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ORIGINAL PAPER

Inhibitory effects of *Rosa gallica* on the digestive enzymes

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Abstract The 50% aqueous ethanol extracts of petals of *Rosa gallica* collected in Xinjiang province, China, exhibited potent inhibitory effects against α-amylase and α-glucosidase. As the active principles, seven hydrolysable tannins were isolated from this species for the first time and elucidated by NMR and ESI-TOF-MS analysis. Quantitative analysis by ultra-performance liquid chromatography demonstrated that the contents of these hydrolysable tannins were 3–5% of the dry weight of the petals, and the hydrolysable tannins must be related to the medicinal utilization of this species.

Keywords *Rosa gallica* · α-Amylase · α-Glucosidase · Inhibitor · Hydrolysable tannin · Polyphenol

Introduction

In Xinjiang province, the westernmost part of China, the Uygur people have a custom of taking a herbal tea whose Uygur name is "*Kizil gul*", made from the blossom or petal of *Rosa gallica*, for the treatment of diabetes [1]. Distilled rose water has also been used in traditional Uygur medicine. The utilization of *R. gallica* in traditional Uygur medicine must be influenced by Arabian medicine, where *R. gallica* has been used traditionally for medicine and aromatic water. The *R. gallica* plants were cultivated in the oasis cities around Tarim basin (Taklamakan Desert). However, there was no scientific report on their medicinal utilization and constituents. In order to clarify the potency of the plant for the treatment of diabetes, we investigated the inhibitory activities of extracts of its petals against α-amylase and α-glucosidase, and these activities were compared with those of acarbose (an anti-diabetic drug used for treating type II diabetes). Moreover, we will report the inhibitory effects of the constituents of its petals against α-amylase and α-glucosidase.

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Result and discussion

Inhibitory effects of extracts of the petals against α-amylase and α-glucosidase

The inhibitory activities of three samples of *R. gallica* collected at Houtan, Niya and Keriya in Xinjiang province, China, were investigated against α-amylase and α-glucosidase. Known inhibitors of α-amylase and α-glucosidase show different inhibitory activities against enzymes from different origins [2, 3]. Therefore, three kinds of α-amylase from different origins were used in our study: bacterial α-amylase from *Bacillus stearothermophilus* (BSA), mammalian α-amylase from porcine pancreas (PPA) and human α-amylase from human saliva (HSA). Similarly, the following three kinds of α-glucosidase were used: yeast α-glucosidase from *Bacillus stearothermophilus* (BSG), *Saccharomyces* sp. (SSG) and mammalian α-glucosidase from rat small intestines (RIG).

Table 1 Inhibitory effects of the extracts of petals of *Rosa gallica* against α-amylase and α-glucosidase from different origins

Collection site	IC_{50} (μg/ml)[a]					
	α-Amylase			α-Glucosidase		
	BSA	PPA	HSA	BSG	SSG	RIG
Houtan	25	20	60	14	14	99
Niya	41	26	63	11	8	94
Keriya	46	34	68	27	14	121
Acarbose	4	38	73	1	38	5

[a] Sample concentration required for 50% inhibition

The inhibitory effects of 50% aqueous (aq.) ethanol extracts of petals of *R. gallica* are shown in Table 1. Compared with the inhibitory effect of acarbose, the extracts exhibited strong inhibitory effects against α-amylase from PPA and HSA and α-glucosidase from *Saccharomyces* sp. (SSG). However the inhibitory effects were different depending on the origin of the enzymes.

The IC_{50} values of the 50% aq. ethanol extracts of the three samples against BSA were 25, 41 and 46 μg/ml, respectively, and the IC_{50} values were nearly ten times higher than that of acarbose. The IC_{50} values of the extracts against HSA were higher than those against BSA, but the IC_{50} values of the extracts against PPA were lower than those against BSA. In the case of PPA, the IC_{50} values were lower than that of acarbose, but those against HSA were almost the same as that of acarbose.

