Antiglycation and antioxidant activity of four Iranian medical plant extracts

Mohammad Reza Safari1, Omid Azizi2, Somayeh Sadat Heidary3, Nejat Kheiripour3, Alireza Pouyandeh Ravan∗1

Abstract

Objective: Diabetes mellitus (DM) is the most common metabolic disorder that defined by chronic hyperglycemia for the deficiency in insulin secretion or resistance. Hyperglycemia could induce non-enzymatic glycation of proteins. It has been suggested that some traditional plants can improve blood glucose and inhibit glycation process. This work evaluates and compares the anti-glycation activities of four Iranian plant extracts in vitro.

Methods: The methanolic extract of “Fumaria officinalis, Stachys lavandulifolia, Salvia hydrangea and Rosa Damascene” was prepared in three different concentrations. Phenolic, flavonoids content and antioxidant activity were evaluated. The multistage glycation markers- fructosamines (early stage), protein carbonyls (intermediate stage) and aggregation of albumin were investigated in the bovine serum albumin (BSA)/glucose system.

Results: All plants showed the high potency of scavenging free radicals and glycation inhibition in the following order: Fumaria officinalis> Rosa Damascene> Stachys lavandulifolia> Salvia hydrangea. There was a significant correlation between antioxidant and anti-glycation activity. Also, the antioxidant and anti-glycation capacity of extracts correlated with total phenolic and flavonoids content.

Conclusion: Our findings demonstrated that the studied plants are good sources of anti-glycation and antioxidant compounds and, these properties can primarily attributable to phenolics, particularly flavonoids.

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia. Prevalence of diabetes and its associated complications has been rising in the last few decades dramatically and leads to substantial morbidity and premature mortality and remains a major risk factor for cardiovascular disease worldwide [1]. Chronic hyperglycemia is the major factor responsible for vascular and organ complications in diabetes[2]. Uncontrolled excess blood glucose reacts with free amino acids of proteins to form a labile Schiff’s base and stabilizes in a compound known as
advanced glycation end products (AGEs) [3]. Glycation of proteins induce several structural modifications and modify the function of many proteins especially albumin. This process affects the affinity of albumin binding activity of drugs, hormones, fatty acids and other small solutes [4, 5]. In addition, it has been shown that albumin-derived AGEs, triggers the generation of intracellular reactive oxygen species (ROS) leading to glucose uptake inhibition and oxidative alteration of intracellular proteins [6]. Albumin has the ability to scavenge free radicals relying on its structure and, this protective feature is lost in the condition of uncontrolled diabetes [7]. Numerous AGEs inhibitors including pharmacological and natural compounds have been investigated for their potentials of impeding diabetes complications but, medicinal plants are proposed to be safer than others and many of them have the property of reducing harmful effects of hyperglycemia [8]. Fumaria officinalis (Fumariaceae) is a widespread perennial herb in Asia and Europe which has played a traditional role in empirical medicine such as treatment of skin diseases, rheumatism, hypertension or infections over centuries. Stachys lavandulifolia is grown in many parts of Iraq, Iran, and Anatolia. This plant is known as Chaye-kuhi in Iran and used as the herbal tea in gastrointestinal disorder [9]. Salvia species such as Salvia hydrangea (endemic to Iran) are rich in phenolic flavonoids and phenolic acids. Many of compounds in the species of this genera have antioxidant properties which act through enzymatic and non-enzymatic pathways [9-11]. Rosa damascene, known as Persian rose, is an erect shrub from the Rosaceae family. The antibacterial, anti-HIV, and antioxidant activities of the essential oil of Rosa damascena have been demonstrated [12]. Fumaria officinalis, Stachys lavandulifolia, Salvia hydrangea and Rosa damascene have long been used in traditional medicine in Iran, and their hypoglycemic effects in diabetes have been proven [13-15].

The objective of present study was to investigate the anti-glycation potential of these plant extracts. The multistage glycation markers fructosamines (early stage), protein carbonyls (intermediate stage) and aggregation of albumin using amyloid-specific dyes—Congo red are investigated. In addition, correlation of antioxidant effects with the extracts total phenolics, and antioxidant potential is also evaluated.

