Maplexins, new α-glucosidase inhibitors from red maple (*Acer rubrum*) stems

By: Chunpeng Wan, Tao Yuan, Liya Li, Vamsikrishna Kandhi, Nadja B. Cech, Mingyong Xie, Navindra P. Seeram

Wan, C., Yuan, T., Kandhi, V., Cech, N. B., Xie, M., Seeram, N. P. (2012). Maplexins, new α-glucosidase inhibitors from red maple (*Acer rubrum*) stems. *Bioorganic and Medical Chemistry Letters, 22(1)*, 567-600. doi:10.1016/j.bmcl.2011.10.073

Made available courtesy of Elsevier: http://dx.doi.org/10.1016/j.bmcl.2011.10.073

*****© Elsevier. Reprinted with permission. No further reproduction is authorized without written permission from Elsevier. This version of the document is not the version of record. Figures and/or pictures may be missing from this format of the document. *****

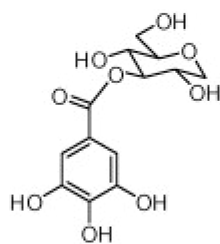
This is the author's version of a work that was accepted for publication in *Bioorganic and Medical Chemistry Letters*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in *Bioorganic and Medical Chemistry Letters*, Volume 22, Issue 1, (2012) DOI: 10.1016/j.bmcl.2011.10.073

Abstract:

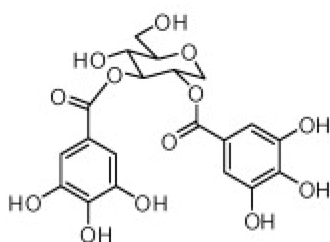
Thirteen gallic acid derivatives including five new gallotannins, named maplexins A–E, were isolated from red maple (*Acer rubrum*) stems. The compounds were identified by spectral analyses. The maplexins varied in number and location of galloyl groups attached to 1,5-anhydro-d-glucitol. The isolates were evaluated for α-glucosidase inhibitory and antioxidant activities. Maplexin E, the first compound identified with three galloyl groups linked to three different positions of 1,5-anhydro-d-glucitol, was 20 fold more potent than the α-glucosidase inhibitory drug, Acarbose (IC_{50} = 8 vs 160 μM). Structure–activity related studies suggested that both number and position of galloyls attached to 1,5-anhydro-d-glucitol were important for α-glucosidase inhibition.

Graphical abstract

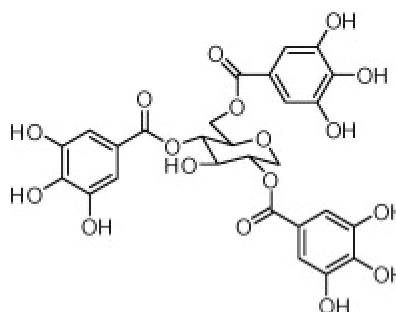
Thirteen gallic acid derivatives, including five new gallotannins, **2**, **3**, **5**, **6**, **9**, assigned the common names of maplexins A–E, respectively, were isolated from red maple stems. The isolates were evaluated for α-glucosidase inhibitory and antioxidant activities in vitro.



Maplexin A (2)



Maplexin C (5)



Maplexin E (9)

Keywords: Acer rubrum | Red maple | Maplexin | Gallotannins | α-Glucosidase inhibitory

Article:

Diabetes is one of the most prevalent human diseases in the world. At least 220 million people suffer from diabetes worldwide and this figure is estimated to double by 2030 (World Health Organization, 2010). Type 2 diabetes mellitus (also called non-insulin-resistant diabetes mellitus) accounts for 90–95% of all diabetes (Centers for Disease Control and Disease Prevention, 2010), which is characterized by insulin resistance, relative insulin deficiency, and hyperglycemia.[1] Type 2 diabetes is associated with chronic complications such as circulatory disease, stroke, hypertension, blindness, kidney failure, uremia and gangrene of the lower limbs.[2] At present, the therapies for type 2 diabetes rely mainly on several anti-diabetic drugs including insulin itself, sulfonylureas, biguanides, thiazolidinediones and α-glucosidase inhibitors. However, several synthetic drugs have limited efficacy, limited tolerability and significant side effects.[3] Therefore, the search for natural products to serve as more effective antidiabetic drugs, with reduced side effects, has attracted scientific interest.[4, 5 and 6]

