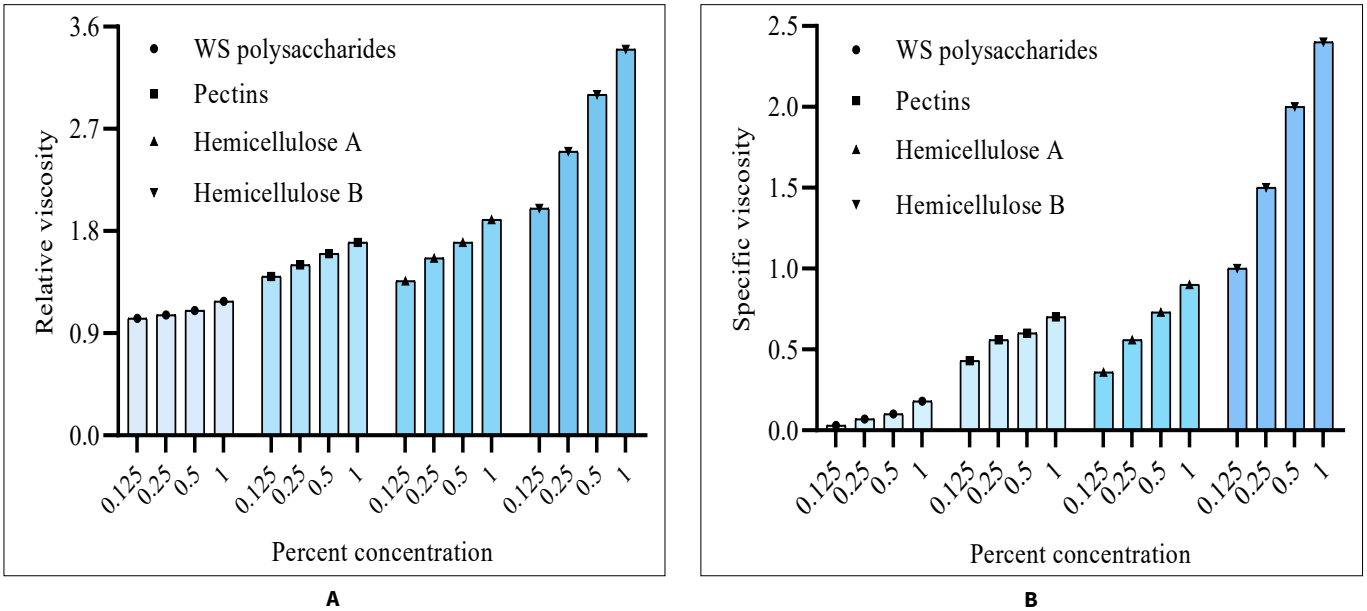
Among the free amino acids isolated from the melon peel, the highest molar ratios were observed for glycine, glutamine, cysteine, phenylalanine and threonine, respectively. The lowest molar ratios were found in tryptophan, lysine, serine, isoleucine, tyrosine and leucine, each present at less than one micromole per one gram of dried peel. The total content of nonessential amino acids was approximately 2.5- times greater than that of essential amino acids (Table 3). These results suggest the need to balance the amino acids profile with essential ones if the raw material is not to be used as a source of dietary amino acids.

Phenolic compounds from melon peel have been reported to exhibit high antioxidative properties, which may be attributed to the high ratio of carbon-carbon double bonds in their structures (24). Among the flavonoids, rutin was identified

Table 2. Monosaccharide composition of the obtained polysaccharides

Types of polysaccharides	Glucose	Arabinose	Xylose	Galactose	Rhamnose	Mannose
Water soluble polysaccharides	1.5	2.0	1.0	2.0	-	-
Pectins	1.0	3.0	2.0	3.0	2.5	-
Hemicellulose A	3.0	3.0	3.5	1.0	1.5	-
Hemicellulose B	3.5	2.5	5.0	2.0	1.0	-

Note: Values are based on single determinations; no statistical analysis was applied



A **B**

Fig. 1. Viscosity of various polysaccharide fractions at different concentrations. A - relative viscosity; B - specific viscosity.

Table 3. Content of free amino acids in melon peel

Essential amino Acids	µM/g	Nonessential amino acids	µM/g
Leucine	0.92	Aspartic acid	1.81
Isoleucine	0.50	Glutamic acid	1.39
Valine	1.00	Serine	0.36
Lysine	0.15	Glycine	7.37
Methionine	1.51	Asparagine	3.68
Threonine	2.96	Glutamine	4.15
Tryptophan	0.13	Cysteine	3.46
Histidine	1.06	Tyrosine	0.79
Phenylalanine	3.27	Alanine	1.49
		Proline	2.39
		Arginine	2.10
Sum (Essential)	11.5	Sum (Nonessential)	28.99
Total free amino acids		**40.49**	

as the most abundant compound. Dihydroquercetin, quercetin and luteolin were found in similar amounts ranging from 0.046-0.087 µM/g. Vitamin B6 was determined to be as the most abundant vitamin, with a concertation of 0.135 mM per 1 g of the raw material. The concentration of other vitamins, including B1, B2, PP and C, ranged from 0.015 to 0.051 mM/g (Table 4).

The composition of metals in melon peel as determined by ICP-MS showed values within expected ranges. Potassium was found to be the most abundant element, accounting for 3.66 % of the dry mass of the peel. However, in the polysaccharides, fractions, its content was only 0.2 %, which may be explained by its retention in the supernatant during polysaccharides precipitated. Similar trends were observed for other metals, including calcium, sodium, zinc, aluminum and iron. Relatively higher percentages of metals in polysaccharides fractions were found for magnesium and strontium. Among rare metals, low concentration were detected in the polysaccharides in all cases except for rubidium (Table 5).

