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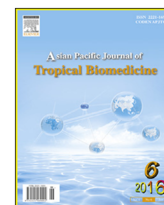

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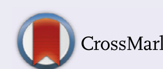
Inhibition of glucose- and fructose-mediated protein glycation by infusions and ethanolic extracts of ten culinary herbs and spices



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ABSTRACT

Objective: To investigate the inhibitory activity of ten culinary herbs and spices namely on glucose-mediated glycation (GMG) and fructose-mediated glycation (FMG) of bovine serum albumin.

Methods: Fluorescence was used as an index of albumin glycation using glucose and fructose as substrates in the presence of infusions and ethanolic extracts of ten culinary herbs and spices. Antioxidant activity of the extracts was evaluated using reducing power, metal ion chelating and superoxide radical scavenging assays. Phytochemicals profile was analysed using 13 standard methods.

Results: FMG was found to be significantly higher than GMG (95 and 84 AU, respectively; $P < 0.05$). Infusions and ethanolic extracts showed significant ($P < 0.05$) inhibitory activity on both GMG and FMG when compared to appropriate controls. No significant difference ($P > 0.05$) was found in the percentage glycation inhibitory activity of infusions compared to ethanolic extracts. The mean percentage inhibitory activity of the extracts for GMG (45.9%) and for FMG (45.1%) was not significantly different ($P > 0.05$). Qualitative phytochemical analysis showed the presence of alkaloids, flavonoids, tannins, terpenoids, anthraquinones, steroids, reducing sugars, proteins, phenols, saponins, phlobatannins, and cardiac glycosides.

Conclusions: The higher rate of fluorescence generation by fructation suggests that glycation by fructose deserves much attention as a glycating agent. Data herein showed that the extracts inhibited GMG and FMG. Thus, these edible plants could be a natural source of antioxidants and anti-glycation agent for preventing advanced glycation end-products-mediated complications.

1. Introduction

Protein glycation is a non-enzymatic reaction between reducing sugars and free amino groups [1], resulting in the formation of reversible intermediates that undergo rearrangements generating advanced glycation end-products (AGEs) [2–5]. The formation of AGEs is also known to result from the action of various metabolites such as fructose, trioses and dicarbonyl compounds [6–9] which participate in the glycation reaction at a much faster rate than glucose [10]. As glucose is the most abundant sugar in blood and elevated in diabetes, most studies have been focused on glycation between glucose and proteins [7]. However, glucose is one of the least reactive sugars [11] and *in vitro* studies suggest that fructose, compared to glucose, is a more potent initiator of glycation [9–15]. Although direct evidence is limited [7], it is likely that the high reactivity of fructose and its metabolites may substantially contribute to the formation of AGEs *in vivo* [12–15]. Hence, further insight into its possible role in AGEs-mediated pathologies is needed to appraise the significance of fructation in healthy people and in diabetic complications

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especially where the sorbitol pathway is active [16]. Several studies have shown the role of AGEs in protein modification leading to physiopathological changes such as diabetes [10,17,18]. Much interest has been devoted to using anti-glycation agents for alleviating diabetic complications [19,20], however, drugs like aminoguanidine have shown serious side effects [2]. Therefore, much effort has been extended to search for dietary plants that could effectively inhibit AGE formation and also have antioxidant properties [19–24]. A great number of aromatic, spicy, medicinal and other plants contain chemical compounds including tannins, alkaloids, terpenoids, steroids and flavonoids exhibiting antioxidant properties and demonstrate such protective effects [25–30].

However, there are little studies supporting the ability of culinary herbs and spices from Mauritius in the prevention of fluorescent AGE formation. Besides initiating investigation on the inhibitory activity of dietary plants on protein glycation [31] and their antioxidant properties [32], this study also investigated the effects of the extracts against glucose- and fructose-mediated glycation of bovine serum albumin (BSA). Since fluorescence generation through the Maillard reaction has been correlated with long-term complications of diabetes [12] and it is known that fructose is a more potent glycating agent than glucose [7], the participation of glycation by fructose (fructation) deserves further exploration. In addition, the phytochemical contents and supplementary antioxidant activities including the reducing power, metal ion chelating and superoxide radical scavenging activities were also evaluated. Dietary plants might be promising anti-glycation agents for the prevention of diabetic complications via inhibition of AGE and oxidation-dependent protein damage.

