

SUPPLEMENTARY MATERIAL

Antioxidant and α -glucosidase inhibitory ingredients identified from Jerusalem artichoke flowers

Yan-Ming Wang^{a,1}, Jian-Qiang Zhao^{b,1}, Jun-Li Yang^a, Pema Tsering Idong^b, Li-Juan Mei^b, Yan-Duo Tao^{b,*} and Yan-Ping Shi^{a,*}

^aCAS Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, PR China; ^bKey Laboratory of Tibetan Medicine Research, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810008, PR China; ¹ These authors contributed equally

Abstract: Jerusalem artichoke (JA, *Helianthus tuberosus* L.) has been researched extensively due to its wide range of uses, but there are limited studies on its flowers. In this study we report the first detailed phytochemical study on JA flowers, which yielded 21 compounds. Compound **4** was identified as a major water soluble yellow pigment of JA flowers. In addition, the methanol extract of JA flowers and the isolates were evaluated for their antioxidant and α -glucosidase inhibitory activities. Among the tested compounds, compound **13** showed the strongest ABTS⁺ free radical scavenging activity with SC₅₀ value of 2.30 ± 0.13 μ g/mL, and compound **6** showed most potent α -glucosidase inhibitory activity with inhibition rate of 60.0% ± 10.3% at a concentration of 250 μ g/mL. Results showed that methanol extract of JA flowers exhibited antioxidant and α -glucosidase inhibitory activities which could be attributed to its phenolic ingredients including chlorogenic acid derivatives, flavonoids and phenols.

Keywords: Jerusalem artichoke flowers, antioxidant activity, α -glucosidase inhibitory effect, phenolic compounds

* Corresponding author. Phone: + 86-931-4968208; fax: + 86-931-8277088, e-mail: soqiang.happy@163.com (Y.-D. Tao); shiyp@licp.cas.cn (Y.-P. Shi).

Experimental

General

Optical rotations were acquired on a Jasco P-1020 digital polarimeter (JASCO Co., Tokyo, Japan). Ultraviolet-visible (UV) spectra were measured on a Shimadzu UV2401A ultraviolet-visible spectrophotometer (Shimadzu Co., Tokyo, Japan). Infrared (IR) spectra were recorded on a Bruker Tensor-27 FI-IR spectrometer (Bruker, Germany) with KBr pellets. Nuclear magnetic resonance (NMR) spectra were measured on a Bruker Avance III-400 spectrometer (Bruker, Rheinstetten, Germany), using tetramethylsilane as an internal standard. Chemical shifts were reported in units of δ (ppm) and coupling constants (J) were expressed in Hz. High resolution mass (HRESIMS) spectra were run on a BrukerApex47e spectrometer by ESI (BrukerDaltonics, MA, USA). Column chromatography (CC) were carried out over silica gel (100–200 mesh, Qingdao Haiyang Chemical Co., Shandong, China) and MCI gel CHP 20P (75–150 μm , Mitsubishi Chemical Co., Tokyo, Japan). Pre-coated silica gel plates (Qingdao Haiyang Chemical Co., Shandong, China) were used for thin-layer chromatography (TLC). Detections were done under UV light (254 nm and 365 nm) and by spraying the plates with 10% sulfuric acid ethanol solution followed by heating. An Agilent series 1200 (Agilent Technologies, Palo Alto, California, USA) was used for HPLC analysis. Prep-HPLC was done on a Hanbon series NP7005C (Hanbon Sci & Tech, Jiangsu, China). XCharge C18 and XAqua C18 columns (10 μm , 100 Å, 250 mm \times 20 mm, Acchrom, Beijing, China) were used for the prep-HPLC.

Materials

The flowers of JA (*H. tuberosus*) were collected locally from Weide farms (Xining City, Qinghai province, China) in August 2015 and verified by Professor Li-juan Mei. The voucher specimen (No.20150811) of *H. tuberosus* has been deposited in the Key Laboratory of Tibetan Medicine Research, Northwest Institute of Plateau Biology, Chinese Academy of Science, China. 2,2'-Azino-di(3-ethyl-benzthiazoline sulphonic acid) (ABTS), 2,2-diphenyl-1-picryl-hydrazil radical (DPPH) and α -glucosidase were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the other chemicals used were of analytical grade. The solvents used for high performance liquid chromatography (HPLC) and preparative HPLC (prep-HPLC) were of HPLC grade.

