Determination of Antidiabetic Polysaccharides of *Ocimum basilicum* Seeds Indigenous to Xinjiang of China by High-Performance Thin-Layer Chromatography–UV/Vis–Mass Spectrometry

Abulimiti Yili, Hurxid Yimamu, Saideiahmat Ghulameden, Zhao Hai Qing, Haji A. Aisa, and **Gertrud E. Morlock***

Key Words

Planar chromatography
Derivatization
Biopolymer
Polymer plant extracts
Dietary fiber
PTP1B
Aliivibrio fischeri

Summary

The seeds of *Ocimum basilicum* have traditionally been used for the prevention and treatment of a number of diseases in Xinjiang of China. In this study, six polysaccharide extracts were isolated from the seeds of *O. basilicum* by sequential extraction and purified. After methanolysis, the monosaccharide compositions of the six polysaccharide extracts were analyzed by high-performance thin-layer chromatography (HPTLC) on HPTLC plates silica gel 60 with a mixture of isopropyl acetate–ethyl acetate–methanol–water (5:4:1:0.1, *v*/*v*). After derivatization with the aniline diphenylamine *o*-phosphoric acid reagent, densitometric quantitation was performed by absorbance measurement at 370 or 630 nm. The results revealed that the polysaccharides in *O. basilicum* seeds consisted primarily of fructose (hR_F 80), glucuronic acid (hR_F 58), galacturonic acid (hR_F 51), rhamnose (hR_F 40), xylose (hR_F 25), arabinose (hR_F 18), and galactose (hR_F 9). Xylose, glucuronic acid, and fructose were the three major components found and account for 45, 31, and 21%, respectively. All extracts contained uronic acids, ranged 3 to 24%. An unknown monomeric unit above glucuronic acid was characterized by mass spectrometry (MS) to be a hexuronic acid, and HPTLC–MS proved to be a well suited method for characterization of polysaccharide-based biopolymers and assignment of its monomers. The polysaccharide extracts (aqueous cold, aqueous hot, acidic, and alkaline) showed inhibitor activities of protein tyrosine phosphatase 1B *in vitro* with half maximal inhibitory concentration (IC_{50}) values of 8.2, 2.2, 70.9, and 0.8 µg mL^{-1}, respectively. For the first time, a molecular basis was provided to explain the hypoglycemic effect of the seeds of *O. basilicum* that has been used as antidiabetic adjuvant in traditional Chinese medicine.

1 Introduction

Uighur medicines are one of the important elements in traditional Chinese medicine (TCM). Therapies using them can be traced back for more than 2500 years. As for TCM, Uighur medicines have developed further and collected a wealth of human pharmacological information and experience and, thus, formed an integrated theory system [1, 2]. However, a lowered product quality as well as a restricted fundamental research limited its further development and international acceptance. Seeds of *Ocimum basilicum* as traditional Chinese medicine are selectively extracted and employed as described in the *Uighur* Medicine of Drug Standard of the Ministry of Public Health of the People's Republic of China as well as in the ancient China medical book Compendium of Materia Medica [3]. As main components of traditional Uighur medicine prescription, seeds of *O. basilicum* have been used for prevention and treatment of various cardiovascular diseases. They are described for example as anti-inflammatory, anticancer, antibacterial, antidiabetic, and as weight loss agent [4, 6, 7]. However, polysaccharides from the seed of the Lamiaceae family, to which *O. basilicum* belongs, are practically unstudied.

The goal of this research was the systematic extraction and isolation of six polysaccharide fractions from the seeds of *O. basilicum,* the screening of the antidiabetic activity *in vitro,* as well as the characterization of the unknown polysaccharide profile of *O. basilicum* seeds. Biopolymer analysis is a challenging field of analysis, and a recently developed rapid HPTLC method for analysis of biopolymers [8] was preferred to gas chromatography or high-performance liquid chromatography for characterization of the polysaccharides after methanolysis. HPTLC seemed to be well-suited for reliable biopolymer profiling due to the selective derivatization and matrix-tolerance [9–11], but also the capability for high sample throughput and its cost-effectiveness [12, 13] might attract plant analysts [14].

A. Yili, S. Ghulameden, Z.H. Qing, and H.A. Aisa, The Key Laboratory of Plant Resources and Chemistry of Arid Zone, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi, Xinjiang 830011, China; H. Yimamu, Graduate University, Chinese Academy of Sciences, Beijing 100039 and School of Pharmacy, Xinjiang Medical University, Urumqi, Xinjiang 830054, China; and G.E. Morlock, Food Science, Justus Liebig University Giessen, Institute of Nutritional Science, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany.
E-mail: Gertrud.Morlock@ernaehrung.uni-giessen.de

2 Experimental

2.1 Materials

The following materials were used:

– Servacel anion exchanger with diethylaminoethyl groups (DEAE-23SN): Reanal, Budapest, Hungary

– Diethyl ether, trichloroacetic acid, sodium hydroxide, methanol, ethanol, chloroform, *n*-butanol, sodium chloride, sulfuric acid, oxalic acid, ammonium oxalate, and sodium borohydride (all analytical grade): Tianjin Guangfu Fine Chemical Research Institute, Tianjin, China