2. Materials and methods

2.1. Chemicals

BSA (BSA; fraction V, fatty acid free, low endotoxin), D-glucose, sodium azide, phosphate buffered saline (PBS), aminoguanidine, trichloroacetic acid (TCA), ethanol, iron (III) chloride 6-hydrate ($FeCl_3$), concentrated hydrochloric acid (HCl), benzene, ammonia (NH_3), sulphuric acid (H_2SO_4), chloroform, copper acetate, acetic acid, glacial acetic acid, Mayer's reagent, Wagner's reagent, Dragendroff's reagent, Hager's reagent, Molisch's reagent, Millon's reagent, iodine, Fehling A, Fehling B, Benedict's reagent, NaOH, sodium nitroprusside, magnesium ribbon, lead acetate, zinc powder, boric acid, HNO_3, ninhydrin, potassium ferricyanide, butylated hydroxytoluene (BHT), ethylenediaminetetracetic acid (EDTA), potassium dihydrogen phosphate, dipotassium hydrogen phosphate, iron (II) chloride, ferrozine, nicotinamide adenine dinucleotide, phenazine methosulphate, nitroblue tetrazolium (NBT), potassium bismuth iodide, picric acid, copper (II) sulphate, potassium sodium tartrate, anhydrous sodium carbonate, sodium citrate, copper (II) sulphate pentahydrate were purchased from Sigma–Aldrich, USA.

2.2. Plant materials and preparation of extracts

Culinary herbs and spices were purchased from a local market, Rose Hill, Mauritius. Ten commercially available herbs and spices were tested, namely, garlic (*Allium sativum* L.), ginger (*Zingiber officinale* Rosc.), thyme (*Thymus vulgaris* L.), parsley (*Petroselinum crispum* Mill.), curry leaves (*Murraya koenigii* L. Spreng), peppermint (*Mentha piperita* L.), turmeric (*Curcuma longa* L.), onion (*Allium cepa* L.), green onion scallion (*Allium fistulosum* L.) and coriander (*Coriandrum sativum* L.). The study was limited to plants that are widely available to the public and are in routine use for daily food cooking in Mauritius. Fresh culinary herbs and spices were ground into a paste and 5 g of the latter were extracted with ethanol (50%) at a ratio of 10 mL/g at room temperature (28 ± 2) °C for 1 week. The extracts were centrifuged at 1 000 *g* for 10 min to remove precipitate. For preparing infusions 5 g of the ground paste were infused into boiling distilled water for 30 min at a ratio of 10 mL/g and filtered through Whatman No. 4 paper [23].

2.3. In vitro glycation of BSA with fructose and glucose

BSA was glycated as described previously with minor modifications [33]. Briefly, BSA (1 mg/mL) was incubated with 200 mmol/L fructose or glucose in 0.2 mol/L PBS, pH 7.4 containing 0.01% sodium azide in darkness at 37 °C for 3 weeks in the absence or presence of the ten plants extracts separately. The assay was conducted using 100 µL of the plants of either ethanolic extracts or infusions in a final volume of 5 mL. Aminoguanidine was used as a positive control. After three weeks incubation, the reactions were stopped by adding 10 µL of 100% (w/v) TCA and after 10 min the mixture was centrifuged at 2 000 r/min for 15 min. The precipitate was re-dissolved in alkaline PBS. The fluorescent AGEs were determined spectrofluorometrically (PerkinElmer) using fluorescence intensity at an excitation wavelength 370 nm and emission wavelength 440 nm. The inhibitory effect of the extracts and aminoguanidine was evaluated by the calculation of percentage inhibition compared with maximum glycation elicited by BSA-glucose or BSA-fructose [3].

2.4. Reducing power assay

The reducing power of samples was determined by the method of Oyaizu [34]. An aliquot of the sample (1.0 mL) was mixed with phosphate buffer (0.2 mol/L, pH 6.6, 2.5 mL) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 50 °C for 20 min. After adding 10% TCA (2.5 mL), the mixture was centrifuged at 650 r/min for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and 0.1% iron (III) chloride (0.5 mL), and the absorbance was measured at 700 nm using an appropriate blank. Assays were carried out in triplicate. BHT was used as a control.

2.5. Metal ion chelating assay

The ability of samples to chelate iron (II) ions was estimated using the method reported by Gulcin (2006) [35] and compared with that of the reference chelator agent EDTA. Samples were added to a solution of 2 mmol/L iron (II) chloride (1 mL). The reaction was initiated by the addition of 5 mmol/L ferrozine (1 mL) and the volume of the mixture was finally adjusted to 4 mL with ethanol, shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was

measured spectrophotometrically at 562 nm. All assays were done in triplicate.