Extraction and isolation

JA flowers (0.922 kg, dry weight) were extracted exhaustively with 95% methanol (3 \times 4 L) by ultrasonic method. After concentration in vacuo, a dried methanol extract (172 g) was obtained. The methanol extract (172 g) was chromatographed on a MCI gel CHP 20P column

eluting with water, 50% MeOH, and 90% MeOH in order to afford four sub-fractions (Fr1–Fr4) based on TLC analyses. Fr1 was separated by silica gel CC, eluting with a CHCl₃/MeOH/H₂O gradient system (9:1:0.1–6:4:1, v/v/v), to give three sub fractions (Fr11–13). Compound **18** (2 mg) was obtained from Fr11 through prep-HPLC on XAqua C18 column eluting with MeCN/H₂O (10/90, v/v, 15 mL/min). Compound **20** (50 mg) was yielded by prep-HPLC on XAqua C18 column eluting with MeCN/H₂O (3/97, v/v, 15 mL/min) from Fr13. Fr2 was separated by silica gel CC eluting with a CHCl₃/MeOH/H₂O gradient system (9:1:0.1–7:3:0.5, v/v/v) to give five sub fractions (Fr21–25). Fr21 was further purified through prep-HPLC on XCharge C18 column eluting with MeCN/H₂O (10–50%, v/v, over 80 min, 15 mL/min) to get **13** (43 mg). Fr22 was purified by the same method to give compounds **14** (7 mg), **15** (12.7 mg), and sub fraction Fr223. Fr223 was purified by prep-HPLC on XCharge C18 column eluting with MeCN/H₂O (25/75, v/v, 15 mL/min) to obtain **12** (10 mg). Fr23 was separated by prep-HPLC on XCharge C18 column eluting with MeCN/H₂O (7/93, v/v, 15 mL/min) to yield **21** (18 mg). Fr24 was separated by prep-HPLC on XCharge C18 column eluting with MeCN/H₂O (7/93, v/v, 15 mL/min) to yield **4** (108 mg), **5** (760 mg), and sub fractions Fr243 and Fr244. Fr243 was purified through prep-HPLC on XCharge C18 column eluting with 10–20% MeCN/H₂O over 10 min, then eluting with 20% MeCN/H₂O isocratic elution (15 mL/min) to yield compounds **11** (124 mg), **6** (25.9 mg), and **7** (107 mg). Fr244 was chromatographed by prep-HPLC via XCharge C18 column eluting with MeCN/H₂O (13/87, v/v, 15 mL/min) to obtain **9** (37 mg) and **10** (12.5 mg). Fr25 was separated by prep-HPLC via XCharge C18 column eluting with MeCN/H₂O (18/82, v/v, 15 mL/min) to obtain compounds **8** (50 mg) and **2** (32 mg). Fr3 was subjected to silica gel CC eluting with a CHCl₃/MeOH/H₂O gradient system (9:1:0.1–7:3:0.5, v/v/v), to give six sub fractions (Fr31–36). Fr31 was purified through prep-HPLC via XAqua C18 column eluting with MeCN/H₂O (28/72, v/v, 15 mL/min) to get compound **17** (22.5 mg). Compound **19** (4.5 mg) was obtained from Fr32 by prep-HPLC via XAqua C18 column eluting with MeCN/H₂O (30/70, v/v, 15 mL/min). Fr36 was purified by prep-HPLC via XCharge C18 column eluting with MeCN/H₂O (7/93, v/v, 15 mL/min) to obtain compound **4** (900 mg) and two sub fractions Fr362 and Fr363. Compound **3** (68 mg) was yielded from Fr362 through prep-HPLC via XCharge C18 column eluting with MeCN/H₂O (15/85, v/v, 15 mL/min). Compound **1** (813 mg) was obtained by prep-HPLC on XCharge C18 column eluting with MeCN/H₂O (13/87, v/v, 15 mL/min) from Fr363. Fr4 was chromatographed through silica gel CC eluting with a petroleum ether/EtOAc gradient system (9:1–6:4, v/v), then with a CHCl₃/MeOH/H₂O gradient system (9:1:0.1–7:3:0.5, v/v/v) to give compound **16** (96 mg).