2. Materials and Methods

2.1. Chemical

All chemicals were of analytical grade: Bovine serum albumin (BSA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 4-dinitrophenyldrazine (DNPH), Thiobarbituric acid (TBA), Congo red, Trichloroacetic acid (TCA), Ethyl acetate and Gentamicin sulfate were purchased from Sigma Aldrich Chemical Company (Poole, UK). Glucose, Urea, and Ethyl alcohol were obtained from Carlo Erba (Carlo Erba Reagenti, Rodano, Milano, Italy). Methanol, Ethylenediaminetetraacetic acid (EDTA) and Hydrochloric acid (HCl) were purchased from Merck (Darmstadt, Germany).

2.2. Plant material and extract preparation

The plants were purchased from the medical and clinical centers. Aerial parts of plants collected and impurities removed, 500 g of the plants were washed, dried at room temperature, ground into powder and mixed with methanol 75% by the ratio of 1 to 10. Twenty-four hours later, the solution was filtered and dried at 37°C. Concentrations of 0.5, 0.25 and 0.12 g/dl in distilled water were prepared and used for subsequent measurements [15].

2.3. Antioxidant activity determinations

The antioxidant activity of the extracts was measured by investigating their ability to scavenge the DPPH free radical method as described previously with slight modifications [16]. Briefly, 0.1 ml of each extract at different concentrations (in distilled water) was added to 4 ml of a DPPH solution (50 μM in ethanol) as the free radical source and incubated for 10 min at RT. The reduction in the solution absorbance, due to proton donating property by component(s), was measured at 517 nm using UV-VIS spectrophotometer (α-1860 Split beam UV/Visible spectrophotometer, China). The DPPH radical reduction percentage was calculated from the following formula:

%scavenging [DPPH] = [(A0- A1)/A0] × 100

A0: The absorbance of sample without extract, A1: The absorbance of sample in the presence of extract

2.4. Determinations of total phenolic

Total phenolic contents were determined by Folin–Ciocalteau method as described previously with some modifications [17]. Briefly, extracts were reconstituted in d H2O (1 mg/ml). 500 μl of each extract or standard solution was added to 2.8 ml of d H2O and 100 μl of freshly prepared 50% Folin–Ciocalteau phenol reagent. Rapidly 1 ml of 20% Na2CO3 was added to the mixture. The test tubes were incubated in a water bath at 30°C for 90 min. Absorbance was measured at 750 nm using a UV-visible spectrophotometer. Distilled water was used as the reagent blank. For quantification of the total phenolic in the extracts, the Gallic acid standard curve was obtained from the absorbance of different concentrations (0-200 mg/l). Total phenolic content of the extracts was expressed as mg of Gallic acid equivalent (GAE) per 100 grams of extracts (mg GAE/100 g EX).

2.5. Determinations of flavonoid content

Flavonoids content determination of each extract was performed according to the Aluminum chloride colorimetric method with some modifications [17]. Briefly, 1 ml of d H2O was added to 100 μl of each extract or standard solution and 100 μl of 5% NaNO2. After 6 min, 100 μl of 10% AlCl3 was added and was incubated at room temperature for 6 min, and then 100 μl of NaOH (1 M) was added to the mixtures. The volume of reaction mixtures was adjusted to 2.5 ml with d H2O. The test tubes were thoroughly mixed and the absorbance of the developed pink color was measured at 510 nm. Using a point standard curve of Quercetin (0–50 mg/l), total flavonoid content was determined and expressed as mg quercetin per 100 g of extracts (mg QE/100 g EX).
2.6. Albumin glycation

Albumin glycation was performed, according to the method described by McPherson et al. with slight modifications [18]. Briefly, a 3.5 ml of total volume of glycation reaction solution includes different extract solutions (0.5 ml), 5% BSA (1 ml), glucose (1 ml, 166.5 mM) and gentamicin (1 ml, 20mg/dl in phosphate buffer 0.01M and pH: 7.4). All solutions were sterilized through 0.22 μm pore size filter. Following incubation at 37 °C for 72h, all the solutions were ensured to be free of microbiological contamination. Finally, the solutions were dialyzed extensively against phosphate buffer and the extra-unbound glucose and any other impurities were removed from the solutions.

2.7. Estimation of fructosamine production

To evaluate the fructosamine production, Thiobarbituric acid (TBA) assay was used according to the protocol described previously [15]. For this purpose, 1 ml of 20% Trichloroacetic acid (TCA) was added to 1 ml of glycated samples, the precipitate washed three times at 6000g for 10 min, solubilized in 1ml phosphate buffer and added to 0.5 ml 40% TCA. Following centrifugation at 6000g for 10 min, 0.5 ml of supernatant was mixed with TBA (0.05 M). The mixtures were incubated in a boiling water bath for 20 min, cooled to room temperature and the absorbance was read at the 443 nm. Inhibition of fructosamine was calculated as a percentage using the following formula:

\[
\text{Inhibitory activity (\%) = \frac{\text{A}_0 - \text{A}_1}{\text{A}_0}} \times 100
\]

\(A_0:\) absorbance of positive control group, \(A_1:\) absorbance in presence of the extract sample.

