Title:

Relationship between antioxidant and antiglycation ability of saponins, polyphenols, and polysaccharides in Chinese herbal medicines used to treat diabetes

Yun-Fang Chen[1], Hsiao-Yuh Roan[1], Chong-Kuei Lii[2], Yuan-Ching Huang[1], Tsu-Shing Wang[1*]

1. Department of Biomedical Sciences, Chung Shan Medical University, Taichung 402, Taiwan, Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan
2. Department of Nutrition, China Medical University, Taichung, Taiwan

* Address correspondence to: Dr. Tsu-Shing Wang, Department of Biomedical Sciences, Chung Shan Medical University, No 110, Sec. 1, JianGuo N. Rd., Taichung 402, Taiwan; Fax: (886) 04-2475-7412; E-mail: tsushing@csmu.edu.tw

Short title: Antioxidant and antiglycation activities of Chinese herbal medicines

Abstract Advanced glycation end-products and oxidative stress have been implicated in the pathogenesis of diabetic complications. Both are known to interact with each other. Therefore, natural compounds or extracts that possess both antioxidant and antiglycation activities might have great therapeutic potential for treating diabetic complications. The main purpose of this study was to evaluate the antiglycation and antioxidant properties of aqueous extracts from 22 Chinese herbal medicines (CHMs) that are commonly used to treat diabetes. The total polysaccharide, polyphenol, and saponin contents in the CHM extracts were determined by use of standard methods to establish their contribution to the antioxidant and antiglycation properties. Our results showed that (1) half CHMs evaluated in this study possessed both high antiglycation and high antioxidant activities, and (2) the total saponin content was the major compound responsible for the antiglycation activity. The total saponin content may also contribute significantly to antioxidant activity. We conclude that the 11 CHMs with both high antiglycation and high antioxidant activities are potential bioactive compounds in the treatment of diabetes.

Keywords: Chinese herbal medicines · Antiglycation · Antioxidation · Polyphenols · Polysaccharides · Saponins

INTRODUCTION

Advanced glycation end-products (AGEs) are generated in the diabetic milieu as a result of chronic hyperglycemia and enhanced oxidative stress (Nakamura et al., 2007; Yamagishi et al., 2008a; Gul et al., 2009). Via direct and receptor-dependent pathways, AGEs promote the development and progression of diabetic complications, including neuropathy, nephropathy, and cardiovascular disease (Sugimoto et al., 2008; Fukami et al., 2008; Yamagishi et al., 2008b). AGEs can accumulate at many sites of the body in diabetes, including the heart and large blood vessels. Because the abundance of AGEs has direct relevance to the pathogenesis of diabetic complications, a clear understanding of the factors contributing to AGE formation may help in ameliorating tissue damage.

Recent reports have suggested that metal-catalyzed oxidation reactions play a major role in accelerating the rate of AGE formation (Chetyrkin et al., 2008; Argirova and Ortwerth , 2003; Price et al., 2001). Therefore, agents with antiglycation and antioxidant properties may retard the process of AGE formation by preventing further oxidation of Amadori products and metal-catalyzed glucose oxidation. In fact, inhibitors of AGEs that have antioxidant activity may act as preventive agents against diabetic complications. There is a long history of treating type II diabetes with Chinese herbal medicines (CHMs) in the Orient; however, few of the CHMs have had their pharmacological properties proved *in vitro* and *in vivo*. In the present study, we attempted to analyze the antioxidant and antiglycation activities of 22 CHMs popularly used in the clinical context. We also studied the relative influence of bioactive components (phenolics, polysaccharides, and saponins) in the antiglycation and antioxidant activities of selected CHMs.

MATERIALS AND METHODS

Preparation of the aqueous extracts

All CMHs were purchased from a Chinese drug store (Nanto, Taichung). Dry herbs were minced with a crushing machine (Yu Chi Machinery, Co., Taiwan). To prepare the aqueous extracts used for the *in vitro* studies, 0.5 g of minced dry herbs was mixed with 10 mL MQ water and stirred for 30 min at room temperature. The mixtures were filtered through Whatman no. 41 filter paper, and the filtrates were transferred to 15-mL conical tubes and spun at 3500 rpm for 30 min at 4°C. The supernatants were collected, aliquoted, and stored at –20°C until used. This solution was considered to be 100% aqueous extract and freshly diluted with MQ water to obtain the desired concentration in each experiment.

