Journal of Pharmaceutical Research International

17(3): 1-8, 2017; Article no.JPRI.34049
Previously known as British Journal of Pharmaceutical Research
ISSN: 2231-2919, NLM ID: 101631759

Assessment of Antiglycation Effects of Some Plants Used in Hypolipidaemic Formulations or as Spices

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Authors' contributions

This work was carried out in collaboration between all authors. Author HKIP involved in the concept and design of the study, overall supervision, collection and interpretation of data, literature survey and writing the manuscript. Author WKVKP carried out the experiments. Authors WITF and JAVPJ collaborated with the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2017/34049
Editor(s):
(1) R. Deveswaran, M.S. Ramaiah College of Pharmacy, Bangalore, India.
Reviewers:
(1) Prasong Srihanam, Mahasarakham University, Thailand.
(2) Abdullahi M. Nuhu, Cst. Kaduna Polytechnic, Kaduna, Nigeria.
(3) Mohini Chetan Kuchekar, Pune University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/19565

Original Research Article

Received 11th May 2017
Accepted 3rd June 2017
Published 16th June 2017

ABSTRACT

Aims: Diabetes mellitus is characterized by hyperglycemia, which leads to acceleration of protein glycation. Some glycation products form inter molecular cross-links, contributing to chronic diabetic complications. Determination of protein glycation inhibitory potential of medicinal plants may offer safe therapeutic avenues in preventing diabetic complications. The aim of this study was to analyze the antiglycation effects of nine plants which included spices and some medicinal plants used in hypolipidaemic formulations.
Study Design: Experimental.
Place and Duration of Study: Department of Biochemistry, Faculty of Medicine, University of Peradeniya, Sri Lanka between March 2014 and March 2015.

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Methodology: Crude methanol extracts of nine, dried plant parts namely, *Brassica juncea* (BJ) seed, *Coscinium fenestratum* (CF) root, *Cuminum cyminum* (CC) seed, *Cyperus rotundus* (CR) root, *Foeniculum vulgare* (FV) seed, *Picrorhiza kurroa* (PK) root, *Acorus calamus* (AC) rhizome, *Trachyspermum roxburghianum* (TR) leaf and *Trigonella foenum-graecum* (TF) seed and standard inhibitor aminoguanidine (AG) (1 mg/mL) were used. Inhibitory effects of plant extracts (0.5/ 2/ 5 mg/mL) on fructosamine formation, glycation and glycation induced cross-linking were assessed.
Results: Fructosamine formation was significantly inhibited ($p<0.001$) in the presence of CF, CR, PK and AC (2 mg/ ml). Antiglycation effects of these four plants were demonstrated when the extent of glycation was assessed using polyacrylamide gel electrophoresis. Protein cross-linking inhibitory effects of AC, CF, CR and PK extracts (2 mg/ ml) were revealed using sodium dodecyl polyacrylamide gel electrophoresis. At 0.5 mg/ ml, this inhibitory effect remained only in CR and PK. When 5 mg/ ml was used, BJ showed inhibitory effects while CC, TR and TF without an effect. Accordingly, the highest inhibitory effects were seen in CR and PK.
Conclusion: Antiglycation effects of root extracts of AC, CF, CR and PK were demonstrated with better effects in CR and PK.

Keywords: Antiglycation; cross-linking; Cyperus rotundus; Picrorhiza kurroa.

1. INTRODUCTION

Diabetes mellitus has affected 415 million people in 2015, among which most affected people are in their productive years of lives and from low and middle income countries [1]. Diabetes mellitus is characterized by hyperglycemia, which leads to acceleration of non enzymatic protein glycation. Amadori products such as fructosamine are formed during early glycation reactions [2]. Further reactions which occur over a period of time result in the production of a complex array of stable, irreversible products known as advanced glycation end-products (AGEs) [3]. During later stages, some AGEs lead to intra or inter molecular cross-linking [4]. Long lived proteins such as collagen are particularly vulnerable for cross-linking. Hence, AGEs are responsible for causing permanent structural and functional damage to the affected molecules [5]. Increase in AGE production associated with diabetes is repeatedly been reported as a central cause in the development of diabetic microvascular and macrovascular complications [6]. Oxidative stress and free metal ions also enhance the AGE formation.