– Insulin, p-nitrophenyl phosphate (pNPP), dithiothretol (DTT), 4-(2-hydroxyethyl)-1-piperazineethansulfonic acid (HEPES), and bovine serum albumin (BSA): Sigma, Shanghai, China

– Quick-change site-directed mutagenesis kit: Stratagene, Cedar Creek, TX, USA

– Protein tyrosine phosphatase 1B (PTP1B) inhibitor compound-2 (positive control): CalBioChem, Shanghai, China

– Ethyl acetate, isopropyl acetate, magnesium chloride (all analytical grade), *o*-phosphoric acid (85%), diphenylamine (≥98%), D(–)-fructose (>99%), D(+)-glucose-1-hydrate (DAB), D(+)-galactose (≥98%), D(+)-mannose, L(+)-rhamnose (>99%), D(+)-xylose (>99%), D(+)-galacturonic acid (monohydrate), and HPTLC plates silica gel 60 (20 × 10 cm, layer thickness 0.20 mm): Merck, Darmstadt, Germany

– Bi-distilled water (produced by Heraeus Destamat Bi-18E) and aniline (≥99.9%): Thermo Fisher Scientific, Schwerte, Germany

– Methanol (≥99.8%, used for chromatography and MS), hydrochloric acid (37%) and pyridine (≥99%): Sigma Aldrich, St. Louis, USA

– Alginic acid (Ph. Eur.): FMC BioPolymer, Ayrshire, United Kingdom

– L(–)-Fucose (>99%), D-glucuronic acid (>97%), and acetyl chloride (>98%): Fluka, Buchs, Switzerland

– L-Iduronic acid (sodium salt): Santa Cruz Biotechnology, Santa Cruz, CA, USA

– L(+)-Arabinose (≥99%): Acros Organics, Geel, Belgium

2.2 Extraction and Isolation of Crude Polysaccharides

In August 2010, *O. basilicum* seeds were collected in Hoten, China. The seeds were identified by Professor Shen Guan Mian from the Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, as seeds of *O. basilicum* L. The fresh seeds of *O. basilicum* (300 g) were ground in a blender (XFB-400, Hunan Pharmaceutical Machinery Factory, Changsha, China) and submitted to enzyme inactivation with methanol–water (4:1, *v*/*v*) under reflux for 20 min. After centrifugation of the cooled extract at 12,000 rpm for 20 min with the Allegra 25R centrifuge (Beckman Coulter, Krefeld, Germany), the sediment was washed with ethanol, dried under vacuum (BL-1180 suction flask, Henan Yi Hua Gong Yi Instrument, Gong Yi, China), milled (Henan Yi Hua Gong Yi Instrument), and defatted with chloroform–methanol (2:1, *v*/*v*) in a Soxhlet apparatus (X0155-1100, Hangzhou Kuang Wei Laboratory Equipment, Hangzhou, China) for 4 h. Successive extractions were performed with different solvents (described in the following) in the mechanical blender (YK120, Shanghai Yi Kong Mechanical and Electrical, Shanghai, China), and after each one, centrifugation was carried out as described above and the residue was submitted to the next extraction. The first extraction of the residual raw material with water at room temperature (120 min) produced the cold water-soluble polysaccharide extract (WSPS-C; No. 1) and the second with boiling water under reflux (180 min), the hot water-soluble polysaccharide extract (WSPS-H; No. 2). Thirdly, pectin substances (PS; No. 3) were extracted by a mixture of oxalic acid solution and ammonium oxalate solution (0.5% each, 1:1, *v*/*v*) at room temperature for 90 min, and thereafter hemicelluloses (HC; No. 4) with a 5% sodium hydroxide solution in the presence of 0.001% $NaBH_4$ (room temperature, 120 min). Extracts Nos. 1–3 were concentrated to 200 mL and treated with ethanol (4-fold excess of ethanol) in order to precipitate the polysaccharides, which were then washed three times with 10 mL ethanol and dried under vacuum. The HC extract No. 4 was neutralized with 50% aqueous acetic acid, the resulting supernatant was dialyzed with a cut-off of 3500 Da (cellulose dialysis bags 3500, Shanghai Yuan Ye Biological Technology, Shanghai, China), and then precipitated with ethanol (4-fold excess) to obtain the HC fraction. All extracts were dissolved in 600 mL water and deproteinized 10 times in an automatic shaker (HZQ-C, Harbin Dong Ming Medical Instrument Factory, Harbin, China) with 200 mL chloroform–*n*-butanol (4:1, *v*/*v*) [15]. The resulting aqueous phase was dialyzed (cut-off 3500 Da), concentrated to 100 mL, and then further precipitated with ethanol (4-fold excess). After centrifugation (3000 rpm, 10 min, 20°C), the precipitates were washed with anhydrous ethanol and then dissolved in 10 mL water and lyophilized (FDU-210, Eyela, Tokyo, Japan) to yield the crude biopolymers.