ABTS assay

The ABTS assay (Re et al., 1999) was used to measure the antioxidant activity of the aqueous extracts. ABTS was dissolved in MQ water to a concentration of 7 mM, and potassium persulfate was added to a concentration of 2.45 mM. The reaction mixture was allowed to stand at room temperature for 16 h in the dark before use. The resulting intensely colored ABTS•+ radical cation was diluted with 0.01 M PBS (phosphate-buffered saline), pH 7.4, to give an absorbance value of ~0.70 at 734 nm. Two microliters of ABTS•+ cation solution was mixed with 50 μL aqueous extract solution (0.12% final concentration) in a disposable microcuvette with a 1 cm path length. Absorbance was measured at 734 nm after reaction for 6 min. The assay was first carried out on glutathione, which served as a standard. Controls without ABTS•+ were used to allow for any absorbance of the extracts themselves. The ABTS scavenging activity was calculated as inhibition % = [1-(test sample absorbance / blank sample absorbance)] x 100. The reduced glutathione was used as positive

control.

Antiglycation assay

The antiglycation assay was performed according to the methods reported by Matsuura and colleagues with slight modifications (Matsuura et al., 2002). In all experiments, the final reaction volume was 0.5 mL and the reactions were performed in 1.5-mL microcentrifuge tubes. Albumin (4 mg/mL final concentration) was incubated with glucose (400 mM final concentration) in the presence of Chinese herbal medicines extracts (3% final concentration), aminoguanidine (positive control), or PBS as the control buffer at the specified concentration. The reaction was allowed to proceed at 60°C for 48 hours. The reaction mixture was allowed to cool at room temperature, and then 0.2 mL reaction mixture was transferred to a new tube and the reaction was stopped by adding 10 μL of 100% (w/v) trichloroacetic acid (TCA). The TCA-added mixture was kept at 4°C for 10 minutes before centrifugation at 15000 rpm. The precipitate was redissolved with 0.8 mL alkaline PBS (pH 10), and the relative amount of glycated bovine serum albumin (BSA) was immediately determined on the basis of fluorescence intensity by use of a spectro fluorometer F-4500 (Hitachi, Tokyo, Japan). The excitation and emission wavelengths used were at 370 nm and 440 nm, respectively. Results are expressed as percentage inhibition of formation of the glycated protein. % inhibition = $[1- (F_{BSA + glucose + CHM\ extract} - F_{BSA + CHM\ extract}) / (F_{BSA + glucose} - F_{BSA})] \times 100$, where F is fluorescence intensity. Aminoguanidine, a typical glycation inhibitor was used as positive control.

Determination of total polyphenols

The total content of polyphenols in the aqueous CHM extracts was quantitated by using the Folin-Ciocalteau colorimetric reaction. Aliquots of 0.5 mL of extract were

mixed with 150 mL 20% $NaCO_3$ and 50 μL of Folin-Ciocalteau reagent (Sigma) by vortex. Samples were then incubated in darkness at room temperature for 30 min. Absorbance was read at 730 nm by use of a spectrophotometer. Tannic acid was used as a reference standard, and the total polyphenol content was expressed as tannic acid equivalents (TAE, mg/mg extract).

Determination of total polysaccharides

Total polysaccharide contents in aqueous extracts from CHMs were determined by a phenol-sulfuric acid colorimetric assay (Dubois et al., 1951) with some modifications. Briefly, aqueous extract of CHM 0.2 mL and 0.8 mL of 99.5% ethanol were combined in a plastic tube and incubate in an ice bath for 1 hour. This mixture was centrifuged at 13000 rpm for 10 min, and the supernatant was removed without disturbing the pellet. The pellet was washed once with 0.8 mL of 99.5% ethanol, air dried and dissolved in a final volume of 0.6mL MQ water. Transfer 0.1 mL of sample to an equal volume of 4% (w/v) phenol and add 0.5 mL of concentrated sulfuric acid on ice in a 1.5-mL microcentrifuge tube. The reaction mixture was kept in a constant temperature water bath at 80°C for 30 min. After cooling to room temperature, the absorbance of the reaction mixture was determined at 492 nm by a Beckman DU800 spectrophotometer (Beckman Coulter, Fullerton, CA). The polysaccharide content was estimated by comparison with a standard curve generated from the analysis of glucose.

Determination of total saponins

The total saponin content of each aqueous CHM extract was estimated by using the method described by Hiai et al. (1976) with some modifications. The aqueous extracts of CHMs (0.1 mL) were mixed with vanillin (8% w/v, 0.1 mL) and sulfuric acid (72% w/v, 1 mL). The mixture was incubated at 60°C for 10 min and cooled in an ice bath for 15

min, and absorbance was read at 560 nm. Aescin was used as a reference standard, and the total saponin content was expressed as aescin equivalents (AE, μg/mg extract).

Statistical analysis

All data are expressed as the mean ± standard error from at least three independent experiments. Correlations of any parameters were analyzed by Pearson's correlation coefficient in bivariate correlation and linear regression analysis by using Microsoft Excel 2007 and Sigmaplot 10.0, respectively.

