Research Paper

Effects of Some Boraginaceae Species Extracts on Albumin, Hemoglobin and Crystalline Glycation Reaction

Z. FAEZIZADEH*, A. GHARIB AND MASOUD GODARZEE[1]

Department of Laboratory Sciences, [1]Department of Biology, Borujerd Branch, Islamic Azad University, Borujerd, Iran

Non-enzymatic glycation of proteins is the major cause of diabetic complications and therefore, inhibition of this reaction could reduce the morbidity of this disease. The aim of this study was to evaluate the *in vitro* effects of aqueous and hydroethanol extracts of *Echium italicum* L., *Anchusa arvensis* L. and *Trichodesma incanum* (Bunge) A. DC. of Boraginaceae on albumin, hemoglobin, and crystalline glycation reaction. The results showed that the hydroethanol extract of *Echium italicum* in the concentrations of 100, 50 and 10 mg/dl could reduce the rate of albumin glycation to 85.10±1.85, 64.14±1.12 and 40.13±1.17%, respectively, and also in the same conditions, rates of hemoglobin glycation were reduced to 69.20±1.71, 46.13±1.93 and 27.73±0.90%, respectively. Moreover, extracts of three tested plants have antiglycation effects on crystalline glycation reaction. Under the identical conditions, the inhibitory effects of hydroethanol extracts of *Echium italicum* on the albumin, hemoglobin, and crystalline glycation reaction were higher than other extracts. The findings showed that the extracts of tested species, particularly in the hydroethanol form, could be used to prevent and treat complications of diabetes mellitus.

Key words: *Echium italicum* L., *Anchusa arvensis* L., *Trichodesma incanum* (Bunge) A. DC., albumin, hemoglobin, glycation

Members of the family Boraginaceae include herbs, shrubs and trees totaling about 146 genera and 2000 species in the worldwide[1]. Some of these plants grow in Iran and used widely in Iran's folk medicine[2,3]. It has been reported that Borage (*Borago officinalis* L.) was useful for its antipyretic, antihypertensive, antispasmodic, aphrodisiac and diuretic properties[4,5]. Previous findings showed that some species of Boraginaceae were used for the treatment of hemorrhoids[6]. Furthermore, it has also been observed that various parts of *Echium* species could be used for their antiinflammatory, antibacterial, antidepressant, antiproliferative, antiviral, antioxidant, cytotoxic and anxiolytic properties[2,3]. Cyanidin, a natural phenolic compound is present in many kinds of plants, such as grapes, pomegranate and some species of Boraginaceae and according to the previous reports, this effective compound has antioxidant and antidiabetic potential[7-10].

Diabetes mellitus is a common endocrine disease and a leading cause of morbidity and mortality in the world[11]. In this disease, the macro and microvascular complications lead to severe effects on the eyes, central nervous systems and kidneys[12]. Evidence from *in vitro* and *in vivo* experiments indicated that diabetic hyperglycemia could increase the risk of complications[13,14]. It seems that non-enzymatic glycation plays an important role in progression of diabetic complications[11]. Due to prolonged hyperglycemia, the non-enzymatic glycation of proteins could significantly change their structural and functional properties[12]. Previous studies demonstrated that the protection of proteins from glycation could reduce the diabetic complications as well[15,16]. The use of glycation inhibitors is considered due to their therapeutic potential in patients with diabetes[17,18]. The

***Address for correspondence**
E-mail: faezizadeh@gmail.com

Accepted 15 August 2016
Revised 12 August 2016
Received 24 May 2016
Indian J Pharm Sci 2016;78(4):486-491

investigation of new drugs with antiglycation activity has attracted the interest of various researchers[19,20]. Herbal medicines are nowadays gaining importance for treating diabetes mellitus due to their less toxicity, significant results as compared to allopathic medicine[21,22]. It is reported that some medicinal plants and their derivatives could inhibit the glycation of proteins as well[17,18].