The red maple species (*Acer rubrum* L.) is native to eastern North America and has been used for medicinal purposes by the Native Americans.[7] Both the red and sugar maple (*Acer saccharum*) species are widely regarded for their sap which is concentrated to produce maple syrup, a natural sweetener. Previous phytochemical studies of some maple species have resulted in the isolation of gallotannins with α-glucosidase inhibitory activities. [8, 9, 10, 11, 12 and 13] However, the red maple is yet to be investigated for such effects. Recently various plant part extracts of the red maple have been shown to have potent antioxidant activities but the active compounds were not identified. [14]

Our laboratory has reported on the identification and α-glucosidase inhibitory activities of phenolic compounds isolated from maple syrup from Canada, from where the majority (ca. 85%) of the world's supply originates.[15, 16, 17 and 18] In our ongoing efforts to discover anti-diabetic compounds from natural sources, we initially screened plant part extracts of various North American maple species for α-glucosidase inhibitory activities. The red maple stems/twigs extract inhibited α-glucosidase with IC_{50} values ranging from 4–10 μg/mL, prompting the current study.

Here we report the isolation and characterization of thirteen gallic acid derivatives from red maple stems that include five new gallotannins, compounds **2**, **3**, **5**, **6**, **9**, assigned the common names of maplexins A–E, respectively (Fig. 1). Also, the antioxidant and α-glucosidase inhibitory properties, as well as structure–activity relationship (SAR) studies of the isolates are described herein.

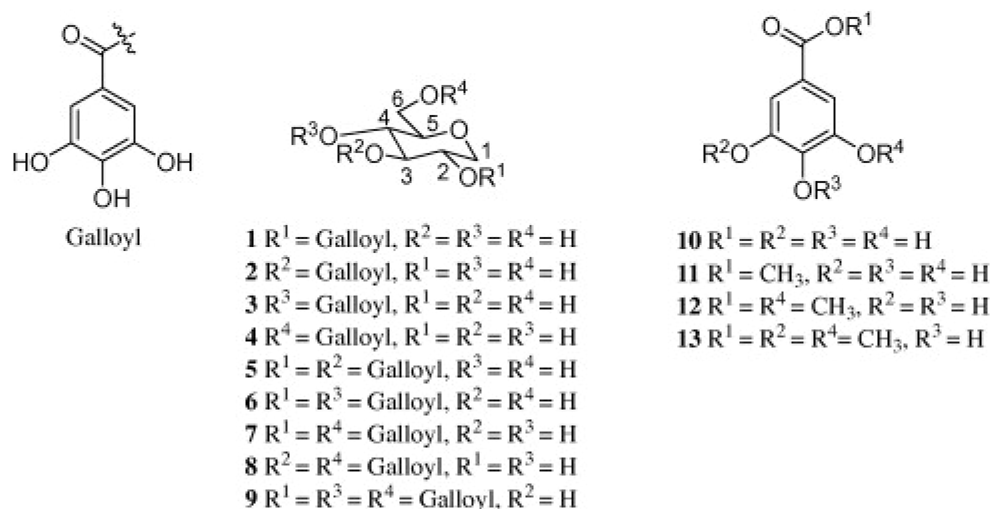


Figure 1. Structures of compounds **1**–**13**.

The stems of red maple were extracted with methanol and partitioned sequentially with *n*-hexanes, EtOAc, and *n*-butanol. From the EtOAc extract, thirteen gallic acid derivatives were isolated by chromatographic methods, [19] and their structures were characterized using physicochemical and spectroscopic methods. The detailed structural elucidation of the new compounds **2**, **3**, **5**, **6**, **9** are described below and their ^{1}H and^{13}C NMR data are shown in Table 1 and Table 2, respectively.

