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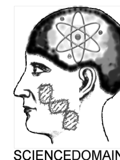

Strong Protein Glycation Inhibitory Potential of Clove and Coriander

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Authors' contributions

This work was carried out in collaboration between both authors. Author HKIP designed the study, wrote the protocol, obtained grants, supervised the project, managed literature search, carried out experiments with 0.5 mg/ ml and lower concentrations of extracts, collected data, analyzed and interpreted the results, wrote the first draft of the manuscript and revised the manuscript. Author DCRW collected plant material, carried out experiments with 5 mg/ ml extracts. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: Protein glycation mediates almost all chronic diabetic complications associated with hyperglycaemia. In view of searching for safe antiglycating agents from nature, the protein glycation inhibitory potential of three spices was determined using a novel, electrophoresis based method.
Study Design: Dry plant parts, standard inhibitor, extraction, *in vitro* glycation model, gel electrophoresis.
Place and Duration of Study: Department of Biochemistry, Faculty of medicine, University of Peradeniya, Sri Lanka, between June 2012 and December 2013.
Methodology: Methanolic extracts of dried *Coriandrum sativum* (Coriander) seeds, *Cinnamomum zeylanicum* (Cinnamon) bark and *Syzygium aromaticum* (Clove) flower buds were used. Bovine serum albumin (BSA) was incubated with fructose in phosphate buffer (pH 7.4) at 37ºC for 30 days in the presence or absence of the extracts. Corresponding blanks, controls and the standard glycation inhibitor aminoguanidine were included. Aliquots were collected at intervals and analyzed

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using polyacrylamide gel electrophoresis under non denaturing conditions (PAGE). Antiglycating effects of the extracts were assessed based on the decrease in migration of BSA.
Results: Previously, we have demonstrated using PAGE, that the increase in BSA migration, in comparison to the blank is proportionate to the degree of glycation. In presence of *C. sativum* and *S. aromaticum*, migration of BSA was reduced with extract concentrations as low as 10 µg/ ml, compared to its migration in absence of the extract (positive control), indicating strong glycation inhibitory effects of the extracts. *C. zeylanicum* showed antiglycating effects at 5 mg/ ml but not at 0.5 mg/ ml.
Conclusion: This study revealed strong protein glycation inhibitory effects in *C. sativum* and *S. aromaticum. C. zeylanicum* showed a comparatively lower inhibition.

Keywords: Glycation inhibitors; PAGE; cinnamon; clove; coriander.

1. INTRODUCTION

Protein glycation is initiated with a non-enzymatic reaction between a carbonyl group of a reducing sugar and amino nitrogen of a protein. Early glycation and oxidation processes give rise to Schiff bases and Amadori products. Highly reactive α-dicarbonyl compounds such as methylglyoxal, accumulate during the reorganization of Amadori products [1]. Further reactions take place over a period of time with molecular rearrangements, leading to the generation of advanced glycation end products (AGEs). AGEs are a complex family of stable, heterogeneous group of compounds. Key factors that determine AGE formation include the degree of hyperglycaemia, rate of protein turnover and the degree of oxidative stress [1]. Hence, glycation of proteins is accelerated under a hyperglycaemic state, associated with diabetes mellitus and under oxidative stress, causing irreversible functional and structural damage to affected protein molecules.

Some AGEs are fluorescent products and some produce reactive oxygen species, that further increase oxidative stress. AGEs may bind to specific cell surface receptors, initiating intracellular signaling pathways that activate pro-oxidant and pro-inflammatory events [2]. Some AGEs result in the formation of intra molecular or inter molecular cross-links. Formed between key molecules in the basement membranes, extra cellular matrix and vessel wall components, these cross-links permanently alter the structure and function of the affected tissues [1]. Cross-linking of extra cellular protein collagen, results in decreased vascular elasticity and reduced vascular compliance [3]. A large body of evidence suggests that AGEs mediate almost all chronic diabetic complications such as retinopathy, nephropathy and cardiovascular diseases [1,2,3,4].

