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Quantification of sucrose in the root of *Geum iranicum* Khatamsaz

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Abstract

Geum iranicum Khatamsaz (Rosaceae) is an endemic plant in Iran. The infusion and decoction of the plant have been used by local people for medicinal purposes. Our previous work on phytochemical studies on *G. iranicum* showed that the root was rich in sugars and sucrose was identified as a major one in it. In this study, the content of sucrose in the hydro-alcoholic (1:1) extract of the root of *G. iranicum* was analyzed using HPLC. The amount of sucrose has been evaluated as 31.75% in the extract and 8.16% in the dried root. As a result, the presence of high amount of sucrose in the root of *G. iranicum* can be applicable for preparation of any pharmaceutical formulations of this plant.

Keywords: *Geum iranicum*, root, sucrose, quantification, HPLC

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Introduction

Geum iranicum is an endemic rhizomatous herb in Iran that belongs to the Rosaceae family (1, 2). In Iranian folk remedy, infusion and decoction of the root of this plant are employed for gastrointestinal disorders like diarrhea and by mixing with flour as a poultice for frost bite (3). Few biological and phytochemical studies have been carried out on *Geum* species in Iran. Our previous work on phytochemical analysis of *G. iranicum* using column chromatography showed that the root was rich in sugars and sucrose was identified as a major sugar in this plant (4). Sugars as primary messengers in signal transduction regulate many important processes along the whole life cycle of the plant (5). Sucrose is the most abundant disaccharide and the major transported sugar in plants (6). Based on previous studies, the role of sucrose as a signaling molecule in plants has been accepted. Sucrose plays an important role in the regulation of some metabolic processes which its role cannot be replaced by other sugars like glucose and also takes part in a wide range of developmental processes (7). In a research, sugars especially sucrose induced the accumulation of anthocyanins and glucosinolates in broccoli sprouts. The genes involved in the biosynthesis and transcriptional regulation of these compounds were up-regulated after the sucrose treatment (8).

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The sucrose content of soybean seeds has an important role to improve the flavor and aroma of soy-based products. For this reason, Teixeira *et al* developed the simple spectrophotometric method to quantify sucrose in soybean grains (9). Several studies reported the pain reducing effect of oral sucrose in neonates (10). Since the infusion and decoction of *G. iranicum* have been used by local people, this work concentrates to quantification of sucrose as one of the major carbohydrates in this plant using HPLC method for the first time.

Materials and methods

Plant material

G. iranicum was collected from Gloul-Sarani protected area 75km north of Shirvan, Khorasan-e- Shomali province, Iran, at an altitude 2460m. The voucher specimen (6714 THE) was deposited at the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Chemicals

Purified sucrose from the root of this plant in our previous work (4) was used as standard material. All solvents were obtained from Merck (Germany).

Instrumentation

HPLC analysis was performed on a Knauer Wellchrom system connected to a photodiode array detector (smart line system, Germany) with the detection wavelength set at 246 nm. The samples were applied to an RP-C18 silica gel column eluted with deionized water and methanol.

Preparation of standard solutions

Stock solution (20 mg/ml) of sucrose was prepared in H_2O. Different concentrations (4, 2, 1, 0.5 mg/ml) were made from stock solution to plot the calibration curve of sucrose.

Sample preparation

Dried roots of *G. iranicum* (50 g) were cut into small pieces and extracted by soxhlet extracter with methanol-water (1:1) for 4 h. 20 mg of dried extract added to volumetric flask and diluted to 5 ml with methanol-water (2.5:2.5) (4 mg/ml). The sample was injected (20 µl) to HPLC with a gradient program that is shown in Table 1.

Table1 HPLC elution condition of hydro-alcoholic extract of *G. iranicum*

Time (min)	Flow (ml/min)	Water %	Methanol %
0	1	80	20
5	1	80	20
35	1	0	100
40	1	0	100
41	1	80	20
45	1	80	20

Results

In this study, the purified sucrose (O-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside, Fig.1) from the root of *G. iranicum* was used as standard sucrose. The NMR data were as follows: ^{1}H-NMR (D_2O, 500 MHz): δ_H= 3.31 (1 H, t, J= 9.5 Hz, H_{Glc}-4), 3.4 (1 H, dd, J= 10, 3.8 Hz, H_{Glc}-2), 3.51 (2 H, s, H_{Fru}-1), 3.59 (1 H, t, J= 9.5 Hz, H_{Glc}-3), 3.66 (4H, m, H_{Glc}-6 and H_{Fru}-6), 3.69 (1 H, m, H_{Glc}-5), 3.72 (1H, m, H_{Fru}-5), 3.89 (1 H, t, J= 8.5 Hz, H_{Fru}-4), 4.04 (1 H, d, J= 8.7 Hz, H_{Fru}-3), 5.25