Number of synthetic compounds with antiglycation effects including the extensively studied aminoguanidine (AG) has being withdrawn from the clinical trials due to safety issues [7]. Determination of protein glycation inhibitory potential of medicinal plants may offer safer therapeutic alternatives in preventing diabetic complications [8]. Medicinal plants are being used since ancient times without side effects and spices are consumed in the daily diet especially in the Asian countries. These plants may offer a gentle and safe means of managing glycation induced molecular damage. However, adequate work has not yet been done especially in Sri Lanka, to prove the antiglycation effects of medicinal plants. Studies carried out on protein glycation inhibitors reported the use of expensive specialized techniques such as high performance liquid chromatography, mass spectrometry, fluorescence spectrometry and specific ELISA assays [9].

The objectives of this study were to analyze the inhibitory effects of nine plants (which included spices and plants used in antihyperlipidaemic preparations) on fructosamine formation, glycation and glycation induced protein cross-linking using recently established methods which do not require sophisticated instruments.

2. MATERIALS AND METHODS

2.1 Plant Material

Crude methanol extracts of nine, dried plant parts namely, *Brassica juncea* (*Aba*) (BJ) seed, *Coscinium fenestratum* (*Vanival*) (CF) root, *Cuminum cyminum* (*Suduru*) (CC) seed, *Cyperus rotundus* (*Kaladuru*) (CR) root, *Foeniculum vulgare* (*Maduru*) (FV) seed, *Picrorhiza kurroa* (*Katukarosana*) (PK) root, *Acorus calamus* (*Wadakaha*) (AC) rhizome, *Trachyspermum roxburghianum* (*Asamodagam*) (TR) leaf and *Trigonella foenum-graecum* (*Uluhal*) (TF) seed were prepared below 50℃.

2.2 Assessment of Inhibitory Effects of Extracts on Fructosamine Formation

Relative concentration of fructosamine was determined using the method as described by Perera et al. [10]. Briefly, chicken egg lysozyme was incubated with 500 mM fructose (pH 7.4) in the presence or absence of extracts (2 mg/ ml) at 37°C for 8 days. Methanol extracts were added at the beginning of incubations. Aminoguanidine (1 mg/mL) (AG) was used as the positive control. Corresponding blanks were prepared in the absence of fructose. Aliquots collected on day 8 were mixed with the sodium carbonate buffer (pH 10.35). Fructose was added to the blanks prior to the assay. Nitroblue tetrazolium in sodium carbonate buffer was added to all tubes and incubated for 15 minutes at 37℃. Absorbance at 530 nm was measured immediately after incubation. Percentage inhibition of the relative fructosamine concentration in the presence of extracts and AG was calculated using following formula.

$$\text{Percentage inhibition} = 100 - \left\{ \frac{\text{(Absorbance of Test - Absorbance of Test Blank)} \times 100}{\text{(Absorbance of Control - Absorbance of Control Blank)}} \right\}$$

2.2.1 Statistical analysis

Inhibitory effects of extracts on relative fructosamine formation were determined three times in duplicate. Statistical analysis was performed using ANOVA to compare the means of different extracts with that of uninhibited control. Values of $p<0.01$ were considered as significantly different.

2.3 Assessment of Glycation Inhibitory Effects of Extracts

Glycation inhibitory effects of extracts were determined using bovine serum albumin (BSA) *in vitro* as described by Wijetunge and Perera [11]. Briefly, BSA was mixed with 500 mM fructose (pH 7.4) in the absence and presence of 2 mg/mL extracts. AG (1 mg/mL) was used as the positive control. Corresponding blanks were prepared in the absence of fructose. Reaction mixtures were incubated at 37ºC for 15 days and aliquots were collected at day 15. Degree of glycation in each aliquot was assessed using polyacrylamide gel electrophoresis (PAGE) under non-denaturing [12]. Changes in the migration position of the BSA bands were monitored after staining gels with Coomassie brilliant blue. Approximate inhibition of glycation was assessed based on the decrease in migration of BSA in comparison to the uninhibited reaction. Experiments were repeated three times.

