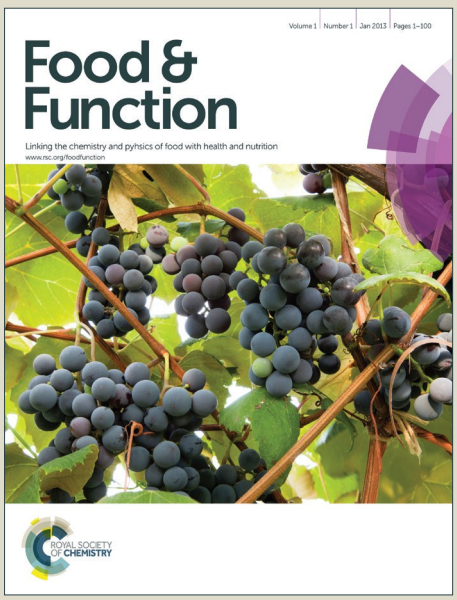
Food & Function

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/foodfunction

Chemical components from the Haulm of *Artemisia Selengensis* and the Inhibitory Effect on Glycation of β-lactoglobulin

Xiaoming Li, Yonglin Lu, Ronghua Deng, Tiesong Zheng, Lishuang Lv*

‡Department of Food Science and Technology, Ginling College, Nanjing Normal University, 122# Ninghai Road, Nanjing, 210097, P. R. China

Corresponding author: Lishuang Lv

Tel.: +86 25 83598286; fax: +86 25 83707623.

E-mail address: lishuanglv@126.com; lulishuang@njnu.edu.cn

ABSTRACT

Artemisia selengensis (AS) has been traditionally used as both food and medicine for thousands of years in China. In our studies, L-tryptophan was firstly isolated from the haulm of AS together with luteolin, rutin, and kaempferol-3-O-glucuronide. Their structures were elucidated by spectroscopic methods including HRMS, 1D and 2D NMR**.** Three flavonoid compounds showed satisfactory suppression effects on formation of advanced glycation end products (AGEs) in β-lactoglobulin-Lactose/MGO/GO model systems, and their anti-glycation activities exhibited a dose-dependent manner. Among those compounds, kaempferol-3-O-glucuronide was demonstrated the strongest inhibitor against formation of AGEs.

KEYWORDS: *Artemisia Selengensis haulm; β-lactoglobulin; advanced glycation end products (AGEs).*

Kaempferol-3-O-glucuronide CID: 44258914; Rutin CID: 5280805;

Luteolin CID: 5280445;

1. Introduction

Artemisia selengensis (AS) is an herbaceous perennial plant of the Compositae family, localized at the water's edge on banks or in swamps, with wild species widely distributed over the Northeast, North, and central China since the Ming dynasty. They're also found in Mongolia, Russia, and Korea. In *Compendium of Materia Medica,* it is recorded that AS has diverse biological activities, e.g. hemostasia, anti-inflammation, relieving cough, reducing sputum, and treatment of acute infectious hepatitis, due to AS's various active compounds. Being a valuable natural product, AS is beneficial to human's health. Some clinical therapies are also reported in the literature [1-3].

In Nanjing, early the 1990's, people started planting AS. Nowadays, AS has become very popular as a healthy food, being delicious and nutritious, for its tender stem is a vegetable with unique flavor, being fragrant, fresh, and crisp. However, the older haulm of AS is usually discarded because of it's inedible. To date, some of its major biologically active components have been isolated, such as flavonoids [4-6], polysaccharides [7], phenolic acids [8] guaianolides [9] and fatty acids [10]. Flavonoids in the haulm of AS comprise more than 1% by weight, but have hitherto been largely ignored in the field of food sciences. Our previous study showed that the flavonoid content of AS increased with the growth of AS stems. With this study, we investigate the chemical composition and biological activities of the AS haulm to promote the utilization of AS in food processing.

Methylglyoxal (MGO) and glyoxal (GO), two major α-dicarbonyl compounds

formed from both glycoxidation and lipoxidation [11], are the pivotal intermediates in the formation of advanced glycation end products (AGEs) *in vivo* [12]. In food processing, the α-dicarbonyl compounds are generated through roasting, baking, broiling, and frying due to caramelization, the Maillard reaction, and lipid oxidation [13]. During such reactions, MGO and GO glycate proteins faster than sugars, causing inter- and intramolecular cross-links of proteins, thus the amount of AGEs sharply increases [14].

β-lactoglobulin (β-lg) is a high quality protein found in a variety of foodstuffs, including infant formulas, baked products, and beverages. The effects of the Maillard reaction and glycation on this protein during heating are well known and replicable, as milk contains high amounts of lactose and lower quantities of other reducing sugars. This replicability makes it a good model with real world-applications to test the anti-AGEs properties of our compounds.

