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Study on the acid polysaccharide from the purslane plant Portulaca olerácea

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Abstract. Nowadays, searching for new raw materials and sources of biologically active substances of plant origin is very important. The wide distribution, relative ease of isolation, and high physiological activity have made the practical application of the acid polysaccharide attractive. An intensive study of the relationship between the structure and physicochemical properties of acidic polysaccharides is currently underway. The objective of the research is to isolate and study the physicochemical properties of the acidic polysaccharide (pectic substances) isolated from the medicinal plant Portulaca (lat. Portúlacaolerácea) growing on the lower part of the Amu Darya. The article presents the results of the isolation and chemical study of the acid polysaccharide by paper and gas-liquid chromatography. It has been established that this carbohydrate complex is represented by pectin substances. Their qualitative and quantitative monosaccharide composition has been established. The physicochemical parameters and monosaccharide composition of garden purslane have been studied. According to chromatography, the monosaccharide composition consists of uronic acid, galactose, glucose, arabinose, xylose, and minor rhamnose. The obtained pectin substances from the garden purslane plant belong to high molecular weight acidic polysaccharides, arabinoglucouranans.

1. Introduction

Portúlaca olerácea has become widespread in Uzbekistan as a weed. Garden purslane (P.oleracea L) is a herbaceous succulent annual plant from the Portulacaceae family, native to the Middle East and India [1,2]. Garden purslane is a highly encouraging pharmacological agent that can be eaten by animals, humans, and in medicine. The World Health Organization lists it as one of the most commonly used herbal remedies and labels it the «Global Panacea» [3]. The calculated carbohydrate content (40.67%) in purslane leaves and stem was considered higher than that of Serratifolia leaves (20%) and Amaranthus Incurvatus leaves (23.7%). The calculated carbohydrate content (40.67%) in purslane leaves and stem was considered higher than that of Sennaobtusfolia leaves (20%) and Amaranthusincurvatus leaves (23.7%). The content of crude fibre in the leaves and stem of purslane. (8.0%) was comparable to reported values (8.50–20.90%) of some Nigerian vegetables [4,5]. One of the discussed disadvantages of using vegetables in human nutrition is their high fibre content, which can cause intestinal irritation and reduced nutrient bioavailability (fungicides and micronutrients 1999). The recommended fibre intake for children, adults and pregnant and lactating mothers is 19-25%, 21-38%, 28% and 29%, respectively. According to reference [5], the leaves and stems of Purslane have the potential to serve as a significant dietary fiber source in human nutrition. Purslane, a herb with origins in traditional Chinese medicine, has achieved global distribution. The active constituents encompass polysaccharides [6], fatty acids, flavonoids, coumarin, and alkaloids [7]. The crop is known for its high content of antioxidant vitamins and omega-3 fatty acids [8]. It is utilized both as a dietary component and for its potential health benefits, primarily in the prevention of specific cardiovascular ailments and the promotion of a robust immune system [9]. The substance in question possesses antibacterial, anti-inflammatory, and antioxidant characteristics, and has been observed to modulate lipid and sugar metabolism within the human body. The inhibitory impact of purslane extract on nodule formation in colon cancer stem cells has been documented [10]. Furthermore, the treatment shown efficacy in

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mitigating ulcerative colitis in both rats and mice, as indicated by previous studies [11,12]. In vivo models demonstrated that the utilization of a distinct polysaccharide component (POP) derived from purslane exhibited an anticancer impact [13].