Several species of Boraginaceae family such as *E. italicum* L. (fig. 1A), *Anchusa arvensis* L. (fig. 1B) and *Trichodesma incanum* (Bunge) A. DC (fig. 1C) are used widely in Iran's folk medicine for their antidiabetic properties. According to our knowledge, there is no scientific research about antidiabetic mechanism of these plants and therefore, in this study, we investigated the antiprotein glycation efficacy of these selected species.

MATERIALS AND METHODS

All the chemicals used were of analytical grade obtained from either Merck (Darmstadt, Germany) or Sigma (St. Louis, MO, USA). Human glycated albumin ELISA kit was obtained from Nacalai Tesque (Kyoto, Japan). Human glycated hemoglobin ELISA kit was obtained from MyBioSource, Inc. (San Diego, California, USA).

The three species of Boraginaceae family, including *E. italicum, A. arvensis* and *T. incanum* were collected during the spring from different regions of Borujerd (Lorestan Province, Iran). These regions have mild climate with mean temperature between 21-25° in the spring. Plant samples were identified and confirmed by comparison with voucher specimens deposited at the Department of Biology, Faculty of Basic Sciences, Borujerd Branch, Islamic Azad University, Borujerd, Iran.

Glycation of proteins:

The glycated human serum albumin, hemoglobin, and crystalline were prepared according to Gharib *et al.* method[17]. In brief, 50 mg/ml of tested proteins were incubated with 40 mmol glucose, 100 µg/ml penicillin, 100 U/ml streptomycin, 2 mg/ml aprotinin, 5 mmol phenylmethylsulfonyl fluoride and 0.5 mg/ml leupeptin in PBS (10 mmol, pH 7.4) at 37° for 8 weeks. Subsequently, the sample was dialyzed extensively against PBS at 4°.

Measurement of glycated albumin, hemoglobin:

The rate of glycated albumin was measured using the human glycated albumin ELISA kit (Nacalai Tesque Company), according to the manufacturer's protocol. In brief, 50 µl of phosphate buffer (10 mmol, pH 7.4) containing 20 µl of the test samples and 0.5 ml/l Tween 20 were added to each well of a 96-well micro titer plate that was coated with antihuman serum albumin antibody. Thereafter, wells were incubated for 20 min and washed three times. Subsequently, the boronate-horseradish peroxidase conjugate in 50 µl of glycine-NaOH (100 mmol, pH 9.0) containing $MgCl_2$ (20 g/l), bovine hemoglobin (3 g/l), and Tween 20 (0.5 ml/l) was added to wells. Again, wells were incubated for

