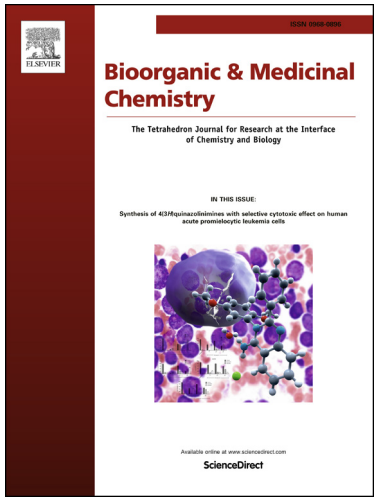


Potential anti-gout constituents as xanthine oxidase inhibitor from the fruits of *Stauntonia brachyanthera*

Dan Liu, Dandan Wang, Wei Yang, Dali Meng

PII: S0968-0896(17)30608-9
DOI: http://dx.doi.org/10.1016/j.bmc.2017.05.010
Reference: BMC 13728

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 27 March 2017
Revised Date: 3 May 2017
Accepted Date: 5 May 2017

Please cite this article as: Liu, D., Wang, D., Yang, W., Meng, D., Potential anti-gout constituents as xanthine oxidase inhibitor from the fruits of *Stauntonia brachyanthera*, *Bioorganic & Medicinal Chemistry* (2017), doi: http://dx.doi.org/10.1016/j.bmc.2017.05.010

Potential anti-gout constituents as xanthine oxidase inhibitor from the fruits of *Stauntonia brachyanthera*

Dan Liu[a], Dandan Wang[a], Wei Yang[a], Dali Meng[a, *]

[a] Key Laboratory of Structure-Based Drug Design and Discovery (Shenyang Pharmaceutical University), Ministry of Education, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China.

* Corresponding author. E-mail address: mengdl@163.com (D.L. MENG)

Abstract

With the aim of finding a better xanthine oxidase inhibitor with potential anti-gout properties, the studies on the fruit of *Stauntonia brachyanthera* were carried out, which led to the isolation of 12 glycosides, including 4 new nor-oleanane triterpenoids. Their structures were determined by comprehensive spectroscopic (NMR and HR MS) analysis. Two compounds (**4** and **11**) exhibited significant inhibitory activities on xanthine oxidase with IC_{50} values of 5.22 and 1.60 μM, respectively. Another five compounds (**1, 2, 3**, **8** and **10**) showed qualified activities. The results suggested that the existences of nor-oleanane triterpenoids and flavonoids in the fruits were responsible for the inhibitory activity on xanthine oxidase that could cut off the production of uric acid. Nor-oleanane triterpenoids, a new leading XO inhibitor, is worthy of further studies on molecular biology level for its mechanisms.

Keywords: *Stauntonia brachyanthera*; fruit; xanthine oxidase inhibitor; nor-oleanane triterpenoids; gout

1. Introduction

Xanthine oxidase (XO) could catalyze the oxidation of hypoxanthine to xanthine and subsequently to uric acid [1] in the purine nucleotides catabolism [2], which is hard to excrete and plays a vital role in producing hyperuricemia and gout. Its re-oxidation involves molecular oxygen which acts as electron acceptor, and during this reaction, superoxide radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) are produced [3]. $O_2^{\bullet-}$ is transformed into H_2O_2 and O_2 either spontaneously or catalyzed by superoxide

dismutase. Thus, the over-activity of XO leads to the deposition of uric acid in the susceptible tissues, which triggers the inflammatory pathways with a concomitant release of reactive oxygen species. Therefore, in order to reduce the serum uric acid level below the threshold, the xanthine oxidase inhibitor (XOI) is the most popular candidate, which can block the biosynthesis of uric acid from purine *in vivo*.

Triterpenoids have been comprehensively reported for their potential bioactivities on gout [4-6]. And Chinese researchers have found the therapeutic effect of triterpenoids on rheumatoid arthritis [7]. Although the mechanism is still unknown, this discovery greatly encouraged our further studies on triterpenoids abundant plant. Fortunately, during investigations, the fruit of *Stauntonia brachyanthera* Hand-Mazz attracted our great interests because of its abundant contents of triterpenoids [8, 9].

S. brachyanthera, an evergreen shrub belonging to the family of Lardizabalaceae, is well-known for its delicious and nutritious fruits [10-12]. The whole plant is demonstrated to possess definite medicinal values during the treatments of cancer, inflammation, pain, and also be used as diuretics in China [13]. Our previous studies had revealed the anti-gout effects of the components of its leaves [14]. Therefore, it could be expected that as another part of the same plant, its fruit might also contain the similar bioactive ingredients.