2.6. Superoxide radical scavenging activity

Superoxide anions were generated using phenazine methosulphate-nicotinamide adenine dinucleotide (PMS-NADH) system. The superoxide anions were subsequently made to reduce NBT which yields a chromogenic product, which is measured at 560 nm [36]. Test solution (1 mL) in 0.1 mol/L phosphate buffer pH 7.4, 1 mL of 468 μmol/L NADH solution, 1 mL of 150 μmol/L NBT solution and 1 mL of 60 μmol/L phenazine methosulphate solution were added to a tube and incubated at room temperature for 5 min. The absorbance was read at 560 nm. All assays were done in triplicate. Gallic acid was used as a positive control.

2.7. Qualitative phytochemical analysis

The plant extracts were subjected to qualitative chemical screening for the identification of various classes of chemical compounds using standard methods [20,37–40]. Preliminary phytochemical screening of the ten plant materials for secondary metabolites was performed as follows.

2.7.1. Determination of tannins

Two millilitre of the plant extracts was stirred with equal volume of distilled water. A few drops of 2% $FeCl_3$ solution was added. The presence of tannins was indicated by the formation of a green precipitate.

2.7.2. Determination of saponins

In foam test, 5 mL of extract was shaken vigorously with 5 mL of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication for the presence of saponins.

In froth test, 2 mL of extract was shaken vigorously with distilled water to froth and was then allowed to stand for 10–15 min. The persistent froth was considered as presence of saponins.

2.7.3. Determination of phlobatannins

Two millilitre of extract was added to 2 mL of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

2.7.4. Determination of anthraquinones

In Borntrager's test, 3 mL of plant extracts were treated with 3 mL of benzene. The mixture was then filtered. The filtrate was then mixed with 5 mL of 10% ammonia solution. The presence of free anthraquinones was indicated by the presence of a pink, red or violet colour in the lower ammonical phase.

Three millilitre of H_2SO_4 was added to 3 mL of extracts and the mixture was boiled then filtered. To the filtrate, 3 mL of benzene was added and shaken. Three millilitre of 10% NH_3 was added to the benzene layer. Anthraquinone derivatives were revealed by a pink, red or violet colouration in the lower ammonical phase.

2.7.5. Determination of terpenoids

Two millilitre of extract was mixed with 2 mL of chloroform and evaporated to dryness. Two millilitre of concentrated H_2SO_4 was then added and heated for about 2 min. A grayish colour indicates the presences of terpenoids.

2.7.6. Determination of diterpenes

Two millilitre of extract were treated with 3–4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

2.7.7. Determination of steroids

Two millilitre of extract was mixed with 2 mL of chloroform and 2 mL of concentrated H_2SO_4. A red colour indicates the presence of steroids.

Two millilitre of extract was mixed with 2 mL of chloroform and treated with H_2SO_4 and acetic acid. The development of a greenish colour indicates the presence of steroids.

2.7.8. Determination of alkaloids

2.7.8.1. Mayer's test

Extracts were mixed with HCl and filtered. Filtrates were treated with Mayer's reagent. Formation of a yellow coloured precipitate indicates the presence of alkaloids.

2.7.8.2. Wagner's test

Extracts were mixed with HCl and filtered. Filtrates were treated with Wagner's reagent. Formation of brown/reddish precipitate indicates the presence of alkaloids.

2.7.8.3. Dragendroff's test

Extracts were mixed with HCl and filtered. Filtrates were treated with Dragendroff's reagent. Formation of red precipitate indicates the presence of alkaloids.

2.7.8.4. Hager's test

Extracts were mixed with HCl and filtered. Filtrates were treated with Hager's reagent. Presence of alkaloids was confirmed by the formation of yellow precipitate.

2.7.9. Determination of carbohydrates and reducing sugars

2.7.9.1. Molisch's test

Two millilitre of Molisch's reagent was shaken with 3 mL of extract. Then 2 mL of concentrated H_2SO_4 was added carefully down the side of the test tube. The presence of carbohydrates was indicated by a violet ring at the interphase.

2.7.9.2. Iodine test

Three millilitre of extract and 1 mL of iodine were mixed together. Presence of carbohydrates was shown by a dark blue or purple colouration.