2.4 Assessment of Glycation Induced Protein Cross-linking Inhibitory Effects of Extracts

Glycation induced protein cross-linking inhibitory effects of extracts were assessed as described by Perera and Ranasinghe [4]. Briefly, chicken egg lyzosyme was incubated with 500 mM fructose (pH 7.4) in the absence and presence of plant extracts (0.5/ 2/ 5 mg/mL) at 37°C for 15 days. Corresponding blanks were prepared in the absence of fructose. Standard inhibitor AG (1 mg/mL) was included. Aliquots were collected at intervals and analyzed using sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) [12]. Gels stained with Coomassie brilliant blue were monitored for the extent of appearance of high molecular weight products of lysozyme. Broad range molecular weight markers were used to assess the approximate size of the high molecular weight products. Experiments were repeated three times.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Inhibitory effects of extracts on fructosamine formation

Fructosamine formation in the presence and absence of extracts was compared using aliquots collected on day 8 of the incubation. Difference between the absorbance of the corresponding test and blank is considered to be proportionate to the relative concentration of fructosamine formed. Fructosamine formation was significantly inhibited in the presence of CF, CR, PK, AC ($p<0.001$) and AG ($p<0.01$) when compared with the uninhibited control (Fig. 1). Mean percentage inhibitions demonstrated by CF, CR, PK and AC were 63.1, 79.6, 94.2 and 46.7% respectively at 2 mg/mL. Low level of inhibition (5.8-11.1%) on the relative fructosamine concentration occurred in the presence of CC, FV, AH and TF.

3.1.2 Glycation inhibitory effects of extracts

The increase in the BSA migration of the test compared to the corresponding blank is reported

to be proportionate to the degree of glycation [11]. When PAGE was conducted, BSA migration was increased (long arrow in Fig. 2A, 2B) in the presence of fructose and absence of extracts (-P). This increase in migration was reduced (short arrow) by approximately 80% in the presence of CF, CR, and AC extracts (2 mg/mL) during the 15 day incubation indicating glycation inhibition (Fig. 2B). Inhibitory effects of PK is somewhat masked at 2 mg/mL as there was an interference in its blank (Fig. 2A). CC, FV and AH (2 mg/mL) showed antiglycation effects to a lesser extent (Fig. 2B).

3.1.3 Glycation induced protein cross-linking inhibitory effects of extracts

When SDS-PAGE was conducted, lysozyme cross-linking was observed as high molecular weight products in the presence of fructose (Figs. 3, 4, 5). The extent of high molecular weight products formed was reported to be proportionate to the degree of glycation induced protein cross-linking [4]. The products formed represented the dimer, trimer and tetramer of lysozyme monomer when compared with molecular weight markers (Fig. 3). Such products