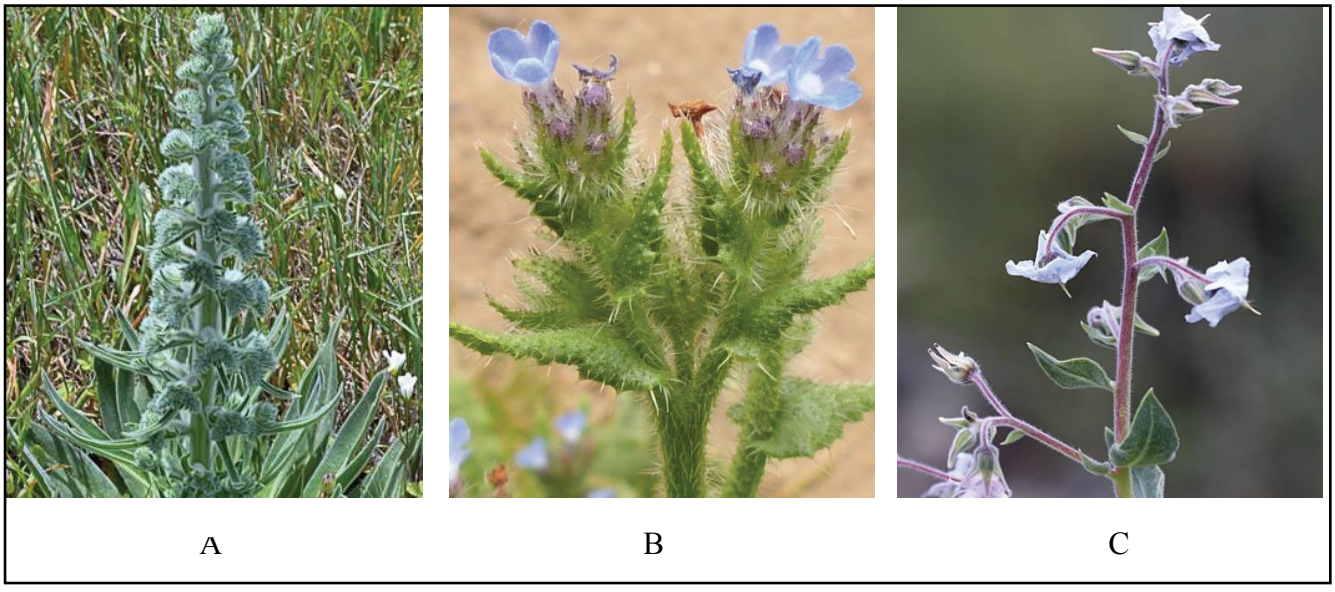

Fig. 1: Images of plants used in study.

(A) *Echium italicum* L., (B) *Anchusa arvensis* L. and (C) *Trichodesma incanum* (Bunge) A. DC.

20 min and the reaction was terminated by adding 50 µl of sulfuric acid (2 mol), and the absorbance at 492 nm was measured using a microplate reader (MTP-120; Corona Electronic). Finally, the percentage rate of glycated albumin in the samples was measured. The rate of glycated hemoglobin was measured using the human glycated hemoglobin ELISA kit (MyBioSource, Inc.), according to the manufacturer's protocol. Furthermore, the rate of crystalline non-enzymatic glycation was measured by the TBA method as described previously[23].

Preparation of plants extracts:

The hydroethanol and aqueous extracts of *E. italicum, A. arvensis* and *T. incanum* were prepared according to the previously reported method[24]. In brief, the air-dried plants were ground into powder using grinder (Moulinex, France), and then 1 g of sample powders was blended with 60 ml of 45% ethanol or water solvents. Subsequently, the blended samples were mixed on a sample stirrer at 900 rpm for 1 min, and then the liquid volumes were increased to 100 ml by solvents. These mixtures were placed in a bath by stirring and cooled in a refrigerator. Subsequently, the mixtures were centrifuged (Shimadzu, Japan) at 2500 rpm for 10 min and vacuum filtered, and the loss solvents were replaced. Finally, the prepared extracts were stored at 4° until used for analysis.

***In vitro* antiglycation studies:**

The percentages of glycated albumin, hemoglobin, and crystalline glycation in the samples were determined after incubation of these tested proteins and glucose in the presence of different concentrations (0, 10, 50, and 100 mg/dl) of each extract for 8 weeks. As positive control, 20 mg/ml aminoguanidine bicarbonate, as an antiglycation drug, was used.

Statistical analysis:

The results were expressed as mean±SD. The statistical analysis was performed using one-way ANOVA followed by Tukey's test and *P*-value less than 0.05 was considered significant. Data were analyzed using SPSS software (version 19.0, SPSS, Inc., IL, USA). All experiments were done in triplicate.