2.7.9.3. Fehling's test

Equal volume of Fehling A and Fehling B reagents were mixed together and 2 mL of it was added to the extract and gently boiled. A brick red precipitate at the bottom of the test tube indicated the presence of reducing sugars.

2.7.9.4. Benedict's test

Two millilitre of extract was mixed with 2 mL of Benedict's reagent and boiled. A reddish brown precipitate indicated the presence of reducing sugars.

2.7.10. Determination of cardiac glycosides (cardenolides)

2.7.10.1. Liebermann's test

Two millilitre of extract was mixed with 2 mL of chloroform and 2 mL of acetic acid and the solution was cooled on ice. H_2SO_4 was then added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus. That is a glycone portion of glycoside.

2.7.10.2. Salkowski's test (detection of phytosterols)

Two millilitre of each plant extract was added to 2 mL of chloroform. The mixture was then shaken gently with 2 mL of H_2SO_4. The presence of a steroidal ring; the glycone portion of glycoside was indicated by a reddish brown colour.

2.7.10.3. Keller–Kilani test

One drop of $FeCl_3$ solution was added to a mixture of 2 mL of extract and 2 mL glacial acetic acid. One millimetre of concentrated H_2SO_4 was then added to the mixture. The presence of a deoxy sugar characteristic of cardenolides was indicated by a brown ring.

Two millilitre of extract was mixed with 10% NaOH and 0.3% solution of nitroprusside. Appearance of transient pinkish red colouration indicates the presence of cardenolides.

2.7.11. Determination of flavonoids and different types of flavonoids

2.7.11.1. Shinoda's test

A piece of magnesium ribbon and HCl were added to the extracts. Purple, red, pink or orange colour confirmed the presence of flavonoids.

2.7.11.2. Alkaline reagent test

Two millilitre of extract was mixed with 2 mL of 2% solution of NaOH. An intense yellow colour which turned colourless on addition of a few drops of dilute acid indicated the presence of flavonoids.

2.7.11.3. Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

2.7.11.4. Test for flavononols

Zinc powder was added with HCl. Development of a deep magenta colour confirmed presence of flavanonols.

2.7.11.5. Test for flavonols

A pinch of boric acid and a few drops of acetic acid were added to the extract. Bright yellow colour with green fluorescence indicated flavonols.

2.7.11.6. Test for flavones and flavanols

H_2SO_4 was added to the extract. A yellow colouration was taken as evidence for the presence of flavones and flavanols. Lively orange to crimson colours indicated the presence of flavanones.

2.7.11.7. Test for flavanones

Few drops of concentrated HNO_3 were added to the extract. Brilliant blue colour confirmed the presence of phloroglucinol derived flavanones.

2.7.12. Determination of simple phenolics

One millilitre of extract was mixed with 1–2 drops of 1% $FeCl_3$. Development of blue-green or black colouration was indicative of the presence of phenol.

2.7.13. Determination of proteins and amino acids

2.7.13.1. Millon's test

Two millilitre of extract was mixed with Millon's reagent. A white precipitate which turned red upon gentle heating confirmed the presence of protein.

2.7.13.2. Ninhydrin test

Two millilitre of extract was boiled with 2 mL of 0.2% solution of ninhydrin. Appearance of a violet colour suggested the presence of amino acids.

2.7.13.3. Xanthoproteic test

The extracts were treated with few drops of concentrated HNO_3. Formation of yellow colour indicates the presence of proteins.

2.8. Statistical analysis

Results were presented as mean ± SD. Difference between groups were compared using paired or unpaired *t*-test with one-tailed or two-tail test. In each analysis $P < 0.05$ was considered statistically significant.

3. Results

3.1. The effects of the extracts on glycation

Formation of AGEs was determined by measuring fluorescence intensity of BSA-glucose or fructose solutions in presence or absence of the extracts (Figure 1). Our findings indicate that glycation of BSA occurred at a significantly higher ($P < 0.05$) rate with fructose (95 AU) than glucose (84 AU) for the controls and also in presence of the extracts. There was significantly less BSA-glycation in the presence of the control aminoguanidine ($P < 0.05$) when compared to BSA-glucose and BSA-fructose solutions. In presence of the extracts, a significantly lower ($P < 0.05$) fluorescence intensity was noted when compared to BSA-sugar solutions.