Flavonoids are common dietary components of plant-derived foods. Several flavonoids: (-)-epigallocatechin 3-gallate (EGCG) from tea [15], phloretin from apple [16], genistein from soybean [17], proanthocyanidins and anthocyanin from berries [18], are known to be scavengers of AGEs by trapping reactive dicarbonyl compounds.

In the present study, we investigate chemical components from AS haulm and their inhibitory activities against the formation of AGEs using the β-lg-lactose/MGO/GO model systems, to find food-derived inhibitors of AGEs. We expect flavonoids to prevent protein glycation during food thermal processing.

2. Materials and Methods

2.1. Materials

The air-dried haulm of *Artemisia Selengensis* was obtained from the "Baguazhou" district in Nanjing. β-lactoglobulin (≥92%) was purified in our lab (Nanjing, Jiangsu, China). The chemical standard kaempferol-3-O-glucuronide, rutin, and luteolin; methylglyoxal (MGO, 40% in water), glyoxal (GO, 40% in water); D_2O, CD_3OD and DMSO-d_6 were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). HPLC-grade solvents and other reagents were obtained from Shanghai Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). HPLC-grade water was prepared using a Millipore Milli-Q purification system (Bedford, MA, USA).

2.2. Extraction and purification procedure

The extraction and purification procedure are shown in Supplemental Figure 1.

The dried powder of AS haulm (1 kg) was extracted with 75% ethanol (7.5 L) at 90°C for 60 min. The solvent was removed by filtration, and then fresh solvent was added to the residue. The extraction process was twice repeated. The combined filtrates were concentrated under reduced pressure at 40 °C by a rotary evaporator (Tokyo Rikakikai Co., Tokyo, Japan).

To remove the pigment, the thick solution was put into a separating funnel for further extraction. Petroleum ether was used as extractant at a 1:3 ratio. These water-soluble fractions were later evaporated and lyophilized.

Dried AS crude extract was re-suspended in 200mL distilled water and loaded onto a glass column (5.8 × 55 cm) packed with AB-8 macroporous adsorption resin. After the sample was loaded, elution was halted for 10 min to facilitate adsorption of

chemicals on resin beads. Five fractions (AS-01~AS-05) were collected by eluting the column with 1.5 L of 0, 10%, 30%, 50%, and 70% ethanol sequentially. The AS-02 fraction (10% ethanol eluted portion) was subjected to an ODS column, eluted with a gradient system: 10%, 20%, 30%, 40%, and 100% MeOH (500 mL for each gradient system). The two fractions yielded, AS-02-1 (10% MeOH eluted) and AS-02-2 (20% MeOH eluted), were further separated on a Sephadex LH-20 column respectively, and eluted with ethanol. Two compounds ASF-1 (from AS-02-1) and ASF-2 (from AS-02-2) were obtained. The AS-03 fraction (30% ethanol eluted portion from AB-8 column) was separated by an ODS column using the same procedure as the AS-02 fraction to obtain ASF-3. The AS-04 fraction (50% ethanol eluted portion from AB-8 column) was chromatographed on silica gel column and eluted with CH_3Cl_3/MeOH by a gradient of 98:2, 20:1, 10:1, 8:1, 6:1, 2:1, and 0:1 to afford ASF-4. The amount of AS-05 fraction (70% ethanol eluted portion from AB-8 column) was too small to abandon.

2.3. Analysis HPLC-MS procedure

The separation of all fractions except ASF-1 and offline data collection for HPLC based activity profiling were carried out with a series Agilent 1200 HPLC system, which consisted of a degasser, a bin pump, a column oven, a diode array detector, and a QQQ mass detector (Agilent, Santa Clara, CA, USA) incorporated with electrospray ionization (ESI) interfaces. A HPLC ZORBAX Eclipse XDB-C_{18} (250×4.6 mm i.d., 5 μm) was used for separation at a flow rate of 0.6 mL/min. The mobile phase fulfilled the following requirements: (1) **Compound ASF-2:** Water with 0.5% formic acid (A)

and Acetonitrile with 0.1% formic acid (B) were used as solvents for a 40 min program: The column was eluted with 12% solvent B for 10 min, followed by linear increases in B to 20% within 10 to 30 min, then to 30% from 30 to 40 min. The UV detector was set at 350 nm. Compound 2 had an intense peek at RT = 37.22 min; (2) **Compound ASF-3:** Water with 0.01% acetic acid (A) and Acetonitrile (B) were used as solvents for a 25 min program: The column was eluted with 90% solvent A for 10 min, followed by linear increases in B to 60% from 10 to 20 min, and then with 60% B from 21 to 25 min. The UV detector was set at 350 nm. Compound 3 had an intense peek at RT = 15.17 min. (3) **Compound ASF-4:** Water with 0.5% formic acid (A) and Acetonitrile with 0.1% formic acid (B) were used as solvents for a 55 min program: The column was eluted with 88% solvent A for 10 min, followed by linear increases in B to 20% from 10 to 30 min, to 30% from 30 to 40 min, to 60% from 40 to 48 min, and then re-equilibrated with 90% B for 6 min from 49 to 55 min. The UV detector was set at 254 nm. Compound 4 had an intense peek at RT = 47.33 min. For MS: The negative ion polarity mode was set for ESI ion source. The typical operating parameters were as follows: spray needle voltage, 5 kV; nitrogen sheath gas, 45 (arbitrary units); auxiliary gas, 5 (arbitrary units).