Inhibition of protein glycation is one of the therapeutic approaches that delay or prevent the progression of diabetic complications. Compounds such as aminoguanidine (AG), which have shown glycation inhibitory effects, failed at clinical trials due to their adverse side effects [5]. Medicinal plants are being used since ancient times to cure diseases. The advantages of using medicinal plants with antiglycation properties, that may offer a gentle and safe means of managing glycation induced molecular damage has been recognized in recent years [6,7]. Spices are consumed in our daily diet without adverse effects and may offer as potential antiglycation agents. However, only a fraction of these medicinal plants have been scientifically validated for their antiglycation potential. Analytical techniques used to identify protein glycation inhibitors, involve expensive specialized techniques such as high performance liquid chromatography, mass spectrometry, fluorescence spectrometry and specific enzyme-linked immuno assays [8].

The objective of the study was to identify the protein glycation inhibitory potential of three spices, using a novel, simple, electrophoresis based method.

2. MATERIALS AND METHODS

2.1 Plant Extracts

Coriandrum sativum (Coriander) seeds (CS), *Cinnamomum zeylanicum* (Cinnamon) bark (CZ) and *Syzygium aromaticum* (Clove) flower buds (SA) were purchased as branded products from the open market. Plant parts were cleaned, dried under the shade and powdered using an electric blender. Dry powder (10 g) was extracted three times with methanol (100 ml) using the ultrasonicator. Methanolic extracts were filtered and the solvent was evaporated by a rotary

evaporator (Buchi RII) at 45-50ºC. Crude extracts were stored at room temperature until further analysis. Extracts were re-suspended in phosphate buffer (pH 7.4) before the experiments.

2.2 Detection of Glycation Inhibitory Potential of Medicinal Plants

Bovine serum albumin (BSA), D-fructose, aminoguanidine hydrochloride (AG) and other reagents were from Sigma. Glycation of BSA was undertaken using a method recently optimized by us [9]. In brief, BSA was incubated with 500 mM fructose in 200 mM phosphate buffer (pH 7.4) containing 0.02% sodium azide in the dark at 37ºC for 30 days. These incubations were conducted in the presence and the absence of plant extracts (5 or 0.5 mg/ml). Lower concentrations (100 or 10 µg/ ml) were used when necessary. BSA incubated with fructose (+ Fructose) in absence of plant extracts (-P) or AG was used as the positive control. AG [10 mM (1.1 mg/ml)] was used as the standard inhibitor. Corresponding blanks were prepared in the absence of fructose (- Fructose). Aliquots were collected at intervals and analyzed for the degree of glycation, using polyacrylamide gel electrophoresis (PAGE) under non-denaturing conditions. Polyacrylamide gels (10%) were prepared according to the standard technique [10]. Samples were loaded under native conditions. Electrophoresis was carried out using the Enduro vertical gel electrophoresis system-E2010-P according to the standard Laemmli method [10]. After separation at pH 8.6, protein bands were visualized by staining with Coomassie brilliant blue. Changes in the migration position of BSA bands in the non-glycated, glycated and inhibited reactions were compared with each other. Approximate percentage inhibition of glycation was assessed, based on the decrease in migration of BSA in the presence of plant extract, in comparison to the uninhibited positive control. Experiments were repeated at least four times.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Glycation inhibitory potential of medicinal plants

Using PAGE, previously, we have demonstrated that the increase in BSA migration (in comparison to the test blank), is proportionate to the degree of protein glycation [9], which occurred with increasing sugar concentration and duration of incubation. AG decreased BSA migration in the presence of sugar, compared to the migration of BSA in the positive control, demonstrating the inhibitory effect of AG on protein glycation [9].

In the present study, we have demonstrated the protein glycation inhibitory potential of CS, CZ and SA using PAGE. All three extracts were tested at 5 and 0.5 mg/ ml. When aliquots collected at day 7 were tested with PAGE (Fig. 1), migration of BSA was considerably reduced in presence of CZ bark extract at 5 mg/ ml (Fig. 1-upward arrow) compared to the movement of BSA in positive control (Fig. 1) indicating antiglycating effects. A reduced BSA movement was also visible in the presence of standard inhibitor AG, but to a lesser extent (Fig. 1). However, at 5 mg/ ml concentration, CS and SA showed an increase in the BSA movement even in absence of fructose (Fig. 1-downward arrow). This interference was minimized by using lower concentrations of CS and SA extracts (0.5 mg/ ml) as seen with the aliquots collected on day 14 (Fig. 2).