Aminoguanidine inhibited the formation of AGEs in BSA/glucose and BSA/fructose by 64.7% and 74.0%, respectively. The percentage inhibition of the ten extracts ranged from 23.8% to 67.8% and 20.2% to 59.4 for the ethanolic extracts and infusions respectively in the BSA/glucose system and from 24.0% to 53.7% and 18.5%–63.2%, respectively for the BSA/fructose system. No significant difference ($P > 0.05$) was found in the inhibitory effects of the ethanolic extracts compared to the infusions and in the percentage inhibitory capacity of the extracts on glycation with glucose compared to fructose.

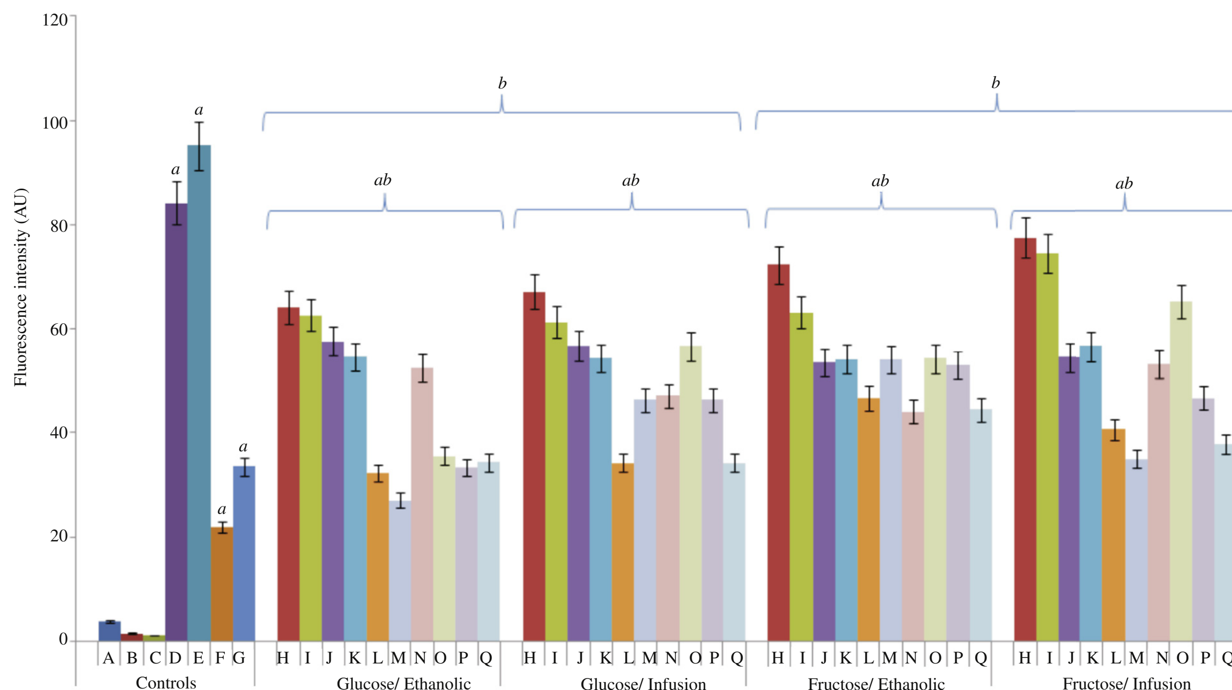

Figure 1. Effects of ten plant extracts on formation of fluorescent AGEs in BSA incubated with glucose or fructose.
A: BSA; B: Glucose; C: Fructose; D: BSA/glucose; E: BSA/fructose; F: Glucose/Aminoguanidine; G: Fructose/Aminoguanidine; H: Garlic; I: Ginger; J: Thyme; K: Parsley; L: Curry leaves; M: Pepper mint; N: Onion; O: Scallion; P: Coriander; Q: Turmeric; Each value represents the mean ± SD ($n = 3$) after 3 weeks incubation at 37 °C; ^{a}: $P < 0.05$ when comparing BSA/glucose to BSA/fructose, BSA/sugar to aminoguanidine control, BSA/glucose to extracts and BSA/fructose to extracts; ^{b}: $P > 0.05$ when comparing ethanolic extracts to infusions and inhibitory effects of extracts on glycation compared to fructation.

3.2. Antioxidant activity of the extracts

3.2.1. Reducing power assay

The reducing power assay showed that all the extracts tested exhibited reducing capacity (Figure 2) in the order of turmeric, coriander, scallion, garlic, curry leaves, ginger, pepper mint, parsley, onion and thyme.