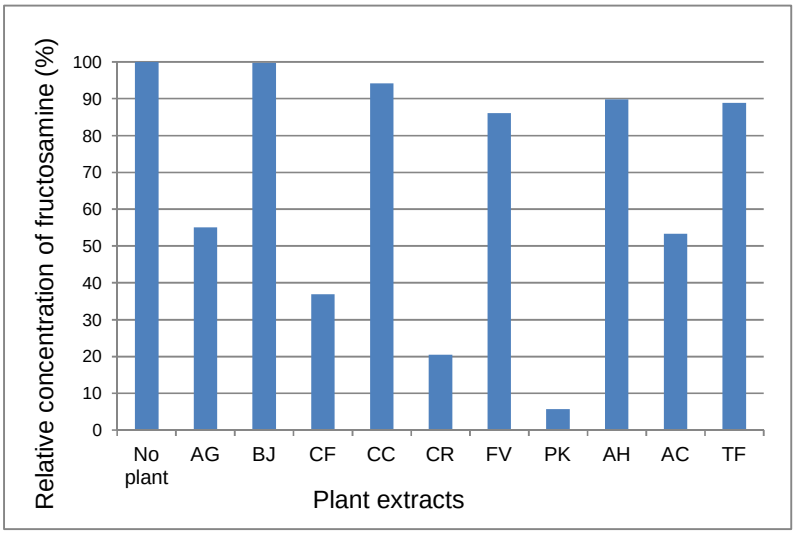

Fig. 1. Relative fructosamine concentration in the presence of extracts

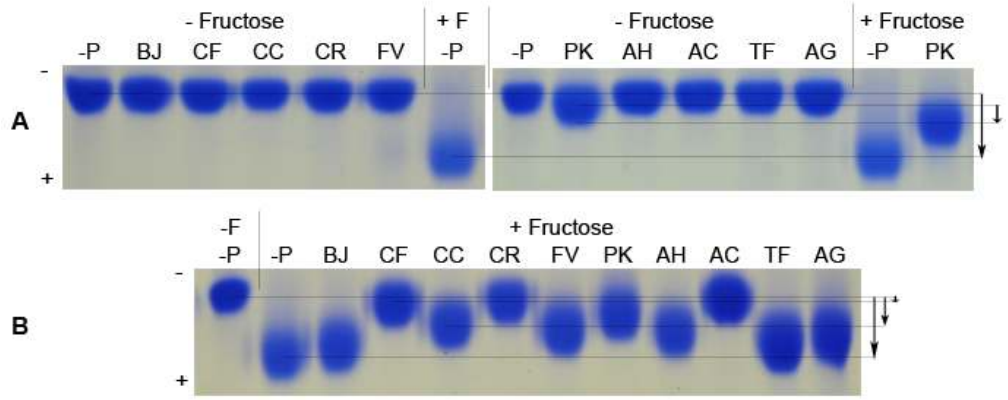

Fig. 2. Effects of extracts on glycation

-F/ - Fructose: In the absence of fructose, +F/ + Fructose: in the presence of fructose, -P: in the absence of plant extract, AG: Aminoguanidine, BJ: Brassica juncea, CF: Coscinium fenestratum, CC: Cuminum cyminum, CR: Cyperus rotundus, FV: Foeniculum vulgare, PK: Picrorhiza kurroa, AC: Acorus calamus, TR: Trachyspermum roxburghianum TF: Trigonella foenum-graecum A: Corresponding blanks in the absence of fructose, B: Corresponding tests in the presence of fructose

were not formed in the absence of fructose (with or without a plant extracts) (Figs. 4A, 5A).

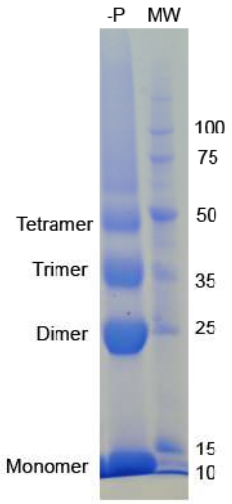

Fig. 3. Formation of high molecular weight products in the presence of fructose
MW: Molecular weight markers (K Da)

Protein cross-linking inhibitory effects were revealed in samples incubated with AC, CF, CR and PK extracts (2 mg/mL) for 1 week (Fig. 4B) and 2 weeks (Fig. 4C). Demonstrated antiglycation potential of the plants at 2 mg/mL was similar to the effect of 1 mg/mL AG. At 0.5 mg/mL this inhibitory effect remained only in CR and PK at the end of week 1 (Fig. 5B) and week 2 incubations (Fig. 5C). BJ, CC, FV, TR and TF did not show any inhibitory effect on cross-linking at 2 mg/mL. BJ showed inhibitory effects when 5 mg/mL extract was used. FV also showed a considerable inhibitory activity at 5 mg/mL (Fig. 5). CC, TR and TF did not show inhibitory effects even at 5 mg/mL (Fig. 5). Accordingly, the highest inhibitory effects were seen in CR and PK among the nine extracts.

3.2 Discussion

Roots of AC, CF, CR and PK are used in the classical reference of *Lekhaneeya Mahakashaya* mentioned in Charaka Samhita Sutra Sthana in which ten plant parts which are very effective in scraping down the excess fat are taken in equal quantities [13]. *Nawarathne Kalka* a poly herbal formula used in Sri Lanka contains components of 14 different plant species including CC seeds, PK root and TR seed [14]. Spices are being used to add flavor to food and for medicinal purposes for thousands of years. Some plant parts which are used in *Lekhaneeya Mahakashaya* and plant parts which are used as spices were selected for the current study. Studies carried out on the plants selected in the present study on likely protective effects against diabetic complications are limited. Glycation induced cross-link inhibitory effects or other antiglycating effects are not reported previously for AC, CF and PK. Present study demonstrated an additional beneficial effect of AC, CF, CR and PK with better antiglycation effects in CR and PK using techniques which do not require sophisticated instruments. Antiglycation effects observed with spices used in this study were much lower compared to that of AC, CF, CR and PK.