The IC_{50} values of the extracts against α-glucosidase were in the range of 11–27 μg/ml for BSG, 8–14 μg/ml for SSG and 94–121 μg/ml for RIG. In the case of BSG and RIG, the IC_{50} values of acarbose (1 and 5 μg/ml, respectively) were less than one tenth of the extracts' values, but that for SSG was higher than those of the extracts.

To clarify the active constituents in the petals, the 50% aq. ethanol extracts were partitioned between ethyl acetate and water, and the inhibitory activities against all the kinds of α-amylase and α-glucosidase were compared. The ethyl acetate-soluble fraction showed lower IC_{50} values compared with those of original extracts. The IC_{50} values of the ethyl acetate fractions of the three samples against α-amylase were lower than those of the extracts, in the range of 7–18 μg/ml, but those of the aqueous fractions were higher, in the range of 41–164 μg/ml. The IC_{50} values of the ethyl acetate-soluble fractions against α-glucosidase were also lower than those of the extracts, in the range of 6–7 μg/ml, and those of the aqueous fractions were higher than those of the ethyl acetate-soluble fractions, in the range of 12–27 μg/ml. Therefore, the constituents in the ethyl acetate-soluble fractions were investigated.

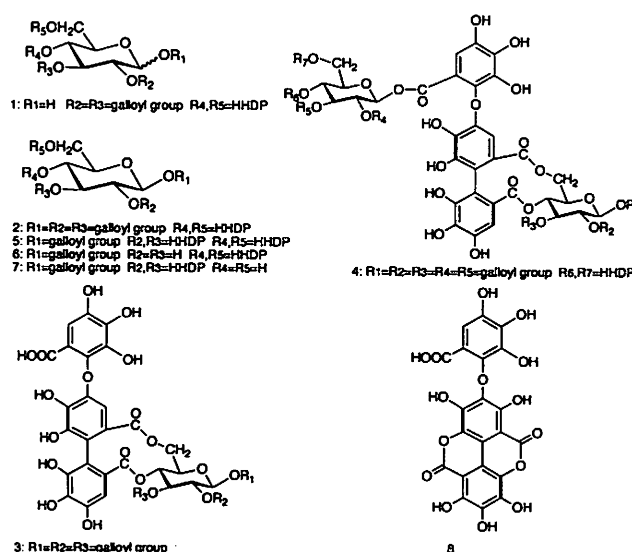

Fig. 1 Structures of hydrolysable tannins isolated from petals of *Rosa gallica*: tellimagrandin I (**1**), tellimagrandin II (**2**), rugosin A (**3**), rugosin D (**4**), casuarictin (**5**), strictinin (**6**), isostrictinin (**7**), valoneoic acid (**8**)

Effects of hydrolysable tannins against α-amylase and α-glucosidase

The ethyl acetate-soluble fraction derived from the sample collected at Houtan was fractionated by using Sephadex LH-20 column chromatography and preparative HPLC, and compounds **1**–**8** were isolated. These compounds were characterized as hydrolysable tannins by analysing their ^{1}H- and ^{13}C-NMR and HR-ESI-TOF mass spectra. The structures were elucidated to be tellimagrandin I (**1**), tellimagrandin II (**2**), rugosin A (**3**), rugosin D (**4**), casuarictin (**5**), strictinin (**6**), isostrictinin (**7**) and valoneoic acid (**8**), respectively (Fig. 1), by comparison of their spectroscopic data with those in the literature [4–11]. These compounds had been isolated from the petals of *Rosa rugosa* [6–9], but this is the first time they have been isolated from *R. gallica*.

The inhibitory activities of these hydrolysable tannins against α-amylase and α-glucosidase were tested along with those of gallic acid and ellagic acid. As shown in Table 2, gallic acid, ellagic acid and valoneoic acid showed almost no inhibition against α-amylase from *Bacillus* sp. (BSA). Strictinin and isostrictinin, hydrolysable tannins with a hexahydroxydiphenoyl (HHDP) group and a galloyl group, also showed no activities. The IC_{50} value of tellimagrandin I, a hydrolysable tannin with an HHDP group and two galloyl groups, was 235 μM. However, the IC_{50} value of tellimagranidin II, a hydrolysable tannin with an HHDP group and three galloyl groups, was 54 μM. The IC_{50} values of casuarictin, rugosin A and rugosin D were 18, 25 and 7 μM, respectively. The IC_{50} value of rugosin D (7 μM) was comparable to that of acarbose (6 μM). These