Based on the above analysis, our group is committed to the studies of natural biological triterpenoids from this plant which might be helpful to reduce the serum uric acid level below the threshold by inhibits the activity of xanthine oxidase. Herewith, the chemical constituents of the fruit of *S. brachyanthera*, as well as their potential anti-xanthine oxidase activities and preliminary structure-activity relationships, will be discussed comprehensively with the aim of finding more promising natural anti-gout resources.

2. Results and discussion

Compound **1-4** were all white amorphous powder obtained from MeOH solvents. On the basis of HR-ESI-TOF-MS, their molecular formulas were deduced to be $C_{40}H_{64}O_{14}$, $C_{41}H_{66}O_{14}$, $C_{48}H_{76}O_{19}$, and $C_{43}H_{68}O_{15}$ from their quasi-molecular ion peak at m/z 767.4241 $[M-H]^-$ (calcd. 767.4223, $C_{40}H_{63}O_{14}$), $[M-H]^-$ *m/z* 781.4396 (calcd.

781.4380), $[M+Na]^+$: *m/z* 979.4872 (calcd. 979.4873), and $[M+H]^+$: *m/z* 825.4634 (calcd. for 825.4631), respectively. After detail analysis of their ^1H and ^{13}C NMR data (**Table 1**-**2**), it could be found that all these compounds had the same aglycon as that of **brachyantheraoside B$_9$** except the sugar units connected to C-24, which were determined to be one *L*-arabinose and one *D*-glucoses in **1**, one *L*-fucose and one *D*-glucose in **2**, one *L*-fucose, one *D*-glucose, and one *L*-arabinose in **3**, and one *D*-glucose and one *L*-fucose in **4** by acid hydrolysis and HPLC analysis.

Table 1 The NMR data of the aglycones of compounds **1**–**4** (600 MHz for ^1H, pyridine-d_5; 150 MHz for ^{13}C, pyridine-d_5. *J* in Hz)

Pos.	1		2		3		4	
	δ H	δ C	δ H	δ C	δ H	δ C	δ H	δ C
1	0.94, 1.48	39.4	0.87, dm (12),	38.8	0.88, m, 1.48,	39.2	0.93, m, 1.46,	39.2
2	1.17, 2.10	28.6	1.76, m, 1.94, m	27.5	1.74, m, 1.94,	28.5	1.18, m, 2.09,	28.6
3	3.54,dd (10.8,	79.8	3.42, dd (10.8,	79.5	3.44, dd (0.8,	79.9	3.46, dd (11.2,	79.6
4		43.6		42.9		43.2		43.4
5	0.89	57.0	0.85, d (10.8)	56.5	0.85, m	57.0	0.91, m	56.9
6	1.35, 1.71	19.7	1.19, m, 1.56, m	19.1	1.37, m, 1.57,	19.7	1.43, m,1.71,	19.9
7	1.30, 1.44	33.9	1.25, m, 1.41, m	33.5	1.24, m, 1.37,	34.0	1.31, m,1.44,	33.9
8		40.1		39.7		40.1		40.1
9	1.61	48.5	1.93, m	48.0	1.90, m	48.5	1.62, m	48.4
10		37.6		37.1		37.6		37.5
11	1.95, 2.20	24.2	1.83, m, 2.14, m	24.0	1.84, m, 2.14,	24.3	1.89, m,2.25,	24.3
12	5.56, brs	122.9	5.52, m	124.3	5.68, m	123.0	5.56, m	122.9
13		144.7		144.3		144.8		144.6
14		42.4		42.1		42.5		42.4
15	1.09, 2.21	28.8	1.15, t (12.6),	28.3	1.14, dm	28.7	1.21, m, 2.17,	28.6
16	1.50, 1.84	24.3	2.00, m, 2.24, m	23.9	2.00, m, 2.23,	24.4	1.49, m, 1.84,	24.3
17		47.1		46.8		47.2		47.0
18	3.37, dd (13.8,	44.8	3.35, dd (12.6,	44.5	3.31, m	44.8	3.37, dd (14.2,	44.7
19	1.94, 2.47	48.4	1.57, m, 2.45, m	48.1	1.57, m; 2.42,	48.5	1.50, m, 2.46,	48.4
20		70.2		69.9		70.3		70.2
21	1.84, 2.08	36.6	1.83, m, 2.08, m	36.2	1.81, m, 2.05,	36.6	1.79, m, 2.07,	36.5
22	1.82, 2.03	35.5	1.82, m, 2.06, m	35.1	1.84, m, 2.05,	35.6	1.82, m,2.03,	35.5
23	1.55, s	23.8	1.51, s	23.5	1.44, s	23.8	1.54, s	23.7
24	3.80, d (12.0),	74.0	4.09, d (9.6),	72.8	4.20, m, 4.23,	73.3	4.12, d (9.7);	73.4
25	0.82, s	16.1	0.73, s	15.7	0.80, s	16.2	0.88, s	16.0
26	0.96, s	17.6	0.92, s	17.2	0.94, s	17.6	0.99, s	17.6
27	1.23, s	26.3	1.21, s	26.0	1.19, s	26.4	1.21, s	26.3
28		180.4		180.0		180.5		180.4
29	1.60, s	26.1	1.58, s	25.7	1.57, s	26.1	1.59, s	26.0