2.3 Preparation of Acidic and Neutral Fractions

Each 50 mg crude polysaccharide was dissolved in 5 mL distilled water and chromatographed on a Servacel DEAE-23SN anion exchange column (2.0 × 20 cm) pre-equilibrated with water (Biologic LP chromatography system, Bio-Rad, Shanghai, China). Neutral polysaccharides WSPS-H-N (No. 2a) and HC-N (No. 4a) were eluted with distilled water. Thereafter, the acidic polysaccharide fractions WSPS-H-A (No. 2b) and HC-A (No. 4b), both adsorbed at the anion exchanger, were eluted with a sodium chloride (NaCl) gradient (0.050–1.5 M). The major acidic polysaccharide fractions were collected with a fraction collector and dialyzed (cellulose acetate membrane MWCO 1000 Da) for 48 h with distilled water for desalination, concentrated at 65°C with the evaporator (R-210, Büchi, Flawil, Switzerland) and then lyophilized.

2.4 Sum Parameters for Carbohydrates, Uronic Acids, and Proteins

The uronic acids' content in the extracts was estimated with the colorimetric *m*-hydroxybiphenyl assay at 520 nm, using glucuronic acid as standard [16]. Neutral carbohydrates in the fractions were measured by the phenol sulfuric acid assay, using glucose as standard [17]. The protein concentration was determined with the Bradford assay using trypsin as standard protein [18].

2.5 Methanolysis of the Extracts for Analysis of Monomeric Units

Each extract (10 mg) was weighted in a 10-mL reaction vial and dissolved in 1 mL methanolic hydrochloric acid (2 mol L^{-1} [8]). After methanolysis at 100°C for 4 h, 50 µL pyridine was added. This neutralized solution was used for HPTLC application. All solutions were stored at –20°C until analysis.

2.6 Standard Mixture Solutions

Each sugar or uronic acid (10 mg) was weighted in a 10-mL reaction vial and dissolved in 1 mL methanolic hydrochloric acid (2 mol L^{-1}). After methanolysis at 100°C for 4 h, 50 µL pyridine was added. Mixtures were composed by adding 30 µL each in a 2-mL measuring flask and filling up to the mark (150 ng μL^{-1}). For qualitative analysis, the derivatives of fructose (Fru, 90 µL), galacturonic acid (GalA), rhamnose (Rha), xylose (Xyl), and galactose (Gal) were added in mixture 1 and those of glucuronic acid (GlcA), fucose (Fuc), arabinose (Ara), mannose (Man), and glucose (Glc) in mixture 2. For quantitative analysis, the derivatives of Fru, GalA, GlcA, Rha, Xyl, Ara, and Gal were composed in mixture 3. The iduronic acid solution (IdoA) was prepared accordingly (0.8 mg/mL).

2.7 HPTLC Method

2.7.1 Application

The samples were applied as 8-mm bands with the Automatic TLC Sampler 4 (CAMAG, Muttenz, Switzerland) on 21 tracks (track distance 9 mm, distance from lower edge 8 mm). The application volume was 0.5–10 µL for samples and 2–15 µL for standards (0.3 to 2.1 µg $band^{-1}$, in mixture 1 for methylated fructose 0.9 to 6.3 µg $band^{-1}$). For overlayed application, 12 µL *O. basilicum* seed extract and 20 µL alginic acid were applied, each as 16 mm bands (track distance 24 mm). Between those 18 µL, IdoA was applied as 24 mm band resulting in an 8-mm overlay on both sides.

2.7.2 Chromatography

Chromatography was performed in the ADC 2 (CAMAG) with 10 mL isopropyl acetate–ethyl acetate–methanol–water 5:4:1:0.1 (*v*/*v*) up to a migration distance of 60 mm. The drying time was 30 s before and 2 min after development.

2.7.3 Derivatization and Documentation

The HPTLC plate was immersed in the aniline diphenylamine *o*-phosphoric acid reagent (20% *o*-phosphoric acid [85%] added to 1:1 mixture of 2% solutions of diphenylamine and aniline in acetone) using the TLC Immersion Device (immersion time 1 s, speed 3.5 cm s^{-1}, CAMAG) and heated on the TLC Plate Heater (CAMAG) at 110°C for 5 min. The reagent stored in the refrigerator was stable for months. The chromatograms were documented under white light illumination (transmission mode) using the TLC Visualizer (CAMAG).

2.7.4 Densitometry

Absorbance measurement was performed at 370 and 630 nm (multi-wavelength scan with the deuterium and tungsten halogen lamps) with the TLC Scanner 3 and winCATS software (CAMAG). The slit dimension was 6 mm × 0.45 mm at a scanning speed of 20 mm s^{-1}.

2.7.5 Recording of Mass Spectra

The TLC–MS Interface (CAMAG) was equipped with the oval elution head (2 × 4 mm) and coupled to the electrospray ionization (ESI) interface of the single-quadrupole mass spectrometer (expression CMS, Advion, Ithaca, NY, USA). An inline filter (Upchurch Scientific A-356; Techlab, Erkerode, Germany) with a 0.5-µm frit (Upchurch Scientific PEEK-Frit Blue UP A-703; Techlab) was integrated in the transfer tube to the ESI source. The zone was eluted with methanol at a flow rate of 100 µL min^{-1} (pump of the HP 1100 ChemStation, Agilent, Waldbronn, Germany). For positive ionization, the MS system was operated in the full scan mode (total ion current [TIC] chronogram) between *m*/*z* 100 and 600 with the following parameters: detection gain of 1050, ESI voltage of 3 kV, capillary voltage of 150 V, source voltage of 25 V, source voltage dynamic of 25 V, nebulizer gas pressure of 60 psig, dissolution gas flow rate of 4 L min^{-1}, capillary temperature, and source gas temperature of 250°C each. Data processing and evaluation for MS measurement were performed with Mass and Data Express 1.1.22.15 (Advion). A plate background at a migration distance comparable to the analyte zone was substracted from the analyte spectrum.