In contrast, fraction ASF-1was carried out with a series Agilent 1290 Infinity 6224 TOF system (Agilent, Santa Clara, CA, USA). A HPLC ZORBAX Eclipse XDB-C_{18} (250×4.6 mm i.d., 5 μm) was used for separation at a flow rate of 0.8 mL/min. The column was eluted with 40% solvent B (Acetonitrile) and 60% solvent A (water with 0.01% acetic acid) for a 15 min. The UV detector was set at 280 nm. Compound 1 had

143 an intense peek at RT = 3.77 min. For MS: The negative ion polarity mode was set for
144 ESI ion source. The typical operating parameters were as follows: spray needle
145 voltage, 5 kV; nitrogen sheath gas, 45 (arbitrary units); auxiliary gas, 5 (arbitrary
146 units).

147 The structural information of compounds 1-4 was obtained by tandem mass
148 spectrometry (MS/MS) through collision-induced dissociation (CID) with a relative
149 collision energy setting as 35%. Data acquisition was performed with Qualitative
150 Analysis of Mass hunter (Agilent, Santa Clara, CA, USA).

151 **2.4. NMR Analysis**

152 NMR ^{1}H (400 MHz), ^{13}C (100 MHz), and 2D NMR spectra were obtained on an
153 AVANCE 400 (Bruker Daltonics Co., Bremen, Germany) spectrometer with TMS as
154 internal reference.

155 **2.5. Inhibition of Compounds (ASF-2~ASF-4) against formation of AGEs in**
156 **β-lactoglobulin glycation systems**

157 β-lactoglobulin (0.083 mmol/L) was incubated with lactose (0.083mol/L) or
158 MGO/GO (1.5 mmol/L) at chosen ratios (1:1000, 1:18) in the presence or absence of
159 Compounds 1-4 (0.05, 0.1, 0.5 mmol/L) in phosphate buffer (pH 6.5) at 85 °C. Then,
160 the sample was collected at selected time points (0, 15, 30, 45, 60, 90, 120 min) and
161 stored at -80°C. A multimode microplate reader (BioTek, Winooski, VT) was used for
162 the quantification of AGEs. The % inhibition of AGEs formation= [1-(fluorescence of
163 the test group / fluorescence of the control group)] ×100% [19]. Each sample was

164 performed in triplicate and the experiment was done three times with comparable
165 results.

166 **3. Results**

167 **3.1 Structure elucidation**

168 Analysis of the ethanol extract obtained from the haulm of AS by repeated column
169 chromatography (AB-8, ODS, and Sephadex LH-20 or silica gel) led to the isolation
170 and identification one compound (ASF-1), along with three flavonoid compounds
171 (ASF-2~ASF-4).

172 The three flavonoids, kaempferol-3-O-glucuronide (ASF-2) [20],
173 rutin (ASF-3) [21] and luteolin (ASF-4) [22], were identified by comparison of their
174 spectroscopic data with those reported in the literature.

175 Compound ASF-1 had a molecular formula of $C_{11}H_{12}N_2O_2$ as determined by
176 ESI-HRMS, NMR ^1H,^{13}C, HMBC, HMQC data（Supplemental Figure 2-3). The
177 negative ESI-MS of compound ASF-1 showed a molecular ion peak at m/z 203.1000
178 $[M-H]^-$, indicating a molecular weight of 204. The ^1H NMR of data (Table 1) revealed
179 four aromatic H-atoms, whose signal from one proton in downfield was observed at d
180 7.62 (d, J = 8.0 Hz), was identified as H-4, while one proton at d 7.42 (d, J = 8.1Hz)
181 was evidenced to be H-7; and there were two other signals, one proton at d 7.17 (d,
182 8.1), the other at d 7.09 (t, J = 7.7 Hz), which were assigned to H-5 and H-6,
183 respectively. One proton resonance multiplet indicative of a methine group was
184 observed at δ_H 3.93(m), and two one-proton double doublets as methylene groups
185 attached to carbonyl moieties [δ_H 3.18 (dd, J = 7.2, 8.0Hz); δ_H 3.37 (dd, J = 10.6, 4.7