RESULTS

Antioxidant activity

ABTS is a stable radical that is often used to evaluate the antioxidant activity of natural compounds (Re et al., 1999). The activities of the extracts studied in scavenging free radicals are shown in Table 1. The definitions of the abbreviations used for the extracts are also given in Table 1. Twelve extracts (LC, AC, RG, AD, SB, PSu, LL, CM, CO, EFo, GU, and PSe) showed significant ABTS scavenging activity (% inhibition > 50%). However, the extracts of CL, PT, and PC had very low ABTS radical scavenging activity (% inhibition < 5).

Antiglycation activity

Antiglycation activity was determined by thermal glycation of BSA by D-glucose. These results are shown in Table 1. Fifteen extracts (LC, AMe, CP, AC, RG, AD, AO, SB, PSu, LL, CM, CO, EFo, EFe, and GU) had strong inhibitory activity of D-glucose-induced AGE formation (% inhibition > 50%). Among them, the extracts of AMe, CP, AO, and EFe had low ABTS radical scavenging activity. The 22 CHMs were divided into 5 groups according to their antiglycation and antioxidant activity (Figure 1). Group I that included 11 different CHM extracts possessed both high antiglycation and high antioxidant activities. Group II, with 4 different CHM extracts, possessed profound antiglycation activity but low antioxidant activity. Group III that has only 1 CHM extract possessed profound antioxidant activity but low antiglycation activity. Group IV possessed low antioxidant and low antiglycation activities, it included 5 different CHM extracts. Group V possessed neither antioxidant nor antiglycation activities, it included only 1 CHM extract. The percentages of extracts for each group were 52% for group 1, 18% for group II, 4.5% for group III, 22% for group VI, and

4.5% for group V. About 68% of the extracts possessed high antiglycation activity, and 55% of the extracts had high antioxidant activity. These results indicate that more than 50% of anti-diabetic CHMs tested in this study simultaneously possess high ABTS scavenging activity and high antiglycation activity. Among them, PSe was the only extract with high ABTS scavenging activity but low antiglycation activity. Moreover, these results also suggest that both antiglycation and antioxidant properties of CHMs may play a major role in controlling and preventing diabetes.

Total polyphenol, polysaccharide, and saponin contents

Total polyphenol, polysaccharide, and saponin contents were determined by Folin-Ciocalteau, phenol-sulfuric acid, and vanillin-sulfuric acid colorimetry assays, respectively. A wide range of total phenolic, polysaccharide, and saponin contents was found in the studied CHMs, as shown in Table 1. The total polyphenol content of the CHMs investigated in this study ranged from 0.3 to 49.5 mg/g tannic acid equivalent (TAE) of dried samples, with an average of 9.2 ± 2.7 mg/g TAE. Six CHM extracts (AC, SB, RG, PSe, EFo, and PSu) had a total of polyphenol content higher than the average value. The total polysaccharide content of the CHMs investigated in this study ranged from 0.5 to 175 mg glucose equivalents (GE) of dried samples, with an average of 19.7 ± 8.0 mg/g GE. Five CHM extracts (CP, CO, AMa, RG, and EFo) had a total of polysaccharide content higher than the average value. In addition, the total polysaccharide content of the CP extract was extremely high compared with the other CHM extracts. The total saponin content of the CHMs investigated in this study ranged from 0.9 to 121.7 mg ascein equivalents (AE) of dried samples, with an average of 37.9 ± 7.4 mg/g AE. The nine CHM extracts (AC, CP, RG, PSe, GU, CM, PSu, LL, and AMe) had higher total saponin content than the average value. Among the CHMs investigated in this study, only the RG extract contained a high total content

of polyphenol, polysaccharide, and saponin simultaneously. It also showed potent antiglycation and antioxidant activities when compared with the other CHM extracts.

DISCUSSION

Several previous studies have shown a close positive relationship between the total polyphenol content of plant extracts and antioxidant activities (Malencić et al., 2008; Alali et al., 2007; Kiselova et al., 2006; Maksimović et al., 2005). We used Pearson correlation and linear regression analysis to determine whether there was any correlation between antiglycation and antioxidant activities and total phenolic, polysaccharide, and saponin contents in the CHM extracts investigated in this study. The correlations are shown in Table 2. The Pearson correlation analysis indicated a weak correlation between antiglycation activity and total saponin content. The Pearson's correlation coefficient was 0.41 ($p = 0.07$). The analysis indicated a significant correlation between antioxidant activity and total phenolic and saponin contents. The Pearson's correlation coefficients between antioxidant activity and total phenolic and total saponin contents were 0.58 ($p = 0.004$) and 0.52 ($p = 0.013$), respectively.