The linkages of these sugars were determined respectively through their HMBC spectra. In the HMBC spectrum of **1**, the long range correlations of H-1 (δ 5.29) of α-arabinose with C-3 (δ 86.9) of β-glucose, H-1 (δ 4.91) of β-glucose with C-24 (δ 74.0) of aglycone, and H-24 (δ 3.80, 4.59) of the aglycone with C-1 (δ 105.3) of

β-glucose finally lead to the formation of the sugar chains. The *β*-anomeric configurations for glucoses and the *α*-anomeric configuration for arabinose were determined from their coupling constants [(7.8, glc), (7.2, ara)].

Table 2 The NMR data of the sugar moieties of compounds **1**–**4** (600 MHz for ^{1}H, pyridine-d_5; 150 MHz for ^{13}C, pyridine-d_5. *J* in Hz)

Pos.	1		2		3		4	
	δ H	*δ* C	*δ* H	*δ* C	*δ* H	*δ* C	*δ* H	*δ* C
	inner-glc		inner-glc		inner-glc		inner-glc	
1′	4.91, d	105.9	4.87, d	103.7	4.77, d	103.8	4.81, d	103.6
2′	3.99	74.8	4.18, m	81.3	4.06, dd	78.6	4.02, m	82.4
3′	4.24	86.9	4.26, t	78.2	4.16, m	86.6	4.12, m	78.2
4′	3.93	69.6	4.20, m	71.5	3.89, t (9.0)	69.8	3.89, m	71.4
5′	3.96	78.7	3.85, m	78.5	4.12, m	76.2	4. 21, m	78.2
6′	4.71, dd	62.9	4.34, dd	62.6	4.66, dd	64.4	1.64, d	64.1
	terminal-ara		terminal-fuc		middle-fuc		terminal-fuc	
1″	5.29, d	106.0	5.30, d	105.6	5.44, d	104.2	5.25,d	106.5
2″	4.19	73.1	4.34, dd	73.8	4.33, dd	73.8	4.45,dd	73.4
3″	4.63	74.8	4.06, dd	75.4	3.98, dd	75.2	4.10,dd	75.6
4″	3.87	69.8	3.99, d	73.2	3.93, d (3)	73.9	4.02, m	73.4
5″	3.78,4.34	67.5	3.82, q	71.8	3.75, q	71.8	3.80,t	71.4
6″			1.62, d	17.3	1.53, d	17.7	1.54, br.s	17.6
					terminal-ara			
1‴					5.16, d	105.9		
2‴					4.46, t (7.8)	73.4		
3‴					3.84, m	75.0		
4‴					4.22, m	70.1		
5‴					3.72, d	68.3		
-COCH3					1.93, s	171.4, 21.2	1.93, s	171.1, 21.1

For compound **2**, the HMBC correlations of the H-1'' (*δ* 5.44) of the fucose and the C-2' (*δ* 81.3) of the glucose, H-1' (*δ* 4.87) of the glucose and the C-24 (*δ* 72.8) of the aglycone, the coupling constants of 7.2 Hz (glc) and 7.5 Hz (fuc), as well as the ^{13}C NMR data of fucose confirmed the composition of the sugar moiety.

The HMBC correlations of compounds **3** between the H-1'' (*δ* 5.44) of the fucose and the C-2' (*δ* 78.6) of the glucose, the H-1''' (*δ* 5.16) of the arabinose and the C-3' (*δ* 86.6) of the glucose, and H-1' (*δ* 4.77) of the glucose and the C-24 (*δ* 73.3) of the aglycone finally led to the formation of its sugar moiety. Meanwhile, the characteristic signals of *δ* 171.4 for the carbonyl carbon, *δ* 64.4 for C-6' of the glucose, as well as the HMBC correlation between the methyl group at *δ* 1.93 (3H, s) and the *δ* 64.4 of C-6' indicated the acetylation of C-6' of the glucose in compound **3**. The *β*-anomeric configuration of the glucoses and fucose and the *α*-anomeric

configuration of the arabinose were determined from their coupling constants [J = 7.8 Hz (glc), 7.8 Hz (fuc), 7.2 Hz (ara), respectively] in ^{1}H NMR and the ^{13}C NMR data of fucose.