2.8. Estimation of carbonyl groups

The method of Uchida et al was chosen for albumin carbonyl compounds estimation with slight modifications [19]. In brief, an equal volume (0.5 ml) of glycated samples and DNPH (10 mM in HCL 2.5 M) were placed in glass tubes and incubated for one hour at room temperature. Proteins of the solution were precipitated by adding 0.5 ml of TCA 20% and the pellet was washed by ethanol-ethyl acetate (1:1 v/v) for three times and dissolved in 1 ml of 6M urea. The UV absorbance was recorded at 365 nm and the carbonyl concentration was calculated by using the molar extinction coefficient of DNPH (6365nm = 21 mM per cm). The percentage of inhibition was calculated as follow:

\[
\text{Inhibition \% = \frac{1 - \text{A}_0 - \text{A}_1}{\text{A}_0}} \times 100
\]

\(A_0:\) absorbance of positive control group, \(A_1:\) absorbance in presence of the extract sample.

2.9. Estimation of amyloid β-structures

Congo red binding to β-amyloid aggregation in the samples was estimated by measuring absorbance at 530 nm [20]. Accordingly, 0.5 ml of glycated albumin was mixed with 100 μl of Congo red (100 Mm solution in phosphate-buffered saline, 10% ethanol) and incubated for 20 min at room temperature. The absorbance was measured at 530 nm. The percentage of inhibition measured by the following equation:

\[
\text{Inhibition \% = \frac{1 - \text{A}_0 - \text{A}_1}{\text{A}_0}} \times 100
\]

\(A_0:\) absorbance of positive control group, \(A_1:\) absorbance in presence of the extract sample.

2.10. Total inhibition of glycation reaction

To evaluate the total inhibition of glycation for each extract, IC50 was calculated for TBA, DNPH and Congo red dye methods and the average was expressed as Mean ± S.E.M.

2.11. Statistical analysis

All experimental data were obtained from at least 3 independent tests for each extract and presented as mean ± SEM. The significance of differences between treatments was tested by ANOVA (Tukey test), using SPSS 16 software. Correlation between two variants was analyzed by Pearson test. Significance value was set at \(p < 0.05\).

3. RESULTS

Total phenol and flavonoid content and, the antioxidant activity of the extracts are summarized in Table 1. The most amounts of phenol and flavonoid content were found in Fumaria officinalis and the lowest amount was obtained from Salvia hyangrane. The result of the antioxidant capacity evaluation of each plant extract by DPPH radical cleaning method was expressed as IC50. Similar to phenol and flavonoid results, Fumaria officinalis and Salvia hyangrane showed the highest (IC50: 122.38 ±8.57) and lowest (234.38± 13.73) antioxidant capacity respectively. The results shown in Table 2 indicate that all extracts inhibited albumin glycation at three stages: fructosamine generation (TBA), carbonyls formation (DNPH) and β-amyloid aggregations (Congo red). The manner of inhibition was dose-dependent and the most effective dose for each extract was 0.5 gr/ dl. In addition, at the same concentration, the inhibitory results were significantly different for each extract as compared to the others. Accordingly, it was found that Fumaria officinalis had the maximum (IC50: 1.04 ±0.05), IC50: 1.44 ±0.02 and Salvia hyangrane exerted the minimum inhibitory effects (IC50: 1.66± 0.02, IC50: 2.91± 0.15) on fructosamine and carbonyls formation respectively. Interestingly, in the case of β-amyloid aggregations, results were different. The highest inhibition of β-amyloid aggregations belonged to Fumaria officinalis again (IC50: 0.002 ± ±0.002), but at this stage, Stachys lavandulifolia have the lowest inhibitory effects (IC50: 1.41± 0.5).

Total inhibitory property of glycation for each extract regardless the level and rate of inhibition were shown in table 3.