We then divided the 22 CHMs into high and low activity groups according to antiglycation and antioxidant activity to compare the total contents of polyphenols, polysaccharides, and saponins; and a t test for comparison of two means was carried out. Figure 2 shows the mean values and standard errors, as well as the significance level reached. Although there was no significant difference among the total polyphenol, polysaccharide, and saponin contents between the groups of CHM extracts, the high antiglycation activity group of CHM extracts had a higher level of total polyphenol, polysaccharide, and saponin contents than did the low antiglycation activity group. Furthermore, it is important to note here that the difference in total saponin content was marginally significant ($p = 0.072$) between the high and low antiglycation activity groups of CHMs tested. These results indicated that saponin compounds might make a major contribution to the antiglycation activity of the CHM

extracts investigated in this study.

According to antiglycation activity, total polyphenol and saponin contents were significantly higher in the high antioxidant activity group of CHM extracts. However, the total polysaccharide content was less in the high antioxidant activity group than in the low antioxidant activity group. Obviously, these results again had confirmed that polyphenols are considered important contributors to the overall antioxidant activity of various plant extracts. Furthermore, these results also indicated that saponins in the CHM extracts could simultaneously act as both a free radical scavenger and a glycation inhibitor.

To investigate whether there was any correlation between antiglycation activity and antioxidant activity, we plotted free radical scavenging capacity and BSA glycation inhibition percentage (Figure 1). We found no significant linear relationship between the antiglycation activity and the antioxidant activity of the CHMs, which suggests that these two activities may be a result of unrelated mechanisms.

The biological functions of some CHM extracts examined in this study with both high antioxidant and high antiglycation activities have also been reported previously, including *Rehmannia glutinosa, Scutellaria baicalensis, Paeonia suffruticosa, Ligustrum lucidum, Chrysanthemum morifolium, and Glycyrrhiza uralensis* (Ha do et al., 2009; Liu et al., 2007, Liu et al., 2008; Waisundara et al., 2008; Gao et al., 2007; Yu et al., 2006; Kim and Lee, 2005; Li and Wang, 2004; Tang et al., 2004; Woźniak et al., 2004; Lee et al., 2003; Liu and Ng, 2000; Gao et al., 1999). Most of those studies were analyses of free radical scavenging activity or other antioxidant activities, however; fewer studies have been conducted on antiglycation or antidiabetic activity (Table 3). The consistency of findings between our study and others showed that our findings are reliable and applicable for further research. Furthermore, our results of this study showed for the first time that the aqueous extract of *Ligusticum chuanxiong,*

Artemisia capillaries, Angelicae dahuricae, Cassia obtusifolia, and *Eupatorium fortunei* possesses both significant antioxidant and antiglycation activity *in vitro*.

The hyperglycaemic state seen in diabetes mellitus is associated with the development of diabetes-specific microvascular complications and accelerated macrovascular disease (Hudson et al., 2002). Evidence implicates the formation and subsequent effects of advanced glycation endproducts (AGEs) as a contributing cause. The recent data further pointed out an increased oxidative damage in the vicinity of the glycated residues of histones (Gudes et al., 2010). These finding strongly suggest that initial glycation and thereby oxidation may interactive synergistically in the development of diabetic complications. Therefore, supply CHM extracts that combines these two types of properties may offer a novel therapeutic strategy for diabetes treatment. Although the correlation data were not strong enough to conclude which composition unequivocally correlated with the antiglycation activity, the marginally significant positive correlations between total saponin content and antiglycation activity were clearly higher than the correlations with the other two bioactive components within the CHM extracts. Therefore, we postulate that the saponins in the CHMs we tested may be the key compound for antiglycation activity. In fact, saponins as the key ingredients in Chinese medicines responsible for both antiglycation and antioxidant activities had also been addressed in few studies (Wang et al., 2011; He et al., 2010; Xi et al., 2010, 2008; Li et al., 2009; Liu and Henkel, 2002).

A recent report showed that the antioxidant and antiglycation activities of 11 antidiabetic traditional Chinese medicines are positively correlated with the extracted total saponin content (Xi et al., 2008), which is in agreement with our above postulation. Using similar analytical methods, including free radical scavenging and the BSA-glucose assay, Xi and his colleagues showed that *Acanthopanax senticosus*

had both potent antioxidant and antiglycation activities, similar to our findings. In addition to antiglycation and antioxidant activities, saponins from a variety of sources have been shown to have hypoglycemic activity (Francis et al., 2002; Abdel-Hassan et al., 2000; Rao & Gurfinkel, 2000), although the detailed molecular mechanism of this activity is still unclear. Recent clinical trials have also found that combined therapy of total saponins with sulfonylureas, a class of antidiabetic drugs, as a hypoglycemic drug could lower the blood glucose level and ameliorate clinical symptoms in the treatment of type II diabetic patients whose blood glucose levels were not well controlled by oral hypoglycemic agents (Lu et al., 2008). Furthermore, the therapy was relatively safe. Taken together, these findings suggest that combinations of single or multiple crude saponins from the CHMs we found to have both high antiglycation and high antioxidant activities might be a safe and effective therapeutic composition for diabetes treatment. Nevertheless, the detailed molecular mechanism of the antiglycation activity of the CHMs tested and the possible synergistic antiglycation activity between these CHMs and the pharmacological activities of the saponins derived from these CHMs need further investigation.