As for **4**, its HMBC correlations between H-1" (δ 5.25) of the fucose and the C-2' (δ 82.4) of the glucose, and H-1' (δ 4.81) of the glucose and the C-24 (δ 73.4) of the aglycone finally confirmed its sugar moiety. At the same time, the diagnostic signals of the acetyl carbons (δ 171.1, 21.1) and the down-field shifted C-6' of the glucose (δ 64.1), as long as the HMBC correlation between the methyl group at δ 1.93 (3H, s) and the C-6' indicated the acetylation of C-6' of the glucose in compound **4**. The β-anomeric configuration for the glucoses and fucose were determined from their coupling constants [J = 7.7 Hz (glc), 7.5 Hz (fuc), respectively] in ^{1}H NMR and the ^{13}C NMR data of fucose.

According to the above analysis, the structures of **1-4** were finally defined as 3β, 20α, 24-trihydroxy-29-norolean-12-en-28-oic acid 24-*O*-α-*L*-arabinopyranosyl-(1→3)-β-*D*-glucopyranoside (**1**), 3β, 20α, 24-trihydroxy-29-norolean-12-en-28-oic acid 24-*O*-β-*L*-fucopyranosyl-(1→2)-β-*D*-glucopyranoside (**2**), 3β, 20α, 24-trihydroxy-29-norolean-12-en-28-oic acid 24-*O*-β-*L*-fucopyranosyl-(1→2)-[α-*L*-arabinopyranosyl-(1→3)]-6-*O*-acetyl-β-*D*-glucopyranoside (**3**), and 3β, 20α, 24-trihydroxy-29-norolean-12-en-28-oic acid 24-*O*-β-*L*-fucopyranosyl-(1→2)-6-*O*-acetyl-β-*D*-glucopyranoside (**4**) (**Fig. 1**), which were given the trivial names as brachyantheraoside B_1, B_2, B_3 and B_8, respectively.

By comparing their physical and spectroscopic data with those of the literatures reported, the rest compounds were identified as YM_9 (**5**) [15], YM_{10} (**6**) [15], brachyanin F (**7**) [16], 1′-*O*-β-D-(3,4-dihydroxyphenyl)-ethyl-6′-*O*-vanilloylglucopyranoside (**8**) [17], brachyanin C (**9**) [16], saponarin (**10**) [18], isoquercitrin (**11**) [19], and vitexin (**12**) [20] (**Fig. 2**).