Assay of DPPH and ABTS⁺ radical-scavenging

DPPH and ABTS⁺ radical scavenging assay was performed as described in a previous paper

(Zhao et al. 2017). Ascorbic acid was used as positive control. In DPPH radical scavenging assay, DPPH was dissolved in ethanol to a concentration of 250 μM and the mixture was allowed to stand in the dark at room temperature for 12–16 h. The DPPH stock solution was diluted with ethanol to an absorbance of 0.700 (\pm 0.020) at 517 nm before use. The radical scavenging activity was determined by the following equation: DPPH radical scavenging activity (%) = $[A_{\text{blank}} - A_{\text{sample}}]/A_{\text{blank}} \times 100$. A_{sample} is the absorbance of diluted DPPH radical solution mixed with the test compound measured at 517 nm, and A_{blank} is the absorbance of DPPH radical solution mixed with methanol solution measured at 517 nm. The SC_{50} values were obtained through GraphPad Prism 6.02 and defined as the concentration of sample required to scavenge 50% of DPPH radicals. In ABTS^+ radical scavenging assay, ABTS was dissolved in water to a 7 mM concentration, and then potassium persulfate was added to a final concentration of 2.45 mM, allowing the mixture to stand in dark at room temperature for 12–16 h to obtain ABTS radical cation (ABTS^+). The ABTS^+ solution was diluted with ethanol to an absorbance of 0.700 (\pm 0.020) at 734 nm before use. ABTS^+ radical scavenging activity (%) = $[A_{\text{blank}} - A_{\text{sample}}]/A_{\text{blank}} \times 100$, A_{sample} is the absorbance of diluted ABTS^+ solution mixed with the test compound measured at 734 nm and A_{blank} is the absorbance of ABTS^+ radical solution mixed with methanol solution measured at 734 nm. The scavenging rate and SC_{50} value were calculated.

α -Glucosidase assay

The α -glucosidase inhibition assay was adapted as described previously (Zhao et al. 2017). 0.26 U of α -glucosidase from *Saccharomyces cerevisiae* was diluted with 0.1 M phosphate buffer consisting of Na_2HPO_4 and KH_2PO_4 (pH 6.5). The 25 μL test compound and 25 μL α -glucosidase were preincubated in 96-well plates at 37 $^\circ\text{C}$ for 15 min. The reaction was initiated by adding 50 μL of 0.3125 mM pNPG (4-nitrophenyl α -D-glucopyranoside) as substrate. The plate was incubated for another 15 min at 37 $^\circ\text{C}$, followed by adding 50 μL of 0.2 M Na_2CO_3 to stop the reaction. All test compounds were dissolved in DMSO, and then diluted with buffer. The final concentrations of tested compounds were between 0.03 and 250 $\mu\text{g}/\text{mL}$. The reaction was monitored by the change of absorbance at 405 nm using a Dimension RxL Max clinical chemistry system Enspire MP150 (Siemens Healthineers, Germany).

Spectral data of compound 10

Cis-caffeic acid 3-*O*- β -glucopyranoside (**10**). White powder. $[\alpha]_{\text{D}}^{25.7} -44.5$ (c 0.02, MeOH); UV (MeOH) λ_{max} nm (log ϵ): 210 (4.06), 288 (3.91), 302 (3.89); IR (KBr) ν_{max} : 3418, 2923, 1688, 1632, 1604, 1558, 1513, 1436, 1339, 1283, and 1071 cm^{-1} ; HRESIMS m/z : 365.0837 $[\text{M}+\text{Na}]^+$ (calculated for $\text{C}_{15}\text{H}_{18}\text{O}_9\text{Na}$ 365.0843); NMR spectroscopic data: ^1H NMR (400

MHz, CD₃OD) δ_{H} 7.93 (brs, 1H, H-2), 7.15 (d, $J = 7.4$ Hz, 1H, H-6), 6.79 (d, $J = 8.2$ Hz, 1H, H-5), 6.73 (d, $J = 12.8$ Hz, 1H, H-7), 5.79 (d, $J = 12.8$ Hz, 1H, H-8), 4.82 (d, $J = 7.2$ Hz, 1H, H-1'), 3.94 (d, $J = 11.9$ Hz, 1H, H-6a'), 3.76 (d, $J = 11.1$ Hz, 1H, H-6b'), 3.55–3.40 (m, 4H, H-3'–H-5'); ¹³C NMR (101 MHz, CD₃OD) δ_{C} 171.6 (C-9), 149.5 (C-4), 146.1 (C-3), 142.5 (C-7), 128.5 (C-1), 128.2 (C-6), 120.3 (C-2), 118.7 (C-8), 116.5 (C-5), 104.0 (C-1'), 78.1 (C-3'), 77.6 (C-5'), 74.8 (C-2'), 71.2 (C-4'), 62.4 (C-6').