In this study, glucose-induced BSA glycation was under a high-temperature condition (60°C). Under such circumstances, the rate of BSA glycation was potently enhanced by glucose. The glycation at physiological 37°C proceeded at a much slower rate than at 60°C; thus, the incubation period was prolonged to at least 14 days (Choi et al., 2008; Li et al., 2008; Farsi et al., 2008; Xi et al., 2008). This condition may directly restrict the antiglycation activity of the extracts used at physiological 37°C. Although we did not run the same experiment at physiological 37°C, a recent report suggested that 60°C did not affect the antiglycation activity of a typical antiglycation agent, aminoguanidine (Jesdsadayammata, 2005). This study also showed clearly that the thermal-catalyzed formation of AGEs is a good rapid

method for defining the antiglycation activity of plant extracts *in vitro*.

The aqueous CHM extracts used in this study contained different amounts of natural compounds. Intrinsic fluorescence and fluorescence quenching by these natural materials may affect the detection of fluorescent AGE formation, especially when the fluorescent AGE generation is very low (Matsuura et al., 2002). In general, the extract samples showed significant background fluorescence intensity, and the levels were further increased when the extracts were co-incubated with BSA at 60°C for 48 hours (Figure 3). This increase in fluorescence intensity may have been due to the direct binding of the auto-fluorescent materials to BSA or may have been indirectly induced by the intrinsic sugars in the extracts. Although these interferences can be removed by the TCA precipitation procedure in the assay method of Matsuura that we adopted in this study, the auto-fluorescent material in the extract samples was not completely removed. However, the background fluorescence derived from the extract alone or from that co-incubated with BSA did not influence the determination of AGE formation by glucose in this study, because the background fluorescence from the appropriate control was subtracted. As a result, high antiglycation activity was detected in 68.2% (15/22) of the CHM extracts evaluated. This phenomenon indicates that Matsuura's assay *is an easy and reliable* m*ethod* of screening for inhibitors of protein glycation. Even though the TCA precipitation removes the interfering substances, the antiglycation inhibition percentage of several CHM extracts was higher than 100%. Thus, we could not totally exclude the false positive that results from the quenching effect of the CHM extracts on the fluorescence intensity of AGEs-BSA. Further refinement of this assay is needed to eliminate the false positives due to the fluorescence quenching by the CHM extracts.

CONCLUSION

We evaluated the antiglycation and antioxidant activities and total polyphenol, polysaccharide, and saponin contents of 22 CHMs commonly used to treat diabetes. In general, the aqueous extracts derived from these 22 CHMs showed a wide range of antiglycation and antioxidant activities and a wide range of total polyphenol, polysaccharide, and saponin contents. Eleven CHM extracts possessed both strong antiglycation and strong antioxidant activities *in vitro*. Moreover, our study revealed six different CHMs, including *Ligusticum chuanxiong*, *Codonopsis pilosula*, *Angelicae dahuricae*, *Chrysanthemum morifolium*, *Eupatorium fortune*, and *Euryale fero* displayed significant anti-diabetic potential for the first time. This information could be helpful to develop new medicinal preparations for diabetes and related symptoms.

In addition, the correlation analysis between the phytochemical composition of the extracts and their antiglycation and antioxidant activity indicated that the total saponin and polyphenol contents may be responsible for the antiglycation and antioxidant activities. Thus, the natural products derived from the 11 CHMs with high antiglycation and high antioxidant activities may offer remarkable prospects for the preventive treatment of diabetic complications. Our findings also provide a strong rationale for further investigation to understand the molecular mechanism of the antiglycation activity of these CHMs with both high antioxidant and high antiglycation activities and explore the possible synergistic antiglycation between them.

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FIGURE LEGENDS

Figure 1. Correlation between the antioxidant activity and the antiglycation activity of extracts of 22 Chinese herbal medicines (R^2 = 0.225). These data were obtained from the experiments reported in Table 1. Each CHM was specified with its own serial number as defined in table 1.

Figure 2. Comparison of the content of bioactive components between the low and high antiglycation or antioxidant activity groups. The extracts of each of the 22 Chinese herbal medicines from Table 1 were sub-grouped on the basis of the values observed for their antioxidant activity and antiglycation activity as being higher (■) or lower (□) than 50% inhibition.

Figure 3. Autofluorescence of extracts derived from Chinese herbal medicines in the absence or presence of bovine serum albumin incubated at 60°C for 48 hours. After incubation, fluorescence intensity was determined by antiglycation assay as described in “Materials & Methods”. Values are the mean from three independent experiments. The definitions of the abbreviations used for the extracts were given in Table 1.