Hz)]. The ^{13}C NMR spectrum of ASF-1 (Table 1) indicated the presence of a carbonyl group (δ_C 174.4), an un-substituted aromatic ring (δ_C 111.9, 119.4, 122.1, 118.4), a tetra-substituted aromatic ring (δ_C 136.3, 126.6), a methylene group (δ_C 26.3), a methine group (δ_C 55.8), as well as two olefin carbons (δ_C 125.0, 107.5). This spectroscopic information was used to establish its core structure as an aromatic ring fused to a heterocyclic ring, which was confirmed by the HMBC correlation data (Figure 2): H-11 [δ_H 3.93 (m)] and H-10 [δ_H3.18 (dd, J =7.2, 8.0 Hz); δ_H 3.37 (dd, J = 4.7, 10.6 Hz)] were correlated with C-12 (δ_C 174.5) and the quaternary carbon at δ_C 107.5 (C-3), which confirmed the connection of CH-CH_2 and C=O moieties. Additionally, H-7 [δ_H 7.42 (d, 8.1)] was correlated with C-9 (δ_C 126.6) and C-6 (δ_C 119.4); H-4 [δ_H 7.62 (d, J = 8.0 Hz)] was correlated with C-5 (δ_C 122.1) and C-9 (δ_C 126.3). This suggested that C-7 and C-4 at the aromatic ring connected with a heterocyclic ring. Thus, ASF-1 was determined as L-tryptophan (Figure 1).

3.2. Inhibitory Effects of Compounds (ASF-2~ASF-4) on the Formation of AGEs

The flavonoid compounds (ASF-2~ASF-4) showed satisfactory suppression against the formation of AGEs in the β-lg-lactose/MGO/GO model systems (Figure 2). Increasing the concentration (0.05-0.5mmol/mL) of each compound gradually augmented the inhibitory activities against the formation of AGEs. Among these compounds, kaempferol-3-O-glucuronide exhibited the strongest anti-glycation capacity at the same concentration in three different β-lactoglobulin systems. The abilities of the four compounds to inhibit AGEs were: kaempferol-3-O-glucuronide > luteolin > rutin.

In the β-lg-lactose system, the respective inhibition ratios of kaempferol-3-O-glucuronide, rutin, or luteolin (0.05 mmol/L) against AGEs were 33.56%, 23.75%, and 22.38% at 120 min (Figure 2), as AGEs were slowly generated throughout the reaction. With an increase to 0.5 mmol/L, the inhibition ratios increased to 60.35%, 54.32% and 33.56% respectively. This suggests that the anti-glycation activities of these compounds have a positive correlation with concentration.

In the β-lg-MGO/GO systems (Figures 3 and 4), these three compounds also showed significant inhibition at 0.05mmol/L, although highly active MGO or GO led to more AGEs than lactose. Of these compounds, kaempferol-3-O-glucuronide is the most efficient AGEs inhibitor in different β-lactoglobulin systems. For 60% inhibition, 0.5mmol/L kaempferol-3-O-glucuronide was needed in the β-lg-lactose system at 85 °C for 120 min; while only 0.1mmol/L kaempferol-3-O-glucuronide needed in the β-lg-GO system and only 0.05mmol/L was needed the in β-lg-MGO system (Figures 2B, 3B, and 4B).

4. Discussion

In this study, a compound L-tryptophan was firstly isolated from AS haulm together with three flavonoid compounds. The structure of L-tryptophan was determined by HRMS spectra, 1D and 2D NMR data**.** The flavonoids showed powerful inhibitory effects against the formation of AGEs in β-lactoglobulin glycation.

Recent studies have shown that the flavonoids, e.g., flavanol (EGCG), chalcone

(phloretin and phloredzin), and isoflavone (genistein), rapidly traps MGO at C-6, C-8 unsubstituted carbons at the A ring and form mono- and di-MGO adducts, thus inhibiting the formation of AGEs [15-17].

The structures of these compounds can account for the difference in their activities. Kaempferol-3-O-glucuronide is the glycoside of the flavonol kaemoferol. Rutin is the glycoside between flavonol quercetin and disaccharide rutinose. As flavonols, they both have the same aromatic A ring, B ring, and a heterocyclic C ring. We found that kaempferol-3-O-glucuronide exhibits higher activity in the anti-glycation of β-lactoglobulin, although rutin has one more -OH than kaempferol-3-O-glucuronide at C-5′ on the B ring. However, if flavonoids possess the same A and C rings, a difference in the number of hydroxyl groups on the B ring does not play a significant role on trapping efficacy [23]. The greater efficiency of kaempferil-3-O-glucuronide is likely due to less steric hindrance from its single glycoside versus rutin's disaccharide, rutinose. The difference between rutin and luteolin is that luteolin is an aglycone of flavone, and there are no hydroxyl groups or glucosides at C-3 on luteolin's C ring. And our results indicate that luteolin has higher inhibitory effects than rutin. This is consistent with current literature that luteolin and rutin exhibit significant inhibitory effects of 82.2% and 77.7% in the BSA glycation system at pH 7.4 and 37 °C. We predict that the reason for this phenomenon is that luteolin lacks steric hindrance in the trapping reaction.