Table 2 Inhibitory effects of hydrolysable tannins isolated from *Rosa gallica* against α-amylase and α-glucosidase

Compound	IC_{50}^{a}			
	α-Amylase[b]		α-Glucosidase[b]	
	μM	μg/ml	μM	μg/ml
Acarbose	6	4	59	38
Rugosin D (4)	7	13	2	3
Rugosin A (3)	23	25	8	9
Casuarictin (5)	18	17	22	21
Tellimagrandin I (1)	235	185	13	10
Tellimagrandin II (2)	54	51	6	6
Strictinin (6)	>158	>100	181	115
Isostrictinin (7)	>158	>100	199	126
Gallic acid	>5882	>1000	841	143
Ellagic acid	>166	>50	1847	558
Valoneoic acid (8)	>106	>50	642	302

[a] Sample concentration required for 50% inhibition

[b] α-Amylase from *Bacillus* sp., α-glucosidase from *Saccharomyces* sp.

results indicated that bulky hydrolysable tannins showed strong inhibitory activities against α-amylase. The inhibitory activity of tannic acid against α-amylase was studied in 2004 [12]; however, this is the first time that this hydrolysable tannin has been found to have potent inhibitory activity against α-amylase from BSA.

In the case of α-glucosidase from *Saccharomyces* sp. (SSG), the IC_{50} values of rugosin D, tellimagrandin II, rugosin A, tellimagrandin I and casuarictin were 2, 6, 8, 13 and 22 μM, respectively, and these values were lower than that of acarbose (59 μM). The IC_{50} values of strictinin and isostrictinin were also higher than that of acarbose, while gallic acid, ellagic acid and valoneoic acid showed almost no inhibition. The number of galloyl groups on the glucose core of the hydrolysable tannins increased their inhibitory activities against α-glucosidase, and one dimeric hydrolysable tannin (4) was shown to be a potent inhibitor of α-glucosidase from SSG.

The inhibitory effects of hydrolysable tannins against rat intestinal α-glucosidase have been reported, and an increase in the number of galloyl groups in the hydrolysable tannins enhanced their inhibitory activity against α-glucosidase [13, 14]. This trend was the same in the case of α-glucosidase from *Saccharomyces* sp. and rugosin D, a dimeric hydrolysable tannin, that showed the strongest activity, which was almost thirty times stronger than that of acarbose.

Quantitative determination of hydrolysable tannins

The contents of hydrolysable tannins (1–5) in the 50% aq. ethanol extracts of the petals were quantitatively

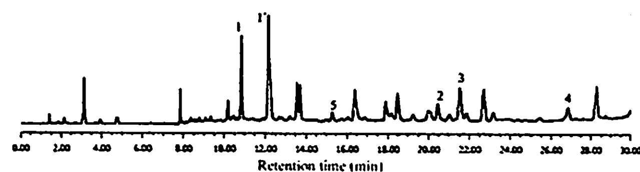

Fig. 2 UPL chromatogram (280 nm) of 50% ethanol extract of the petals of *Rosa gallica* collected at Niya in Xinjiang province, China: 1,1′ tellimagrandin I, 2 tellimagradin II, 3 rugosin A, 4 rugosin D, 5 casuarictin

Table 3 Contents of hydrolysable tannins in the petals of *Rosa gallica*

Collected place	Hydrolysable tannins (mg/g dry weight)					
	Tellimagrandin I	Tellimagrandin II	Rugosin A	Rugosin D	Casuarictin	Total
Houtan	39.7	2.7	6.3	3.0	1.0	52.7
Niya	33.1	3.7	5.8	3.7	0.5	46.8
Keriya	25.4	3.4	3.7	3.0	0.4	35.9

determined by ultra-performance liquid chromatography (UPLC). The UPL chromatogram of the 50% aq. ethanol extract of petals from *R. gallica* collected at Niya is shown in Fig. 2. The purity of peaks in each chromatogram was confirmed by comparison of these spectra measured by using a photodiode array (PDA) detector with those of the isolated samples.