Table 1. Antiglycation and antioxidant activities and composition of aqueous extracts derived from 22 Chinese herbal medicines

Chinese herbal medicine	AntiG[a] activity (%)	AntiO[b] activity (%)	TPP[c] (mg TAE[f]/g dry wt)	TPS[d] (mg GE[i]/g dry wt)	TS[e] (mg AE[j]/g dry wt)
1. Ligusticum chuanxiong (LC)	149.2 ± 16.1	100.7 ± 2.3	8.3 ± 0.6	6.1 ± 0.6	33.1 ± 0.7
2. Astragalus membranaceus (AMe)	131.7 ± 20.0	39.1 ± 11.2	4.3 ± 0.3	3.9 ± 0.3	39.7 ± 1.8
3. Codonopsis pilosula (CP)	130.1 ± 6.3	24.7 ± 1.1	1.7 ± 0.1	175.0 ± 4.5	97.4 ± 0.3
4. Artemisia capillaries (AC)	120.5 ± 4.2	101.1 ± 0.1	49.5 ± 1.0	10.1 ± 0.3	121.7 ± 4.0
5. Rehmannia glutinosa (RG)	110.9 ± 19.1	100.6 ± 0.5	17.1 ± 0.1	32.1 ± 3.8	90.8 ± 0.5
6. Angelicae dahuricae (AD)	104.5 ± 24.9	94.9 ± 1.6	3.8 ± 0.2	6.0 ± 0.4	15.4 ± 0.1
7. Alisma orientalis (AO)	100.2 ± 39.6	38.3 ± 1.0	2.1 ± 0.3	3.3 ± 0.2	21.9 ± 0.8
8. Scutellaria baicalensis (SB)	100.0 ± 10.8	97.3 ± 0.2	40.4 ± 4.0	8.8 ± 0.7	24.6 ± 0.7
9. Paeonia suffruticosa (PSu)	99.7 ± 9.4	101.1 ± 1.5	10.2 ± 0.1	2.4 ± 0.6	49.2 ± 3.5
10. Ligustrum lucidum (LL)	91.5 ± 26.0	99.3 ± 1.7	7.7 ± 0.2	7.0 ± 0.7	41.9 ± 5.0
11.Chrysanthemum morifolium (CM)	88.0 ± 6.6	81.2 ± 1.6	8.9 ± 0.3	15.1 ± 2.9	56.6 ± 9.7

12. Cassia obtusifolia (CO)	76.4 ± 1.1	81.2 ± 0.6	3.5 ± 0.7	53.1 ± 0.4	15.5 ± 5.5
13. Eupatorium fortunei (EFo)	69.6 ± 19.1	100.1 ± 0.5	11.1 ± 0.7	29.5 ± 1.7	27.6 ± 7.5
14. Euryale ferox (EFe)	62.1 ± 4.5	16.1 ± 2.2	0.7 ± 0.0	2.2 ± 0.5	1.6 ± 0.1
15. Glycyrrhiza uralensis (GU)	60.4 ± 0.9	91.3 ± 8.6	7.7 ± 0.0	6.7 ± 0.2	66.5 ± 4.8
16. Coix lacrymajobi (CL)	48.6 ± 5.6	3.4 ± 0.4	0.5 ± 0.0	6.4 ± 1.6	4.4 ± 0.1
17. Pinella ternate (PT)	42.0 ± 8.2	0.8 ± 0.6	0.6 ± 0.0	10.1 ± 0.3	1.2 ± 0.0
18. Radix trichosanthis (RT)	41.4 ± 1.1	32.8 ± 0.5	1.2 ± 0.1	1.6 ± 0.2	3.1 ± 0.2
19. Atractylodes macrocephala (AMa)	14.0 ± 0.2	29.1 ± 0.6	1.5 ± 0.1	41.3 ± 3.5	28.9 ± 9.9
20. Radix Puerariae (RP)	2.2 ± 25.3	32.7 ± 4.9	4.6 ± 0.2	1.9 ± 0.7	9.3 ± 2.3
21. Periploca sepium (PSe)	-9.5 ± 1.5	97.4 ± 0.9	16.3 ± 0.6	9.6 ± 1.9	81.4 ± 1.4
22. Poria cocoa (PC)	-118.3 ± 52.8	0.7 ± 2.0	0.3 ± 0.0	1.5 ± 0.9	0.9 ± 0.0

a: antiglycation; b: antioxidant; c: total polyphenols; d: total polysaccharides; e: total saponins; f: tannic acid equivalent; i: glucose equivalent; j: aescin equivalent. Percent inhibition (%) of antiglycation and antioxidant activities was determined for antioxidant activity and antiglycation activity at a concentration of 0.12% and 3%, respectively. The antiglycation activity (%) of positive control 1 mM glutathione and the

antiglycation activity (%) of the positive control mM aminoguanidine was 77.7 ± 3.0 and 71.7 ± 4, respectively.