3.2.2. Metal ion chelating assay

The results obtained for metal ion chelating assay is shown in Figure 3. Onion, scallion and thyme showed the highest activity; curry leaves, pepper mint and coriander showed average activity; garlic, ginger and parsley showed very little activity whereas only a slight activity was observed with turmeric.

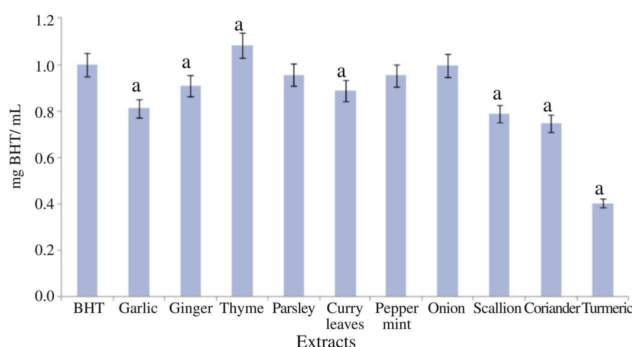

Figure 2. Reducing power of the ten extracts.
Results are presented as mean ± SD ($n = 3$). ^{a}: $P < 0.05$ when compared to control BHT.

3.2.3. Superoxide radical scavenging activity

The result for superoxide radical scavenging activity is shown in Figure 3. All the extracts showed scavenging activity in the order of garlic < turmeric < ginger < parsley < coriander < pepper mint < thyme < scallion < curry leaves < onion.

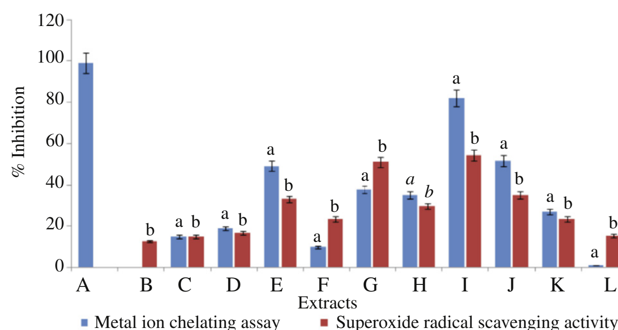

Figure 3. Metal ion chelating assay and superoxide scavenging activity of the ten extracts.
A: EDTA; B: Gallic acid; C: Garlic; D: Ginger; E: Thyme; F: Parsley; G: Curry leaves; H: Pepper mint; I: Onion; J: Scallion; K: Coriander; L: Turmeric; Results are presented as mean ± SD ($n = 3$); ^{a}: $P < 0.05$ when compared to control EDTA; ^{b}: $P < 0.05$ when compared to control gallic acid.

3.3. Phytochemical analysis of the extracts

Qualitative evaluation of the chemical constituents of the selected extracts showed the presence of various secondary

Table 1
Phytochemical profile of ten extracts.

Phytochemicals		Garlic	Ginger	Thyme	Parsley	Curry leaves	Pepper mint	Onion	Scallion	Coriander	Turmeric
Tannins		+	+	+	+	+	+	+	+	+	+
Saponins	Foam	+	+	+	+	+	+	+	+	+	+
	Froth	+	+	+	+	+	+	+	+	+	+
Phlobatannins		−	−	−	−	−	−	+	−	−	−
Anthraquinones	Free	−	−	−	−	−	−	−	−	−	−
	Derivatives	−	−	−	−	−	−	+	−	−	−
Terpenoids		−	+	+	−	−	−	−	+	+	+
Diterpenes		−	+	−	+	+	+	−	+	+	+
Steroids		−	+	−	−	−	−	−	−	−	+
Simple phenolics		+	+	+	+	+	+	+	+	+	+
Detection of alkaloids	Mayer's	+	+	+	+	−	−	−	+	−	+
	Wagner's	−	−	−	−	−	−	−	−	−	−
	Dragendroff's	−	−	−	−	−	−	−	−	−	−
	Hager's	−	−	−	−	−	−	−	−	−	−
Protein and amino acids	Millon's	+	−	−	−	−	−	+	−	+	+
	Xanthoproteic	+	+	+	+	+	+	+	+	+	+
	Ninhydrin	+	+	+	+	+	+	+	+	+	+
Detection of carbohydrates and reducing sugars	Molisch's	+	+	+	+	+	+	+	+	+	+
	Iodine	−	−	−	+	+	+	+	+	+	−
	Fehling's	+	+	−	−	−	−	+	+	−	−
	Benedict's	+	−	−	−	−	−	+	−	−	−
Detection of glycosides	Liebermann's	+	−	+	+	+	+	+	+	+	+
	Phytosterols	+	+	+	−	+	+	+	+	−	−
	Deoxy sugar	+	+	+	+	+	+	+	+	+	+
	Cardenolides	−	−	−	−	−	−	−	−	−	−
Detection of flavonoids	Shinoda's	−	−	−	−	−	−	−	−	−	−
	Alkaline	+	+	+	+	+	+	+	+	+	+
	Lead acetate	+	+	+	+	+	+	+	+	+	+
	Flavanonols	−	−	−	−	−	−	−	−	−	−
	Flavonols	+	−	+	+	+	−	−	+	−	−
	Flavones	+	+	+	+	+	+	+	+	+	+
	Flavanones	−	−	−	−	−	−	−	−	−	−