Our data highlight the effects of these compounds on glycation of β-lactoglobulin via α-dicarbonyl compounds. α-dicarbonyl compounds arise from free sugar, the

initial Schiff bases, Amadori and other intermediates [24-26]. Since α-dicarbonyl compounds and glucose can potentially damage different subsets of proteins, experimental outcomes may be different. While in the β-lg-lactose system, these flavonoid compounds also exhibited significant inhibition against glycation of β-lactoglobulin. The mechanism of inhibition of glucose-mediated glycation needs further discussion.

In addition, the high percentage of L-tryptophan in AS haulm could also contribute to the inhibitory effect on the glycation of β-lactoglobulin in β-lg-MGO/GO/lactose systems via the free amino group or imino group reacting with the reactive carbonyl.

Recently, flavonoids have shown more and more potential as strong inhibitors against the formation of AGEs *in vivo*, but there only a few studies that involved flavonoids in the anti-glycation of food proteins at high temperatures. We suggest that the flavonoids from various foods and natural plants can be used as effective AGEs inhibitors in food thermal processing. Our results in this study pave the way for further investigation of these compounds from AS haulm in real food matrices to confirm the inhibitory activities against the formation of AGEs.

Appendix

Supplemental Figure 1. Procedure of extraction and purification of flavones from *Artemisia Selengensis* haulm

Supplemental Figure 2. The HPLC-MS spectra of compound ASF-1. (A) HPLC−DAD chromatograms spectrum, (B) HRMS spectrum

Supplemental Figure 3. The ^{1}H NMR spectra of compound ASF-1. (A). ^{1}H NMR spectrum; (B). ^{13}C NMR spectrum; (C). HMQC spectrum; (D). HMBC spectrum

Acknowledgements

This work was supported by NSF of Jiangsu province of China (Project BK2012850) and Natural Science Foundation of Zhejiang province of China (Project LY12C15001). Our thanks to Aaron Yerke from North Carolina Agricultural and Technical University for his suggestions on revisions and editing.

Conflict of interest

There is no conflict of interest for all authors.

References

1. C. Deng, X. Xu, N. Yao, N. Li and X. Zhang, *Analytica Chimica Acta*, 2006, 556, 289-294.
2. L. Peng, Y. Wang, H. Zhu and Q. Chen, *Food Chemistry*, 2011, 125, 1064-1071.
3. Y. Zhu and M. Qin, *Chinese Archives Of Traditional Chinese Medicine*, 2006, 24, 1749-1786.
4. L. Peng, X. Jia, Y. Wang, H. Zhu and Q. Chen, *Food Analytical Methods*, 2009, 3, 261-268.
5. J. Zhang and L. Kong, *Chinese Traditional and Herbal Drugs*, 2008, 39, 23-26.
6. J. Zhang, Y. Lin and L. Kong, *Chinese Traditional and Herbal Drugs*, 2004, 35, 979-980.
7. K. A. Koo, Kwak, J.H., Lee, K.R., Zee, O.P., Woo, E.R., Park, H.K., Youn, H.J., *Archives of Pharmacal Research* 1994, 17, 371-374.
8. Z. Tu, L. Zhang, H. Wang, Y. Ye and W. Liu, *Science and Technology of Food Industry*, 2012, 33, 239-242.
9. X. F. Jinfeng Hu, *Chinese Chemical Letters*, 1998, 9, 829-832.
10. L. Zhang, Z.-c. Tu, T. Yuan, H. Wang, Z.-f. Fu, Q.-h. Wen and X.-q. Wang, *Industrial Crops and Products*, 2014, 56, 223-230.
11. E. B. Frye, T. P. Degenhardt, S. R. Thorpe and J. W. Baynes, *Journal of Biological Chemistry*, 1998, 273, 18714-18719.
12. P. Beisswenger, S. Howell, R. Nelson, M. Mauer and B. Szwergold, *Biochemical Society Transactions*, 2003, 31, 1358-1363.
13. J. Degen, M. Hellwig and T. Henle, *Journal of Agricultural and Food Chemistry*, 2012, 60, 7071-7079.
14. N. Rabbani and P. J. Thornalley, *Biochemical Society Transactions*, 2008, 36, 1045-1050.
15. S. Sang, X. Shao, N. Bai, C.-Y. Lo, C. S. Yang and C.-T. Ho, *Chemical research in toxicology*, 2007, 20, 1862-1870.
16. X. Shao, N. Bai, K. He, C.-T. Ho, C. S. Yang and S. Sang, *Chemical research in toxicology*, 2008, 21, 2042-2050.
17. L. Lv, X. Shao, H. Chen, C. T. Ho and S. Sang, *Chemical research in toxicology*, 2011, 24, 579-586.
18. W. Wang, Y. Yagiz, T. J. Buran, C. d. N. Nunes and L. Gu, *Food Research International*, 2011, 44, 2666-2673.
19. A. J. Furth, *Analytical biochemistry*, 1988, 175, 347-360.
20. N. Castillo-Muñoz, S. Gómez-Alonso, E. García-Romero, M. V. Gómez, A. H. Velders and I. Hermosín-Gutiérrez, *Journal of Agricultural and Food Chemistry*, 2008, 57, 209-219.
21. F. Fathiazad, A. Delazar, R. Amiri and S. D. Sarker, *Iranian Journal of Pharmaceutical Research*, 2006, 3, 222-227.
22. S. J. Kellam, K. A. Mitchell, J. W. Blunt, M. H. Munro and J. R. Walker, *Phytochemistry*, 1993, 33, 867-869.
23. X. Shao, H. Chen, Y. Zhu, R. Sedighi, C.-T. Ho and S. Sang, *Journal of Agricultural and Food Chemistry*, 2014, 62, 3202-3210.
24. E. Fujimori, *Biochim Biophys Acta*, 1989, 998, 105-110.
25. S. P. Wolff and R. T. Dean, *Biochemical Journal*, 1987, 245, 243-250.
26. J. V. Hunt, R. T. Dean and S. P. Wolff, *Biochemical Journal*, 1988, 256, 205-212.

