DOI 10.1007/s10600-015-1218-7
Chemistry of Natural Compounds, Vol. 51, No. 1, January, 2015

BRIEF COMMUNICATIONS

HPTLC STUDY OF THE MONOSACCHARIDE COMPOSITION OF A POLYSACCHARIDE FROM *Apocynum venetum* LEAVES

L.-J. Shi,[1,2] H. Yimamu,[2] A. Kawuli,[1] Saideaihemati,[1] H. Q. Zhao,[1] A. Yili,[1*] G. Morlock,[3] and H. A. Aisa[1*]

High-performance thin-layer chromatography (HPTLC) is used for rapid analysis of biopolymers [1], has a high throughput, and is economically efficient [2–4]. Currently, monosaccharides are often determined by HPLC although a single analysis including sample preparation and chromatography is lengthy, as a rule, 30 min and more. Moreover, in our opinion, HPTLC is a promising method for determining monosaccharides because a series of samples can be analyzed simultaneously under identical conditions. The method does not require complicated sample preparation and can be used for qualitative and quantitative analysis of the monosaccharide composition of polysaccharides from various medicinal plants. Therefore, the goals of the present communication were to separate water-soluble polysaccharides (WSPS) from *Apocynum venetum* leaves and to study their monosaccharide composition using HPTLC.

The perennial herbaceous plant *Apocynum venetum* L. (Apocynaceae) is broadly distributed in northwestern China. Its leaves are used in China as a tea for treating neurasthenia, heart palpitation, insomnia, and hypertonia [5–7]. Also, the literature teaches that extracts of *A. venetum* leaves have antidepressant and antioxidant properties and exhibit a calming effect [8–10]. With respect to chemistry, it was found that leaves of this plant contain flavonoids, glycosides, proteins, and tanning agents. However, the polysaccharides have not been studied.

Raw material was collected in August 2012 in the Altai of Xinjing–Uyghur Autonomous Region, China.

The carbohydrate complex from *A. venetum* leaves was isolated as before [11]. For this, dry leaves (0.5 kg) were inactivated for 1 h in refluxing $CHCl_3$–MeOH (1:1). Then, polysaccharides were extracted by hot water (90°C, 3 × 1:6 ratio) for 6 h. The extract was centrifuged, evaporated, and precipitated by EtOH (1:4). The resulting precipitate was separated by centrifugation; rinsed successively with alcohol, Me_2CO, and Et_2O; and dried in a vacuum cabinet. The yield of crude polysaccharides was 13.9% of the air-dried raw material.

Polysaccharides were purified of proteins by dissolving in distilled H_2O and treating several times (9 × 200 mL) with $CHCl_3$–BuOH (4:1) as previously reported [12] until a Bradford test was negative and absorption at 280 and 260 nm was minimal. The yield of purified WSPS was 6.2%.

WSPS were an amorphous reddish-brown powder that was readily soluble in H_2O and gave a negative reaction with I_2.

The monosaccharide composition was established by methanolysis (1 mL of 1 M HCl in MeOH) of WSPS (100 mg) at 100°C for 4 h and addition of Py (50 μL) followed by qualitative determination of the monosaccharide composition by HPTLC on Silica gel 60 chromatography plates (20 × 10 cm, Merck). The solution (2–15 μL) was placed on the chromatography plate. The bands were 9 mm wide with a distance of 8 mm between them.

The eluent was the solvent system *i*-PrOAc–EtOAc–MeOH–H_2O (5:4:1:0.1).

The plates were developed by a mixture of aniline, diphenylamine, and orthophosphoric acid [H_3PO_4 solution (20%) treated with a mixture of Me_2CO solutions (2%) of diphenylamine and aniline (1:1)] using a TLC Immersion Device (immersion time 1 s, speed 3.5 cm/s) and heated at 110°C for 5 min. Monosaccharides were identified by comparing them with standard galactose, arabinose, xylose, rhamnose, galacturonic acid, glucuronic acid, and fructose.

Chromatography plates were scanned at 370 nm on a CAMAG TLC Scanner 3 instrument using the WinCATS program.

1) Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, 830011, Urumqi, Xinjiang, China, e-mail: haji@ms.xjb.ac.cn; 2) Pharmacy School, Xinjiang Medical University, 830054, Urumqi, Xinjiang, China; 3) Institute of Food Chemistry, Justus Liebig-University Giessen, Heinrich-Buff-Ring 26, D-35392 Giessen, Hessen, Germany.
Translated from *Khimiya Prirodnykh Soedinenii*, No. 1, January–February, 2015, pp. 114–115. Original article submitted October 2, 2013.