RESULTS AND DISCUSSION

In this research, the effects of the various concentrations of tested plant extracts on rates of albumin, hemoglobin, and crystalline glycation were studied. Under the identical conditions, the inhibitory effects of hydroethanol extracts of tested plants on glycation rates of three tested proteins were higher than those of aqueous extracts (Tables 1, 2 and 3). In all conditions, the albumin, hemoglobin, and crystalline glycation rates were significantly inhibited compared to the negative control group. Moreover, treatment with 100 mg/ml hydroethanol extracts of *E. italicum, A. arvensis* and *T. incanum* could reduce the rate of hemoglobin glycation to 69.20±1.71 and 41.90±1.25 and 27.85±1.30%, respectively (Table 2). As positive control aminoguanidine bicarbonate could decrease the rate of albumin, hemoglobin, and crystalline glycation to 56.12±1.39, 39.15±0.50 and 34.10±1.35%, respectively. Compared to control group, the rate of albumin glycation with 100 mg/ml hydroethanol extracts *E. italicum, A. arvensis* and *T. incanum* were reduced to 85.10±1.85, 53.10±0.69 and 38.13±1.81% at 8 weeks, respectively (Table 1). Under the identical conditions, the inhibitory effects of *E. italicum* extracts on albumin, hemoglobin, and crystalline glycation were significantly higher than those of other tested plants. Furthermore, the antiglycation rates of extracts on crystalline were lower than those of other tested proteins (Table 3).

In diabetes mellitus, the nonenzymatic glycation of proteins occurs in a wide variety of proteins,

TABLE 1: EFFECT OF HYDROETHANOL AND AQUEOUS EXTRACTS ON *IN VITRO* ALBUMIN GLYCATION REACTION

Plant scientific name	Extracts	% Inhibition of albumin glycation		
		Concentrations of extracts (mg/dl)		
		10	50	100
.*Echium italicum* L	Hydroethanol	40.13±1.17*	64.14±1.16*	85.10±1.85*
	Aqueous	34.15±1.35*	57.10±1.56*	73.74±1.24*
.*Anchusa arvensis* L	Hydroethanol	32.50±2.85*	38.08±1.30*	53.10±0.69*
	Aqueous	25.58±0.90*	31.30±1.37*	48.20±1.35*
Trichodesma incanum (Bunge)	Hydroethanol	28.18±1.90*	30.33±1.60*	38.13±1.81*
A. DC.	Aqueous	21.60±1.80*	24.17±1.21*	34.28±1.65*

Data presented as mean±SD. Values significantly different from negative control (*P<0.05)

TABLE 2: EFFECT OF HYDROETHANOL AND AQUEOUS EXTRACTS ON *IN VITRO* HEMOGLOBIN GLYCATION REACTION

Plant scientific name	Extracts	% Inhibition of hemoglobin glycation		
		Concentrations of extracts (mg/dl)		
		10	50	100
Echium italicum L.	Hydroethanol	27.73±0.90*	46.13±1.93*	69.20±1.71*
	Aqueous	25.28±0.65*	30.90±1.10*	61.93±1.10*
Anchusa arvensis L.	Hydroethanol	19.28±0.65*	30.90±1.10*	41.90±1.25*
	Aqueous	17.23±0.51*	22.14±0.90*	32.10±1.13*
Trichodesma incanum (Bunge) A. DC.	Hydroethanol	15.13±0.54*	19.93±1.10*	27.85±1.30*
	Aqueous	10.38±0.43*	13.19±0.75*	21.15±0.83*

Data presented as mean±SD. Values significantly different from negative control (*P<0.05)

TABLE 3: EFFECT OF HYDROETHANOL AND AQUEOUS ON *IN VITRO* CRYSTALLINE GLYCATION REACTION

Plant scientific name	Extracts	% Inhibition of crystalline glycation		
		Concentrations of extracts (mg/dl)		
		10	50	100
Echium italicum L.	Hydroethanol	24.13±1.10*	47.25±0.86*	64.75±0.43*
	Aqueous	21.35±0.70*	43.68±1.20*	58.77±0.25*
Anchusa arvensis L.	Hydroethanol	15.83±0.53*	26.53±1.80*	39.87±1.45*
	Aqueous	13.12±0.70*	19.78±0.72*	26.18±1.20*
Trichodesma incanum (Bunge) A. DC.	Hydroethanol	15.18±0.84*	15.18±0.80*	23.35±1.10*
	Aqueous	7.18±0.32*	9.98±0.38*	18.13±0.75*

Data presented as mean±SD. Values significantly different from negative control (*P<0.05)

and this phenomenon could enhance diabetic complications[25]. Therefore, in medicine, the inhibition of protein glycation has been proposed as an important strategy for the restriction of diabetic complication development[26]. The use of some medicinal plant extracts to reduce of diabetic complications has been widely investigated[17,27].