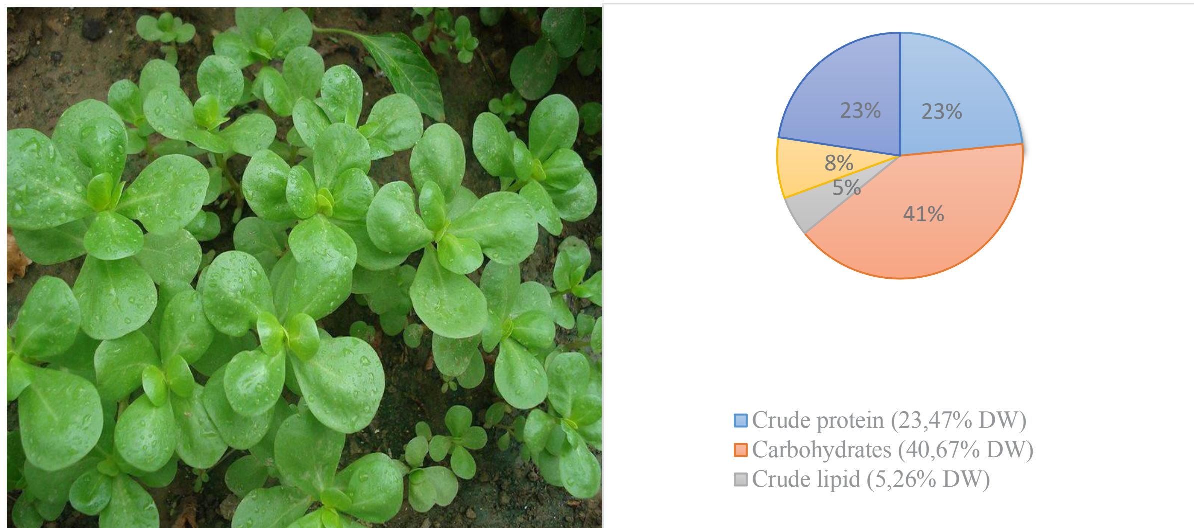

Fig. 1. The concentration of the food component [4,5]

Purslane is used as a choleretic, antipyretic agent for inflammation of the liver, kidneys, bladder, intestinal ulcers and bloody diarrhoea in Uzbekistan. The plant is also used in the treatment of intestinal infections (Kh.Kh. Kholmatov, 1964). The protective effects of an aqueous extract derived from Portulaca oleracea have been seen in type 2 diabetic db/db mice. This extract has demonstrated the ability to prevent diabetic vascular inflammation, hyperglycemia, and diabetic endothelial dysfunction. These findings suggest that Portulaca oleracea may play a beneficial role in mitigating the effects of diabetes and its associated vascular problems [14]. The plant's crude polysaccharide extract has been found to exhibit hypoglycemic effects and regulate lipid and glucose metabolism in alloxan-induced diabetic mice [15]. Additionally, it has been observed to reduce levels of total cholesterol, triglycerides, and fasting blood glucose in diabetic-2 mice [15,16]. The herb extract sharply raises blood pressure due to the fact that it contains a large amount of norepinephrine. Established hemostatic effect in internal bleeding [17].

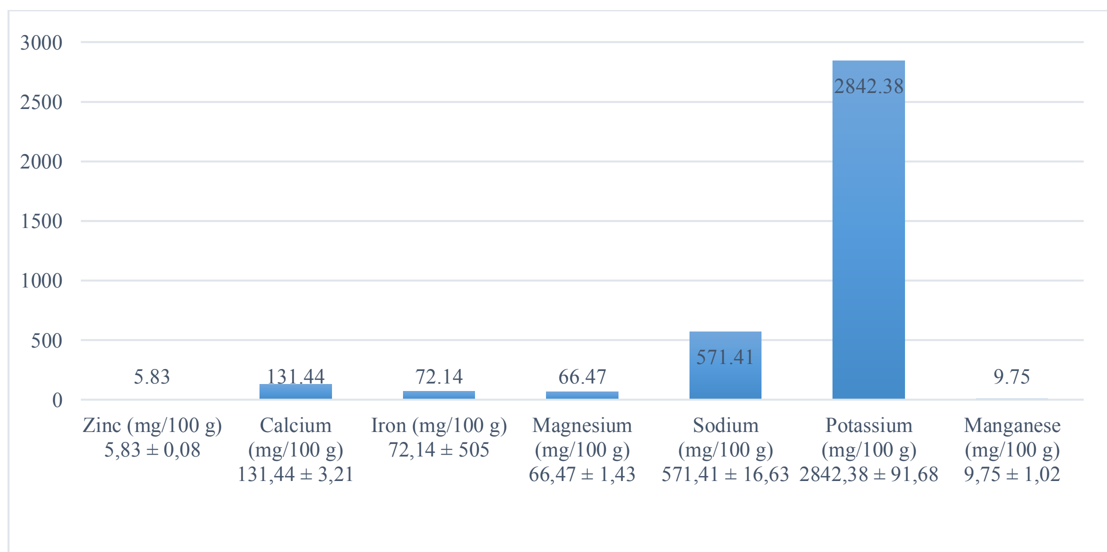

Fig. 2. Micro and macro elements of purslane

The purpose of this work is to isolate and study the physicochemical properties of an acidic polysaccharide (pectic substance) isolated from a medicinal plant garden purslane (lat. Portúlacaolerácea) growing in the lower part of the Amu Darya.

