Stevia residue extract increases intestinal uric acid excretion via interacting with intestinal urate transporters in hyperuricemic mice

Arshad Mehmood[1 2], Lei Zhao[1 2 *], Chengtao Wang[1 2 *], Imam Hossen[1 2], Rifat Nowshin Raka[12], Huimin Zhang[1 2]

[1] Beijing Advance Innovation Center for Food Nutrition and Human Health, Beijing Technology and Business University, Beijing 100048, China

[2] Beijing Engineering and Technology Research Center of Food Additives, School of Food and Chemical Technology, Beijing Technology and Business University, Beijing 100048, China

Preparation of STVRE

The Stevia leaves powder was extracted twice with 18 volumes of distilled water at 65°C for 1.5 hr and then filtrated. The filtrate was mixed with ferrous sulfate (0.9% w/v, final concentration) and adjusted to pH 9.0–10.0 with calcium hydroxide. After flocculation at 50°C for 30 min, the mixture was filtrated to obtain the Stevia residue. The Stevia residue was then extracted three times with acidified 95% (v/v) acetone (pH 1.5) at 45°C for 1 hr and filtrated. The filtrate was evaporated under reduced pressure to remove acetone, and thereafter purified by extraction with ethyl acetate. The partially purified extract was applied to an HZ841 macroporous resin column, and then eluted it with 2 bed volume (BV) of distilled water, followed by 2 BV of alkaline water (pH 9.5) at a flow rate of 1 BV/hr to remove impurities. Subsequently, the adsorbed components were eluted with 3 BV of 17% (v/v) ethanol, followed by 1.5 BV of 50% (v/v) ethanol. The eluents were concentrated and lyophilized to obtain SRE1 and SRE2, respectively. The resulting STVRE powder was stored at −20°C prior to use (1, 2).

Qualitative and quantitative analysis of the major bioactive compounds in SREs

The major bioactive compounds in SREs were identified by HPLC– ESI–QTOF–MS/MS (6530 HPLC-MS system; Aglient Technologies, Santa Clara, CA, USA) and quantified by HPLC (1290 HPLC system; Aglient Technologies, Santa Clara, CA, USA). The HPLC parameters were as follows: chromatographic column, Agilent Eclipse Plus C18 column (50 mm × 2.1 mm i.d., 1.8 μm; Aglient Technologies, Santa Clara, CA, USA); mobile phase, 0.1% formic acid in water (A) and methanol (B); flow rate, 0.1 mL/min; elution procedure, 0–10 min, 10%–50% B; 10–12 min, 50%–

10% B; 12–15 min, 10% B; detection wavelength, 320 and 360 nm; column temperature, 30°C; volume of sample injection, 1 μL. Authentic compounds (3-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, caffeic acid, quercetin, 3,4-O-dicaffeoylquinic acid, 3,5-O-dicaffeoylquinic acid, 4,5-O-di-caffeoylquinic acid and quercetin-3-O-rhamnoside) were dissolved in methanol; the concentrations were adjusted to 6.25, 12.5, 25, 50, 100, 200 and 400 μg/mL, respectively, and the calibration curves were established under the same determination conditions. Mass spectrometric detection was set as follows: Negative ion mass spectra of the column eluate were recorded in the range of m/z 100–1500 (1, 2).

Supplementary Table 1. Retention times (Rt), MS and MS2 fragmentation ions of the phenolic compounds in Stevia residue extract (STVRE).

Compounds	Rt (min)	Pseudomolecular ion $[M-H]^-$ (m/z)	MS2 (m/z)	Tentative identification	mg/ml
1	3.8	353.0878	191.0571 (100), 179.0357 (72), 135.0451 (9)	3-O-Caffeoylquinic acid	1.68 ± 0.75
2	4.2	353.0878	191.0567 (100), 179.0353 (76), 173.0431 (4), 135.0442 (13)	4-O-Caffeoylquinic acid	3.79 ± 0.41
3	4.4	179.0350	135.0456 (100)	Caffeic acid	8.54 ± 0.82
4	7.9	515.1195	353.0916 (100), 335.0823 (1), 191.0575 (12), 179.0361 (9), 135.0443 (2)	3,4-O-Dicaffeoylquinic acid	232.75 ± 2.17
5	8.0	515.1195	353.0914 (100), 335.0771 (1), 191.0564 (5), 179.036 (14), 173.0467 (24), 135.0440 (2	3,5-O-Dicaffeoylquinic acid	92.43 ± 0.97
6	9.1	515.1195	353.0921 (100), 191.0587 (4), 179.0365 (21), 173.0462 (19), 135.0459 (2)	4,5-O-Dicaffeoylquinic acid	265.94 ± 2.63
7	9.3	447.0933	301.0373 (100)	Quercetin-3-O-rhamnoside	125.97 ± 1.13
8	11.0	301.0354	107.0139 (8), 121.0301 (11), 151.0042 (100), 178.9994 (72)	Quercetin	3.05 ± 0.63
				Total chlorogenic acids	605.13 ± 5.66
				Total flavonoids	129.02 ± 20.61

Adopted from (1 and 2) with permission Wiley publisher

Supplementary Table 2. List of primer sequences uses in this study

Gene Name	forward	reverse
ABCG2	TAAATGGAGCACCTCAACCT	GAGATGCCACGGATAAACTG
GLUT9	GAGATGCTCATTGTGGGACG	GTGCTACTTCGTCCTCGGT
GAPDH	GTGAAGGTCGGTGTGAACGG	GTGATGGCATGGACTGTGGTC

Supplementary Table 3. Effect of STVRE on body weight of fructose-PO-YE induced hyperuricemic mice (n = 8)

Treatments groups	Body Weight (g)
GN	52.23 ± 2.35
GB	49.86 ± 4.56
GM	50.56 ± 1.93
GS1	51.53 ± 3.57
GS2	50.08 ± 2.98

GN: normal group, GM: model group, GB: benzbromarone group (20 mg/kg bw), GS1: stevia residue extract (200 mg/kg bw) and GS2: stevia residue extract (400 mg/kg bw)

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compare with GM, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compare with GN

1. L. Zhao, X. Wang, M. Xu, Y. Lian, C. Wang, H. Yang, A. Mehmood, Dietary intervention with Stevia residue extracts alleviates impaired glucose regulation in mice. J Food Biochem, 2018, 42, e12651.

2. L. Zhao, H. Yang, M. Xu, X. Wang, C. Wang, Y. Lian, *Stevia* residue extract ameliorates oxidative stress in d-galactose-induced aging mice via Akt/Nrf2/HO-1 pathway. J Funct Foods, 2019, 52, 587-595.