Table 1. ^{1}H NMR [δ, (multiplicity, J_{HH} in Hertz)] spectroscopic data for compounds **2**,**3**, **5**, **6** and **9** [a]

No.	**2**	**3**	**5**	**6**	**9**
1_{ax}	3.27 (1H, dd, 10.3, 9.9)	3.24 (1H, dd, 10.8, 10.0)	3.45 (1H, dd, 10.9, 10.6)	3.40 (1H, dd, 10.9, 10.5)	3.46 (1H, dd, 11.5, 10.5)
1_{eq}	3.98 (1H, dd, 10.3, 5.8)	3.99 (1H, dd, 10.8, 5.0)	4.20 (1H, dd, 10.9, 5.3)	4.18 (1H, dd, 10.9, 5.7)	4.22 (1H, dd, 11.5, 5.0)
2	3.71 (1H, ddd, 9.9, 9.3, 5.8)	3.58 (1H, m)	5.08 (1H, m)	4.99 (1H, m)	5.02 (1H, m)
3	5.04 (1H, dd,	3.45 (1H, m)	5.40 (1H, dd,	3.99 (1H, dd,	4.03 (1H, dd,

	9.4, 9.3)		9.5, 9.3)	9.4, 9.3)	9.3, 9.2)
4	3.52 (1H, dd, 9.5, 9.4)	4.89 (1H, overlap)	3.69 (1H, dd, 10.0, 9.5)	5.02 (1H, dd, 9.4, 8.9)	5.22 (1H, dd, 9.8, 9.3)
5	3.29 (1H, m)	3.58 (1H, m)	3.39 (1H, m)	3.56 (1H, m)	3.85 (1H, m)
6	3.85 (1H, dd, 11.9, 1.8)	3.58 (1H, m)	3.88 (1H, brd, 11.6)	3.61 (1H, brd, 9.9)	4.40 (1H, brd, 11.6)
	3.66 (1H, dd, 11.9, 5.4)	3.49 (1H, dd, 11.3, 6.2)	3.72 (1H, dd, 11.6, 5.4)	3.56 (1H, dd, 9.9, 5.6)	4.19 (1H, dd, 11.6, 5.4)
2′, 6′	7.14 (2H, d, 1.5)	7.09 (2H, d, 1.4)	7.05 (2H, d, 1.2)	7.09 (2H, d, 1.1)	7.11 (2H, d, 2.1)
2″, 6″	—	—	6.96 (2H, d, 1.4)	7.11 (2H, d, 1.0)	7.11 (2H, d, 2.1)
2‴, 6‴	—	—	—	—	7.08 (2H, d, 2.0)

[a] Data were measured in CD_3OD at 500 MHz.

Table 2. ^{13}C NMR (δ values) spectroscopic data for compounds **2**, **3**, **5**, **6** and **9** [a]

No.	**2**	**3**	**5**	**6**	**9**
1	69.5	69.6	66.4	66.6	66.6
2	68.5	70.2	70.0	71.9	71.8
3	79.8	79.5	76.5	73.5	73.5
4	68.6	71.4	68.5	71.4	71.1
5	81.1	76.4	81.3	79.6	76.8
6	61.3	61.4	61.2	61.2	62.7
1’	120.5	119.6	119.2	119.7	119.6
2′, 6′	108.9	108.9	108.9	108.9	108.9
3′, 5′	145.0	145.1	144.9	145.1	145.0

4′	140.5	138.4	138.4	138.6	138.6
7′	167.1	166.3	166.0	166.3	166.2
1″	—	—	120.0	119.6	119.6
2″, 6″	—	—	108.9	108.9	108.8
3″, 5″	—	—	145.0	145.1	145.0
4″	—	—	138.7	138.6	138.5
7″	—	—	166.8	166.2	166.0
1‴	—	—	—	—	119.8
2‴, 6‴	—	—	—	—	109.0
3‴, 5‴	—	—	—	—	145.1
4‴	—	—	—	—	138.7
7‴	—	—	—	—	166.7

[a] Data were measured in CD_3OD at 125 MHz.