In presence of SA and CS at lower concentrations of the extract (0.5 mg/ ml), migration of BSA was reduced (Fig. 2- arrow) compared to the migration of BSA in the positive control, when aliquots collected at day 14 were analyzed using PAGE indicating antiglycating effects (Fig. 2). CZ failed to show glycation inhibitory effects at 0.5 mg/ ml (Fig. 2).

BSA migration was reduced even in presence of 10 µg/ ml CS and SA extracts (Fig. 3- small arrow) indicating strong glycation inhibitory effects when the aliquots collected on day 30 were tested using PAGE (Fig. 3). This inhibition was nearly 50% at 10 µg/ ml concentration, compared to the migration of BSA in the positive control. SA at 100 µg/ ml showed further reduction in BSA migration (Fig. 3- arrow) indicating a dose-dependent increase in inhibition (Fig. 3). The ability of SA and CS extracts to inhibit sugar modifications to BSA seem to be comparatively similar, considering their retention position in the gel (Figs. 2 and 3).

3.2 Discussion

We have previously established a simple method to identify plant based inhibitors of protein glycation, using PAGE. When conducted under

native conditions, this method showed an increase in the BSA movement towards the anode, under conditions that increase glycation such as presence of sugar, increase in sugar concentration, presence of more reactive sugars such as ribose, increase in incubation temperature and increase in the duration of incubation [9]. Glycation affects the net charge of a protein as the positively charged free amino groups are targets of glycation, to which carbonyl groups of reducing sugars are attached. Thereby, the net negative charge of the protein is increased which increases its migration towards the anode. This increase in BSA migration occurred to a lesser degree when glycation was inhibited [9,11]. PAGE method can be described as a qualitative, semi quantitative and a simple method useful in identification of relative differences in glycation inhibition.

At higher concentrations of extract (5 mg/ ml), the inhibitory effects of CS and SA appear to be masked, as BSA movement increased with these extracts even in the absence of fructose. We have observed similar findings previously too with plant extracts having strong antiglycation potentials [11]. These extracts seem to contain phytochemicals which increase the net negative charge of BSA or alter the pH of the reaction solution, when used at higher concentrations. However, this interference was minimized by reducing the concentration of plant extract.

Spices have contributed to the flavor, colour and fragrance of food and have been used for medicinal purposes for thousands of years. Although the beneficial effects of spices have been studied for their hypoglycaemic effects, studies carried out on their likely additional protective effects against diabetic complications are few. In the present study, our results demonstrated the glycation inhibitory effects of CS, CZ and SA using the PAGE method. Among the three spices, the inhibitory effects shown were higher with CS and SA compared to that of CZ as seen when lower extract concentrations were used. Results of another study (unpublished data), on the inhibitory effects of glycation induced protein cross-linking using a different method were comparable with results of the present study, providing evidence for a higher efficacy of CS and SA in inhibiting protein glycation and glycation induced cross-linking.

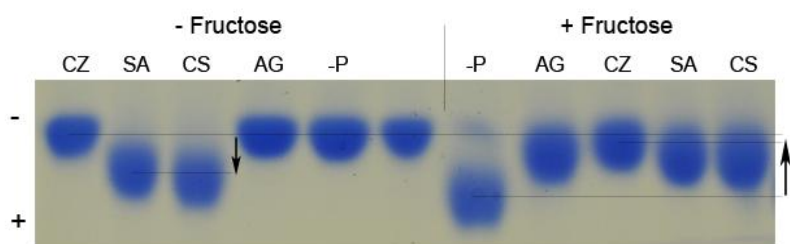

Fig. 1. Effect of plant extracts on BSA migration at 5 mg/ ml
AG: with aminoguanidine, CS: with Coriandrum sativum, CZ: with Cinnamomum zeylanicum, SA: with Syzygium aromaticum, -P: without plant extract, - Fructose: without fructose, + Fructose: with fructose