 0009-3130/15/5101-0130

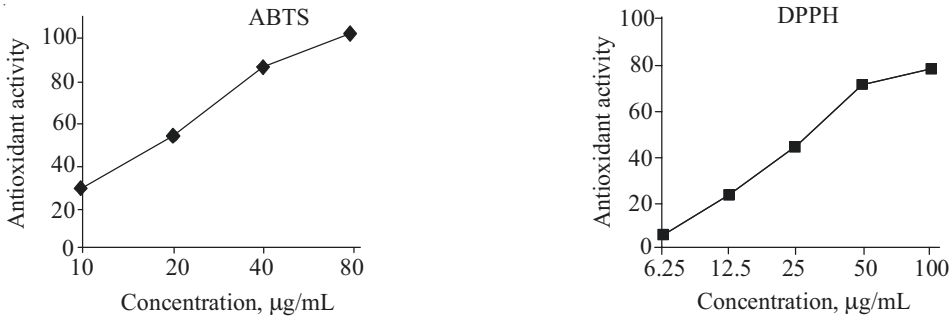

Fig. 1. Antioxidant activity of WSPS from *A. venetum*.

The analytical results showed that the polysaccharide obtained from *A. venetum* leaves was a heteropolysaccharide at the monosaccharide level. The mobile phase used for HPTLC provided good separation of galacturonic acid (R_f 0.58), rhamnose (0.48), xylose (0.29), arabinose (0.23), and galactose (0.11). Arabinose and galacturonic acid were the dominant monosaccharides in the WSPS. The composition was arabinose (2.92%), galacturonic acid (2.23), galactose (1.39), and rhamnose (1.65). Xylose was present in insignificant amounts.

Antioxidant properties of the WSPS were determined by the ABTS and DPPH methods as reported before [13]. The total activity was expressed as percent scavenging of DPPH or ABTS radicals and was calculated using the formula

$$\text{DPPH or ABTS inhibition (\%)} = [(\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sam}})/(\text{Abs}_{\text{blank}})] \times 100,$$

where $\text{Abs}_{\text{blank}}$ is the absorption of ABTS or DPPH radical + EtOH and Abs_{sam}, absorption of ABTS or DPPH radical + sample.

Figure 1 shows that the antioxidant activity increased with increasing concentration of polysaccharides. The half-maximum of the inhibitory concentration (IC_{50}) of the polysaccharides from *A. venetum* was 16.33 ± 0.13 μg/mL and 33.07 ± 0.99 μg/mL according to the ABTS and DPPH methods, respectively, which was 2.961 ± 0.380 and 5.34 ± 0.42 less than the values for vitamin C. These results showed that the polysaccharide from *A. venetum* had antioxidant properties and is being further investigated.

ACKNOWLEDGMENT

The work was sponsored by the programs Research Center for Development of Drugs of Central Asia (Chinese Academy of Sciences) and Development Program for Young Inventive Scientists of Xinjiang-Uyghur Autonomous Region (Grant No. 2013721044).

REFERENCES

1. G. E. Morlock and F. Gamlich, *J. Planar Chromatogr.–Mod. TLC*, **25**, 244 (2012).
2. G. E. Morlock and G. Sabir, *J. Liq. Chromatogr. Relat. Technol.*, **34**, 902 (2011).
3. A. Yili, H. Yimamu, S. Ghulameden, Z. H. Qing, H. A. Aisa, and G. E. Morlock, *J. Planar Chromatogr.–Mod. TLC*, **27**, 11 (2014).
4. G. Morlock and W. Schwack, *LCGC Eur.*, **21**, 366 (2008).
5. *Pharmacopoeia of People's Republic of China*, National Pharmacopoeia Committee, Chemical Industry Press, 2010.
6. Q. B. Xiong, W. Z. Fan, Y. Tezuka, I. K. Adnyana, P. Stampoulis, M. Hattori, T. Namba, and S. Kadota, *Planta Med.*, **66**, 127 (2000).
7. T. Kagawa, Y. Nakazawa, E. Tagashira, Y. Takahashi, S. Onizuka, and S. Nishibe, *J. Nat. Med.*, **58**, 299 (2004).
8. V. Butterweck, S. Nishibe, T. Sasaki, and M. Uchida, *Biol. Pharm. Bull.*, **24**, 848 (2001).
9. T. Yokozawa, Y. Kashiwada, M. Hattori, and H. Y. Chung, *Biol. Pharm. Bull.*, **25**, 748 (2002).
10. O. Grundmann, J.-I. Nakajima, S. Seo, and V. Utterweck, *J. Ethnopharmacol.*, **110**, 406 (2007).
11. H. A. Aisa, M. Abdurahim, A. Yili, L. Xui, R. K. Rakhmanberdyeva, and B. T. Sagdullaev, *Chem. Nat. Compd.*, **42**, 347 (2006).
12. A. M. Staub, *Methods Carbohydr. Chem.*, **5**, 5 (1965).
13. Y. Zhao, J. Dou, T. Wu, and H. A. Aisa, *Molecules*, **18**, 958 (2013).