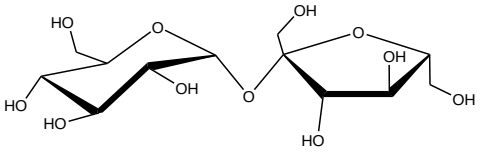

Figure 1 The structure of sucrose

(1H, d, J= 3.8 Hz, H_{Glc}-1).[13]C-NMR (D_2O, 500 MHz): δ_C= 60.1 (C_{Glc}-6), 61.3 (C_{Fru}-1), 62.3 (C_{Fru}-6), 69.2 (C_{Glc}-4), 71.0 (C_{Glc}-2), 72.4 (C_{Glc}-5), 72.5 (C_{Glc}-3), 73.9 (C_{Fru}-4), 76.3 (C_{Fru}-3), 81.3 (C_{Fru}-5), 92.1 (C_{Glc}-1), 103.6 (C_{Fru}-2) (4). The content of sucrose in the hydro-alcoholic (1:1) extract of the root of *G. iranicum* was analyzed using HPLC. Four dilutions of the standard, purified sucrose from the same plant were used for plotting a calibration curve (Fig.2). In this study, a gradient elution of methanol-water was used to achieve complete separation of sucrose in the extract. Purity of sucrose peak in HPLC chromatograms was confirmed with photodiode array detector. Sucrose was identified in the chromatogram of the extract by comparing the UV spectra with those of the standard (Fig.3).

The amount of sucrose has been evaluated as 31.75% in the extract and 8.16% in the dried root.

Discussion

Sorbitol and sucrose are the two main translocatable carbon forms in the Rosaceae family (11). Sorbitol normally occurs in all subfamilies of the Rosaceae, except rosoideae (12). Since the genus *Geum* belongs to rosoideae (13), our results confirmed the presence of high levels of sucrose in *G. iranicum.* In folk medicine the root of *Geum* is employed as a tonic (14,15). It seems that the significant amount of sucrose in the root is one of the important factors that cause this effect. Sucrose is a pharmaceutical excipient which widely used in oral formulations. It is used as a binding, bulking, suspending and coating agents and also sweetener (16). Furthermore, Sucrose is considered for its bacteriostatic, bactericidal and wound healing properties (17,18). Sugar cane and sugar beet plants contain 15-20% and 10-17% sucrose respectively (16). Based on our findings, the amount of sucrose in the root of *G. iranicum* was 8.16% in the dried root which

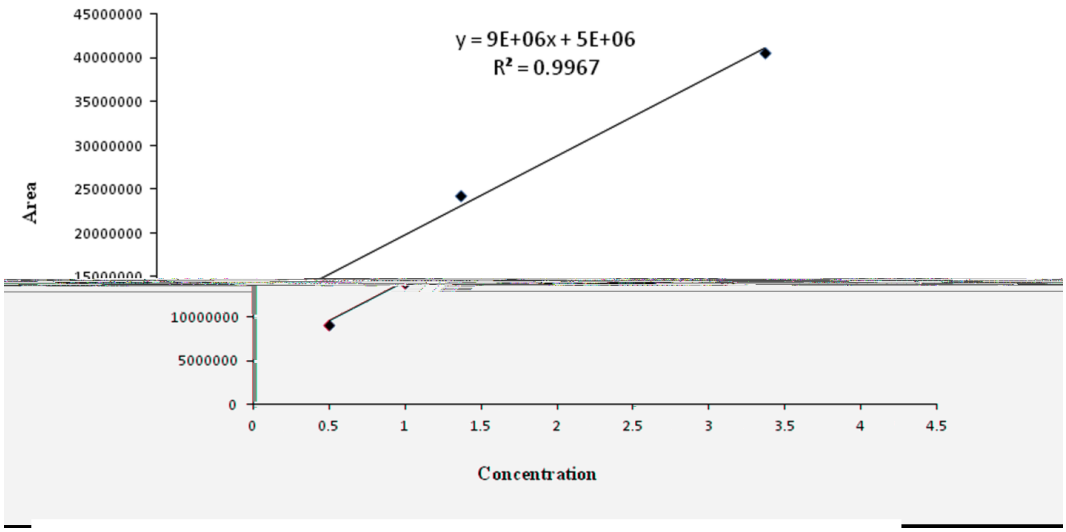

Figure 2 Calibration curve for standard sucrose (concentration vs. peak area)

