

## POLYSACCHARIDES FROM *Cichorium glandulosum* SEEDS

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The herb *Cichorium glandulosum* (chicory, Compositae) is used in Chinese folk medicine to treat liver diseases [1]. We isolated previously from this plant coumarins [2]. Herein we report results from a study of carbohydrates from *C. glandulosum* seeds. Low-molecular-weight and dye components were removed by defatting dried seeds (100 g) with  $\text{CHCl}_3$  in a Soxhlet apparatus for 8 h. Then the residue was extracted with alcohol (80%). The remaining raw material was extracted three times with cold water to isolate water-soluble polysaccharides (WSPS-1). The resulting extracts were combined, centrifuged, and dried. The extract was purified of proteins by the literature method [3] and precipitated by alcohol in a 1:3 ratio. The resulting precipitate was separated, washed with alcohol, defatted with acetone, and dried in vacuo over  $\text{P}_2\text{O}_5$ . The yield of WSPS-1 was 1.06%. Then the residue was extracted with hot water at 80°C to afford WSPS-2 in 1.39% yield. Extracts WSPS-1 and WSPS-2 gave a negative reaction for starch.

WSPS-1 and WSPS-2 were light-brown powders that dissolved in water. Their hydrolysate ( $\text{H}_2\text{SO}_4$ , 2 N, 8 h, 100°C) contained acidic and neutral sugars in various ratios according to PC using  $\text{BuOH:Py:H}_2\text{O}$  (6:4:3) with development by anilinium acid phthalate. The quantitative monosaccharide composition of the hydrolysates was analyzed using GC with a PE-Turbomass aidosystem XI mass spectrometer, PESS quartz column ( $30 \times 0.25$  mm), 200°C, 35 mL/min,  $\text{N}_2$  carrier gas. Sugar samples were analyzed as aldonitrile acetates [4, 5]. Table 1 gives the monosaccharide composition of the polysaccharides. WSPS-1 were a mixture of acidic and neutral polysaccharides. The dominant monosaccharides of WSPS-1 were rhamnose, xylose, and galactose; of WSPS-2, mainly mannose and galactose.

After removing WSPS, the remaining raw material was extracted twice with oxalic acid and ammonium oxalate solutions (0.5%, 1:1) for 2 h at 70°C. The extracts were combined, centrifuged, dialyzed, dried, and precipitated by alcohol (1:2). The precipitate was removed, washed with alcohol, and dried in vacuo over  $\text{P}_2\text{O}_5$ . The yield of pectinic substances (PS) was 2.28%.

PS were a white powder that was soluble in water. Its hydrolysate ( $\text{H}_2\text{SO}_4$ , 2 N, 42 h, 100°C) contained predominantly rhamnose, mannose, and galactose according to GC. The contents of free ( $\text{C}_f$ , 18.9%) and esterified carboxylic groups ( $\text{C}_e$ , 9.5%) were determined by titration. The degree of esterification ( $\lambda$ ) was 33.5% [6]. Therefore, PS were low esterified pectins.

Hemicellulose (HC) was isolated after removal of PS by extraction with base solution (5%) at room temperature. The extract was neutralized by acetic acid. The resulting precipitate was removed to afford HC-A in 0.92% yield. The supernatant solution was condensed and precipitated with alcohol. The resulting precipitate (4.28% yield) was designated HC-B. The predominant monosaccharides in the hydrolysates ( $\text{H}_2\text{SO}_4$ , 2 N, 72 h, 100°C) were mannose (HC-A) and arabinose and xylose (HC-B).

The biological activity was studied using inhibition by polysaccharides of nonenzymatic glycosylation of protein *in vitro* and antioxidant effects (ability to damage hydroxyl free radicals) [7]. Table 2 presents the results.

Table 2 shows that WSPS-1, WSPS-2, and PS had various biological activities. WSPS-1 were the best for inhibiting nonenzymatic glycosylation of protein; WSPS-2, for inhibiting peroxide oxidation of lipids.

PS were the best for destroying hydroxyl radicals.

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TABLE 1. Content and Monosaccharide Composition of Carbohydrates from *Cichorium glandulosum* Seeds

| Polysaccharide | Yield, % | Monosaccharide composition, % |       |       |       |      |       |      |
|----------------|----------|-------------------------------|-------|-------|-------|------|-------|------|
|                |          | Rha                           | Xyl   | Ara   | Man   | Glc  | Gal   | UAc  |
| WSPS-1         | 1.06     | 20.2                          | 10.88 | 7.90  | 4.23  | 1.88 | 21.29 | 0.98 |
| WSPS-2         | 1.39     | 6.40                          | 13.95 | 3.26  | 35.48 | 1.42 | 16.02 | 0.71 |
| PS             | 2.28     | 27.83                         | 0.87  | 3.49  | 22.81 | 3.06 | 18.51 | 2.72 |
| HC-A           | 0.92     | 2.30                          | 4.02  | 10.75 | 48.92 | 4.20 | -     | 0.50 |
| HC-B           | 4.28     | 2.91                          | 4.47  | 18.98 | 2.29  | 2.42 | -     | -    |

TABLE 2. Screening for Biological Activity of Polysaccharides

| Polysaccharide | Inhibition of nonenzymatic protein glycosylation, % | Peroxide oxidation of lipids, % | Ability to destroy hydroxyl radicals, % |
|----------------|-----------------------------------------------------|---------------------------------|-----------------------------------------|
| WSPS-1         | 48                                                  | 41                              | 24                                      |
| WSPS-2         | 15                                                  | 48                              | 34                                      |
| PS             | 43                                                  | 32                              | 52                                      |

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