Figure Caption

Figure 1. Structures of compounds ASF-1~ASF-4. A. L-tryptophan (compound ASF-1); B. kaempferol-3-O-glucuronide (compound ASF-2); C. rutin (compound ASF-3); D. luteolin (compound ASF-4).

Figure 2. Key HMBC correlations of compound ASF-1.

Figure 3. Inhibitory effects of Compounds ASF-2~ASF-4 on AGEs' formation in β-lg-MGO model under the ratio 1:18 at 85 ℃ for 120 min. Data are presented as the means ± SD of three replications. A. kaempferol-3-O-glucuronide (compound ASF-2); B. rutin (compound ASF-3); C. luteolin (compound ASF-4). Data are presented as the means ± SD of three replications.

Figure 4. Inhibitory effects of Compounds ASF-2~ASF-4 on AGEs' formation in β-lg-GO model under the ration 1:18 at 85 ℃ for 120 min. A. kaempferol-3-O-glucuronide (compound ASF-2); B. rutin (compound ASF-3); C. luteolin (compound ASF-4). Data are presented as the means ± SD of three replications.

Figure 5. Inhibitory effects of Compounds ASF-2~ASF-4 (A-D) on AGEs' formation in β-lg-Lactose model under the ratio 1:1000 at 85 ℃ for 120 min. A. kaempferol-3-O-glucuronide (compound ASF-2); B. rutin (compound ASF-3); C. luteolin (compound ASF-4). Data are presented as the means ± SD of three replications.

347 **Table 1**

348 ^{1}H and ^{13}C NMR chemical shift data [a] of ASF-1, ^{13}C-^{1}H long-range correlation signals

349 in the HMBC spectra (δ in ppm, J in Hz).

	δ_H (∫ , m, J Hz)	δ_C	HMBC ($^{1}H \rightarrow ^{13}C$)
2	7.20 (s)	125.0	C-3, C-9, C-10, C-11
3		107.4	
4	7.62 (d, 8.0)	118.4	C-5, C-9, C-3,C-8
5	7.17 (d, 8.1)	122.1	C-4, C-9, C-3
6	7.09 (t, 7.7)	119.4	C-7
7	7.42 (d, 8.1)	111.9	C-9, C-6
8		136.3	
9		126.6	
10	3.18 (dd, 7.2, 8.0); 3.37 (dd, 4.7,10.6)	26.3	C-3, C-11, C-12, C-2, C-9
11	3.93 (m)	55.0	C-10, C-3, C-12
12		174.4	

350 [a] Data was recorded in D_2O.