2. Materials and methods

2.1. Aerial parts of garden purslane (lat. Portúlaca olerácea) were collected in early June in the lower part of the Amu Darya, Khorezm region. Standard methods were used to conduct a general analysis of the main class of compounds [19]. The analysis was carried out not only on the leaf but also on the aerial part. According to the weight method, the amount of pectin was determined (when precipitated with alcohol). A hydrochloric acid solution is used as an extractant. To isolate pectin substances, the raw materials were sequentially treated with hydrochloric acid pH-2.5 at a temperature (of 65-70°C), and after 3-12 hours, pectin substances were isolated. The combined extracts were concentrated and precipitated with a threefold volume of 96% ethyl alcohol in a ratio of 1:5. The resulting precipitates were filtered off, washed with ethyl alcohol, dried and weighed [19]. The resulting pectin is a pink powder, and with iodine, it gives a negative reaction, which indicates the absence of starch. Odourless and slimy when tested on the tongue.

2.2 Viscosity. The relative viscosity (η_{rel}) of the isolated HP was determined using the Oswald viscometer with a capillary diameter of 0.73 mm. at a temperature of 25º C. To determine the viscosity, a transparent 1% pectin solution is prepared and the calculation is made according to the following formula:

$$\eta_{rel} = {t_2}/{t_1}$$

t_1- solvent flow time (seconds); t_2- polysaccharide solution flow time (seconds).

2.3. The molecular weight was determined. Size exclusion chromatography (SEC) was performed on an Agilent 1260 Infinity high-speed liquid chromatograph (USA) with a refractometric detector. TSKGMPWXL (ToyaSoda, Japan) with a linear calibration dependence is used as a sorbent. The eluent flow rate was 0.8 ml/min. The volume of the injected sample is 25 μL [23]. Chromatographic data are processed using the Windows Chemstation 7 program.

2.4. The monosaccharide composition of pectin substances is determined by paper and gas-liquid chromatography. Paper chromatography (PC) uses Filtrak-FN 13, 18 paper (Germany). GC analysis of the samples was carried out on a ShimadzuGC-2010 chromatograph with a flame ionization detector, a ShimadzuRxi-624SilMS quartz capillary column (30mx0.25mmx1.40μm), a mobile phase rate (N_2) of 1.5 ml/min, an injector temperature of 260°C, and a detector temperature of 280°C and column temperature 230°C.

2.5. Analysis of pectin substances by IR spectroscopy. IR spectra of pectins were taken on a Perkin-Elmer FT-IR/NIR Spectrometer Fourier IR spectrometer. Spectrum 3. UniversalATRSamplingAccessory absorption area (range) 530-3600 cm^{-1}.[26]

2.6. Complete acid hydrolysis of PS. To determine the monosaccharide composition, complete acid hydrolysis was carried out. The isolated polysaccharides (100 mg each) were hydrolyzed with 3 ml of 1N H_2SO_4 solution, at 100°C. PS within 24 h. After the time had elapsed, the hydrolyzate was placed in a beaker and is neutralized via barium carbonate. The formed precipitate was filtered off, the filtrate was deionized with a KU-2 cation exchanger, evaporated to a small volume (0.5 ml), and chromatographed on FN-18 paper in the butanol-1–pyridine–water (6:4:3) system with known monosaccharides (witnesses). The chromatograms were dried and developed with acid aniline phthalate, followed by heating in an oven at 110°C for 1–2 min. In the monosaccharide composition of polysaccharides, galactose, glucose, arabinose and xylose were identified [20,21]. According to gas-liquid chromatography, samples were taken in the form of aldononitrile acetates [22].

Preparation of aldonitrile acetates. The hydrolyzate after complete acid hydrolysis of pectin is evaporated to dryness. The mixture of monosaccharides is dissolved in 2 ml of freshly distilled pyridine and 100 mg of hydroxylamine hydrochloric acid is added. The solution is heated in a water bath for 1 hour. 2 ml of freshly distilled acetic anhydride is added to the cooled solution and heated for 1 hour at a temperature of 90^0C. After cooling, 25 ml of water is added to the mixture, the acetylated aldonitriles are extracted with chloroform. The extract is washed with water to remove pyridine and acetic acid. It is then dried over anhydrous sodium sulfate and evaporated in vacuo to a volume of about 2 ml. The resulting solution is dissolved in pure acetone and injected into the chromatograph using a microsyringe.