2.7.6 Bioluminescence Detection with Aliivibrio fischeri *Bacteria*

The developed plate was automatically dipped into the luminescent bacteria suspension using the TLC Chromatogram Immersion Device III (immersion speed 3 cm s^{-1}, immersion time 0 s). The suspension was prepared according to DIN EN ISO 11248-1 (2009), section 5. For imaging, the HPTLC plate was placed into the compartment of the BioLuminizer (CAMAG). Ten images were taken in time intervals of 3 min. The exposure time was 50 s for each image.

2.8 Inhibition of Protein Tyrosine Phosphatase 1B

The enzyme activities were measured using pNPP as substrate in the microplate spectrophotometer (Bio-Rad). Two millimolar pNPP and enzyme in a buffer (containing 50 mM HEPES, pH 7.3, 100 mM NaCl, 0.1% BSA, and 1 mM DTT) were added with or without test compounds to each 96-well microplate. Following incubation at 30°C for 30 min, the reaction was terminated with 3 M NaOH. The amount of *p*-nitrophenol formed was measured via its absorbance at 405 nm and corrected by the pNPP hydrolysis generated without enzyme [19, 20].

3 Results and Discussion

3.1 Isolation and Characterization of the Crude Polysaccharides

For isolation of the polysaccharides, successive extractions were performed. They included extractions of the completely defatted seeds with water, first at room temperature and then under reflux, precipitation of the polysaccharides by alcohol and intermediate purification by centrifugation. The yield of the polysaccharide extracts in the air-dry seeds **(Table 1)** were 2.5 g/100 g

Table 1

Polysaccharide yield as well as sum parameters of proteins, uronic acids, and sugars in the crude polysaccharide extracts obtained from air-dry *Ocimum basilicum* seeds (assignments in section 2.2) with regard to PTP1B inhibition activity.

No.	Extract	Polysaccharide yield (%)	Proteins (%)	Uronic acids (%)	Neutral sugars (%)	Sum (%)	PTP1B IC_{50} (µg mL^{-1})
1	WSPS-C	2.5	8.0	23.6	57.5	81.1	8.2
2	WSPS-H	2.6	9.0	16.3	49.3	65.6	2.2
3	PS	7.7	1.4	5.5	35.2	40.7	70.9
4	HC	4.7	10.0	3.2	47.4	50.6	0.8
	Sum (%)	17.5	28.4				

(2.5%) and 2.6 g/100 g (2.6%) for WSPS-C and WSPS-H extracts, respectively. The PS extract (via complex formation with oxalic acid) yielded 7.7 g/100 g (7.7%) and the HC extract (via alkaline extraction) 4.7 g/100 g (4.7%). Consequently, the results showed that the total polysaccharide content of air-dry *O. basilicum* seeds was 17.5% (*w*/*w*).

In the individual extracts, the sum parameters of uronic acids and neutral sugars determined by colorimetric assays ranged from 3.2 to 23.6 and 35.2 to 57.5%, respectively. However, the colorimetric assay might vary up to ±5% due to interferences caused by the complex structure of neutral and acidic sugars in the same solution as well as using glucose as standard since various monosaccharides differ in absorbance in the phenol sulfuric assay [17, 21].

Each of the four crude extracts (WSPS-C, WSPS-H, PS, and HC) contained 17.0% protein according to the Bradford assay [18]. All extracts were deproteinized until the Bradford assay responded negatively, and no absorption was detected at 280 and 260 nm.

In order to gain purified, acidic polysaccharide fractions, the relatively stronger PTP1B-inhibiting polysaccharide extracts HC and WSPS-H were further fractionated by anion-exchange chromatography. The respective polysaccharide fraction that was not adsorbed to the anion-exchange column was regarded as neutral polysaccharide fraction (WSPS-H-N and HC-N), whereas the adsorbed polysaccharide fraction was regarded as acidic polysaccharide fraction (WSPS-H-A and HC-A). After elution, all fractions were subjected to further HPTLC analysis to characterize their respective monomeric units.