Total glycation inhibition indicates the overall inhibitory results were significantly different for each extract as compared to the others. Accordingly, it was found that Fumaria officinalis had the maximum (IC50: 1.04 ±0.05), IC50: 1.44 ±0.02 and Salvia hyangrane exerted the minimum inhibitory effects (IC50: 1.66± 0.02, IC50: 2.91± 0.15) on fructosamine and carbonyls formation respectively. Interestingly, in the case of β-amyloid aggregations, results were different. The highest inhibition of β-amyloid aggregations belonged to Fumaria officinalis again (IC50: 0.002 ± ±0.002), but at this stage, Stachys lavandulifolia have the lowest inhibitory effects (IC50: 1.41± 0.5).

Total inhibitory property of glycation for each extract regardless the level and rate of inhibition were shown in table 3. Total glycation inhibition indicates the overall inhibition of extract obtained from TBA, DNPH and Congo red methods. Cumulatively, the most promising effect against glycation was observed in the presence of Fumaria officinalis (IC50: 0.83 ±0.01) and in Salvia hyangrane least anti-
glycation activities were detected (IC50: 1.95 ± 0.05).
The correlation analysis demonstrated that total anti-
glycation activity or TBA, DNPH and Congo red reactions for
a particular extract was strongly correlated with its antiox-
idant capacity (DPPH-radical scavenging) (Table 4). Like
antioxidant capacity, anti-glycation activity was strongly
correlated with phenolic and flavonoid contents (table 5).

4. DISCUSSION

Long-standing hyperglycemia in DM can change proteins
structures in both tissues and serum. Alteration in protein
structure is due to the non-enzymatic glycation activity of
proteins and leading to malfunction of proteins [6]. Many
attempts have been made to prevent the process of pro-
glycation and some of them were made in the field of
herbal medicines. In traditional medicine, some medici-
val plants have been observed to attenuate hyperglycemia
in diabetes mellitus [21]. In the present study, we evalua-
ted inhibition activity of four Iranian medicinal plant ex-
tracts on albumin glycation, in vitro. First, we examined
the antioxidant capacity. Our findings showed that Fu-
maria officinalis, Rosa Damascene, Stachys lavandulifol-
ia and Salvia hydrangea have the highest amount of phe-
nolic and flavonoid compounds and antioxidant capacity
respectively. In addition, phenol concentration was more
than flavonoids in all extracts. Pearson correlation analysis
indicated that there is a significant correlation between the
antioxidant capacity and the phenolic (P< 0.000) and flavo-
noid content (P<0.001) in the extracts. Persistent hypergly-
cemia induces a condition of oxidative stress by oxidization
of excess glucose and generation of ROS [22]. Protein de-
rivatives such as carbonyls and modified amino acids arise
from direct attack of ROS. The strategy of applying anti-
dioxidant has been extensively studied and their protective
effects against free radical damages are approved [23]. In our
investigation, all extracts showed antioxidant activity but,
the best potential of antioxidant and scavenging free radici-
cals were achieved with Fumaria officinalis. This was con-
sistent with the results of other studies. Ivanov et al. found
that officinalis has the highest amount of phenolic content
and antioxidant properties among other species of Fuma-
ria [24]. Also, Rosa damascene, Stachys lavandulifolia, and
Salvia hydrangea have been demonstrated to be a good re-
source of antioxidants due to the content of phenolic and
flavonoids compounds [25-27].

Phenols and flavonoids are categorized as antioxidants
and their protective effects in diabetes have been report-
ed. Some studies indicate the more content of phenol and
flavonoids have more effects on hyperglycemic protection
[28]. The findings of our study showed such effects. Glyca-
tion of albumin including the formation of fructosamine,
carbonyl groups, and amyloid β-structures, significant-
lly attenuated in the presence of extracts. The initial step
of AGEs formation also known as the Maillard reaction
starts up through nucleophilic attachment reaction of
free amino groups of proteins with the carbonyl group of
reducing sugars to form reversibly a Schiff base product,
which in turn convert to stable fructosamine residue (ke-
toamine) by an Amador rearrangement. Schiff’s base and
fructosamines have been named early glycation products.