Table 2. The Pearson correlation coefficient between antioxidant or antiglycation activity and the content of bioactive components in water extracts derived from 22 Chinese herbal medicines

Activity (%)	Total content of		
	Phenolics (mg TAE/g dry wt)	Polysaccharides (mg GE/g dry wt)	Saponins (mg AE/g dry wt)
Antiglycation	0.29 *p* = 0.187	0.23 *p* = 0.296	0.40 *p = 0.065*
Antioxidant	0.58* *p = 0.004*	-0.12 *p = 0.604*	0.52* *p = 0.013*

* was considered statistically significant ($p < 0.05$). The significance (two-tailed probability values) of a Pearson correlation coefficient *r* was calculated by use of free online *p*-value calculators (http://www.danielsoper.com/statcalc/calc44.aspx).

Table 3. Previously reported studies on the antiglycation and free radical scavenging activity in Chinese herbal medicine extracts that possessed both potent antiglycation and free radical scavenging activity in the present study

CHM	Free radical scavenging activity	Antiglycation or antidiabetic activity	Reference
Ligusticum chuanxiong	Yes	ND	Jeong et al., 2009
Artemisia capillaries	ND	ND	
Rehmannia glutinosa	Yes	Yes	Yu et al., 2006 Liu et al., 2008 Waisundara et al., 2008
Angelicae dahuricae	Yes	ND	Piao et al., 2004
Scutellaria baicalensis	Yes	Yes	Gao et al., 1999 Woźniak et al., 2004 Waisundara et al., 2008
Paeonia suffruticosa	Yes	Yes	Lee et al., 2003 Liu & Ng, 2000 Liu et al., 2007 Ha do et al., 2009
Ligustrum lucidum	Yes	Yes	Li & Wang, 2004 Gao et al., 2007
Chrysanthemum morifolium	Yes	ND	Kim & Lee, 2005
Cassia obtusifolia	ND	ND	
Eupatorium fortunei	ND	ND	
Glycyrrhiza uralensis	Yes	Yes	Tang et al., 2004

ND: No data.

Figure 1

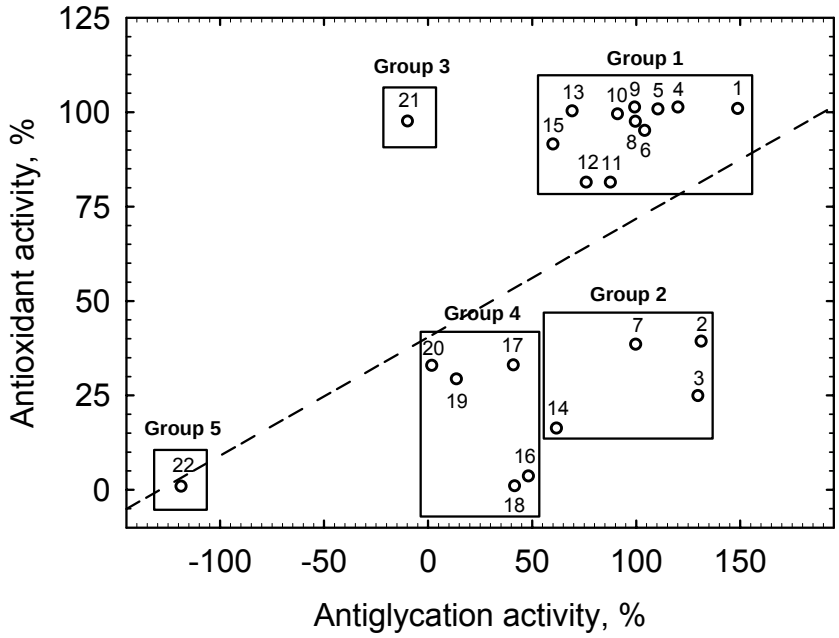

Antioxidant activity, %
Antiglycation activity, %
125
100
75
50
25
0
-100
-50
0
50
100
150
Group 1
Group 2
Group 3
Group 4
Group 5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