Compound **2**,[20] a colorless amorphous solid, had a molecular formula of $C_{13}H_{16}O_9$ determined by HRESIMS at *m/z* 315.0717 $[M-H]^-$ (calcd for $C_{13}H_{15}O_9$, 315.0716). Its IR absorptions implied the presence of ester carbonyl (1693 cm^{-1}) and aromatic ring (1610 cm^{-1}). Inspection of the NMR data showed signals indicative of galloyl moiety at δ_H 7.14 (s, 2H) and δ_C 167.1, 145.0 (2 × C), 140.5, 120.5, and 108.9 (2 × C). Eight proton signals at δ_H 3.27–5.04 indicated the presence of a sugar substructure. Apart from the galloyl carbon signals, six oxygenated carbon signals at δ_C 81.1, 79.8, 69.5, 68.6, 68.5 and 61.3 were observed in the ^{13}C NMR spectrum, which also supported the presence of a sugar moiety. Further combined analysis of the ^1H–^1H COSY, HSQC and HMBC spectra allowed the establishment of the structure of **2**. The HSQC spectrum allowed the assignment of all the protons attached to their corresponding carbons. From the ^1H–^1H COSY spectrum, a 1-deoxysugar moiety (i.e., C-1 to C-6; Fig. 2a) was established, and their relative stereochemistry was determined by the proton coupling constants ($J_{1ax,2}$ = 9.9 Hz, $J_{2,3}$ = 9.3 Hz,$J_{3,4}$ = 9.4 Hz, $J_{4,5}$ = 9.5 Hz). Thus, a 1-deoxysugar moiety was determined as 1,5-anhydro-glucitol. The HMBC correlations between H-3 and the ester carbonyl (C-7′) indicated that the galloyl group was linked at C-3 of the 1,5-anhydro-glucitol moiety. Acid hydrolysis of **2** afforded 1,5-anhydro-d-glucitol, which was identified by direct co-TLC comparison with an authentic sample. Therefore, compound **2** was elucidated as 3-*O*-galloyl-1,5-anhydro-d-glucitol, assigned the common name of maplexin A.

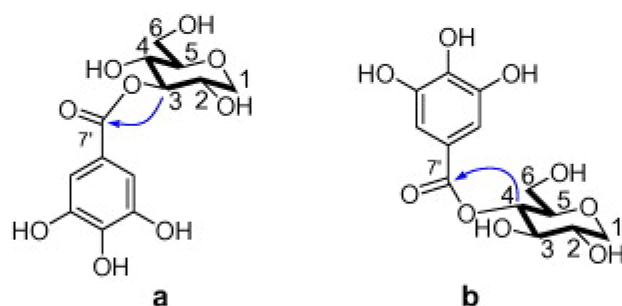


Figure 2. (a) ^{1}H–^{1}H COSY (_) and key HMBC correlations (H→C) of **2**; (b) ^{1}H–^{1}H COSY (_) and key HMBC correlations (H→C) of **3**.

Compound **3**,[21] had the same molecular formula as compound **2** (i.e., $C_{13}H_{16}O_9$ as per HRESIMS data) as well as similar UV and IR data. The ^{1}H and ^{13}C NMR spectra indicated the presence of similar galloyl and 1,5-anhydro-glucitol substructures as for compound **2**. Further analyses of the ^{1}H–^{1}H COSY, HSQC and HMBC data showed that the only difference between **2** and **3** was the linkage position connecting the galloyl to the 1,5-anhydro-glucitol moiety. The galloyl was eventually deduced to be attached to C-4 of the glucitol substructure by the HMBC correlations from H-4 to C-7′ (Fig. 2b). The d-configuration of the glucitol was determined by the similar acid hydrolysis method as described for compound **2**. Compound **3** was thus determined as 4-*O*-galloyl-1,5-anhydro-d-glucitol assigned the common name of maplexin B.