These adducts could undergo subsequent oxidation, re-
arrangement, dehydration, and cyclization to form sta-
ble agents called advanced glycation end products AGEs.
AGEs are extremely reactive irreversibly bound com-
plex structures that continue to react with nearby amino
groups of proteins to generate intra- and intermolecular
crosslinks [29-32]. It has been demonstrated that AGEs in-
hibitors can impede the generation of reactive dicarboxyls
and oxygen species [6]. Antioxidants such as phenols and
flavonoids have been suggested that inhibit AGEs forma-
tion and such properties have been attributed to the struc-
ture of these compounds. Actually, adjacent OH-groups
identified to be responsible for their antioxidant and an-
ti-glycation activity [33, 34]. The association between an-
tioxidant and antglycation activity was apparent in the
way that maximum glycation inhibition was assigned to
Fumaria officinalis which showed the highest antioxidant
property. Salvia hydrangea exhibited the lowest power in
the prevention of fructosamine and carbonyl formation,
so we expected to observe the similar effect in Congo
red experiment but, the result was different and Stachys
lavandulifolia with more antioxidant capacity than Salvia
hydrangea, showed the lowest ability to prevent amyloid
β-structures formation. This property can be justified due
to some evidence. It has been shown that despite the ex-
istence of an association between antioxidant content and
antiglycation capacity [15, 35, 36], some plants with strong
antiglycation activity possess low antioxidant activity [36].
The result of our study indicates that all extracts were able
to remove free radicals and prevent albumin glycation
and these properties were strongly correlated with each
other. We also examined three different concentrations of
0.5, 0.25 and 0.12 gr/dl in all Experiments. Our findings
demonstrated that more concentration for each extract
will result in more inhibition of albumin glycation. There-
fore, it seems that the ability of antioxidant and antiglyca-
tion of plants was due to phenolic and flavonoid concen-
tration. Previous, Fathiazad, Gholamhoseinian, and Zarei et al
showed that administration of Fumaria parviflora, Rosa
damascene, and Salvia hydrangea has a significant hypo-
glycemic effect on streptozotocin-induced diabetic rats
[13, 27]. Also, Rahzani et al suggested that Stachys lavan-
dulifolia has a strong capacity in reducing oxidative stress
and managed to be useful in diseases related to the accu-
mulation of free radicals [37]. According to these results
and medical history of plants studied in the present study,
positive effects of the extracts on reducing albumin glyca-
tion were not unexpected and their antiglycation activity
was demonstrated.

Although for several synthetic AGEs inhibitors hopeful
advances have been obtained in vitro and in animal mod-
els, still most of the clinical trials have been more or less
unsatisfactory in part because of unpredicted side-effects.
Thus, petition for natural or herbal agents has increased in
diabetics.

5. Conclusion

In conclusion, our result’s support the opinion that anti-
diabetic herbs are reasonable agents to inhibit oxidative stress and glycation. Based on their anti-glycation capacities, we suggest that the consumption of plant extracts may promote the health of the diabetics by discounting AGEs-induced complications. These new outcomes offer evidence for a novel mechanism by which antidiabetic traditional plants might have extra protective effects and lay the foundation for future in vivo investigations against diabetic complications.

Acknowledgements

This study was financially supported by Hamadan University of Medical Sciences (Grant No. 9303131155).

Conflict of Interests

The authors declared no competing interests.

Table 1 Total phenols, flavonoids concentration and antioxidant activity of plant extracts.

| Plant extract          | Phenol total (mg GAE/100 g EX) | Flavonoid total (mg QE/100 g EX) | Radical clearance DPPH IC50 |
|------------------------|---------------------------------|----------------------------------|-----------------------------|
| *Fumaria officinalis*  | 64.10± 1.77                     | 18.13± 2.27                     | 122.3± 8.57                 |
| *Rosa Damascene*       | 51.05± 2.50                     | 13.84± 0.98                     | 148.14± 13.20               |
| *Stachys lavandulifolia* | 40.50±1.43                   | 11.53± 0.55                     | 189.10± 17.96               |
| *Salvia hydrangea*     | 16.05±2.29                      | 8.84± 0.83                      | 234.63± 13.73               |

The results were calculated as Mean ± SEM.

Table 2 The percentage of glycation inhibition expressed as TBA, DNPH and Congo red in different concentration of plant extracts as