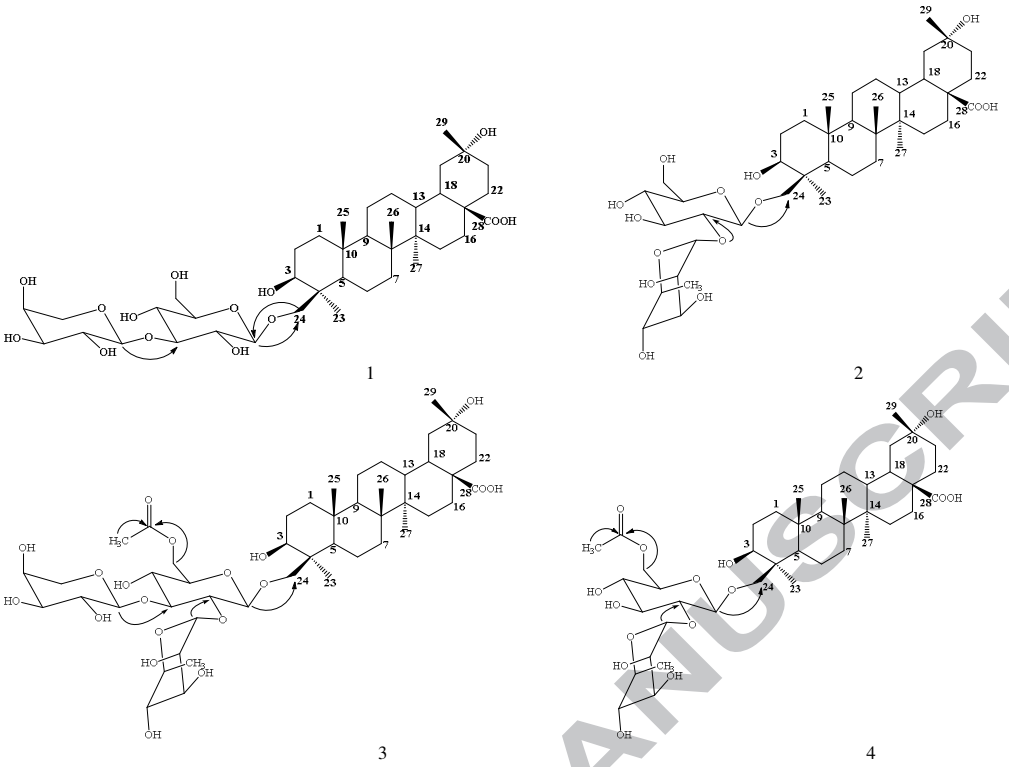


Fig. 1 The structures and the key HMBC correlations of compounds **1-4**

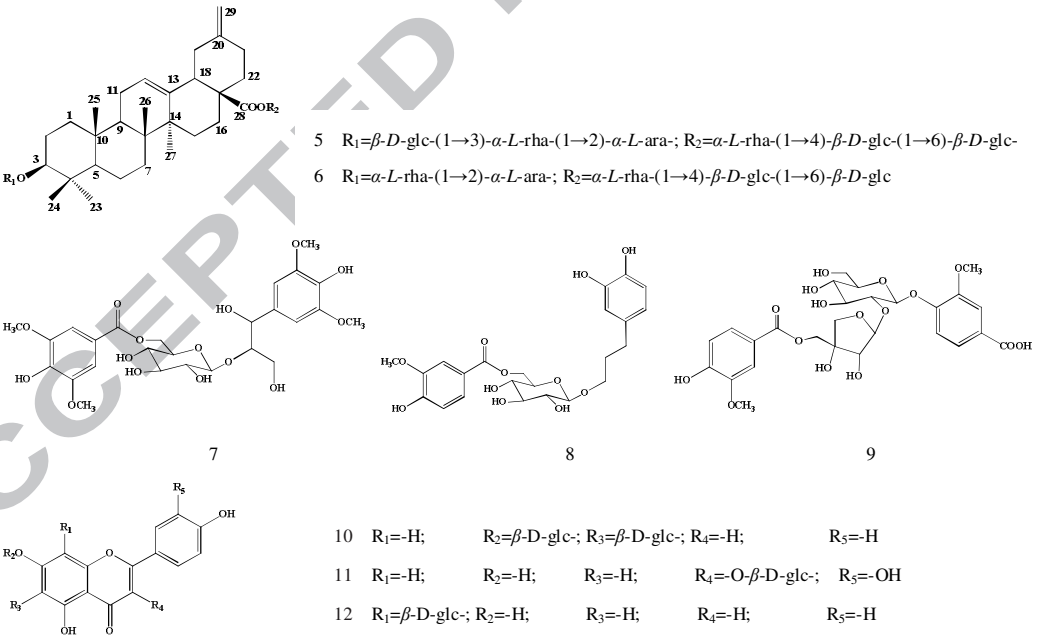


Fig. 2 The structures of compounds **5-12**

The xanthine oxidase inhibitory activities of all the glycosides were evaluated *in vitro* and the results were listed in **Table 3**. Allopurinol was used as the positive control. Among them, **4** and **11** exhibited significant inhibitory activities with the IC_{50}

values of 5.22 and 1.60 μM, respectively, which were all stronger than that of positive control (IC_{50} 50.15 μM). In addition, compounds **1**, **2**, **3**, **8** and **10** also showed the inhibitory activities against XO in a certain degree with IC_{50} values of 57.42, 56.68, 33.70, 61.10 and 50.24 μM, respectively, which were close to that of the positive control.

Table 3 The inhibitory activities of the isolated compounds on XO *in vitro*

Compoounds.	**IC_{50} (μM)**
1	57.42
2	56.68
3	33.70
4	5.22
5	-
6	97.30
7	86.22
8	61.10
9	-
10	50.24
11	1.60
12	-
allopurinol	50.15

* IC_{50} > 100 μM

These compounds could be divided into two groups, nor-oleanane glycosides and phenol glycosides. From **Table 3**, it could be found that the nor-oleanane glycosides had better inhibitory behaviors than those of the others. By comparing the structures of **1**, **2**, **3** and **4** to those of the others (**5** and **6**), it could be confirmed that the inhibitory activities will be significantly weakened when the C-28 was substituted by the sugar moieties and the free carboxyl group at C-28 was very necessary to the anti-gout activity [14]. The acetyl substitution on sugar moieties could lower down the polarity of the molecule and subsequently reduce the abilities, such as **3** and **4**. And the more glycosyls it had in the structure, the less inhibition it produced (**3** and **4**).

Flavonoids, phenols, and their glycosides have been reported to have inhibitory effects on XO [21-23]. Among the six phenolic glycosides obtained in our present studies, the XO inhibitory effect of **11** (IC_{50}=1.60 μM) was significantly higher than the others, and **10** (IC_{50}=50.24 μM) also expressed a considerable capability. The results indicated that flavonoids were much powerful than the simple phenolics. Preliminary

structure-activity relationships analysis showed that the unsubstituted C-8 was necessary for the flavonoids to practice as a XOI, and the sugar substitution at C-3 could enhance the inhibition capability. Among the simple phenolic glycosides, **7** and **8** exhibited different activities, while **9** had no effect at all. So, the oxidation on phenolic structure would increase the XO inhibitory effect, which was consistent with the previous report [24]. From an overall perspective, compared with nor-oleanane glycosides, the activities of phenolic glycosides didn't perform a good XOI activity. But, as a main constituent in the fruits of *S. brachyanthera*, this type of chemical constituent would also play an active role for the XO inhibitory effects of the fruit *in vivo* due to their multiple-factors and multiple-targets properties.