Compound **5**,[22] obtained as a colorless amorphous solid, had a molecular formula of $C_{20}H_{20}O_{13}$ as determined by HRESIMS at *m/z* 467.0826 $[M-H]^-$ (calcd for $C_{20}H_{19}O_{13}$, 467.0826). The ^{1}H and ^{13}C NMR data were similar to those of compounds **2** and **3**, indicating that the structures of both compounds were closely related, and the only difference was likely the presence of an additional galloyl moiety in **5**. Further analysis of the 2D NMR data allowed the establishment of the structure of **5**. In the HMBC spectrum, the correlations from H-2 to C-7′, and from H-3 to C-7″ indicated that the two galloyl groups were linked at C-2 and C-3 of 1,5-anhydro-glucitol, respectively. The d-configuration of the glucitol was determined by the same method as for compound **2**. Compound **5** was therefore elucidated as 2,3-di-*O*-galloyl-1,5-anhydro-d-glucitol assigned the common name of maplexin C.

Compound **6**,[23] had the same molecular formula (i.e., $C_{20}H_{20}O_{13}$) as compound **5** based on the HRESIMS at *m/z* 467.0821 $[M-H]^-$ (calcd for $C_{20}H_{19}O_{13}$, 467.0826). The IR and UV spectra were also similar to **5**. Initial analyses of the ^{1}H and ^{13}C NMR data revealed the presence of two galloyl groups and a 1,5-anhydro-glucitol moiety. The only difference between **6** and **5** was the linkage position of the galloyl to the 1,5-anhydro-glucitol group. The two galloyl groups were finally assigned to attachment at C-2 and C-4 of the 1,5-anhydro-glucitol on the basis of the HMBC correlations from H-2 to C-7′ and from H-4 to C-7″, respectively. The d-configuration of the glucitol was determined similar to that for compound **2**. Compound **6** was thus elucidated as 2,4-di-*O*-galloyl-1,5-anhydro-d-glucitol assigned the common name of maplexin D.

Compound **9**,[24] obtained as a colorless amorphous solid had a molecular formula of $C_{27}H_{24}O_{17}$ as determined by HRESIMS at *m/z* 619.0916 $[M-H]^-$ (calcd for $C_{27}H_{23}O_{17}$, 619.0935). From the NMR spectra, three sets of signals for galloyl moieties, eight proton signals at δ_H 3.46–5.22, and six oxygenated carbon signals at δ_C 76.8, 73.5, 71.8, 71.1, 66.6 and 62.7 were observed. The aforementioned data suggested that compound **9** was similar to the above compounds, the only difference being the presence of three galloyl groups attached to the 1,5-anhydro-glucitol moiety. The HMBC correlations from H-2 to C-7′, from H-4 to C-7″, and from H_2-6 to C-7‴ indicated that the three galloyl groups were linked at C-2, C-4, and C-6 of the 1,5-anhydro-glucitol, respectively. The d-configuration of the glucitol was determined similar to that described for compound **2**. Compound **9** was thus elucidated as 2,4,6-tri-*O*-galloyl-1,5-anhydro-d-glucitol assigned the common name of maplexin E. To the best of our knowledge, this is the first gallotannin reported to date that has three galloyl groups attached to three different positions of a 1,5-anhydro-glucitol moiety.

Maplexins A–E (each 2 mg), were individually added to a mixture of concentrated HCl (0.5 mL), H_2O (2 mL) and dioxane (3 mL) and refluxed for 2 h. After completion of the reaction (monitored by TLC), the mixture was evaporated to dryness. The dry reaction mixture was partitioned between $CHCl_3$ and H_2O (3×5 mL). The aqueous layer was neutralized with Na_2CO_3 and then concentrated to dryness. The concentrate was dissolved in methanol and purified by Sephadex LH-20 chromatography to give 1,5-anhydro-d-glucitol, which was identified by co-TLC and specific rotation with an authentic standard ($R_f = 0.43$, $CHCl_3$–MeOH, 10:1 v/v, positive value for optical rotation). The ESIMS and NMR data further supported these results.

Apart from the maplexins reported here, eight known compounds were identified as ginnalins B (**1**),[25] C (**4**),[25] and A (**7**),[26] 3,6-di-*O*-galloyl-1,5-anhydro-d-glucitol (**8**), [12] gallic acid (**10**), [27] methyl gallate (**11**), [27] 3,4-dihydroxy-5-methoxybenzoic acid methyl ester (**12**) [28] and methyl syringate (**13**) [29] on the basis of NMR and ESIMS data.

