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EXTRACTION OF AN ANTIDIABETIC POLYSACCHARIDE FROM SEEDS OF *Ocimum basilicum* AND DETERMINATION OF THE MONOSACCHARIDE COMPOSITION BY PRECOLUMN HIGH-EFFICIENCY CAPILLARY ELECTROPHORESIS[a]

H. Imam,[1,2] S. Lian,[2] R. Kasimu,[2*] R. K. Rakhmanberdyeva,[3] and H. A. Aisa[4] UDC 547.917

Ocimum basilicum is a dwarf plant of the family Lamiaceae and possesses many useful properties. Fruit of this plant is known to be rich in minerals, vitamins, carotinoids, and aminoacids. The decoction of fruit is used in folk medicine to improve vision and kidney functioning and to maintain good physical condition [1]. It exhibits hemostatic properties and is used to treat dysentery, cholic, and diarrhea [2]. It has been found that fruit of *O. basilicum* exhibits antioxidant, antiseptic, anti-inflammatory, and bactericidal activity; functions as a diuretic; and is used to treat angiocardiopathy [3–6]. However, polysaccharides from seeds of this plant have not been studied.

Herein we communicate results from a study of water-soluble polysaccharides (WSPS) from seeds of *O. basilicum*, the determination of its monosaccharide composition by precolumn high-efficiency capillary electrophoresis, and *in vitro* screening for antidiabetic activity.

Powder of dried seeds (1 kg) was refluxed (3×) at 60°C with 10 volumes of EtOH (95%) for 3 h in order to remove lipids. The remaining raw material was dried in air and extracted (3×) with distilled H_2O (1:10 ratio) for 24 h at room temperature. The aqueous extracts were combined, filtered, concentrated, and precipitated by EtOH (95%). The final concentration of EtOH was 80%. The resulting precipitate was separated, dissolved in H_2O (600 mL), treated with $CHCl_3$:BuOH (200 mL, 4:1), and purified of protein by the literature method [7]. Then, the aqueous fraction was dialyzed against tapwater (removing WSPS with MW 3,500 Da) and distilled H_2O and again precipitated with 5 volumes of EtOH. The resulting precipitate was centrifuged (3000 rpm, 10 min, 20°C), washed with EtOH, dissolved in water, and lyophilized. The yield of starting polysaccharide was 25.3 g.

The monosaccharide composition of the polysaccharide was determined by acid hydrolysis (2 M H_2SO_4, 8 h, 110°C) and neutralization by NaOH solution (4 M). The mixture was diluted with de-ionized H_2O (5 mL) and centrifuged (1000 rpm, 5 min). The obtained supernatant was used to prepare the 1-phenyl-3-methyl-5-pyrazolone (PMP) [8].

The polysaccharide hydrolysate (200 μL) was placed into a centrifuge tube, treated with methanolic PMP (100 μL, 5 M) and aqueous NaOH (100 μL, 3 M), held for 30 min at 70°C on a water bath, cooled to room temperature, and neutralized with HCl (100 μL, 0.3 M). The resulting solution underwent liquid–liquid extraction with an identical volume of *iso*-amylacetate (2×) and $CHCl_3$. The excess of reagent was removed from the organic phase after vigorous shaking and centrifugation. The aqueous layer was filtered through a membrane (0.45 μm) and diluted with H_2O before analysis by HPLC. Standard samples of monosaccharides were obtained in an analogous manner. The analysis was carried out on a Beckman P/ACETM MDQ HPLC under the following conditions: borate buffer (35 mM, pH 10.02), capillary temperature 25°C, potential 20 kV, detection at 254 nm, injection pressure 0.5 psi for 5 s, fused quartz capillary 48.5/58.5 cm, separation temperature 25°C.

The results showed that WSPS from seeds of *O. basilicum* consisted of glucose, galacturonic acid, rhamnose, mannose, arabinose, glucuronic acid, and galactose in mole ratio 3.54:2.56:1.56:0.46:0.21:0.99:0.12, respectively. The dominant monosaccharide in WSPS isolated by cold water was glucose. The monosaccharide could not be detected directly by absorption because of the lack of chromophores in its molecule.

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1) Graduate University, CAS, Beijing, 100039, P. R. China; 2) School of Pharmacy, Xinjiang Medical University, Urumqi, Xinjiang, 830054, P. R. China; 3) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences, Republic of Uzbekistan, Tashkent, fax: (99871) 120 64 75: 4) Xinjiang Technical Institute of Physics & Chemistry, CAS, Urumqi, Xinjiang, 830011, P. R. China. Translated from *Khimiya Prirodnykh Soedinenii*, No. 4, July–August, 2012, pp. 587–588. Original article submitted March 5, 2012.

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It is known from the literature that seeds of *O. basilicum* exhibit antitumor and antidiabetic activity [9]. However, the biological activity of seeds growing in Xinjiang (China) has not been studied.

The antidiabetic activity of WSPS from seeds of *O. basilicum* was determined by measuring the inhibitory activity for protein tyrosine phosphatase 1B *in vitro*. The inhibitory activity for protein tyrosine phosphatase 1B was IC_{50} = 8.20 μg/mL [10]. The reagent 1-phenyl-3-methyl-5-pyrazolone (PMP) was developed earlier for precolumn derivatization in order to increase the yield of single highly absorbing derivatives [11]. Based on this, we developed a new method for analyzing monosaccharide components by preparing derivatives with precolumn HPLC. The advantage of this method relates to the speed, high sensitivity, and good reproducibility that enable it to be used for qualitative monitoring of polysaccharides from seeds of *O. basilicum*.

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