As mentioned above, the leaves of the plant had been reported in our previous study for its anti-gout bioactivity due to the existence of nor-oleanane triterpenoids [14]. However, besides nor-oleanane triterpenoids, the flavonoids in the fruits also contribute a lot to the effect. Therefore, combined the studies on its leaves and fruits, it could be believed that both nor-oleanane triterpenoids and flavonoids are naturally occurring bioactive compounds that exerts prominent anti-gout effects, which enrich the structures and resources of gout suppressants.

3. Conclusion

The current study reported four new and eight known compounds from the delicious fruit of *S. brachyanthera*. Seven of them showed obvious inhibitory activities on xanthine oxidase and the effects of two ones were even stronger than that of positive control. Therefore, this fruit could exert its XO inhibitory functions with the existences of nor-oleanane triterpenoids and flavonoids. Especially nor-oleanane triterpenoids, a new leading XO inhibitor, is worthy of further studies from molecular biology level for its mechanism.

4. Experimental section

4.1 Instruments and reagents

The nuclear magnetic resonance (NMR) spectra were acquired using Bruker ARX-600 spectrometer (Bruker Biospin, Rheinstetten, Germany). Chemical shifts (δ ppm) are relative to TMS (Tetramethylsilane) as an internal standard. HR-TOF-MS

was measured on a micro TOFQ Bruker mass spectrometer. Analytical HPLC was performed on a kromasil-ODS column (4.6×200 mm, AkzoNobel Global, Bohus, Sweden) consisting with a refractive index detector (Shimadzu RID-10A, Kyoto, Japan). Signs of optical rotation were observed by an optical rotation detector (Jasco LC2000P, Tokyo, Japan). Preparative HPLC was equipped with an YMC-ODS column (YMC-Pack ODS-A, 250×20 mm, 5 μm) and a refractive index detector (Shimadzu RID-10A). Column chromatographic (CC) purifications were performed on silica gel (200-300 mesh, Qingdao Haiyang Chemical Group Corporation, Qingdao, China), as well as Sephadex LH-20 (GE Healthcare, Uppsala, Sweden), and Macroporous resin HPD100 (Cangzhou Bon adsorber Technology Co., Ltd., Cangzhou, China). Xanthine Oxidase from milk (X4875-10UN, Grade IV, ammonium sulfate suspension, 0.1-0.4 units/mg protein), *D*-glucose, *L*-fucose, *L*-arabinose, and *L*-rhamnose were all purchased from Sigma-Aldrich (Sigma-Aldrich China, Shanghai, China). Silica gel GF_{254} (Qingdao Haiyang Chemical Group Corporation, Qingdao, China) were used for analytical and preparative TLC. And xanthine was purchased from Beijing Coupling Technology Co., Ltd. All solvents and chemicals used here were analytical grade, while other reagents were of HPLC grade or of the highest grade commercially available.

4.2 Plant material

The plants of *S. brachyanthera* were collected on October, 2009 in Hunan Province, and were identified by Prof. Jincai Lu, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, China. The specimen (NO.HLG-0910) was deposited in the School of Traditional Chinese Material Medica, Shenyang pharmaceutical University, China.

4.3 Extraction and isolation

The air-dried fruits of *S. brachyanthera* (7.0 kg), whose seeds were separated, were crushed into good pieces and were decocted with EtOH-H_2O (7:3, v/v, 35 L) under refluxing for 4 hours for 4 times, and the total extraction was retrieved after filtered. After a recuperation of the solvent from the mixture *in vacuo*, the final aqueous fractions (2.7 kg) were dissolved in water and passed through macroporous

adsorptive resin (HPD100), eluted seriatim with H_2O, 30% EtOH, 60% EtOH and 90% EtOH, respectively. The 60% EtOH eluates (210 g) were dealt with silica gel column chromatography (CC) (1000 mm×100 mm i.d.) with a successive CH_2Cl_2-MeOH system (100:1-0:100, v/v) to give eight fractions (1-8). **Comp. 12** (3.4 mg) was obtained directly from **Fr. 5** by the recrystallization method; **Comp. 1** (54.1 mg) and **11** (10.6 mg) were separated from **Fr. 6** employing ODS CC (300 mm×50 mm i.d.) with MeOH-H_2O (33:67, v/v) as eluant; **Comp. 5** (20.0 mg), **6** (14.3 mg), and **10** (155.5 mg) were all obtained from **Fr. 7** by the same method as **11**. **Comp. 2-4** (5.2, 14.9, 15.4, 6.6 mg, respectively) and **7-9** (10.9, 11.1, 8.7 mg, respectively) were purified from **Fr. 4** and **Fr. 8**, respectively, employing ODS CC (500 mm×60 mm i.d.) with MeOH-H_2O (30:70-45:55, v/v) as eluant and followed by preparative HPLC with MeOH-H_2O (80:20-55:45, v/v) as mobile phase.