We investigated the in vitro α-glucosidase inhibitory[16] properties and the structure–activity relationship (SAR) of all thirteen isolates. Compounds **5**–**9** and **11** were found to be inhibitors of α-glucosidase enzyme in a concentration-dependent manner (Table 3). Compounds **1**–**4**, which possess one galloyl group each, did not show any activity in this assay, while compounds **5**–**8**, which possess two galloyl groups each, showed moderate α-glucosidase inhibitory activity. This is in agreement with a recent report suggesting that the α-glucosidase inhibitory activities of gallotannins are influenced by the number of their galloyl groups.[10]Remarkably, maplexin E (**9**), which was the only isolate that contained three galloyl groups, showed powerful α-glucosidase inhibitory activity. Maplexin E was 20 fold more potent than the known α-glucosidase inhibitory drug, Acarbose (IC_{50} = 8.26 vs161.38 μM, respectively).

Table 3. Antioxidant and α-glucosidase inhibitory activities of compounds **1**–**13**

Compounds	IC_{50} (μM)[a]	
	DPPH	α-Glucosidase
1	32.70 ± 0.48	n.d.
2	47.99 ± 1.11	n.d.
3	45.57 ± 1.45	n.d.
4	30.49 ± 0.80	n.d.
5	18.80 ± 0.77	1745.78 ± 168.05
6	18.59 ± 0.77	1221.84 ± 16.30
7	17.74 ± 0.21	95.38 ± 11.65
8	18.52 ± 0.44	88.42 ± 6.94
9	13.06 ± 0.16	8.26 ± 0.37
10	20.39 ± 0.34	n.d.
11	16.49 ± 0.26	317.39 ± 3.70
12	116.50 ± 4.98	6541.11 ± 19.90
13	990.57 ± 80.60	n.d.
Vitamin C [b]	71.02 ± 1.61	—
BHT[b]	1634.09 ± 16.07	—
Acarbose[b]	—	161.38 ± 5.5

n.d. = not detected; BHT = butylated hydroxytoluene. [a] IC_{50} values are shown as mean ± S.D. from three independent experiments. [b] Positive control.

Based on the α-glucosidase inhibitory activities of compounds **5**–**8** (IC_{50} = 1745.78, 1221.84, 95.38 and 88.42 μM, respectively), which have two galloyl groups each, SAR inferences could be made. Compounds **7**and **8** showed stronger activities than compounds **5** and **6**, which suggested that the α-glucosidase inhibitory activities of these gallotannins were influenced by both the number and positions of the galloyl groups. Thus, it was apparent that a galloyl group attached at the C-6 position of the 1,5 anhydro-glucitol moiety increased activity.

The antioxidant activities of compounds **1**–**13** were evaluated in the diphenylpicrylhydrazyl (DPPH) free radical scavenging assay.[17] All of the isolates except **12** and **13** showed better antioxidant activities than the positive controls, vitamin C, and the commercial synthetic antioxidant, butylated hydroxytoluene (Table 3). The IC_{50} values of compounds **1**–**4** ranged from 30.49 to 47.99 μM, compounds **5**–**8** ranged from 17.74 to 18.80 μM, and compound **9** was 13.06 μM. These results suggested that the antioxidant activity of these gallotannins were influenced mainly by the number of the galloyl groups, while the location of the galloyl group on the 1,5-anhydro-d-glucitol moiety was less important for antioxidant potential.

In conclusion, we have identified thirteen compounds including five new gallotannins, named maplexins A–E, from red maple with α-glucosidase inhibitory potential. A new α-glucosidase inhibitory gallotannin, named pycnalin, has recently been identified from the Japanese red maple, *Acer pycnanthum*. [10] Our SAR results are in agreement with that study [10] suggesting that the number of galloyl groups attached to the 1,5-anhydro-d-glucitol are important for inhibition of α-glucosidase. However, we have now demonstrated that both number and location of the galloyl groups on the 1,5-anhydro-d-glucitol moiety are important for activity. Thus, synthetic manipulation of these gallotannins may result in compounds with enhanced α-glucosidase inhibitory potential. However, whether these natural compounds could serve as potential therapeutic agents for type-2 diabetes would require further studies.