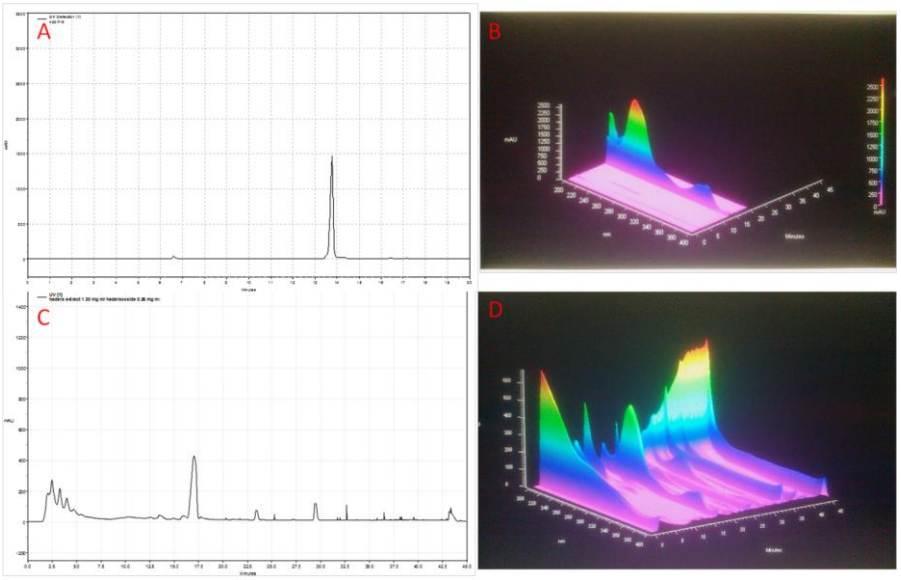

Figure 3 HPLC profiles of sucrose and the hydroalcoholic extract of *G. iranicum* A: HPLC chromatogram of standard sucrose, B: UV spectrum of sucrose, C: HPLC chromatogram of the extract, D: UV spectrum of the extract

was considerable. Some *Geum* species like *G. rivale*, *G. urbanum* and *G. japonicum* have long been used in folk medicine as a diuretic and astringent (19,20). Recently, much attention has been paid to the *Geum* genus for their multifaceted activities (21).

Previous studies on *Geum* species have shown various biological effects such as antioxidant, antimicrobial and anti-inflammatory activities (15,22,23). Literature review revealed several studies on *G. japonicum* which is native in East Asia. Anti viral activity against human immunodeficiency virus (HIV), herpes simplex virus (HSV) and cytomegalo virus (CMV) and also anticoagulant activity have been reported from *G. japonicum*. Furthermore, this plant showed potent effect on angiogenesis and cardiomyogenesis in acute myocardial infarction in an animal model (24-28). There are five species of the genus *Geum* in Iran (1). Few studies have been conducted on this genus in our country. In a research the extract and essential oil of *G. kokanicum* showed antimicrobial activity and in another study a potent inhibitory effect on matrix metalloproteinase activity was reported from this plant (29,30). In our previous works we reported the presence of terpenoid, steroid, phenylpropanoid, tannin and sugar in the extract and high amount of eugenol in the essential oil of the roots of *G. iranicum* (4,31). In addition, the aqueous fraction obtained from the root extract of *G. iranicum* showed a potent antibacterial activity against resistant clinical isolates of *Helicobacter pylori* (4). Osato *et al* reported that the osmotic effect of honey's sugar content was the most important factor for observed anti *H. pylori* activity (32). For this reason, it seems that the high amount of sugar in the root of *G. iranicum* might be one of the important compounds responsible for the antibacterial activity.

Conclusion

As mentioned above, sucrose is a pharmaceutical excipient and antibacterial, wound healing and pain reducing effects have been reported from this compound. Also it can improve the flavor and aroma of any products. As a result, the presence of sucrose in high levels in the root of *G. iranicum* can be applicable for preparation of any pharmaceutical formulations of this plant.

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Conflict of interest

The authors declared no potential conflict of interest with respect to the authorship, and/or publication of this study.

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