4.4 Acid hydrolysis

The operation was carried out with a modified method reported [8, 16]. In brief, the solution of sample (each about 4.0 mg) is homogenized in methanol (2.5 mL), associated with 1 M H_2SO_4 (2.5 mL). Then the solution was heated at 90 ℃ for 7 h (kept sealed) and was neutralized with 1 M NaOH to give cloudy solution. An extraction with EtOAc (saturated with H_2O, 5 mL×2) was done when the mixture was cool down, of which the aqueous solution was retrieved under reduced pressure. The H_2O fraction was diluted and passed through a Sep-Pak C_{18} cartridge, which then analyzed by HPLC under the following method: column, Capcell Pak NH_2 UG80; solvent, MeCN-H_2O (3:1); flow rate, 0.5 mL/min; detection, RI and OR. The identifications of *D*-glucose, *L*-arabinose, *L*-rhamnose, and *L*-fucose presenting in the polysaccharide parts were recognized by the polarities with those of authentic samples. They were detected at 589 nm on a polarimeter that the optical rotation of 0.2% (w/v) of *D*-glucose, *L*-arabinose, *L*-rhamnose, and *L*-fucose consisting with a homologous standard distilled water solution.

4.5 In vitro xanthine oxidase inhibitory activity

The XO inhibition assay was carried out as the modified method reported [14, 25, 26]. The uric acid concentration was measured at 290 nm by spectrophotometric on 96

well plates (Fisher Science, US). The compounds were previously prepared into 1.5, 0.15, 0.015, 0.0015 mg/mL solutions, respectively. The tubes were incubated at 37 °C for 15 min, then, 60 μL of xanthine solution was added into *control* (10 μL PBS (0.2 M, pH 7.4) solution and 30 μL xanthine oxidase solution (0.48 M)) and *test* groups (10 μL compound solution and 30 μL xanthine oxidase solution), respectively, while 60 μL of PBS was added into *sample controls* (10 μL compound solution and 30 μL PBS). The absorption was then measured immediately. The XO inhibition rate was determined by measuring the difference in absorbance that correlates with uric acid formation. Allopurinol was used as positive control. All experiments were performed in triplicate and averaged.

XO inhibition rate = $(1-(A_t-A_s)/A_c) \times 100\%$, where A_c, A_s, and A_t are the absorbances of the *control*, *sample control*, and *test groups*, respectively.

4.6 Statistical analysis

All computations were made with the statistical software of *Statistical Product and Service Solutions* (SPSS, version 17.0), and the level of significance was set at $P<0.05$.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No.81073154, 81374061, 81573694), the Program for Innovative Research Team of the Ministry of Education and Program for Liaoning Innovative Research Team in University, and the Program for Innovation team of Liaoning province (Grant No. LT2015027).

References

1. Mittal, A.; Phillips, A. R. J.; Loveday, B.; Windsor, J. A. *World. J. Surg.* **2008**, *32 (2)*, 288-295.

2. Emmerson, B. *Clin. Biochem.* **1997**, *30(3)*, 253.

3. Kelley, E. E.; Khoo, N. K. H.; Hundley, N. J.; Malik, U. Z.; Freeman, B. A., Tarpey, M. M. *Free. Radic. Biol. Med.* **2010**, *48(4)*, 493-498.

4. Liu, C. H.; Yen, M. H.; Tsang, S. F.; Gan, K. M.; Hsu, H. Y.; Lin, C. N. *Food. Chem.* **2010**, *118*, 751-756.

5. Nkanwen, E. R. S.; Gojayev, A. S.; Wabo, H. K.; Bankeu, J. J. K.; Iqbal, M. C.; Guliyev, A. A.; Tane, P. *Fitoterapia*, **2013**, *86*, 108-114.

6. Lin, K. W.; Maitraie, D.; Huang, A. M.; Wang, J. P.; Lin, C. N. *Fitoterapia*, **2016**, *108*, 73-80.

7. Chinese herbal medicine company, China traditional Chinese medicine resources, (1st ed.), Beijing: The Science Publishing Company, **1994**, 373.