Determination of the total number of carboxyl groups of pectin substances. Quantitative determination of the functional groups of pectin substances (free carboxyl, total carboxyl) was carried out by the titrimetric method [24,25]. To do this, 0.05 g of pectin materials are mixed with 20 ml of water and left for two hours. A few drops of phenolphthalein indicator were added, then titrated with 0.1 N NaOH until a pink tint was formed. The number of free carboxyl groups is calculated by the formula:

$$K_f = \frac{V_1}{m} \times 0.45\%$$

V_1 - amount of 0.1N NaOH used.

Determination of esterified carboxyl groups (K_e). 0.1 N NaOH - 10 ml was added to the above reaction mixture to de-esterify the carboxyl groups. The mixture was left for two hours. Then 10 ml of 0.1 N HCl was added to the reaction mixture - the pink colour disappears. Next, a few drops of phenolphthalein indicator were added. Titrate with 0.1 N NaOH until a pink tint appears, spent 0.1 N NaOH - V_2 is determined. The calculation of degree of esterification of the carboxyl group (COOH) is calculated by the following formulas:

$$K_e = \frac{V_2}{m} \times 0.45\%; \qquad K_t = K_f + K_e; \qquad D_e = \frac{K_e}{K_t}\%$$

V_2 – the amount of 0.1 N NaOH used.

3. Results and discussion

At the first stage of the study, polysaccharides were isolated from the aerial part of the purslane plant, and some physical and chemical properties were studied.

Table 1. The pectic substance of the purslane plant

Above-ground parts of plants	Extraction temperature	Time/ hour	medium pH	Substance output
Purslane (Portúlacaolerácea) leaves and stems	65-70 ^{0}C	4	2.5 pH	3.5 - 4 %
Galega orientalis (Galega orientalis) from leaves and stems[27]	45 °C	2	2.6 pH	3.4 %
leaves of cultivated varieties of Alcea rosea red (in % air-dry weight)[28]	70-80 °C	4	2.4 pH	10.5 %

At the second stage, the average molecular weight of pectin substances was determined, which was 18.5 kDa. The physical and technological properties of pectin substances depend on

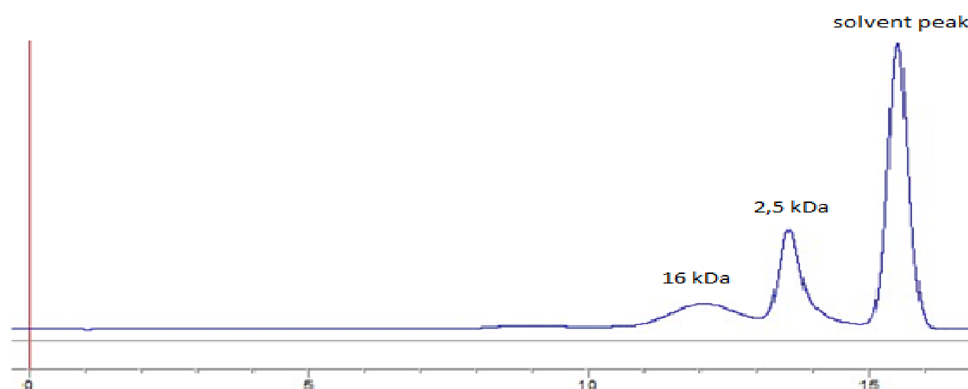

Fig. 3. The molecular weight of pectin substances

their molecular weight. From the figures, the sample consists of two or three fractions, and the molecular weight of pectin substances is 18.5 kDa. Thus, pectin substances from the purslane plant are high-molecular.

In the third stage, the monosaccharide composition of pectin substances is determined by paper and gas-liquid chromatography with complete acid hydrolysis. According to paper chromatography, the monosaccharide composition of HP is galactose, glucose, arabinose, xylose, and galacturonic acid. The monosaccharide composition of pectin consists of galactose, glucose, arabinose, and xylose in ratios of 2.3:15.0:2.3:1.0 and a small amount of

rhamnose and galacturonic acid. The data is given in Table 2.