351

352 **Supplemental Figure Caption**

353 Supplemental Figure 1 Procedure of purification

354 Supplemental Figure 2 HPLC-HRMS Spectrum of ASF-1

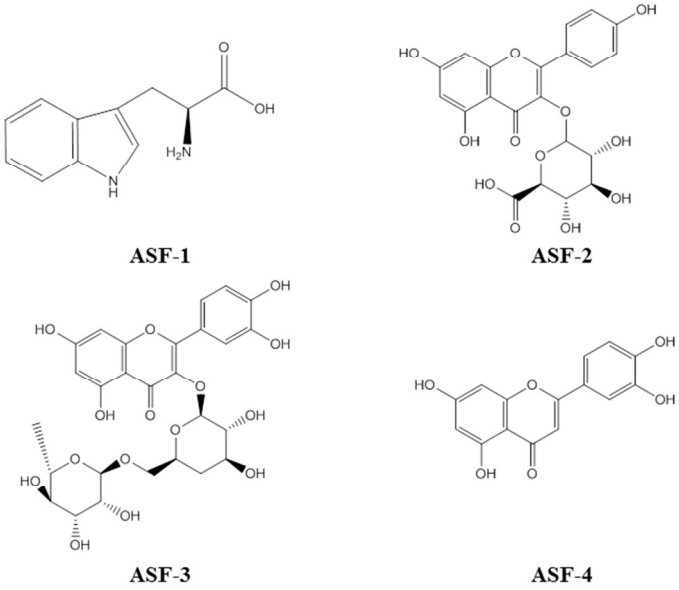
355 Supplemental Figure 3-A ^{1}H-NMR Spectrum of ASF-1

356 Supplemental Figure 3-B ^{13}C-NMR Spectrum of ASF-1

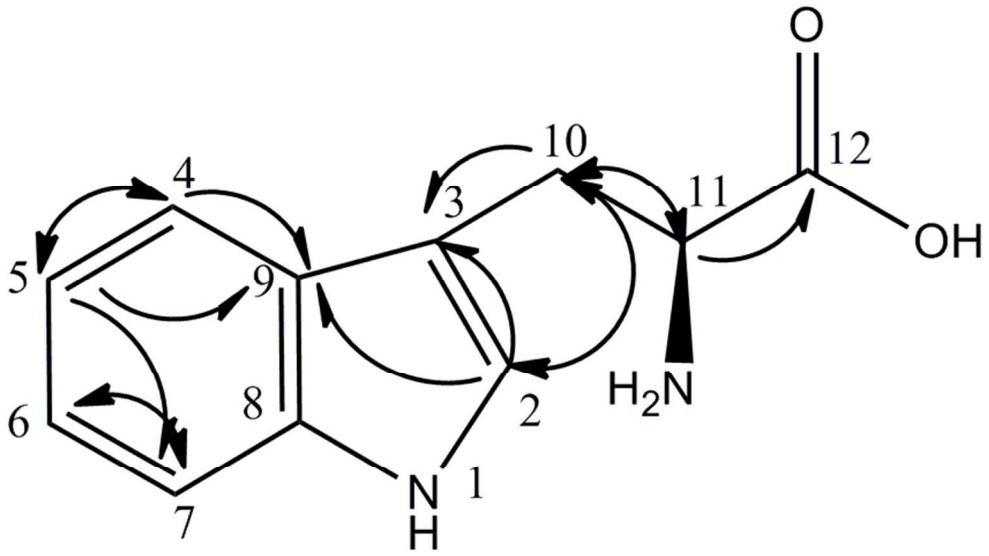
357 Supplemental Figure 3-C HMBC Spectrum of ASF-1