8. Chen, C.; Meng, D. L. *Biochem. Syst. Ecol.* **2013**, *48*, 182-185.

9. Meng, D. L.; Xu, L. H.; Chen, C.; Yan, D.; Fang, Z. Z.; Cao, Y. F. *J. Funct. Foods.* **2015**, *16*, 28-39.

10. Liu, Y.; Liu, X. W.; Fan, C. S. *Journal of Jiangxi University of TCM*, **2006**, *18(5)*, 41-42.

11. Liu, S. B.; Peng, X. L.; Tian, X. R.; Liu, Z. X. *Guizhou Agricultural Sciences,* **2010**, *38(9)*, 207-208.

12. Wang, Z.; Mei, S. M.; Zhen, L.; Xiao, C. Y. *Chinese Patent*, **2012** CN101376872A.

13. Wang, Z.; Mei, S. M.; Zhen, L.; Xiao, C. Y. *Chinese Journal of Ethnomedicine and Ethnopharmacy*, **2007**, *4*, 031.

14. Liu, X. L.; Li, S.; Meng, D. L. *Bioorg. Med. Chem. Lett.* **2016**, *26(12)*, 2874-2879.

15. Wang, Z.; Mei, S. M.; Zhen, L.; Xiao, C. Y. *Chinese Patent*, **2009**, CN101376872A.

16. Zhao, J.; Guo, J.; Zhang, Y.; Meng, D. L.; Sha, Z. *J. Funct. Foods.* **2015**, *14*, 374-383.

17. Zheng, X. K.; Liu, Y. B.; Li, J.; Feng, W. S. *Acta Pharmaceutica Sinica*, **2004**, *39(9)*, 716-718.

18. Içli, S. *Magn. Reson. Chem.* **2005**, *12(3)*, 178-182.

19. Kim, A. R.; Ko, H. J.; Chowdhury, M. A.; Chang, Y. S.; Woo, E. R. *Arch. Pharm. Res.* **2015**, *38(6)*, 1059-1065.

20. Hooper, A. M.; Caulfield, J. C.; Hao, B.; Pickett, J. A.; Midega, C. A. O.; Khan, Z. R. *Phytochemistry*, **2015**, *117*, 380-387.

21. Arimboor, R.; Rangan, M.; Aravind, S. G.; Arumughan, C. *J. Ethnopharmacol.* **2011**, *133*, 1117-1120.

22. Liu, H. X.; He, M. T.; Tan, H. B.; Gu, W.; Yang, S. X.; Wang, Y. H.; Li, L.; Long, C. L. *Phytochem. Lett.* **2015**, *12*, 133-137.

23. Dong, Y.; Huang, H. H.; Zhao, M. M.; Sun-Waterhouse, D. X.; Lin, L. Z.; Xiao, C. Q. *J. Funct. Foods.* **2016**, *24*, 26-36.

24. Toshiya, M.; Yoshimi, S.; Chizuru, T.; Miyuki, I.; Yukari, M.; Sari, H.; Akiko, M. *Free. Radical. Bio. Med.* **2014**, *69*, 300-307.

25. Smelcerovic, A.; Rangelov, M.; Smelcerovic, Z.; Veljkovic, A.; Cherneva, E.; Yancheva, D.; Nikolic, G. M.; Petronijevic, Z.; Kocic, G. *Food. Chem. Toxicol.* **2013**, *55*, 493-497.

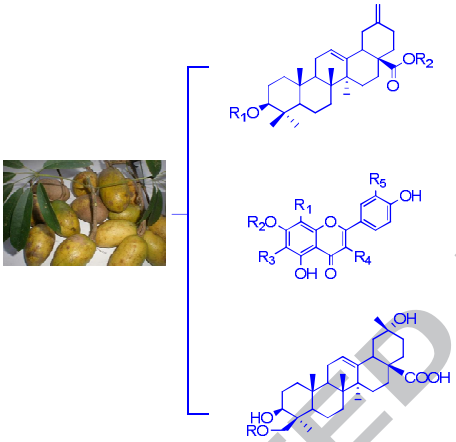
26. Smelcerovic, Z.; Veljkovic, A.; Kocic, G.; Yancheva, D.; Petronijevic, Z.; Anderluh, M.; Smelcerovic, A. *Chem-Biol. Interact.* **2015**, *229*, 73-81.

Potential anti-gout constituents as xanthine oxidase inhibitor from the fruits of *Stauntonia brachyanthera*

Dan Liu[a], Dandan Wang[a], Wei Yang[a], Dali Meng[a, *]

[a] Key Laboratory of Structure-Based Drug Design and Discovery (Shenyang Pharmaceutical University), Ministry of Education, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China.

* Corresponding author. E-mail address: mengdl@163.com (D.L. MENG)



Xanthine Oxidase

Table 3 The inhibitory activities of the isolated compounds on XO *in vitro*

Compoounds.	IC_{50} (μM)
1	57.42
2	56.68
3	33.70
4	5.22
5	-
6	97.30
7	86.22
8	61.10
9	-
10	50.24
11	1.60
12	-
allopurinol	50.15

* IC_{50} > 100 μM