+: Presence of constituent; −: Absence of constituents.

metabolites (Table 1). Phytochemical analysis showed the presence of bioactive constituents such as carbohydrates, proteins, steroids, alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, phlobatannins, tannins, terpenoids and diterpene.

4. Discussion

The present study was undertaken to compare the inhibitory effect of ten culinary herbs and spices on glucose- and fructose-mediated BSA glycation. Based on the fluorescence property, we studied the influence of ten plant extracts on the formation of AGEs *in vitro*. Fructose was found to be significantly more effective than glucose in the glycation of BSA *in vitro*. Our results showed that the culinary herbs and spices efficiently inhibited AGE formation. Glycation of proteins is a major cause of chemical modification [3] disrupting molecular conformation of polypeptides involved in pathogenesis of age-related disorders including Alzheimer's disease and diabetic complications [2,10,17]. In principle, all reducing sugars whether aldoses or ketoses and even molecules related to sugars can initiate the reaction *in vivo* [17]. As in the case of glucose, the reducing free carbonyl group of fructose may react non-enzymatically with amino groups in a process known as fructation or fructosylation [3,12]. The fact that elevated fructose and fructose metabolites can initiate deleterious glycation involved in development of metabolic diseases is of much concern [14,41] as long-term consumption of fructose has been associated with hyperlipidaemia, impaired glucose tolerance and increased insulin resistance [12]. Thus, there are some evidence to highlight the importance of fructose as an effective glycating agent with respect to AGE formation and should be given due consideration. Though extracellular concentration of fructose is normally lower than that of glucose; its high reactivity suggests that fructose is a strong candidate for fructation *in vivo* [13,42]. Fructose and its metabolites are believed to be important precursors in intracellular formation of AGEs and participate in glycation at a much faster rate than glucose [2]. The rate of glycation is directly proportional to the percentage of sugar in the open-chain (acyclic) form [14] and fructose open chain population is approximately 8 times that of glucose [13]. In the context of AGE formation, the formation of glyceraldehyde from fructose is very reactive in the Maillard reaction as compared with that of glyceraldehyde-3-phosphate from glucose [13]. Also, fructose is formed from sorbitol through glucose by the polyol pathway [42], one of the mechanisms for the formation of AGEs of particular relevance in diabetics [43]. Intracellularly, fructose is elevated in a number of tissues of diabetic patients in which the polyol pathway is active [41]. In the cells of these tissues, the concentrations of fructose and glucose are of the same magnitude [14]. Fructose consumption has increased during the past decades despite evidence implicating fructose in the development of metabolic disorders, obesity and diabetic complications [7]. Although dietary fructose has some adverse side effects, it is still advocated as a preferred sweetener and as a glucose substitute for diabetics [7]. Fructose found

naturally in honey, fruits, used as sweeteners and as a constituent of sucrose in food products forms a main component of human diets [12]. Consequently, the possibility that increased fructose intake might result in increased tissue concentration potentiating fructose derived glycation reaction should be stressed [14]. Further insight into these processes is needed to properly estimate the relevance of such alterations in healthy and diabetic people.