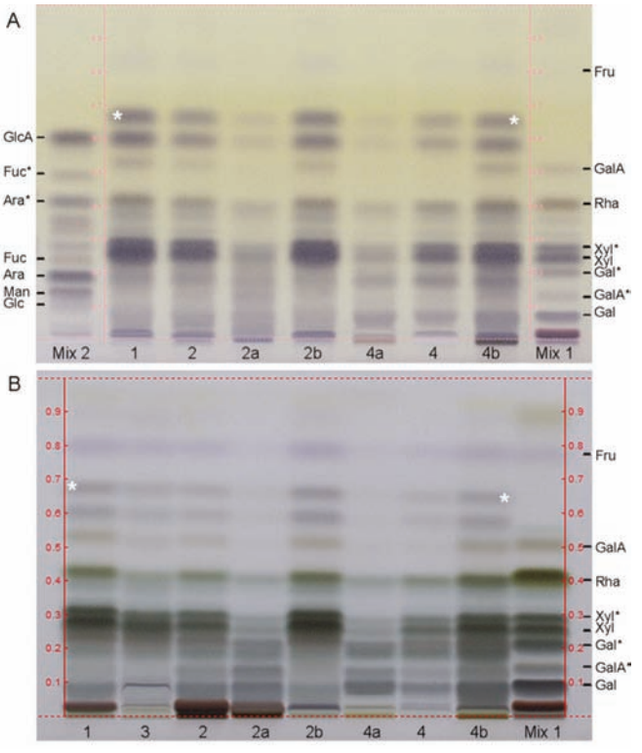

Figure 1

HPTLC separation of the monomeric units of the different polysaccharide extracts from *Ocimum basilicum* seeds after methanolysis and derivatization with aniline diphenylamine *o*-phosphoric acid reagent; application volumes of 4 µL each for visual comparison (A) and different volumes (from left: 4, 15, 4, 3, 4, 4, 3, 4, and 4 µL) (B); assignments in sections 2.2 and 2.3.

3.2 Characterization of the Monomeric Units in the Polysaccharide Extracts by HPTLC

A methanolysis of the various polysaccharide extracts was performed to obtain the respective monomeric units. After neutralization, the derivatives obtained, *i.e.,* methyl glycosides formed from the respective sugars and methyl glycoside methyl esters derived from the respective uronic acids, were separated by normal-phase chromatography within 20 min and documented after derivatization with the aniline diphenylamine *o*-phosphoric acid reagent. In both standard mixtures, the methyl glycosides and methyl glycoside methyl esters derived from the respective sugars and uronic acids were mainly visible, but also minor side products were observed due to different methylation degrees (marked with asterisk in **Figure 1;** standard mixtures were composed with regard to minimal side product interference) [8]. In *O. basilicum* seed extracts, a heteropolysaccharide that was mainly composed of Gal, Ara, Xyl, Rha, GalA, GlcA, and Fru (listed according to ascending hR_F) was evident, if compared to standard mixtures applied aside (Figure 1, **Table 2**).

3.3 Quantitation of the Monomeric Units by HPTLC

For quantitation, standard mixture 3 was freshly prepared containing only the relevant monomeric units found in the present seeds. The new standard mixture 3, a kind of facsimile of the seed extract, was thoroughly compared with the seed extract fingerprint with regard to a broadened band shape or change in color hue to recognize a potential coelution with any further existing monomeric unit. For the given *O. basilicum* seed extracts, the match of the band shapes and color hues was excellent and the separation was found to be reliable. Standard mixture 3 was applied in four different volumes (2, 5, 10, and

Table 2

Performance of the calibration of the monomeric units (for method validation).

Derivative of	Color	hR_F value (mix 3)	Absorbance wavelength (nm)	Calibration	*r*	sdv%
Galactose (Gal)	Grey	9	630	Linear	0.9927	6.9
Arabinose (Ara)	Blue	18	630	Linear	0.9988	3.1
Xylose (Xyl)	Blue	25	630	Linear	0.9997	1.8
Rhamnose (Rha)	Grey	40	370	Polynomial	0.9978	4.1
Galacturonic acid (GalA)	Green	51	630	Linear	0.9741	19.3
Glucuronic acid (GlcA)	Grey	58	630	Linear	0.9834	15.1
Fructose (Fru)	Blue	80	630	Linear	0.9960	6.3

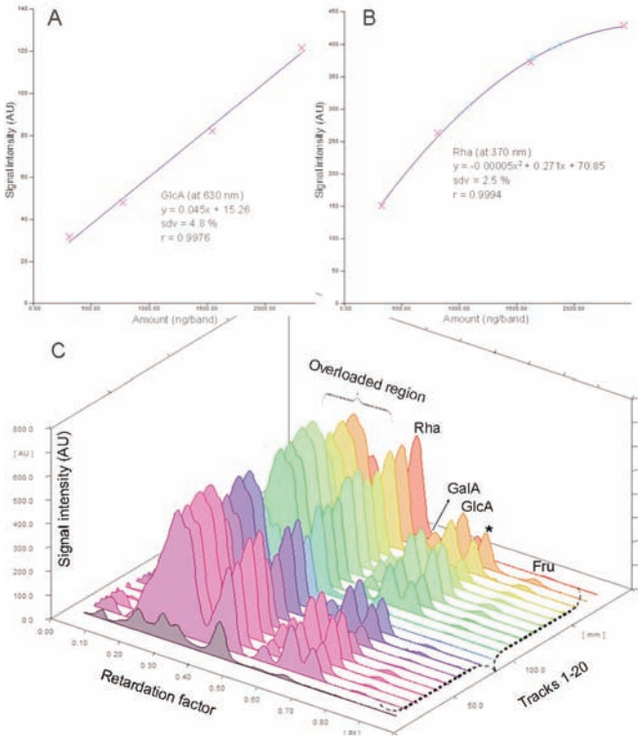

Figure 2

Linear and polynomial calibrations of the methylated/methoxylated and derivatized GlcA (A, at 630 nm) and Rha (B, at 370 nm), respectively (0.3 to 2.1 µg band^{-1}; 2, 5, 10 and 15 µL applied); 3D densitogram (C, at 370 nm) for quantitation of monomeric units > hR_F 40 in Figure 3A.

