

## CARBOHYDRATES FROM THE AERIAL PART OF *Ferula kuhistanica* AND *F. tenuisecta*

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*Water-soluble polysaccharides, pectinic substances, and hemicelluloses were isolated from the aerial part of Ferula kuhistanica and F. tenuisecta. Their physicochemical properties were determined.*

**Keywords:** *Ferula*, water-soluble polysaccharides, pectinic substances, hemicelluloses, monosaccharides.

The flora of Uzbekistan is rich in various medicinal plants that have long been used in folk medicine to treat various diseases. These include representatives of the genus *Ferula* L. (Apiaceae) that includes about 150 species [1], about 45 of which are indigenous to Uzbekistan. Many *Ferula* species have high feed value and are melliferous. Roots of several species are used by local residents as sources of starch [2]. The aerial part of *F. kuhistanica* is the source of an estrogenic phytopreparation that is used to increase egg production in poultry [3].

The goal of our work was to isolate and characterize the structure of the carbohydrate complex from aerial parts of *F. kuhistanica* and *F. tenuisecta*. Pigments and low-molecular-weight compounds were removed from air-dried raw material by extraction with CHCl<sub>3</sub> and MeOH. Then, extraction with EtOH (82%) isolated sugars that were soluble in alcohol, which were identified in both species by paper chromatography as saccharose and fructose.

The remaining raw material was extracted successively with cold and hot water, a mixture of oxalic acid and ammonium oxalate solutions (0.5%) with heating, and base solution (5%) to afford water-soluble polysaccharides (WSPS-C and WSPS-H), pectinic substances (PS), and hemicelluloses (HMC), respectively. Table 1 presents the yields of isolated carbohydrates and their monosaccharide compositions.

Table 1 shows that the carbohydrate contents in the studied species were different, from 0.79 to 9.12%, and that their distributions in leaves and stems were also different. The greatest accumulation of WSPS, PS, and HMC was observed in *F. kuhistanica* leaves. The monosaccharide compositions of the isolated polysaccharides were dominated by the acidic sugars galacturonic and glucuronic acids and by neutral sugars, i.e., they were acidic polysaccharides.

According to GC, the neutral sugars occurred in different ratios. This enabled them to be assigned to one group or another of polysaccharides considering the dominant monosaccharides. For example, WSPS-H from *F. kuhistanica* leaves were glucagalactoarabans according to the monosaccharide composition; WSPS-H from *F. tenuisecta* leaves, glucans.

WSPS of *F. kuhistanica* stems were dominated by glucose, galactose, mannose, and arabinose; of *F. tenuisecta*, galactose and glucose. WSPS of *F. kuhistanica* leaves contained mainly arabinose and galactose; of *F. tenuisecta*, rhamnose and glucose. All isolated WSPS were amorphous powders that were freely soluble in water and had an insignificant viscosity index ( $\eta_{\text{rel}} = 1.1\text{--}1.3$ ).

It was shown using WSPS-C of *F. kuhistanica* stems that they were heterogeneous, i.e., a mixture of various polysaccharides. Precipitation of WSPS from aqueous solution by Fehling solution produced three fractions that differed qualitatively and quantitatively (Table 2).

Table 2 shows that fraction 3 was a galactoaraban, in contrast with fractions 1 and 2, and contained less uronic acids.

The highest amount of PS was observed in leaves and stems of *F. kuhistanica*. The monosaccharide composition of these included arabinose, rhamnose, and galactose. PS of *F. tenuisecta* also contained rhamnose, arabinose and galactose. Pectins were characterized by the presence of galacturonic acid.

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TABLE 1. Qualitative and Quantitative Properties of Carbohydrates from the Aerial Part of *F. kuhistanica* and *F. tenuisecta*

PS type	Yield, %	Monosaccharide composition						
		Rha	Ara	Xyl	Man	Glc	Gal	UAc
<i>F. kuhistanica</i> (leaves)								
WSPS-C	8.12	—	4.5	—	—	1.0	4.3	+
WSPS-H	2.0	—	2.5	—	—	1.0	1.6	+
PS	8.6	—	1.0	1.0	—	—	1.0	+
HMC	8.8	1.0	6.6	5.4	—	—	4.8	+
<i>F. kuhistanica</i> (stems)								
WSPS-C	3.6	2.0	4.3	1.0	6.7	9.5	7.6	+
WSPS-H	1.5	1.4	4.0	1.0	2.0	4.8	5.3	+
PS	10.5	6.2	3.2	—	—	—	1.0	+
HMC	4.5	3.3	—	—	—	—	1.0	+
<i>F. tenuisecta</i> (leaves)								
WSPS-C	6.1	3.1	—	—	—	1.0	—	+
WSPS-H	2.5	1.0	—	—	—	3.6	—	+
PS	2.5	1.0	—	—	—	1.1	—	+
HMC	6.4	3.2	—	—	—	1.0	—	+
<i>F. tenuisecta</i> (stems)								
WSPS-C	1.3	2.9	2.7	—	1.0	3.3	7.8	+
WSPS-H	1.0	1.5	2.4	—	1.0	3.0	5.0	+
PS	2.0	2.7	3.4	—	1.0	—	1.2	+
HMC	5.7	3.0	1.0	9.6	—	—	1.8	+

+ The presence of UAc was determined by PC.