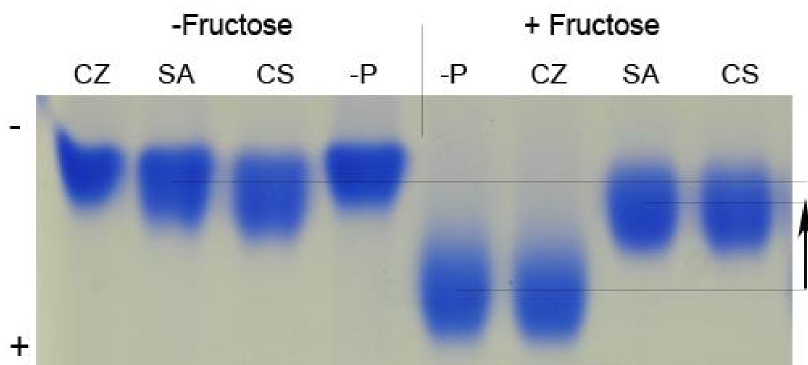

Fig. 2. Effect of plant extracts on BSA migration at 0.5 mg/ ml
CS: with Coriandrum sativum, CZ: with Cinnamomum zeylanicum, SA: with Syzygium aromaticum, -P: without plant extract, - Fructose: without fructose, + Fructose: with fructose

Fig. 3. Effect of CS and SA on BSA migration at 10 µg/ ml

CS: with Coriandrum sativum, SA: with Syzygium aromaticum, -P: without plant extract, - Fructose: without fructose, + Fructose: with fructose., 10: with 10 µg/ ml extract, 100: with 100 µg/ ml extract

Some spices are very rich sources of polyphenolic compounds which are known for their strong antioxidant properties. Recent investigations reveal that foods rich in antioxidants have the potential to inhibit protein glycation [12,13] probably by mopping up free radicals. In spices, a positive correlation was found between the total phenolic content of the extract and their glycation inhibitory potential [12,14].

Dearlove et al. [12] demonstrated inhibition in the formation of fluorescent AGEs with spices. Among the 24 herbs and spices tested, the most potent inhibitory effects were seen with 50% ethanolic extracts of cloves, ground Jamaican allspice and cinnamon. A few studies revealed the protein glycation inhibitory effects of CS, CZ and SA using advanced equipment. By incubating BSA with glucose, Ramkissoon et al. [15,16] reported the inhibitory effects of CS ethanol extracts on the formation of fluorescent AGEs using spectrofluorimetry. In addition, nine other extracts of herbs and spices including garlic, ginger, thyme, parsley, curry leaves, peppermint, turmeric, onion and green onion scallion were tested in these studies.

Rutin, a major polyphenol isolated from CZ is known for its antiglycating effects [17]. Methanolic extracts from eight dried spices, namely, basil, cardamom, cinnamon, cumin, parsely, rosemary, thyme and turmeric, inhibited glycation at various degrees when BSA was glycated with glucose. Among the eight spices used, they demonstrated cinnamon as having the highest antiglycating potential in inhibiting the formation of fluorescent AGEs [14].

A recent study showed that, the aqueous extract of clove inhibited the formation of both fluorescent and non-fluorescent AGEs [8]. At the end of a 4 week incubation of BSA with 500 mM fructose, clove inhibited fluorescent AGE formation by 86.3% and 95.2% at concentrations of 0.25 and 1 mg/ml respectively. Our results with methanolic extracts of clove were compatible with the results of Suantawee et al. [8]. Under similar incubation conditions, 1 mg/ ml clove extract, reduced the formation of carboxymethyllysine, a biomarker of non-florescent AGEs by 72.8% [8]. A potent antioxidant and free radical scavenging activity of clove extract was also revealed in their study, consistent with the findings of previous studies. Polyphenolic compounds such as gallic acid, quercetin glucoside and ellagic acid, present in clove extract [18] are known to have antiglycation effects [6]. Hydroxyl and superoxide scavenging activities of clove, may have contributed to their glycation inhibitory effects [8].

Hypoglycaemic effects of CS, CZ and SA were previously revealed *in vivo*, using streptozotocin-induced diabetic rats [19], patients with type 2 DM [20] and type 2 diabetic mice [21] respectively. Unlike these *in vivo* models, the antiglycating effects observed with CS, CZ and SA in our *in vitro* glycation model, was unaffected by their possible hypoglycaemic effect, as the sugar concentrations used, remained similar and relatively stable in the presence and absence of the plant extracts. Hence, our results suggest an additional beneficial effect of CS, CZ and SA extracts against chronic diabetic complications caused by protein glycation.

The antiglycation potential demonstrated in our study, with CS, CZ and SA extracts, using the native PAGE method, was seen to be comparable with results shown, using established techniques in previous studies. Furthermore, we conclusively revealed a stronger inhibitory effect in CS and SA compared to that of CZ. The therapeutic efficacy and safety of these extracts, as potential antiglycation agents, needs further investigation using *in vivo* experiments.