In folk medicine, Boraginaceae species have been used for the treatment and prevention of many ailments and diseases such as hypertension, diarrhea and diabetes mellitus and have been a tremendous resource for the development of new drugs[28-31]. Many species of Boraginaceae family are grown in Iran and some of them are used in ethnomedicine[4]. However, there were no studies about their antiglycation properties and therefore, in the present study, we investigated the *in vitro* effects of aqueous and hydroethanol extracts of *E. italicum, A. arvensis* and *T. incanum* on albumin, hemoglobin, and crystalline glycation reactions. The results from this study revealed that the all prepared extracts possessed antiprotein glycation. These findings correlated with those reported by other investigators about antiglycation efficacy of some natural plants, such as *Allium cepa* L.[32], *Curcuma longa* L.[33], *Rosmarinus officinalis* L.[34], *Cinnamomum verum*[35] and *Origanum officinalis*[36] under *in vitro* conditions. Under identical conditions, the antiglycation potential of extracts on albumin was higher than hemoglobin, and crystalline glycation, respectively. According to the previous studies, this difference may contribute to the protein structure[37]. The hydroethanol extracts of each tested plant have more inhibitory effects on protein glycation reaction, compared to aqueous extracts. These results may be related to effects of extracting solvents on the isolation of phytochemical constituents[38].

Under identical conditions, the extracts of *E. italicum* have more potent antiglycation activity compared to *A. arvensis* and *T. incanum* extracts. Moreover, our data revealed that the antiglycation potential of *E. italicum* was higher than *A. sativum, Zingiber officinale, A. cepa* and *Thymus vulgaris* that their antiglycation activities were reported previously[33,34]. Thus, the ability of *E. italicum* to modulate glycation might be providing its use as a significant source of the natural product in preventing the diabetic complications.

In summary, the present study was carried out to evaluate the antiglycation potential of *E. italicum, A. arvensis* and *T. incanum* extracts on albumin, hemoglobin, and crystalline glycation reaction. Results indicated that the hydroethanol extracts of these plants might be promising antiglycation agents for the prevention of diabetic complications through inhibition of protein

glycation. Therefore, the prepared extracts could be considered as a promising strategy for developing the antidiabetic drugs.

Acknowledgments:

The authors would like to acknowledge the staff of Islamic Azad University.

Financial support and sponsorship:

This study was supported by Islamic Azad University, Borujerd Branch, Iran.

Conflicts of interest:

There are no conflicts of interest.