There is reported evidence for antiglycating effects of CR (*in vitro*), CC (*in vivo*) and FV (*in vivo*). CR extract (25-250 µg/mL) has significantly decreased the formation of fluorescent AGEs *in vitro* when proteins were incubated with 100 mM fructose for 14 days suggesting the possibility of the use of CR against diabetic complications [15]. Methanolic extract of seeds of CC has declined glycated hemoglobin and collagen linked fluorescence in streptozotocin-diabetic rats, indicating reduction in AGE formation [16]. In the same study, CC extract also has reduced blood glucose and inhibited formation of free radicals. In another study the effect of CC on alloxan-induced diabetes in albino rats was evaluated with a significant decline in glycated proteins and an elevation in reduced glutathione, superoxide dismutase, catalase, and glutathione peroxidase in the pancreas, liver, kidney, intestine and aorta [17]. Prolonged treatment with the petroleum ether fraction of the FV demonstrated improvement in blood glucose and glycated haemoglobin in streptozotocin-induced diabetic rats [18]. BJ leaf extract decreased serum glucose and glycosylated proteins. Whether the BJ seed has the same effect is not shown. Results of the current study tallied with previous findings on CR. We did not see clear evidence on glycation induced protein cross-linking inhibitory effects in CC even at 5 mg/mL extracts. Previous studies have shown reduction in blood glucose and glycated proteins such as HbA_{1c} *in vivo* with CC [16]. The antiglycation effects shown under *in vivo* conditions may have resulted due to the hypoglycaemic effects of extracts or elevation of antioxidant enzyme activity *in vivo*. Therefore a proper comparison between findings of the present study and those of previous *in vivo* studies cannot be made.

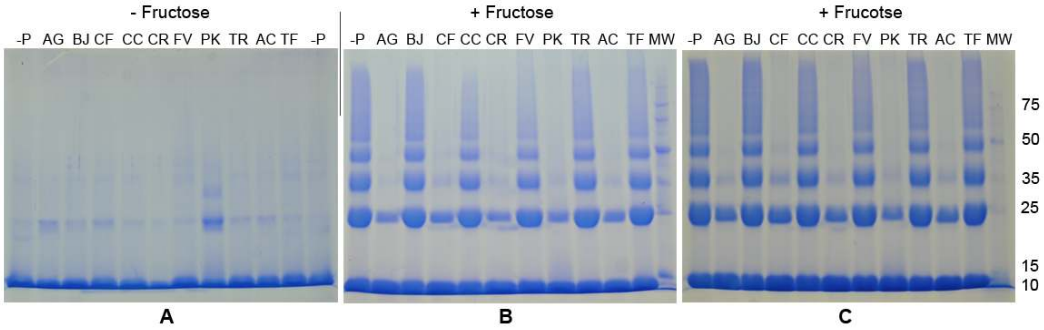

Fig. 4. Effect of plant extracts (2 mg/mL) on glycation induced protein cross-linking

- Fructose: In the absence of fructose, + Fructose: in the presence of fructose, -P: in the absence of plant extract, AG: Aminoguanidine, BJ: Brassica juncea, CF: Coscinium fenestratum, CC: Cuminum cyminum, CR: Cyperus rotundus, FV: Foeniculum vulgare, PK: Picrorhiza kurroa, AC: Acorus calamus, TR: Trachyspermum roxburghianum TF: Trigonella foenum-graecum, MW: Molecular weight markers (K Da)