15 µL). A potential coelution with a minor side product of the methanolysis of monomeric units within the mixture does not impact quantitation because it applies for both, standard and sample zones. Consequently, the quantitative error was considered as negligible if such a coelution with a minor side product occurred.

Absorbance measurement was performed at 630 nm except for Rha, which was recorded at 370 nm due to slightly improved resolution from neighboring peaks (Table 2). For Rha, the regression analysis was polynomial, whereas for the others, a linear calibration was used **(Figure 2A).** Normally, peak height was used for evaluation, except for Fru. The extracts were applied in different volumes to assure the quantitation of the main monomeric units and also of minor monomeric units **(Figure 2B).** Higher extract volumes were applied for evaluation of the monomeric units in the upper hR_F range (>hR_F 40 in **Figure 3A**) in *O. basilicum* seed extracts.

In the WSPS-C extract of *O. basilicum* seeds, the predominant monomeric units were 19.7% for Xyl, 16.7% for GlcA, and 10.0% for Fru. In the WSPS-H extract, 16.7% Xyl, 11.7% GlcA, and 8.7% Fru were obtained. Xyl and GlcA were also major monomeric units in the acidic fractions WSPS-H-A and HC-A (**Table 3,** Figure 3A).

Through the anion exchange chromatography, the GlcA content increased from 11.7% to 17.5% and Xyl from 16.7% to 19.8% in the WSPS-H-A fraction. The GlcA increased from 1.4% to 13.1% and Xyl from 2.5% to 16.5% in the HC-A fraction (Table 3, **Figure 4**). To conclude, after anion exchange chromatography, not only the GlcA and Xyl content increased but also the purity of the extracts (18% versus 51% in the unfractionated fraction).

3.4 Characterization of an Unknown Monomeric Unit by HPTLC–MS

Above the GlcA derivative, an unknown zone at hR_F 67 was visible in the extracts (marked * in Figures 1, 2B, and 3). The zone had the same color hue and color intensity as well as peak performance as the GlcA below. Hence, for further characterization of the unknown monomeric unit, mass spectra were recorded, which showed a pronounced mass signal at *m*/*z* 259 **(Figure 3B).** After methanolysis, methyl glycoside methyl esters were derived from the uronic acids and the mass signal at *m*/*z* 259 could be assigned to a 3-fold methylated hexuronic acid like mannuronic (ManA), guluronic (GulA), or IdoA. For comparison, mass spectra were also recorded from the GlcA zone below. GlcA also showed the base peak at *m*/*z* 259 for the 3-fold methylated uronic acid. Thus, the first assumption that the unknown zone could be an uronic acid monomer (based on the chromatographic and ultraviolet–visible [UV–vis] spectral data before and after derivatization) was confirmed by the mass signal, which indicated a 3-fold methylated hexuronic acid.

As standard compounds of ManA and GulA were not available, the monomeric units of *O. basilicum* seeds were compared with those of alginic acid, which mainly consist of ManA and GulA. The unknown zone in *O. basilicum* seeds did not match to any zone of the alginate's monomeric units (**Figure 5A,** track 2 versus 3), and thus, ManA and GulA could be excluded. IdoA,