Acknowledgments

This project was supported by funding provided by Agriculture and Agri-Food Canada through the Developing Innovative Agri-Products (DIAP) initiative. The Federation of Maple Syrup Producers of Quebec participated in the collection of plant materials from Quebec, Canada. The authors would like to thank Mr. J. Peter Morgan and Dr. Emmanouil Apostolidis for assistance with plant authentication and conduction of the α-glucosidase inhibitory assay, respectively.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2011.10.073

References and notes

1. Moller, D. E. Nature 2001, 414, 821.

2. Israili, Z. H. Am. J. Ther. 2011, 18, 117.

3. Inzucchi, S. E. J. Am. Med. Assoc. 2002, 287, 360.

4. Qi, L. W.; Liu, E.; Chu, C.; Peng, Y. B.; Cai, H. X.; Li, P. Curr. Top. Med. Chem. 2010, 10, 434.

5. Benalla, W.; Bellahcen, S.; Bnouham, M. Curr. Diabetes Rev. 2010, 6, 247.

6. Tundis, R.; Loizzo, M. R.; Menichini, F. Mini-Rev. Med. Chem. 2010, 10, 315.

7. Arnason, T.; Hebda, R. J.; Johns, T. Can. J. Bot. 1981, 59, 2189.

8. Hatano, T.; Hattori, S.; Ikeda, Y.; Shingu, T.; Okuda, T. Chem. Pharm. Bull. 1990, 38, 1902.

9. Honma, A.; Koyama, T.; Yazawa, K. J. Enzym. Inhib. Med. Chem. 2011, 26, 176.

10. Ogawa, A.; Miyamae, Y.; Honma, A.; Koyama, T.; Yazawa, K.; Shigemori, H. Chem. Pharm. Bull. 2011, 59, 672.

11. Honma, A.; Koyama, T.; Yazawa, K. Food Chem. 2010, 123, 390.

12. Kumamoto, O. Chem. Pharm. Bull. 1960, 8, 72.

13. Koyama, T.; Honma, A.; Yazawa, K. J. Wood Sci. 2010, 56, 507.

14. Royer, M.; Diouf, P. N.; Stevanovic, T. Food Chem. Toxicol. 2011, 49, 2180.

15. Li, L.; Seeram, N. P. J. Funct. Foods 2011, 3, 125.

16. Apostolidis, E.; Li, L.; Lee, C.; Seeram, N. P. J. Funct. Foods 2011, 3, 100.

17. Li, L.; Seeram, N. P. J. Agric. Food Chem. 2011, 59, 7708.

18. Li, L.; Seeram, N. P. J. Agric. Food Chem. 2010, 58, 11673.

19. The dried stems (500 g) of Red maple species were ground and extracted exhaustively with methanol. The combined dried methanol extract was resuspended in water and partitioned successively with n-hexanes, EtOAc and nbutanol. The EtOAc fraction (18 g) was subjected to a silica gel column chromatography (CHCl3/MeOH) to yield three fractions (A1–A3). Fraction A3 (8 g) was chromatographed on a Sephadex LH-20 column and eluted with MeOH to give seven sub-fractions (B1–B7). Fraction B4 was chromatographed on a C18 MPLC column eluting with a gradient system of MeOH/H2O (1:9 to 7:3, v/v) to afford 14 sub-fractions (C1–C14). Fraction C2 was separated by semipreparative HPLC eluted with MeOH/H2O (20/80 v/v, 3.2 mL/min) to yield compounds 2 (2.8 mg), 3 (2.5 mg) and 10 (460 mg). Fraction C3 was separated by semi-preparative HPLC eluted with MeOH/H2O (25/75 v/v, 3.2 mL/min) to yield compounds 1 (18 mg) and 4 (9.2 mg). Fraction C5 was separated by semipreparative HPLC eluted with MeOH/H2O (30/70 v/v, 3.2 mL/min) to yield compounds 5 (5.3 mg) and 11 (7.7 mg). Fraction C6 was separated by semipreparative HPLC eluted with MeOH/H2O (27/73 v/v, 3.2 mL/min) to yield compound 6 (25 mg). Fraction C9 was separated by semi-preparative HPLC eluted with MeOH/H2O (25/75 v/v, 3.2 mL/min) to yield compounds 7 (13 mg) and 12 (4.6 mg). Fraction C12 was separated by semi-preparative HPLC eluted with MeOH/H2O (41/59 v/v, 3.2 mL/min) to yield compound 13 (0.8 mg). Fraction B6 was chromatographed on a C18 MPLC column eluting with a gradient system of MeOH/H2O (2:8 to 7:3, v/v) to afford 10 sub-fractions (D1–