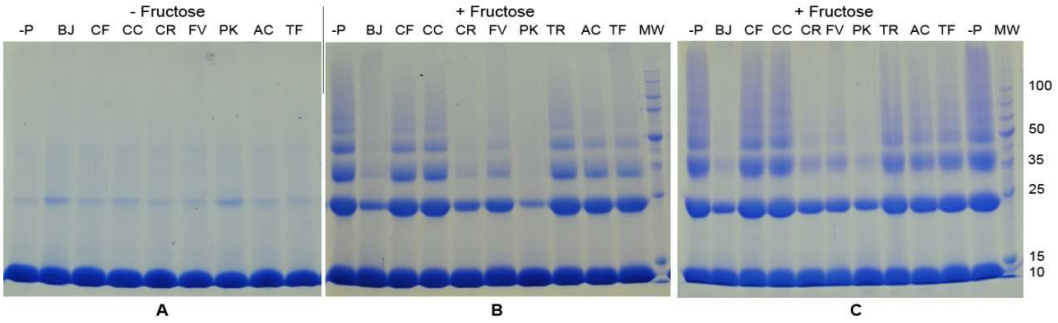

Fig. 5. Effect of plant extracts (0.5 or 5 mg/mL) on glycation induced protein cross-linking

- Fructose: In the absence of fructose, + Fructose: presence of fructose, -P: in the absence of plant extract. AG: Aminoguanidine, BJ: Brassica juncea, CF: Coscinium fenestratum, CC: Cuminum cyminum, CR: Cyperus rotundus, FV: Foeniculum vulgare, PK: Picrorhiza kurroa, AC: Acorus calamus, TR: Trachyspermum roxburghianum TF: Trigonella foenum-graecum, MW: Molecular weight markers (K Da). CF, CR, PK, AC (0.5 mg/mL), BJ, CC, FV, TR, TF (5 mg/mL)

Nawarathne Kalka also showed antiglycation effects in which PK constitutes 1% of the formula. However, main contributor for this effect is not revealed [14].

A good correlation is known to exist between the free radical scavenging and antioxidant activities of the extracts and the glycation inhibitory activity [19]. There is evidence for a role in free radical scavenging of AC, CF, CR and PK. Methanolic extracts of AC rhizome showed good free radical scavenging and metal ion chelating abilities [20]. Administration of CF stem methanolic fractions in streptozotocin-diabetic rats reversed diabetes induced reductions occurred in antioxidant enzymes such as catalase, super oxide dismutase and glutathione S transferase [21]. Antioxidant activity of hydroalcoholic extract CR was evaluated using various antioxidant assays such as DPPH radical scavenging and measuring the effect against metal-catalyzed protein oxidation using a pro-oxidant model (Fe^{2+}/ascorbate) in rat liver homogenates [22]. Antioxidant effect of alcoholic extract of PK rhizome was revealed, showing a very high DPPH scavenging activity with an IC_{50} of 72.6 µg/mL [23].

In the present study, sugar concentration was constant in the incubation mixtures in the presence and absence of the extracts. Hence the antiglycation activities identified in the present study are independent of the possible hypoglycaemic effects of these extracts. Further studies are required to identify the efficacy and safety of these plant extracts *in vivo*.

4. CONCLUSION

According to up to date literature search, antiglycation effects (including inhibitory effects of protein cross-linking) of *Acorus calamus*