Figure 2

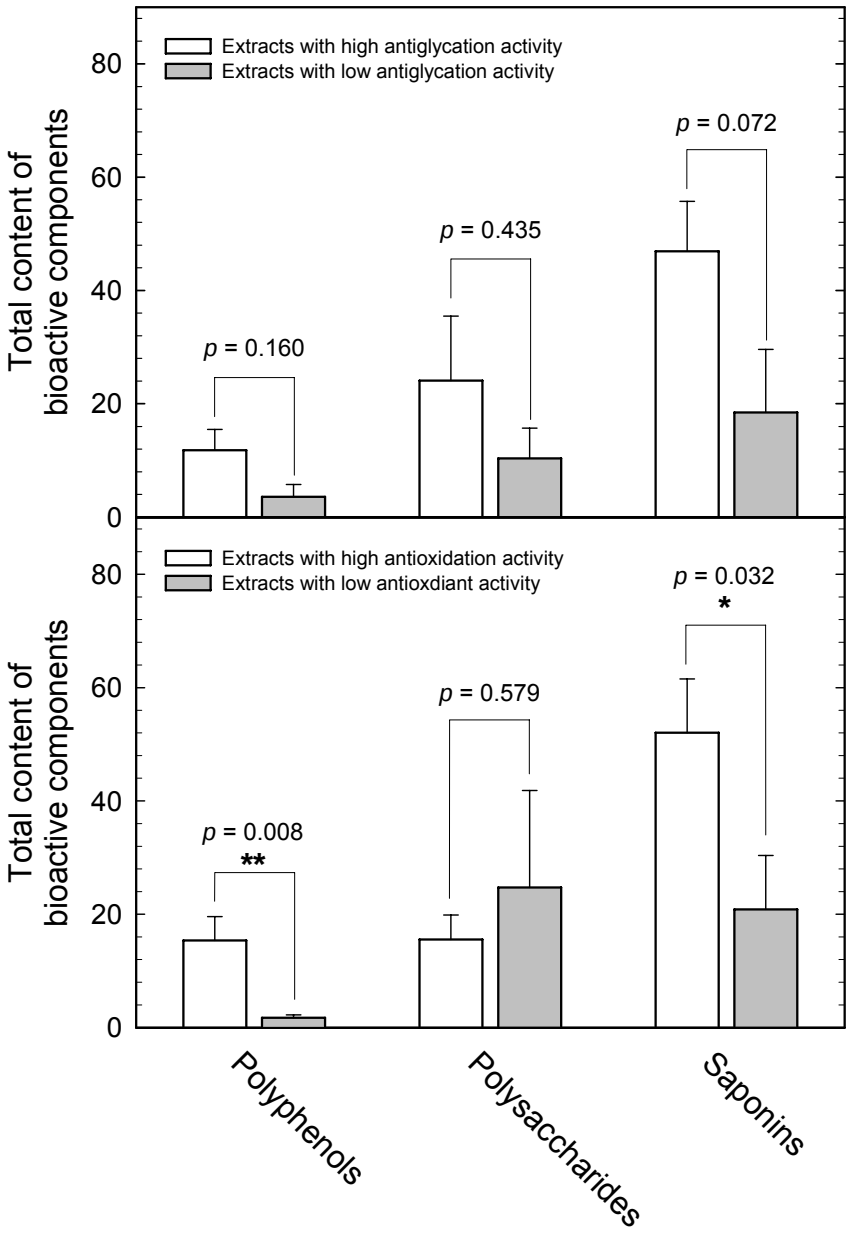
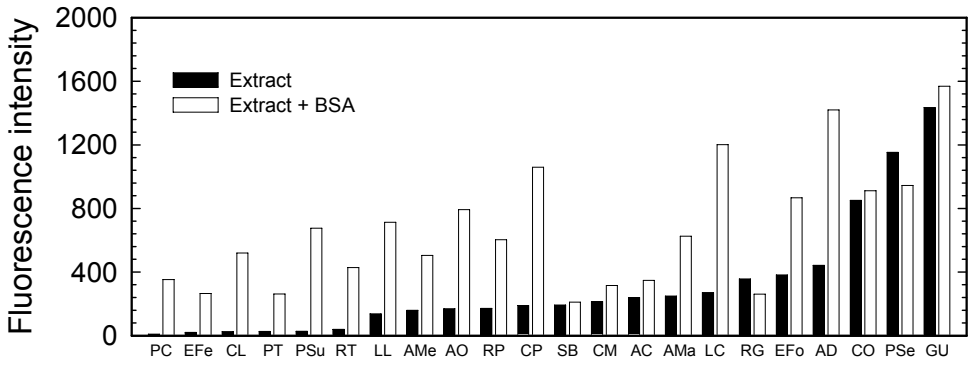

Extracts with high antiglycation activity
Extracts with low antiglycation activity
Total content of bioactive components
p = 0.160
p = 0.435
p = 0.072
0
20
40
60
80
Extracts with high antioxidation activity
Extracts with low antioxdiant activity
Total content of bioactive components
p = 0.008
**
p = 0.579
p = 0.032
*
0
20
40
60
80
Polyphenols
Polysaccharides
Saponins

Figure 3


Fluorescence intensity
2000
1600
1200
800
400
0
Extract
Extract + BSA
PC EFe CL PT PSu RT LL AMe AO RP CP SB CM AC AMa LC RG EFo AD CO PSe GU