| Plant extract          | Inhibition (%) | Concentration (gr/dl) |
|------------------------|----------------|-----------------------|
|                        |                | 0.5                   | 0.25                   | 0.12 | IC50  |
|                        |                |                       |                       |     |      |
| *Fumaria officinalis*  | 94.17± 1.74    | 80.56± 1.83           | 63.15± 1.78            | 1.04± 0.05 |
| *Rosa Damascene*       | 82.84± 1.99    | 70.43± 1.87           | 49.40± 1.74            | 1.20± 0.02 |
| *Stachys lavandulifolia* | 67.98± 2.33   | 62.65± 1.42           | 42.38± 3.09            | 1.29± 0.03 |
| *Salvia hydrangea*     | 48.21± 1.43    | 36.35± 1.91           | 29.06± 2.68            | 1.66± 0.02 |
|                        |                |                       |                       |     |      |
|                        |                | TBA                   | DNPH                   | Congo red |
| *Fumaria officinalis*  | 60.34± 2.13    | 47.92± 2.5            | 37.69± 1.72            | 1.44± 0.02 |
| *Rosa Damascene*       | 45.16± 2.17    | 39.10± 1.85           | 31.11± 2.14            | 1.75± 0.08 |
| *Stachys lavandulifolia* | 37.63± 3.20  | 27.99± 2.01           | 24.74± 1.84            | 2.07± 0.32 |
| *Salvia hydrangea*     | 25.32± 1.86    | 20.22± 1.37           | 18.01± 1.61            | 2.91± 0.15 |
|                        |                |                       |                       |     |      |
| *Fumaria officinalis*  | 93.56± 2.86    | 84.08± 2.39           | 73.25± 1.81            | 0.002± 0.002 |
| *Rosa Damascene*       | 72.16± 1.85    | 58.08± 3.00           | 47.19± 3.49            | 1.27± 0.30 |
| *Stachys lavandulifolia* | 58.94± 4.45   | 49.06± 2.85           | 44.49± 2.21            | 1.41± 0.50 |
| *Salvia hydrangea*     | 64.67± 3.78    | 54.5± 3.53            | 48.34± 1.73            | 1.28± 0.01 |

The results were calculated as Mean ± SEM.
**Table 3** Total inhibition of albumin glycation obtained from TBA, DNPH and Congo red tests

| Plant extract              | IC<sub>50</sub> | Glycation inhibition reaction assay | \( IC_{50} \) | Total inhibition |
|----------------------------|-----------------|-------------------------------------|----------------|-----------------|
|                           | TBA             | DNPH                               | Congo red      |                 |
| *Fumaria officinalis*      | 1.04 ± 0.05     | 1.44 ± 0.02                         | 0.002 ± 0.002  | 0.83 ±0.01      |
| *Rosa Damascene*           | 1.20 ± 0.02     | 1.75 ± 0.08                         | 1.27 ± 0.03    | 1.41 ± 0.01     |
| *Stachys lavandulifolia*   | 1.29 ± 0.03     | 2.07 ± 0.32                         | 1.41 ± 0.05    | 1.59 ± 0.10     |
| *Salvia hydrangea*         | 1.66 ± 0.02     | 2.91 ± 0.15                         | 1.28 ± 0.01    | 1.95 ± 0.05     |

The results were calculated as Mean ± SEM.

**Table 4** Correlation coefficients of TBA, DNPH, Congo red and total glycation inhibition with antioxidant capacity (DPPH)

|                         | DPPH scavenging \( IC_{50} \) | Coefficient (probability) |
|-------------------------|---------------------------------|---------------------------|
|                         | TBA \( IC_{50} \)               | \( r = 0.951^* \)         |
|                         | \( P = 0.000 \) **              |                           |
|                         | DNPH \( IC_{50} \)              | \( r = 0.920^* \)         |
|                         | \( P = 0.000 \) **              |                           |
|                         | Congo red \( IC_{50} \)         | \( r = 0.677^* \)         |
|                         | \( P = 0.016 \) **              |                           |
|                         | Total Antiglycation \( IC_{50} \)| \( r = 0.917^* \)         |
|                         | \( P = 0.000 \) **              |                           |

* Whatever the correlation coefficient is closer to 1, more correlation is observed between properties
** Significant correlation between variants \( P<0.05 \).

**Table 5** Correlation coefficients of total phenolic and flavonoids with DPPH-radical scavenging and antiglycation capacity

|                         | Total phenol Coefficient (probability) | Total flavonoid Coefficient (probability) |
|-------------------------|----------------------------------------|------------------------------------------|
|                         | DPPH-scavenging \( IC_{50} \)          | TBA \( IC_{50} \)                        |
|                         | \( r = -0.947^* \)                     | \( r = -0.940^* \)                      |
|                         | \( P = 0.000 \) **                     | \( P = 0.000 \) **                      |
|                         | DNPH \( IC_{50} \)                     | Congo red \( IC_{50} \)                 |
|                         | \( r = -0.980^* \)                     | \( r = -0.839^* \)                      |
|                         | \( P = 0.000 \) **                     | \( P = 0.001 \) **                      |
|                         | Congo red \( IC_{50} \)                | Total antiglycation \( IC_{50} \)       |
|                         | \( r = -0.962^* \)                     | \( r = -0.944^* \)                      |
|                         | \( P = 0.000 \) **                     | \( P = 0.000 \) **                      |
|                         | Congo red \( IC_{50} \)                | Total antiglycation \( IC_{50} \)       |
|                         | \( r = 0.874^* \)                      | \( r = 0.920^* \)                      |
|                         | \( P = 0.016 \) **                     | \( P = 0.000 \) **                      |
|                         | Total Antiglycation \( IC_{50} \)      |                                          |
|                         | \( r = 0.941^* \)                     |                                          |
|                         | \( P = 0.000 \) **                     |                                          |