The contents of these hydrolysable tannins are shown in Table 3. The total contents of these compounds were 3–5% of the dried weight of the petals. These compounds showed strong inhibitory activity against the digestive enzymes, and also had higher contents in the dried petals of the plant. Therefore, these compounds must be responsible for the activities of the plant, and the traditional tea of the Uygur people must be effective for the treatment of diabetes.

Experimental

General

^{1}H- and ^{13}C-NMR spectra were measured with a JEOL α-500 spectrometer in acetone-d_6 or DMSO-d_6 at 30°C. Chemical shifts were determined by using acetone (δ_H: 2.04 ppm, δ_C: 29.80 ppm) or DMSO (δ_H: 2.49 ppm, δ_C: 39.50 ppm) as the internal reference. HR-ESI-TOF-MS spectra were recorded on a JEOL JMS-T100LC spectrometer. UPLC was performed by using an Acquity Ultra Performance Liquid Chromatography system (Waters, USA) comprising a binary solvent manager, a sample manager, a column heater, a PDA detector, and an Acquity UPLC BEH Phenyl (2.1 i.d. × 100 mm, 1.7 μm) column. Sephadex LH-20 (GE Healthcare, Sweden) was used for

Fig. 3 Morphological characteristics of *Rosa gallica* collected at Houtan in Xinjiang province (China)

open column chromatography. Preparative HPLC was performed by using an FMI pump and a C_{18} column (3.0 i.d. × 30 cm, GL Science, Tokyo, Japan), or a Shimadzu LC-8 pump, an Hitachi L-4250 UV-VIS detector with 2 mm cell and a Inertsil ODS-3 (20 i.d. × 250 mm, GL Science). Gallic acid, ellagic acid and other chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The digestive enzymes α-amylase from *Bacillus* sp., porcine pancreatic and HSA, and α-glucosidase from *Bacillus* sp. and rat intestinal acetone powder were purchased from Sigma-Aldrich Japan Co. (Japan). α-Glucosidase from *Saccharomyces* sp. was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Plant materials

Cultivated *R. gallica* were collected at Houtan, Niya and Keriya around Tarim basin in Xinjiang province, China, on 1–3 May 2007. The petals were separated and air-dried, then kept at room temperature in sealed packages. The rose has the following morphological characteristics (Fig. 3): the plant is an erect shrub, growing to 2 m tall. Leaves are pinnate, compound with 3–5 leaflets and have a stipule. There are 5 sepals, ovate-lanceolate and stipitate glandular. Stipules are mostly adnate to petiole and the free parts are ovate and thin. The style is not exserted to sepals and is free. Flowers are solitary or several and rarely have a bracteole. Leaflets are rugose, not shiny and their length and width are about 3.4–4.8 and 1.5–3.0 cm, respectively, and have acutely serrate margins. There are 30–40 petals, double, dark pink, obovate, and the apex is emarginated.

These characteristics were coincident with those of *R. gallica* according to nomenclature lists [15, 16].

Extraction of materials

The pulverized petals (5 g) were extracted with 50% aq. ethanol (250 ml) at room temperature for 24 h, three times. After removal of ethanol, the combined extract was lyophilized. Then part of the extract was partitioned between ethyl acetate and water, three times. The ethyl acetate layer was evaporated to obtain the ethyl acetate-soluble fraction, and the aqueous layer was lyophilized to obtain the water-soluble fraction.