TABLE 2. Fractional Composition of WSPS-C from *F. kuhistanica* Stems

Fraction	Yield, %	Monosaccharide composition, GC			Uronic acids, %
		Gal	Glc	Ara	
1	30.0	1.0	Tr.	2.2	55.7
2	44.0	1.0	1.0	3.4	35.5
3	20.0	4.1	1.0	5.8	19.2

Tr.: traces.

PS were cream-colored amorphous powders that were freely soluble in water to form viscous solutions.

The yields of HMC from leaves and stems of the two *Ferula* species were in the range 5.7–8.7%. HMC were brown powders that were insoluble in water and freely soluble in dilute bases. HMC from *F. kuhistanica* leaves typically had elevated contents of arabinose, xylose, and galactose; from stems, rhamnose and galactose. HMC from *F. tenuisecta* stems were characterized by primarily rhamnose and xylose; from leaves, rhamnose. Galacturonic and glucuronic acids were observed in all HMC. IR spectra of isolated WSPS, PS, and HMC exhibited absorption bands corresponding to the various functional groups of the acidic and neutral polysaccharides [4].

Thus, carbohydrates in the aerial parts of two *Ferula* species were distributed unevenly with their greatest accumulation in *F. kuhistanica* leaves.

## EXPERIMENTAL

Descending paper chromatography (PC) used Filtrak FN-13,18 paper and solvent system *n*-BuOH:Py:H<sub>2</sub>O (6:4:3) with detection by anilinium biphenylate (aldoses) and urea solution (5%) (ketoses).

GC was performed on a Chrom-5 chromatograph with a flame-ionization detector, glass column ( $150 \times 0.3$  cm), XE-60 (5%) on Chromaton N-AW (200–250 mesh), He carrier gas, and flow rate 60 mL/min.

WSPS were hydrolyzed by  $H_2SO_4$  solution (1 N) for 10 h; PS and HMC, by  $H_2SO_4$  (2 N) for 20 h at 100°C. Hydrolysates were neutralized by  $BaCO_3$ , deionized by KU-2 cation-exchanger ( $H^+$ ), evaporated, and analyzed by PC. Monosaccharides were analyzed by GC as aldononitrile acetates [5].

IR spectra were recorded in pressed KBr pellets on a Perkin–Elmer Model 2000 IR-Fourier spectrometer. Viscosities of polysaccharides were measured using a VPZh-2 capillary viscosimeter with 0.73 mm diameter.

**Inactivation of Raw Material.** Ground raw material (100 g) was treated successively with refluxing  $CHCl_3$  (1:6) and MeOH (1:5) and filtered. The solid raw material was dried.

**Isolation of Sugars Soluble in Alcohol (SSA).** The remaining inactivated raw material was extracted with EtOH (82%, 2×) for 1 h. The extracts were separated, evaporated, and chromatographed.

**WSPS Extraction.** The remaining raw material was extracted with  $H_2O$  at room temperature with constant stirring for 1 h (2×, 1:5 and 1:3). The extracts were combined, evaporated, and precipitated by MeOH (1:3). The solid WSPS-C were separated, washed with MeOH, and dehydrated with  $Me_2CO$ . Then, WSPS-H were extracted analogously at 80°C. Table 1 presents the yields and monosaccharide compositions.

**Isolation of PS.** The remaining raw material was treated twice with a mixture of equal volumes of oxalic acid and ammonium oxalate solutions (0.5%) at 80°C for 2 h (1:10). The extracts were evaporated, dialyzed, precipitated by MeOH (1:10), and dehydrated with  $Me_2CO$ .

**Isolation of HMC.** Raw material from PS extraction was extracted twice with NaOH solution (5%, 1:2 and 1:3) at room temperature for 2 h. The extract was filtered, neutralized with HOAc, dialyzed, evaporated, and precipitated by MeOH (1:3). The solid was separated and dehydrated with  $Me_2CO$ .

**Fractionation of WSPS-C by Fehling Solution.** Polysaccharide (0.5 g) was dissolved in  $H_2O$  (20 mL) and treated with Fehling solution until the precipitation stopped. The precipitate was centrifuged, separated, treated with HOAc solution (50%), washed with MeOH, and dehydrated with  $Me_2CO$ . Yield 0.15 g (fraction 1). The centrifugate was dialyzed. The resulting precipitate was separated and dehydrated with  $Me_2CO$ . Yield 0.22 g (fraction 2). Then, the centrifugate was evaporated and precipitated by MeOH (1:3). The precipitate was separated. Yield 0.1 g (fraction 3).

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