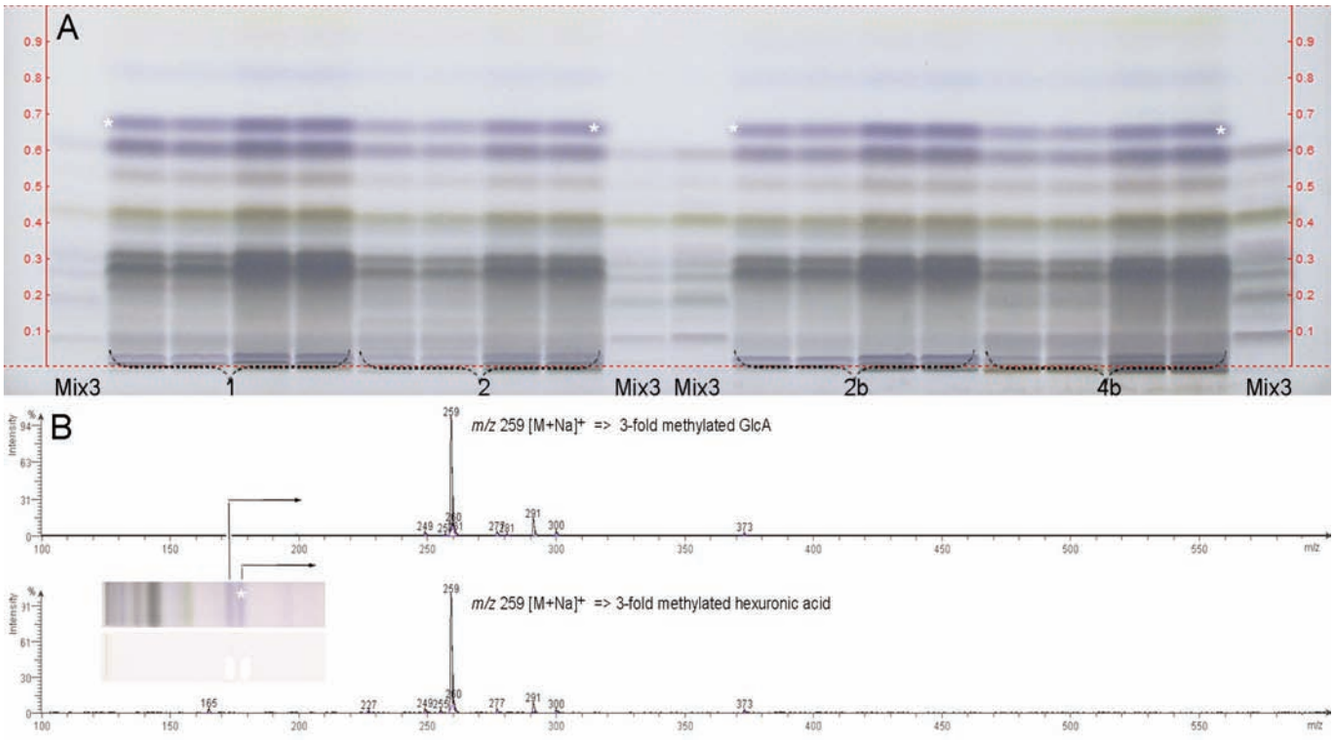

Figure 3

Chromatogram of the monomeric unit contents of the different polysaccharide extracts from *Ocimum basilicum* seeds (A, mix 1: 2, 5, 10, and 15 µL [0.3 to 2.1 µg band^{-1}]; sample extracts: 4 and 8 µL twice each) and mass spectra of GlcA and the upper unknown zone at hR_F 67 (B).

which is an epimer of GlcA, might explain the antiviral effect observed by the traditional use of *O. basilicum* seeds in TCM. IdoA was available as standard compound and investigated; however, it did also not match to the unknown zone. Instead, IdoA showed a similar hR_F value as Fru. For confirmation of the correct assignment of Fru, an overlayed application of the *O. basilicum* seed extract with IdoA (**Figure 5B,** track 1 *versus* track 2, which was applied overloaded to visualize the upper zone) was performed, and a differentiation between both zones was evident in the marked overlay region of interest. Hence, Fru was correctly assigned in *O. basilicum* seed extract. Further, hexuronic acids like D-altruronic acid and L-alluronic acid were not available as standard compounds.

3.5 Bioactivity against *A. fischeri* Bacteria

Previous studies with *Ocimum sanctum* leaves [22, 23] indicated that some of the biological properties of *O. basilicum* seeds may be caused due to the presence of low-molecular weight compounds, such as lignans and sesquiterpene lactones. As beneficial therapeutic effects of the seeds of *O. basilicum* extracts were reported, the *A. fischeri* bioassay was chosen as a first test, indicating potential low-molecular weight bioactive compounds in general. For bioluminescence detection with *A. fischeri* bacteria, ten images were taken in time intervals of 3 min to monitor time-dependent changes. Over the whole period investigated (30 min), any bioactive compounds in the native *O. basilicum* seed extracts (without methanolysis) were not visible in the chromatogram. Also, when the chromatogram was inspected under UV 254 nm, UV 366 nm, and in the visible range, any UV-active, natively fluorescent, or visible compounds were not detected. This proved, at the given sample preparation, the efficient isolation of the pure polymers from *O. basilicum* seeds. Thus, the polysaccharides of *O. basilicum* seed might have potent antidiabetic properties, and effects observed by the TCM use of *O. basilicum* seed extracts might be attributed to the

Table 3

Monomeric unit composition (%) of the polysaccharide fractions obtained from *Ocimum basilicum* seeds (assignments in sections 2.2 and 2.3).

No.	Fraction	Percentage of monosaccharide units in the seed extracts (%)						
		GlcA	GalA	Rha	Xyl	Ara	Gal	Fruc
1	WSPS-C	16.7	4.2	4.9	19.7	8.0	4.4	10.0
2	WSPS-H	11.7	2.0	3.1	16.7	5.1	4.8	8.7
3	PS	1.1	0.3	0.5	6.5	1.5	1.2	1.8
4	HC	1.4	0.2	0.9	2.5	2.5	4.2	0.8
	SUM	30.9	6.8	9.4	45.4	17.1	14.6	21.3
2b	WSPS-H-A	17.5	3.0	5.1	19.8	7.7	4.7	11.9
4b	HC-A	13.1	3.0	5.0	16.5	6.2	9.5	10.6

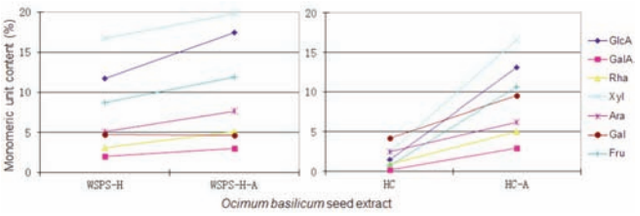

Figure 4

Comparison of the monomeric unit contents of the different polysaccharide extracts from *Ocimum basilicum* seeds before and after ion-exchange chromatography.