Assessment of α-amylase inhibitory activity

Three kinds of α-amylase from *Bacillus* sp. (BSA, 20 unit/mg of protein), porcine pancreatic (PPA, 1370 unit/mg of protein) and HSA (1920 unit/mg of protein) were used, and enzyme activity was measured as follows. Phosphate buffer (0.25 M, pH 7.0, 0.5 ml) containing 0.4 mg/ml soluble starch and test sample was incubated at 37°C for 5 min. The test samples were dissolved in H_2O. After addition of enzyme (200 unit/ml), the reaction mixture was incubated at 37°C for 10 min. Then the enzyme reaction was stopped by cooling to 0°C, and iodine solution (5 mM) was added to the reaction mixture. The amount of residual starch was determined by measuring absorption at 660 nm. The IC_{50} values were estimated graphically by plotting percentage inhibition versus the log of test sample concentrations.

Assessment of α-glucosidase inhibitory activity

Two kinds of α-glucosidase from *Saccharomyces* sp. (SSG, 163 unit/mg of protein), and *Bacillus* sp. (BSG, 103 unit/mg of protein) were used. Crude rat intestinal α-glucosidase was prepared from rat intestinal acetone powder, and showed sucrase (0.16 unit/mg of protein) activities which were measured by using sucrose (20 mM) as a substrate [3]. Their activities were evaluated as follows. The test samples (10 μl, in DMSO) were added to the mixture of phosphate buffer (415 μl, 10 mM, pH 7.0) and 25 μl of 1 M sucrose, and the mixture was incubated at 37°C for 5 min. Then 50 μl of enzyme solution was added to the mixture and was incubated at 37°C for 20 min. The enzyme solution (10 μg/ml) was prepared by dissolving α-glucosidase in 10 mM phosphate buffer (pH 7.0) containing 0.2% BSA (albumin from bovine serum). The reaction was stopped by addition of 10 μl of 10 mM deoxynojirimycin. The residual glucose concentrations in the reaction mixture were measured by using a glucose oxidase (GOD) method (glucose CII-test, Wako Pure Chemical Industries Ltd.). The IC_{50} values were determined graphically by plotting percentage inhibition versus the log of test sample concentrations.

Isolation of bioactive compounds

Pulverized petals of *R. gallica* (100 g) collected at Houtan of Xinjiang province, China, were extracted with 50% aq. ethanol (1 l), three times. The extract was evaporated to remove ethanol, and the aqueous fraction was extracted with ethyl acetate three times. The combined ethyl acetate fraction was evaporated to dryness to obtain the ethyl acetate-soluble fraction (8.4 g). The ethyl acetate-soluble fraction (5 g) was dissolved in ethanol and applied to a column of Sephadex LH-20 (5 i.d. × 18 cm). The column was eluted with a solvent system comprising ethanol/H_2O/acetone to obtain fractions (Fr. 1–10) containing hydrolysable tannins with different degrees of galloylation [17]. These fractions were further purified by reversed-phase chromatography and preparative HPLC. Compounds **6** (10 mg), **7** (8 mg) and **8** (15 mg) were separated from Fr. 4 eluted with ethanol/water (70:30). Compound **1** (47 mg) was isolated from Fr. 5 eluted with ethanol/H_2O (60:40). Compounds **2** (15 mg), **4** (12 mg) and **5** (20 mg) were isolated from Fr. 6 eluted with ethanol/water/acetone (54:36:10). Compound **3** (27 mg) was isolated from Fr. 8 eluted with ethanol/water/acetone (42:28:30).

Compound 1 (tellimagrandin I): light yellow powder. UV λ_{max} (MeOH) nm (log ε): 218 (4.13), 227 (4.04), 252 (4.37), 275 (4.56). HR-ESI-TOF-MS *m/z*: 809.0813 $[M + Na]^+$ (calcd for $C_{34}H_{26}O_{22}Na$: 809.0812). $[\alpha]_D^{26}$ +140.7 (*c* 0.1, acetone). NMR spectral data were coincident with the literature [4].

Compound 2 (tellimagrandin II): light yellow powder. UV λ_{max} (MeOH) nm (log ε): 229 (4.02), 245 (3.99), 278 (4.55). HR-ESI-TOF-MS *m/z*: 961.0923 $[M + Na]^+$ (calcd for $C_{41}H_{30}O_{26}Na$: 961.0906). $[\alpha]_D^{27}$ +56.4 (*c* 0.05, acetone). NMR spectral data were coincident with the literature [4, 5].