*Whatever the correlation coefficient is closer to -1, higher correlation is observed between properties
** Significant correlation between variants \( P<0.05 \).
References

1. Janghorbani M, Van Dam RM, Willett WC, Hu FB. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. Am J Epidemiol. 2007;166:495-505.
2. Ghadermazi R, Khoshjou H, Hoseini Zijod SM, Behrooj H, Kheiripour N, Ganji M, et al. Hepatoprotective effect of tempol on oxidative toxic stress in STZ-induced diabetic rats. Toxicin Rev. 2017;37(1):1-5.
3. Cade WT. Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. Phys Ther. 2008;88(11):1322-35.
4. Lee P, Wu X. Modifications of human serum albumin and their binding effect. Curr Pharm Des. 2015;21(14):1862-5.
5. Anguizola J, Matsuda R, Barnaby OS, Hoy K, Wa C, De-Bolt E, et al. Glycation of human serum albumin. Clin Chim Acta. 2013;425:64-76.
6. Singh VP, Bali A, Singh N, Jaggi AS. Advanced glycation end products and diabetic complications. Korean J Physiol Pharmacol. 2014;18(1):1-14.
7. Garcia-Martinez R, Caraceni P, Bernardi M, Gines P, Arroyo V, Jalan R. Albumin: pathophysiologic basis of its role in the treatment of cirrhosis and its complications. Hepatology. 2013;58(5):1836-46.
8. Peng X, Zheng Z, Cheng K-W, Shan F, Ren G-X, Chen P, et al. Inhibitory effect of mung bean extract and its constituents vetivixin and isovetivixin on the formation of advanced glycation endproducts. Food Chem. 2008;106(2):475-81.
9. Javidnia K, Mojaf M, Mojahedi S. Chemical constituents of the essential oil of Stachys lavandulifolia Vahl from Iran. Iran J Pharm Res. 2004;3:61-3.
10. Perry NS, Bolten C, Perry EK, Ballard C. Salvia for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. Pharmacol Biochem Behav. 2003;75(3):651-9.
11. Nickavar B, Abolhasani L. Bioactivity-guided separation of an ß-amylase inhibitor flavonoid from Salvia virgata. Iran J Pharm Res. 2013;12(1):57-61.
12. Gholamhosseini A, Fallah H, Sharifi far F. Inhibitory effect of methanol extract of Rosa damascena Mill. flowers on a-glucosidase activity and postprandial hyperglycemia in normal and diabetic rats. Phytomedicine. 2009;16(10):935-41.
13. Fatihazad F, Hamedeyazdan S, Kesropanah MK, Khaki A. Hypoglycemic activity of Fumaria parviflora in streptozotocin-induced diabetic rats. Adv Pharm Bull. 2013;3(1):207-10.
14. Nasri H, Shirzad H, Baradaran A, Rafieian-kopaei M. Antioxidant plants and diabetes mellitus. J Res Med Sci. 2015;20(5):491.
15. Ravan AP, Shafiei G, Eftekharian MM, Azizi A, Roohanaei G, Goudarzi F, et al. Inhibition of albumin glycation at different stages by four anti-diabetic plant extracts correlates with polyphenols and antioxidant capacity in vitro. Br J Pharm Res. 2016;12(2):1-8.
16. Tupe RS, Sankhe NM, Shaikh SA, Kemse NG, Khaire AA, Phatak DV, et al. Nutraceutical properties of dietary plants extracts: Prevention of diabetic nephropathy through inhibition of glycation and toxicity to erythrocytes and HEK293 cells. Pharm Biol. 2015;53(1):40-50.
17. Seif M, Fetoui H, Makni M, Zeghal N. Mitigating effects of antioxidant properties of Artemisia campestris leaf extract on hyperlipidemia, advanced glycation end products and oxidative stress in alloxan-induced diabetic rats. Food Chem Toxicol. 2010;48(7):1986-93.
18. McPherson JD, Shilton BH, Walton DJ. Role of fructose in glycation and cross-linking of proteins. Biochemistry. 1988;27(6):1901-7.