358 Supplemental Figure 3-D HMQC Spectrum of ASF-1

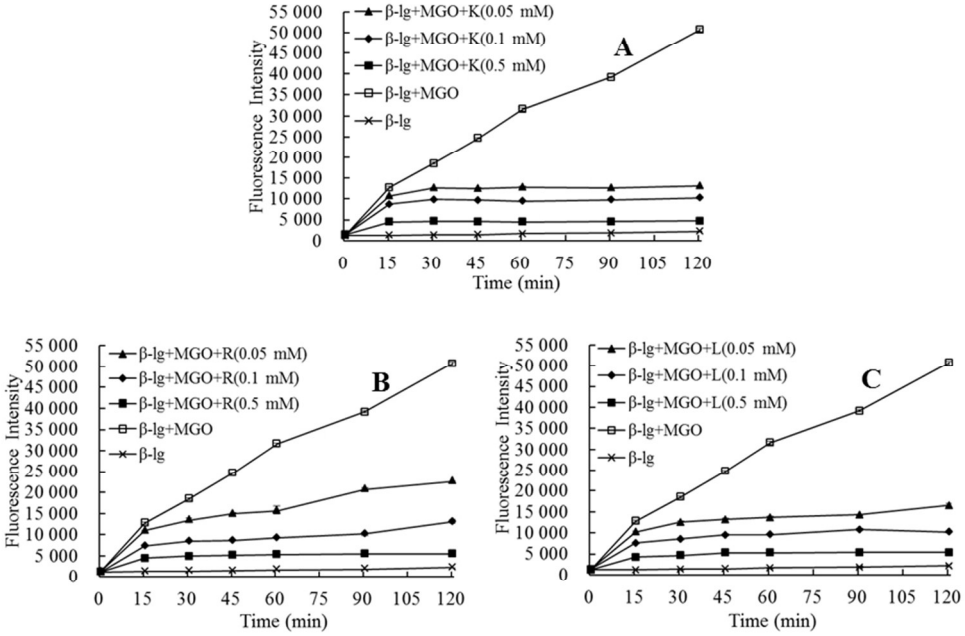
359



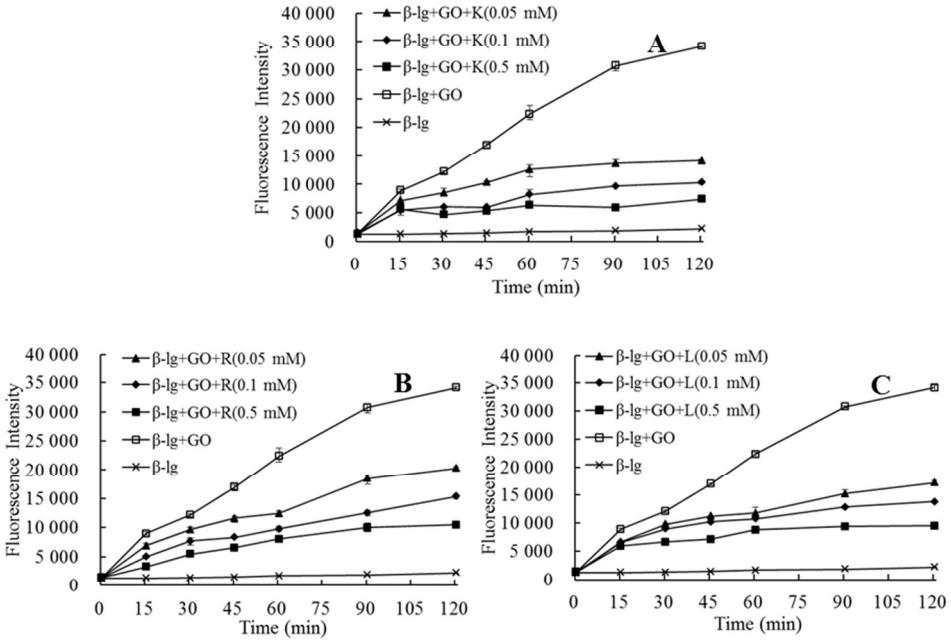
254x190mm (96 x 96 DPI)



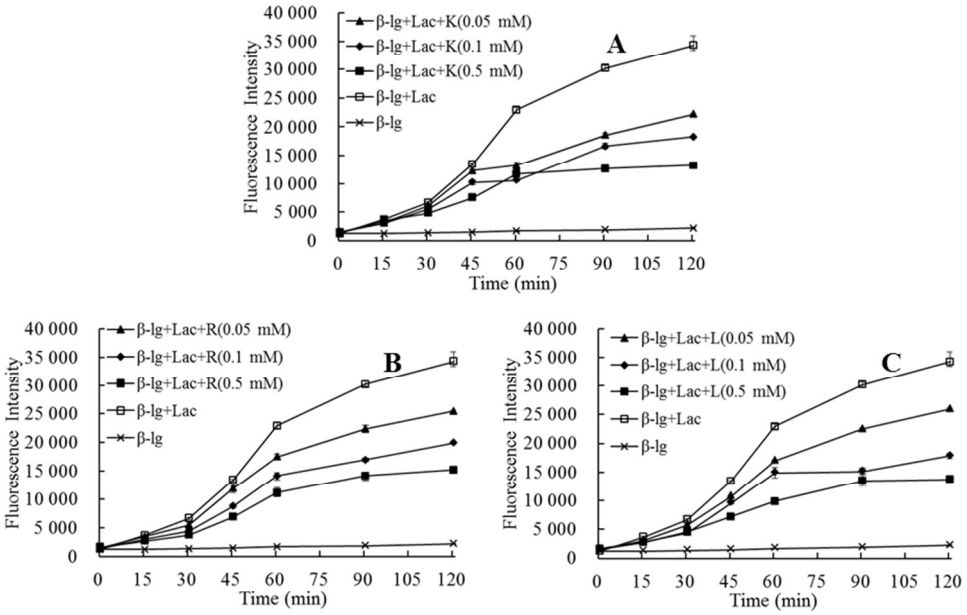
73x41mm (300 x 300 DPI)



254x190mm (96 x 96 DPI)



254x190mm (96 x 96 DPI)



254x190mm (96 x 96 DPI)