Compound 3 (rugosin A): light brown powder. UV λ_{max} (MeOH) nm (log ε): 215 (4.03), 243 (4.18), 276 (4.32). HR-ESI-TOF-MS *m/z*: 1129.0982 $[M + Na]^+$ (calcd for $C_{48}H_{34}O_{31}Na$: 1129.0992). $[\alpha]_D^{26}$ +91.6 (*c* 0.1, acetone). NMR spectral data were coincident with the literature [6, 8].

Compound 4 (rugosin D): light brown powder. UV λ_{max} (MeOH) nm (log ε): 239 (4.75), 278 (5.30). HR-ESI-TOF-MS *m/z*: 1897.1792 $[M + Na]^+$ (calcd for $C_{82}H_{58}O_{52}Na$: 1897.1743). $[\alpha]_D^{27}$ +93.2 (*c* 0.1, MeOH). NMR spectral data were coincident with the literature [7, 9].

Compound 5 (casuarictin): light yellow powder. UV λ_{max} (MeOH) nm (log ε): 204 (4.57), 212 (4.56), 229 (4.09), 246 (4.51), 258 (4.71), 275 (4.32). HR-ESI-TOF-MS *m/z*: 935.0790 $[M + H]^-$ (calcd for $C_{41}H_{27}O_{26}$: 935.0924). $[\alpha]_D^{27}$ +16.2 (*c* 0.1, MeOH). NMR spectral data were coincident with the literature [10].

Compound 6 (strictinin): light yellow powder. UV λ_{max} (MeOH) nm (log ε): 217 (4.53), 233 (4.74), 249 (4.65), 270 (4.66). HR-ESI-TOF-MS *m/z*: 657.0704 $[M + Na]^+$ (calcd for $C_{27}H_{22}O_{18}Na$: 657.0743). NMR spectral data were coincident with the literature [10, 11].

Compound 7 (isostrictinin): light yellow powder. UV λ_{max} (MeOH) nm (log ε): 204 (4.67), 211 (4.57), 244 (4.54), 271 (4.50). HR-ESI-TOF-MS *m/z*: 657.0704 $[M + Na]^+$ (calcd for $C_{27}H_{22}O_{18}Na$: 657.0737). NMR spectral data were coincident with the literature [11].

Compound 8 (valoneoic acid): white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.47, 7.02, 6.93 (each 1H, s). ^{13}C-NMR (DMSO-d_6) δ: 165.8, 159.09, 159.01, 149.8, 149.4, 148.5, 142.9, 140.6 (br), 139.50, 139.46, 139.0, 136.6, 136.1, 135.1, 114.6, 113.8, 112.1, 110.4 (br), 108.4 (br), 108.3 (br), 108.0. HR-ESI-TOF-MS *m/z*: 471.0199 $[M + H]^+$ (calcd for $C_{21}H_{11}O_{13}$: 471.0205).

Quantitative determination of hydrolysable tannins

The amounts of compounds **1–5** in petals were determined by UPLC, using gradient elution. The UPLC analytical conditions were as follows: the column used was an Acquity UPLC BEH Phenyl (2.1 × 100 mm, 1.7 μm) and column temperature was 25°C. The initial mobile phase (A) was 2.5% (v/v) acetonitrile containing 0.1% (v/v) formic acid, and final mobile phase (B) was acetonitrile containing 0.1% formic acid. The gradient conditions were as follows: 0–4 min, 0% B; 10 min, 10% B; 24 min, 15% B; 28 min, 20% B; 29 min, 40% B; 30 min, 100% B. The flow rate was 0.2 ml/min. The 50% aq. ethanol extracts of petals (1.0 mg) was dissolved in 1 ml of 50% methanol, and 1 μl was injected. The isolated hydrolysable tannins were used as standards for quantitative analysis. The detector wavelength was set from 200 to 500 nm, and maximum absorption wavelengths for the isolated compounds were selected as follows: 278.3 nm for tellimagradin II and rugosin D, 276.4 nm for rugosin A, and 274.6 nm for tellimagradin I and casuarictin.

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