REFERENCES

1. Ahmed HO, Kordofani MA. Leaf and stem anatomy of five species from the genus *Heliotropium* L. (Boraginaceae) in Sudan. J Chem Pharm Res 2012;4:4575-81.
2. Sayyah M, Sayyah M, Kamalinejad M. A preliminary randomized double blind clinical trial on the efficacy of aqueous extract of *Echium amoenum* in the treatment of mild to moderate major depression. Prog Neuropsychopharmacol Biol Psychiatry 2006;30:166-9.
3. Abolhassani M. Antiviral activity of borage (*Echium amoenum*). Arch Med Sci 2010;6:366-9.
4. Ghahremanitamadon F, Shahidi S, Zargooshnia S, Nikkhah A, Ranjbar A, Soleimani Asl S. Protective effects of *Borago officinalis* extract on amyloid β-peptide (25-35)-induced memory impairment in male rats: A behavioral study. Biomed Res Int 2014;2014:798535.
5. Arm JP, Boyce JA, Wang L, Chhay H, Zahid M, Patil V, *et al*. Impact of botanical oils on polyunsaturated fatty acid metabolism and leukotriene generation in mild asthmatics. Lipids Health Dis 2013;12:141-52.
6. Tosun A, Akkol EK, Bahadir O, Yeşilada E. Evaluation of antiinflammatory and antinociceptive activities of some *Onosma* L. species growing in Turkey. J Ethnopharmacol 2008;120:378-81.
7. Ahmad S, Ahmad S, Bibi A, Ishaq MS, Afridi MS, Kanwal F, *et al*. Phytochemical analysis, antioxidant activity, fatty acids composition, and functional group analysis of *Heliotropium bacciferum*. Sci World J 2014;2014:829076-84.
8. Kumar MS, Chaudhury S, Balachandran S. *In vitro* callus culture of *Heliotropium indicum* Linn. for assessment of total phenolic and flavonoid content and antioxidant activity. Appl Biochem Biotechnol 2014;174:2897-909.
9. Salem N, Msaada K, Hammami M, Jday A, Salem S, Limam F, *et al*. Variations in Tunisian borage essential oil profiles and their antioxidant activities during flowering. Nat Prod Res 2014;28:1919-22.
10. Fernández LR, Cirigliano A, Fabani MP, Lima B, Alberti S, Kramer F, *et al*. Antioxidant neolignans from *Cordia americana*. Planta Med 2013;79:1724-9.
11. Das S, Haroled Peter PL, Bhavani ML, Naresh P, Ramana MV. Age- and sex-related prevalence and drug utilization pattern in the management of type 2 diabetes mellitus and its comorbidity with cardiovascular diseases: A comparative study. Indian J Pharm Sci 2015;77:478-84.
12. Furusyo N, Hayashi J. Glycated albumin and diabetes mellitus. Biochim Biophys Acta 2013;1830:5509-14.
13. Naderi GH, Dinani NJ, Asgary S, Taher M, Nikkhoo N, Boshtam M. Effect of some high consumption spices on hemoglobin glycation. Indian J Pharm Sci 2014;76:553-7.
14. Mishra MR, Mishra A, Pradhan DK, Panda AK, Behera RK, Jha S. Antidiabetic and antioxidant activity of *Scoparia dulcis* Linn. Indian J Pharm Sci 2013;75:610-4.
15. Grzebyk E, Piwowar A. Glycoxidative modification of albumin in medical research. Pol Merkur Lekarski 2013;34:239-42.
16. Xu W, Chen L, Shao R. Influence of glycation of plasma proteins in diabetes on the binding interaction with polyphenols. Curr Drug Metab 2014;15:116-9.
17. Gharib A, Faezizadeh Z, Godarzee M. Treatment of diabetes in the mouse model by delphinidin and cyanidin hydrochloride in free and liposomal forms. Planta Med 2013;79:1599-1604.
18. Sheikh N, Safari MR, Mani Kashani Kh, Araghchian M, Zeraati F. Study on the effect of garlic on the *in vitro* albumin glycation reaction. Acta Medica Iranica 2004;42:16-8.
19. Meeprom A, Sompong W, Chan CB, Adisakwattana S. Isoferulic acid, a new antiglycation agent, inhibits fructose- and glucose-mediated protein glycation *in vitro*. Molecules 2013;18:6439-54.
20. Mosihuzzman M, Naheed S, Hareem S, Talib S, Abbas G, Khan SN, *et al*. Studies on α-glucosidase inhibition and anti-glycation potential of *Iris loczyi* and *Iris unguicularis*. Life Sci 2013;92:187-92.
21. Elosta A, Ghous T, Ahmed N. Natural products as anti-glycation agents: possible therapeutic potential for diabetic complications. Curr Diabetes Rev 2012;8:92-108.
22. Ahmad MS, Pischetsrieder M, Ahmed N. Aged garlic extract and S-allyl cysteine prevent formation of advanced glycation endproducts. Eur J Pharmacol 2007;561:32-8.
23. Yarat A, Uguz Z, Ustunel A, Emekli N. Lens glutathione, lens protein glycation and electrophoretic patterns of lens proteins in STZ induced diabetic rats. Glycoconj J 1995;12:622-6.
24. Segovia F, Lupo B, Peiró S, Gordon MH, Almajano MP. Extraction of antioxidants from Borage (*Borago officinalis* L.) leaves-optimization by response surface method and application in oil-in-water emulsions. Antioxidants 2014;3:339-57.
25. Cozma AI, Sievenpiper JL, de Souza RJ, Chiavaroli L, Ha V, Wang DD, *et al*. Effect of fructose on glycemic control in diabetes: a systematic review and meta-analysis of controlled feeding trials. Diabetes Care 2012;35:1611-20.
26. Rondeau P, Bourdon E. The glycation of albumin: structural and functional impacts. Biochimie 2011;93:645-58.
27. Safari MR, Sheikh N, Mani-Kashani KH. Study on the effect of vitamin C on the *in vitro* albumin glycation reaction. Iran J Pharm Res 2006;4:275-9.
28. Sousa C, Moita E, Valentao P, Fernandes F, Monteiro P, Andrade PB. Effects of colored and noncolored phenolics of *Echium plantagineum* L. bee pollen in Caco-2 cells under oxidative stress induced by tert-butyl hydroperoxide. J Agric Food Chem 2015;63:2083-91.
29. Aliasl J, Barikbin B, Khoshzaban F, Naseri M, Sedaghat R,