The low metal content in the polysaccharides reactions suggests minimal interaction between melon peel polysaccharides and metal ions. Although some polysaccharides are known to bind various metals in this case, an opposite effect is observed indicating that melon peel polysaccharides bind significantly to lower amount of metals compared to other classes of bioregulators or polysaccharides present in the peel pulp. The results obtained in this work are in consistent with previously reported data, indicating that potassium, calcium, sodium and magnesium are the most abundant metals in the peel composition. The concentration of other metals were several or several dozen fold lower compared to macroelements (15).

The WS polysaccharide fraction was found to be nontoxic. Its oral administration to mice at a dose of 5000 mg/kg caused insignificant physiological changes such as frequent breathing and increased motor activity which lasted 20-30 min. They returned to normal breathing after 5-6 hr and did not differ from the control group of animals. During the observation for 14 days, no acute poisoning effects or death were observed (5/0). During the entire study, changes in the hair, skin and feces were normal in mice administered 5000 mg/kg of polysaccharide and no adverse effects were observed. After 14 days, changes in body weight increased compared to the first day, reaching 13 % in mice treated with polysaccharide concentrate and 18.8 % in mice in the control group. Although the dynamics of body weight in the control group were higher than in the group treated with polysaccharide concentrate, the difference between them was not statistically significant.

We also studied the effects of WS polysaccharide fraction on skin regeneration in mice. The wound-healing properties of the polysaccharide solution were demonstrated by comparing wound area reduction in the treatment and control groups. The treatment of the wound area with a 2.5 % aqueous solution of WS polysaccharides showed approximately two fold faster wound-healing compared to control animals (treated with water) on days 4, 8 and 12. By day 16 complete wound closure was observed in treated group. (Fig. 2). In general, the application of WS polysaccharides effectively accelerated the wound healing process. The treatment stimulated early reduction in wound size and enhanced epithelialization indicating a clear benefit over untreated wounds.

Table 4. Contents of flavonoids and vitamins in melon peel

Flavonoids	µM/g	Vitamins	mM/g
Dihydroquercetin	0.072	Vitamin B1	0.051
Luteolin	0.087	Vitamin B2	0.022
Rutin	0.262	Vitamin B6	0.135
Quercetin	0.046	Vitamin PP	0.017
		Vitamin C	0.015

Conclusion

The results of this study demonstrate that the WS fraction of polysaccharides isolated from Gurvak melon variety can be obtained in yields up to 15 % and possessed significantly wound-healing properties suitable for topical applications. Further in depth research will be necessary to evaluate its potential in functional food development. Comparative studies using alternative extraction techniques, formulations and delivery systems may help broaden the understanding and application of these melon -derived polysaccharides.

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Authors' contributions

SB and AMA prepared and edited the manuscript. GB and SM supervised the work. SB, NM, SF, UI, GR and IMA were involved in the experiments.

Table 5. Percentage of metals in WS polysaccharides extracted from *C. melo* Mill. peel

Metal	In peel	In poly-saccharides	Metal	In peel	In poly-saccharides	Metal	In peel	In poly-saccharides
Potassium	3.66	0.201	Copper	0.00456	0.00199	Vanadium	0.00013	0.0001
Calcium	0.837	0.450	Chromium	0.00210	0.00175	Molybdenum	0.000079	0.000014
Sodium	0.538	0.0334	Titanium	0.00146	0.000099	Zirconium	0.000076	0.000011
Magnesium	0.189	0.3990	Manganese	0.00127	0.001030	Yttrium	0.000024	0.000003
Zinc	0.0295	0.00777	Barium	0.00101	0.000836	Cadmium	0.000019	0.000012
Aluminum	0.0282	0.00112	Nickel	0.000857	0.000192	Ytterbium	0.000008	0.000005
Strontium	0.0118	0.01850	Rubidium	0.000523	0.008190	Scandium	<0.000001	<0.000001
Iron	0.00798	0.00118	Lithium	0.000157	0.000023			

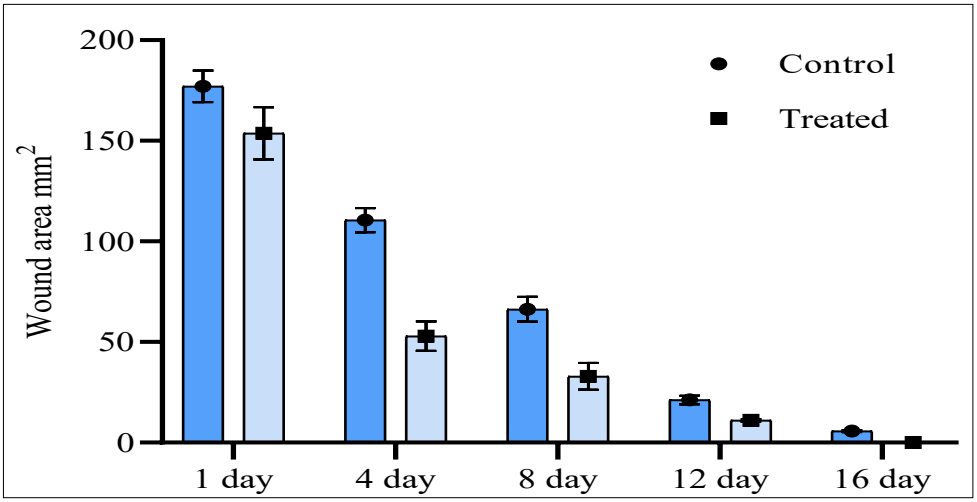

Fig. 2. External wound-healing effect of WS polysaccharide solution in mice (n=5).

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflicts of interest.

Ethical issues: None

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