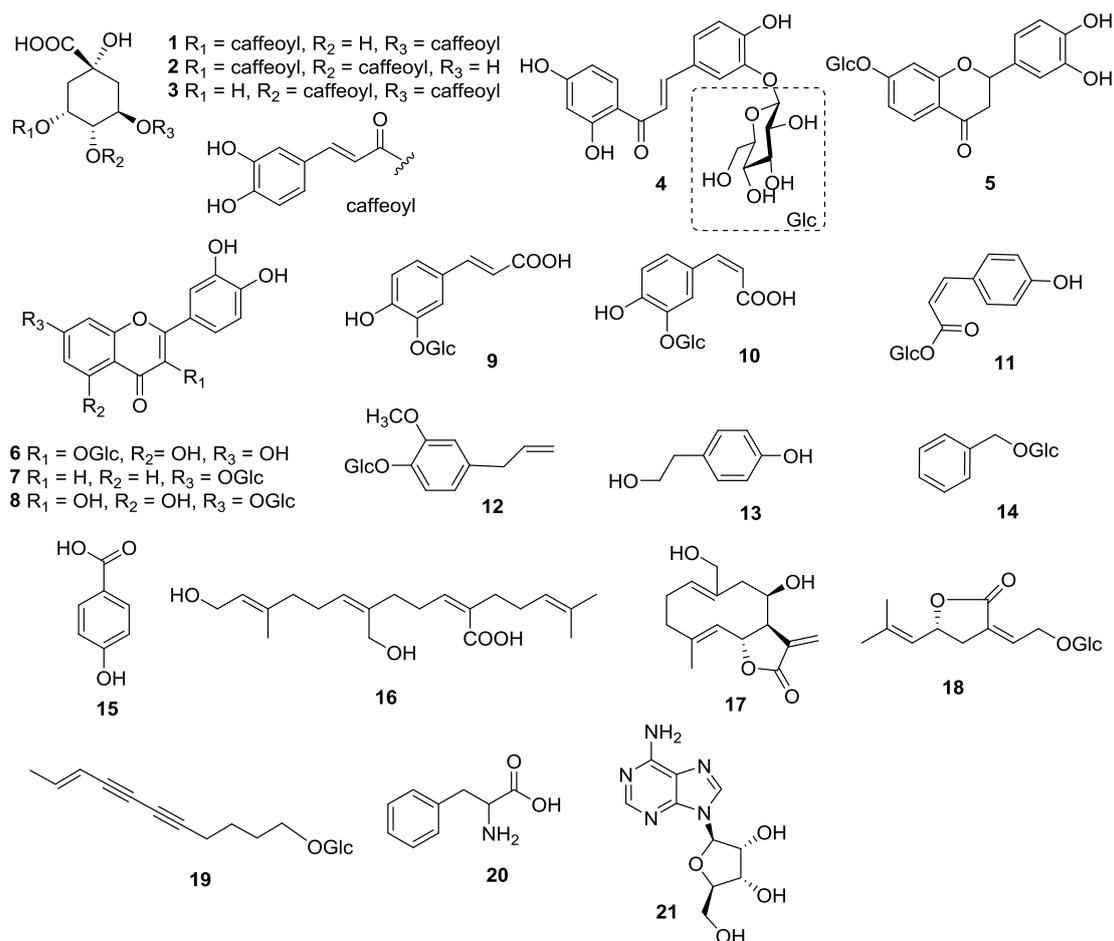


Figure S1. Structures of components 1–21 identified from the flowers of Jerusalem artichoke.