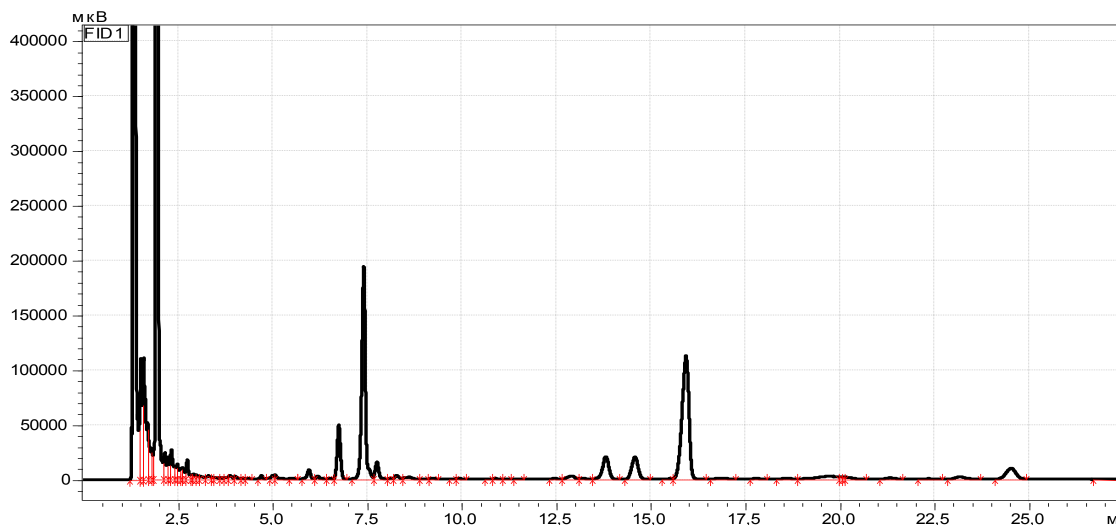

Fig. 4. Gas-liquid chromatography

According to paper and gas-liquid chromatography, it can be seen that pectin substances are arabinoglucouirananes. *At the fourth stage,* work was carried out to determine the viscosity and physicochemical parameters using the method of titration of pectin substances. The data is given in Table 2.

Table 2. Physical and chemical indicators of pectin substances

Type carbohydrates	η rel	MM kDa	Monosaccharide composition						UAc
			Gal	Glc	Ara	Man	Xyl	Rha	
Pectins of Portúlaca olerácea	1.3	18.5	2.3	15.0	2.3	-	1.0	traces	+
Pectic substances of the leaves of cultivated varieties of Alcea rosea[28] red	3.72	24.0	1.0	tr.	3.0	tr.	1.5	7.5	+

η rel - relative viscosity, MM- molecular mass, Gal – galactose, Glc- glucose, Ara – arabinose, Man- mannose, Xyl- xylose, Rha- rhamnose, tr- traces, UAc- uronic acids.

In the fifth stage, using the titration method, the degree of esterification of pectin substances of the studied plant is calculated. Characteristics of indicators of pectin substances are given in Table 3.

Table 3. Indicators of pectin substances

Names of plants	K_f	K_e	K_e	D_e
Purslane garden (Portulaca oleracea)	1,8±0,2 %	27	28,8	93,7
Beetroot [29]	19,2±0,2			45,2±0,8

K_f– free carboxyl groups; K_e- esterified carboxyl groups; K_t- the total amount of carboxyl groups; D_e– degree of esterification.

These indicators indicate that pectin substances are highly esterified acidic polysaccharides.

4. Conclusions

Pectin substances from garden purslane are high molecular weight acidic polysaccharides. The molecular weight of pectin substances is 18.5 kDa. The relative viscosity of a 1% pectin solution is 1.3. The monosaccharide composition of pectin consists of galactose, glucose, arabinose, and xylose in ratios of 2.3:15.0:2.3:1.0 and galactouronic acid. Purslane pectic substances have a high degree of esterification (93.7%) and a low content of free carboxyl groups (1.8%). The absorption band of pectin is characteristic of the - 1631 cm-1 (COO-) carbonyl group in the carboxyl group, and the absorption band - 1372 cm-1 corresponds to the ionized carboxyl group associated with metals. In

further experiments, we will determine the indicators of methoxylated carboxyl groups of pectin substances. pH. Ash content. Dynamics of accumulation of pectin substances in purslane garden plants.

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Conflict of Interest

The author(s) declares no conflict of interest.

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