D10). Fraction D1 was separated by semi-preparative HPLC eluted with MeOH/H_2O (30/70 v/v, 3.2 mL/min) to yield compound 8 (1.4 mg). Fraction D8 was separated by semi-preparative HPLC eluted with MeOH/H_2O (35/65 v/v, 3.2 mL/min) to yield compound 9 (5 mg).

20. 3-O-galloyl-1,5-anhydro-D-glucitol (2): colorless amorphous solid; $[\alpha]_D^{20}$ +25 (c 0.280, MeOH); UV (MeOH) kmax (loge): 276 (4.10), 216 (4.41) nm; IR mmax 1693, 1610 cm1; for 1 H NMR and 13C NMR data, see Tables 1 and 2, respectively; HREIMS at m/z 315.0717 [MH] (calcd for $C_{13}H_{15}O_9$, 315.0716).

21. 4-O-galloyl-1,5-anhydro-D-glucitol (3): colorless amorphous solid; $[\alpha]_D^{20}$ +15 (c 0.060, MeOH); UV (MeOH) kmax (loge): 276 (4.10), 216 (4.41) nm; IR mmax 1690, 1608 cm1; for 1 H NMR and 13C NMR data, see Tables 1 and 2, respectively; HREIMS at m/z 315.0719 [MH] (calcd for $C_{13}H_{15}O_9$, 315.0716). 22. 2,3-di-O-galloyl-1,5-anhydro-D-glucitol (5): colorless amorphous solid; ½a20 D

+13 (c 0.120, MeOH); UV (MeOH) kmax (loge): 276 (4.10), 216 (4.41) nm; IR mmax 1705, 1600 cm1; for 1 H NMR and 13C NMR data, see Tables 1 and 2, respectively; HREIMS at m/z 467.0826 [MH] (calcd for $C_{20}H_{19}O_{13}$, 467.0826).

23. 2,4-di-O-galloyl-1,5-anhydro-D-glucitol (6): colorless amorphous solid; $[\alpha]_D^{20}$ +6 (c 0.170, MeOH); UV (MeOH) kmax (loge): 276 (4.10), 216 (4.41) nm; IR mmax 1703, 1601 cm1; for 1 H NMR and 13C NMR data, see Tables 1 and 2, respectively; HREIMS at m/z 467.0821 [MH] (calcd for $C_{20}H_{19}O_{13}$, 467.0826).

24. 2,4,6-tri-O-galloyl-1,5-anhydro-D-glucitol (9): colorless amorphous solid; $[\alpha]_D^{20}$ +10 (c 0.130, MeOH); UV (MeOH) kmax (loge): 276 (4.10), 216 (4.41) nm; IR mmax 1710, 1598 cm1; for 1 H NMR and 13C NMR data, see Tables 1 and 2, respectively; HREIMS at m/z 619.0916 [MH] (calcd for $C_{27}H_{23}O_{17}$, 619.0935).

25. Song, C.; Zhang, N.; Xu, R.; Song, H. Acta Chim. Sinica 1982, 40, 1142.

26. Bock, K.; Faurschou laCour, N.; Jensen, S. R.; Nielsen, B. J. Phytochemistry 1980, 19, 2033.

27. Cai, B.; Wang, B.; Liang, H.; Zhao, Y. Zhongguo Zhong Yao Za Zhi 2009, 34, 2331.

28. Zhang, X.; Ling, L.; Wang, H. Zhongcaoyao 1991, 22, 299.

29. Tan, J.; Jiang, S.; Zhu, D. Tianran Chanwu Yanjiu Yu Kaifa 2005, 17, 267.