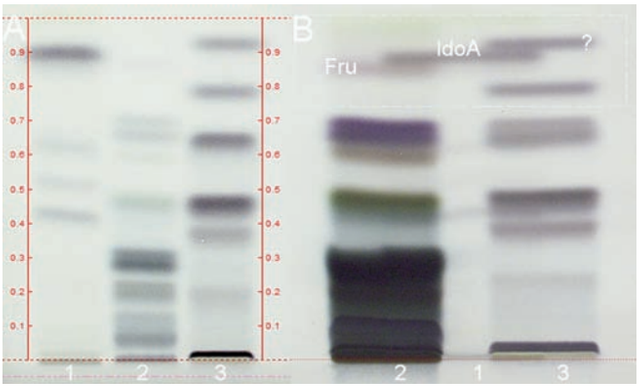

Figure 5

Comparison side by side (A) and overlayed application (B; marked region of interest) of IdoA on track 1 (A: 8 µL [6 µg/8 mm band]; B: 18 µL [14 µg/24 mm band]), alginic acid on track 3 (A: 8 µL [80 µg/8 mm band]; B: 20 µL [200 µg/16 mm band]), and *Ocimum basilicum* seed extracts on track 2 (A: 0.5 µL [5 µg/8 mm band]; B: 12 µL [120 µg/16 mm band] overloaded to visualize the zone assigned as Fru).

biopolymers because small molecules visible under UV/Vis/FLD or *A. fischeri* bioactive molecules were not evident.

3.6 Inhibitory Effects against PTP1B

The antidiabetic activity of the present plant family is reported among others in a recent review [24]. Studies, in which the ethanolic leaf extract of *O. sanctum* was administered to streptozotocin-diabetic rats and which then monitored the levels of plasma glucose, renal, skeletal muscle, and hepatic glycogen [25], were not able to indicate potential single active compounds. It is still unclear, which single compounds cause this antidiabetic effect. Consequently, studying the antidiabetic activity of the seeds' biopolymer fractions was of great interest, as the seeds were also described to have such antidiabetic effects [4–7]. The cytosolic nonreceptor PTPase PTP1B acts as negative regulator of insulin signal transduction, and it might be used as molecular level legitimate therapeutic target in the effective management of type 2 diabetes. Different concentrations of WSPS-C, WSPS-H, PS, and HC were assayed for inhibitory effects against PTP1B *in vitro* [20]. All four extracts were identified as PTP1B inhibitors with half maximal inhibitory concentration values IC_{50} of 8.2, 2.2, 70.9, and 0.8 µg mL^{-1} for WSPS-C, WSPS-H, PS, and HC, respectively (Table 1).

GlcA/Xyl were the dominant monomeric units in the extracts WSPS-C and WSPS-H that represented 54/44% and 38/37% of the sum, respectively (Table 3). Their PTP1B inhibitor activities (IC_{50} 8.2 and 2.2 µg mL^{-1}, respectively; Table 1) were weaker than the HC extract (0.8 µg mL^{-1}), in which Ara and Gal were dominant monosaccharides. The HC extract expressed the strongest inhibitor activity against PTP1B. This indicated that the different polysaccharide structures conferred distinct biological properties between the extracts.

4 Conclusion

Although seeds of *O. basilicum* have widely been used in traditional remedies, little is known regarding the active components responsible for its therapeutic properties. Our results showed that *O. basilicum* seeds contain high amounts of polysaccharides with a total of 17.5%, of which especially the acidic PS extract accounts for 7.7%. In addition, all four crude polysaccharide extracts showed an inhibition of PTPIB *in vitro*. Thus, for the first time, a molecular basis was provided to explain the hypoglycemic effect of the seeds of *O. basilicum* that has been used as an antidiabetic adjuvant in traditional Chinese medicine.

For characterization of the monomeric units and assignment of carbohydrate-based biopolymers, HPTLC was more effective in terms of sample throughput, robustness, pattern recognition, costs, and analysis time if compared to other methods. *In situ* derivatizations are a strong feature of planar chromatography because all chromatographic runs are selectively and simultaneously derivatized and evaluated through the image. At the same time, HPTLC does not require a complex sample preparation. All these features of HPTLC contribute to its well-suited use for quality control as well as to standardization of original medicinal plants by its visually appealing fingerprint.

Acknowledgments

Abulimiti Yili was on leave from Xinjiang Technical Institute of Physics and Chemistry, CAS, Urumqi, China to join the JLU, Giessen, Germany. The study was funded by the Chinese Academy of Sciences for Senior Visiting Scholarship [2012]3217, and "Youth Science and Technology Innovation Talents Project of 2013" of Xinjiang Uyghur Autonomy region of China. Thanks are owed to Stephanie Krüger for instrumental support and recording of mass spectra.

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Ms received: August 9, 2013
Accepted: November 25, 2013