4. CONCLUSION

We have demonstrated strong inhibitory effects on protein glycation in methanolic extracts of *Coriandrum sativum* seeds and *Syzygium aromaticum* flower bud using a PAGE method. *Cinnamomum zeylanicum* bark showed a relatively lower antiglycation potential.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products sparking the development of diabetic vascular injury. Circulation. 2006; 114(6):597-605.
2. Peppa M, Uribarri J, Vlassara H. Glucose, advanced glycation end products, and diabetes complications: What is new and what works. Clinical Diabetes. 2003;21(4): 186-187.
3. Karmakar S, Goswami RP. Advanced glycation end products (AGEs): It's role in the pathogenesis of diabetic complications. Partha Medicine Update. 2012;22:277-82.
4. Monnier VM. Glycation products as markers and predictors of the progression of diabetic complications. Annals of the New York Academy of Sciences. 2005; 1043:567-581.
5. Rahbar S, Figarola JL. Novel inhibitors of advance glycation end products. Archives of Biochemistry and Biophysics. 2003; 419:63-79.
6. Wu C, Huang S, Lin J, Yen G. Inhibition of advanced glycation end product formation by foodstuffs. Food and Function. 2011;2: 224-234.
7. Saraswat M, Reddy PY, Muthenna P, Reddy GB. Prevention of non-enzymic glycation of proteins by dietary agents: prospects for alleviating diabetic complications. British Journal of Nutrition. 2009;101:1714-1721.
8. 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.
9. Wijetunge DCR, Perera HKI. A novel *in vitro* method to detect inhibitors of protein glycation. Asian Journal of Medical Sciences. 2014;5(3):15-21.
10. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970; 227(5259):680-685.
11. Perera HKI, Handuwalage CS. Detection of protein glycation inhibitory potential of nine antidiabetic plants using a novel method. Asian Journal of Medical Sciences. 2015; 6(2):1-6.
12. 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):275-281.
13. Elosta A, Ghous T, Ahmed N. Natural products as anti-glycation agents: Possible therapeutic potential for diabetic complications. Current Diabetes Reviews. 2012;8(2):92-108.
14. Ho SC, Chang PW. Inhibitory effects of several spices on inflammation caused by advanced glycation end products. American Journal of Plant Sciences. 2012;3:995-1002.
15. Ramkissoon JS, Mahomoodally MF, Ahmed N, Subratty AH. Relationship between total phenolic content, antioxidant potential, and antiglycation abilities of common culinary herbs and spices. Journal of Medicinal food. 2012;15(12): 1116-1123.
16. Ramkissoon JS, Mahomoodally MF, Ahmed N, Subratty AH. Antioxidant and anti-glycation activities correlate with phenolic composition of tropical medicinal herbs. Asian Pacific Journal of Tropical Medicine. 2013;6(7):561-569.

17. Rao PV, Gan SH. Cinnamon: A multifaceted medicinal plant. Evidence-Based Complementary and Alternative Medicine. 2014;12. Available:http://dx.doi.org/10.1155/2014/642942
18. Atawodi S, Atawodi J, Pfundstein B, Spiegelhalder B, Bartsch H, Owen R. Assessment of the polyphenol components and *In vitro* antioxidant properties of *Syzygium aromaticum* (L.) Merr. & Perry. Electronic Journal of Environmental, Agricultural and Food Chemistry. 2011;10: 1970-1978.
19. Eidi M, Eidi A, Saeidi A, Molanaei S, Sadeghipour A, Bahar M, et al. Effect of coriander seed (*Coriandrum sativum* L.) ethanol extract on insulin release from pancreatic beta cells in streptozotocin induced diabetic rats. Phytotherapy Research. 2009;23(3):404-406.
20. Sangal A. Role of cinnamon as beneficial antidiabetic food adjunct: A review. Advances in Applied Science Research. 2011;2(4):440-450.
21. Kuroda M, Mimaki Y, Ohtomo T, Yamada J, Nishiyama T, Mae T, et al. Hypoglycemic effects of clove (*Syzygium aromaticum* flower buds) on genetically diabetic KK-Ay mice and identification of the active ingredients. Journal of Natural Medicines. 2012;66(2):394-399.

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