Kamalinejad M, *et al*. Effect of *Arnebia euchroma* ointment on post-laser wound healing in rats. J Cosmet Laser Ther 2015;17:41-5.

30. Kumar N, Kumar R, Kishore K. Onosma L. A review of phytochemistry and ethnopharmacology. Pharmacogn Rev 2013;7:140-51.
31. Ganie SA, Dar TA, Hamid R, Zargar O, Ul Abeer S, Masood A, *et al*. *In vitro* antioxidant and cytotoxic activities of *Arnebia benthamii* (Wall ex. G. Don): A critically endangered medicinal plant of Kashmir Valley. Oxid Med Cell Longev 2014;2014:792574-82.
32. Ramkissoon JS, Mahomoodally MF, Ahmed N, Subratty AH. Relationship between total phenolic content, antioxidant potential, and antiglycation abilities of common culinary herbs and spices. J Med Food 2012;15:1116-23.
33. Ramkissoon JS, Mahomoodally MF, Ahmed N, Subratty AH. Antioxidant and anti-glycation activities correlates with phenolic composition of tropical medicinal herbs. Asian Pac J Trop Med 2013;6:561-9.
34. Hsieh CL, Peng CH, Chyau CC, Lin YC, Wang HE, Peng RY. Low-density lipoprotein, collagen, and thrombin models reveal that *Rosemarinus officinalis* L. exhibits potent antiglycative effects. J Agric Food Chem 2007;55:2884-91.
35. Jin S, Cho KH. Water extracts of cinnamon and clove exhibits potent inhibition of protein glycation and anti-atherosclerotic activity *in vitro* and *in vivo* hypolipidemic activity in zebrafish. Food Chem Toxicol 2011;49:1521-1529.
36. Kim HY, Kim K. Protein glycation inhibitory and antioxidative activities of some plant extracts *in vitro*. J Agric Food Chem 2003;51:1586-1591.
37. Iannuzzi C, Irace G, Sirangelo I. Differential effects of glycation on protein aggregation and amyloid formation. Front Mol Biosci 2014;2:1-9.
38. Perera PR, Ekanayake S, Ranaweera KKD. Antiglycation and antioxidant activities of a ready to serve herbal drink of *Syzygium Cumini* bark extract. Med Aromat Plants 2014;3:1-4.