rhizome, *Coscinium fenestratum* root and *Picrorhiza kurroa* root were demonstrated for the first time. Antiglycation effects of *Cyperus rotundus* root were also observed which tallied with previous findings. Among the four extracts, better effects were observed in *Cyperus rotundus* and *Picrorhiza kurroa*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors want to acknowledge Mr. A.M.P.S.T.M. Bandara for assistance with electrophoresis, Mr. G. Gunasekera for photography.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. International Diabetes Federation. IDF Diabetes Atlas, 7th edition. Brussels, Belgium: International Diabetes Federation; 2015. Available:http://www.diabetesatlas.org/
2. Meeprom A, Sompong W, Chan CB, Adisakwattana S. Isoferulic acid, a new anti-glycation agent, inhibits fructose-and glucose-mediated protein glycation *in vitro*. Molecules. 2013;18(6): 6439-54.
3. Abdallah HM, El-Bassossy H, Mohamed GA, El-Halawany AM, Alshali KZ, Banjar ZM. Phenolics from *Garcinia mangostana* inhibit advanced glycation end products formation: Effect on Amadori Products, cross-linked structures and protein thiols. Molecules. 2016;21(2): 251.
4. Perera HKI, Ranasinghe HASK. A simple method to detect plant based inhibitors of glycation induced protein cross-linking. Asian Journal of Medical Sciences. 2015; 6(1):28-33.
5. Sadowska-Bartosz I, Bartosz G. Prevention of protein glycation by natural compounds. Molecules. 2015; 20(2):3309-34.
6. Goh SY, Cooper ME. The role of advanced glycation end products in progression and complications of diabetes. Journal of Clinical Endocrinology and Metabolism. 2008;93(4):1143-52.
7. Rahbar S and Figarola JL. Novel inhibitors of advance glycation end products. Archives of Biochemistry and Biophysics. 2003;419:63-79.
8. Wu C, Huang S, Lin J, Yen G. Inhibition of advanced glycation endproduct formation by foodstuffs. Food and Function. 2011;2: 224-34.
9. Suantawee T, Wesarachanon K, Anantsuphasak K, Daenphetploy T, Thien-Ngern S, Thilavech T, et al. Protein glycation inhibitory activity and antioxidant capacity of clove extract. Journal of Food Science and Technology; 2014. DOI: 10.1007/s13197-014-1452-1
10. Perera HKI, Premadasa WKVK, Poongunran J. α-Glucosidase and glycation inhibitory effects of *Costus speciosus* leaves. BMC Complementary and Alternative Medicine. 2016;16(1):2-10.
11. Wijetunge DCR, Perera HKI. A novel *in vitro* method to detect inhibitors of protein glycation. Asian Journal of Medical Science. 2014;5(3):15-21.
12. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970; 227(5259):680-85.
13. Bagde AB. Charak samhita-complete encyclopedia of Ayurvedic Science. International Journal of Ayurveda and Alternative Medicine. 2013;1(1):12-20.
14. Fernando CD, Karunaratne DT, Gunasinghe SD, Cooray MD, Kanchana P, Udawatte C, et al. Inhibitory action on the production of advanced glycation end products (AGEs) and suppression of free radicals *in vitro* by a Sri Lankan polyherbal formulation *Nawarathne Kalka*. BMC Complementary and Alternative Medicine. 2016;16(1):197.
15. Ardestani A, Yazdanparast R. *Cyperus rotundus* suppresses AGE formation and protein oxidation in a model of fructose-mediated protein glycoxidation. International Journal of Biological Macromolecules. 2007;41(5):572-78.
16. Jagtap AG, Patil PB. Antihyperglycemic activity and inhibition of advanced glycation end product formation by *Cuminum cyminum* in streptozotocin

induced diabetic rats. Food and Chemical Toxicology. 2010;48(8):2030-36.
17. Surya, D, Vijayakumar R S, Nalini N. Oxidative stress and the role of cumin (*Cuminum cyminum* Linn.) in alloxan-induced diabetic rats. Journal of Herbs, Spices & Medicinal Plants. 2005;11(3): 127-39.
18. Dongare V, Kulkarni C, Kondawar M, Magdum C, Haldavnekar V, Arvindekar A. Inhibition of aldose reductase and anti-cataract action of trans-anethole isolated from *Foeniculum vulgare* Mill. fruits. Food Chemistry. 2012;132(1):385-90.
19. Dearlove RP, Greenspan P, Hartle DK, Swanson RB, Hargrove JL. Inhibition of protein glycation by extracts of culinary herbs and spices. Journal of Medicinal Food. 2008;11(2):275-81.
20. Manju S, Chandran RP, Shaji PK, Nair GA. *In vitro* free radical scavenging potential of *Acorus calamus* L. rhizome from Kuttanad Wetlands, Kerala, India. International Journal of Pharmacy and Pharmaceutical Sciences. 2013;5:376-80.
21. Neethu P, Haseena P, Thomas RS, Goveas SW, Abraham A. Antioxidant properties of *Coscinium fenestratum* stem extracts on streptozotocin induced type 1 diabetic rats. Journal of Applied Pharmaceutical Science. 2014;4(1):29-32.
22. Yazdanparast R, Ardestani A. *In vitro* antioxidant and free radical scavenging activity of *Cyperus rotundus*. Journal of Medicinal Food. 2007;10(4):667-74.
23. Shubha KS, Lakshmidevi N, Sowmya S. *In vitro* antioxidant activity of alcoholic extract of *Solanum xanthocarpum* Sch. and wend and *Picrorhiza kurroa* royal Ex Benth. International Journal of Medical and Pharmaceutical Sciences. 2013;4(4): 12-21.

Peer-review history:
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