19. Uchida K, Kanematsu M, Sakai K, Matsuda T, Hattori N, Mizuno Y, et al. Proc Natl Acad Sci U S A. Proceedings of the National Academy of Sciences. 1998;95:4882-7.
20. Tupe RS, Sankhe NM, Shaikh SA, Phatak DV, Parikh JU, Khaire AA, et al. Aqueous extract of some indigenous medicinal plants inhibits glycation at multiple stages and protects erythrocytes from oxidative damage—an in vitro study. J Food Sci Technol. 2015;52(4):1911-23.
21. Sadowska-Bartosz I, Bartosz G. Prevention of protein glycation by natural compounds. Molecules. 2015;20(2):3309-34.
22. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;107(9):1058-70.
23. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):118.
24. Ivanov I, Vrancheva R, Marchev A, Petkova N, Aneva I, Denev P, et al. Antioxidant activities and phenolic compounds in Bulgarian Fumaria species. Int J Curr Microbiol Appl Sci. 2014;3(2):296-306.
25. Ginova A, Milahel K, Konakov A. Antioxidant Capacity of petals and leaves from different rose (Rosa damascena Mill) Plantations in Bulgaria. Int J Pure App Biosci. 2013;1(2):38-43.
26. Rahimi Khoigani S, Rajaei A, Goli SA. Evaluation of anti hyperglycaemic, anti-inflammatory and antioxidant activities of the methanol extract of Moringa oleifera in diabetes-inflammatory and antioxidant activities of the methanol extract of Moringa oleifera in diabetes-induced nephrotic male wistar rats. Molecules. 2017;22(4):439.
27. Zarei A, Vaezi G, Malekirad AA, Abdollahi M. Effects of ethanol extract of Salvia hydrangea on hepatic and renal functions of streptozotocin-induced diabetic rats. Avicenna J Phytomed. 2015;5(2):138-147.
28. Omodonisi EI, Aboua YG, Oguntileju OO. Assessment of the anti-hyperglycaemic, anti-inflammatory and antioxidant activities of the methanol extract of Moringa oleifera in diabetes-induced nephrotic male wistar rats. Molecules. 2017;22(4):439.
29. Gkogkolou P, Böhm M. Advanced glycation end products: Key players in skin aging? Dermatoendoctriol. 2012;4(3):259-70.
30. Seneviratne C, Dombi GW, Liu W, Dain JA. In vitro glycation of human serum albumin by dihydroxyacetone and dihydroxyacetone phosphate. Biochem Biophys Res Commun. 2012;417(2):817-23.
31. Rondeau P, Bourdon E. The glycation of albumin: structural and functional impacts. Biochimie. 2011;93(4):645-58.
32. Ho S-C, Wu S-P, Lin S-M, Tang Y-L. Comparison of anti-glycation capacities of several herbal infusions with that of green tea. Food Chem. 2010;122(3):768-74.
33. Yeh W-J, Hsia S-M, Lee W-H, Wu C-H. Polyphenols with antiglycation activity and mechanisms of action: A review of recent findings. J Food Drug Anal. 2017;25(1):84-92.
34. Kaewnarin K, Niamsup H, Shank L, Rakariyatham N. Antioxidant and antiglycation activities of some edible and medicinal plants. Chiang Mai J Sci. 2014;41(1):105-16.
35. Chen Y-F, Roan H-Y, Lii C-K, Huang Y-C, Wang T-S. Relationship between antioxidant and antiglycation ability of saponins, polyphenols, and polysaccharides in Chinese herbal medicines used to treat diabetes. J Med Plant Res. 2011;5(11):2322-31.
36. Grzegorczyk-Karolak I, Golią K, Gburek J, Wysokiwska H, Matkowski A. Inhibition of Advanced Glycation End-Product Formation and Antioxidant Activity by Extracts and Polyphenols from Scutellaria alpina L. and S. altissima L. Molecules. 2016;21(6):739.
37. Rahzani K, MalekIrad AA, Zeraatpishe A, Hosseini N, Seify SMR, Abdollahi M. Anti-oxidative stress activity of Stachys lavandulifolia aqueous extract in human. Cell J. 2013;14(4):314.