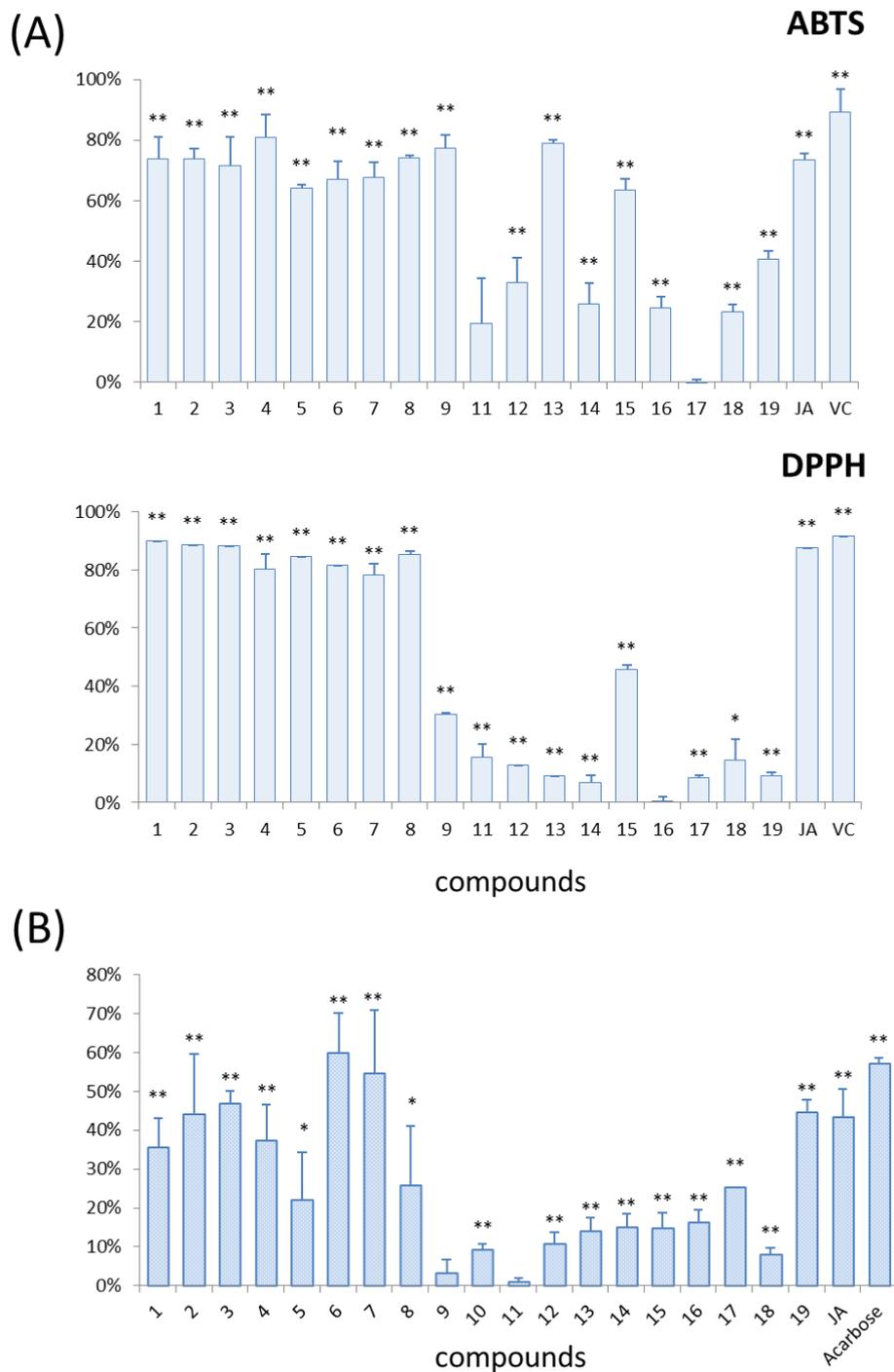


Figure S2. (A) DPPH and ABTS⁺ free radical scavenging rates of compounds **1–9**, **11–19**, and positive control ascorbic acid (VC) at a concentration of 50 $\mu\text{g/mL}$, and JA represented the 95% methanol extract of JA flowers at a concentration of 500 $\mu\text{g/mL}$; (B) Inhibition rates of compounds **1–19**, and positive control acarbose at a concentration of 250 $\mu\text{g/mL}$, and 95% methanol extract of JA flowers (JA) at a concentration of 2.5 mg/mL against the α -glucosidase; Compound **21** showed no α -glucosidase inhibitory activity at a concentration of 250 $\mu\text{g/mL}$; * $p < 0.05$ and ** $p < 0.01$ versus blank control.

Table S1. Radical scavenging activities of the isolated compounds from the flowers of Jerusalem artichoke.

Compounds	SC ₅₀ ^a (µg/mL)	
	ABTS	DPPH
1	5.93 ± 0.84	5.58 ± 0.53
2	6.40 ± 0.29	n.d.
3	5.31 ± 0.34	5.29 ± 0.38
4	3.22 ± 0.37	5.09 ± 0.85
5	8.57 ± 1.57	10.28 ± 0.33
6	11.97 ± 0.38	5.99 ± 0.89
7	7.11 ± 0.29	5.79 ± 0.49
8	15.00 ± 0.16	n.d.
9	18.44 ± 1.72	n.d.
13	2.30 ± 0.13	n.d.
15	23.73 ± 1.47	n.d.
ascorbic acid	5.35 ± 0.36	6.33 ± 0.32

n.d. = not determined.

^a SC₅₀ values are shown as mean ± SD (n = 3).

References

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