Non-enzymatic reactions of fructose compared with glucose are believed to cause higher production of reactive carbonyls and oxygen species (ROS) [7]. Fructose through the polyol pathway when activated by hyperglycemia may lead to increased oxidative stress and formation of AGEs [14]. Glycation is not only a major cause of AGE-mediated protein modification, but it also induces oxidation-dependent tissue damage [2]. ROS including free radicals and reactive carbonyl groups are also generated during glycation and glycoxidation [7]. In addition, transition metals also catalyse auto-oxidation of glucose and further generate reactive carbonyl compounds to form AGEs [18]. Therefore, scavenging free radicals through antioxidants can alleviate oxidative stress and reduce oxidative damage-mediated glycation and glycoxidation [17,44]. Also, metal chelators may retard the process of AGEs by preventing further oxidation of Amadori products and metal-catalysed glucose oxidation [41].

Various methods of accessing antioxidant capacities of the edible plants studied have been reported previously [45]. Here the reducing power assay, metal ion chelating assay and superoxide radical scavenging assay provide additional evidence of their ability to decrease ROS through free radical scavenging properties of the phytochemical compounds detected *in vitro*. The reducing ability of a compound generally depends on the presence of reductones [46]. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity [47]. The antioxidant principles present in the ten extracts caused reduction of Fe^{3+}/ferricyanide complex to the ferrous form, and thus proved the reducing power aptitude. The metal chelating ability of the extracts was measured by the formation of ferrous ion-ferrozine complex. The results of this study demonstrate that the extracts have an effective capacity for iron binding, suggesting their antioxidant potential. Superoxide radical was generated by the PMS-NADH and NBT systems and results revealed that the ten extracts possess antioxidant activity which was similar to previously demonstrated ferric reducing antioxidant power and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity [27,28]. Based on our results, it may be concluded that all the extracts showed strong antioxidant activity; reducing power ability, metal ion chelating ability and free radical scavenging activity when compared to standards such as BHT, EDTA and gallic acid. It has been reported that many antioxidant-containing foods can scavenge free-radicals generated during the glycation process as well as prevent reducing sugars and Amadori products from self-oxidation, thus inhibiting AGE formation [17,48]. Accordingly, the observed anti-glycation activity of the extracts may be explained through mechanisms inhibiting AGE formation by decreasing the ROS formation or by scavenging the ROS formed *in vitro* by auto-oxidation of sugars and/or oxidative degradation of Amadori products. As the ten extracts exhibited different activities, there may be different percentages of phytochemical constituents present in the extracts as demonstrated by the phytochemical evaluation.

The phytochemical analysis of the ten extracts showed the presence of phenolics, carbohydrates, proteins, steroids, alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, phlobatannins, tannins, terpenoids and diterpenes. It has been reported that the antioxidant and protein glycation inhibitory activity of edible plants are related to the free radical scavenging property of phenolic compounds and flavonoids [18]. Our findings indicate that the ten extracts have phenolic compounds and flavonoids which may contribute to the reported antioxidant and anti-glycation activity. Thus, the ability of the extracts to modulate glycation-mediated BSA oxidation might be partly from their antioxidant activity [3]. These *in vitro* assays indicate that the plant extracts have potent antioxidant properties thus providing evidence in support for their use as significant source of natural antioxidant which might be helpful in preventing the progress of various oxidative stress related diseases. Current scientific evidence demonstrates that inhibition of AGEs formation is a therapeutic strategy for diabetic complications [6,35]. Therefore, much effort has been extended to searching for phytochemical compounds from dietary plants, fruits, and herbal medicines that effectively inhibit AGE formation [2,17].

However, the antioxidant activity of the extracts might not be the only reason for their anti-glycation properties. Several biochemical mechanisms of anti-glycation reactions have been proposed [44] such as breaking the cross-linking structures in the formed AGEs, blocking the carbonyl groups and inhibiting the formation of late-stage Amadori products [17]. Other underlying mechanisms for the anti-glycation activity may be relevant. Further comprehensive studies of the extracts are required to evaluate their anti-glycation mechanisms described above. Nonetheless, information gathered on the plants extracts studied provides evidence that may potentially justify their use as traditional treatment of different ailments. Available data allow to postulate that these dietary agents should be further investigated as possible natural protector of AGE formation *in vivo*. By virtue of their antioxidants and anti-glycation effects, these edible plants could be a promising natural source of anti-glycation agents for preventing AGE-mediated diabetic complications through inhibition of AGEs formation and oxidation-dependent protein damage.

In this study, BSA glycation was found to be significantly higher in presence of fructose compared to glucose. Owing to its high reactivity and contribution to AGE formation, fructose should be given due consideration to have further insight to properly estimate the relevance of these properties in diabetic complications. Our findings showed that ethanolic extracts and infusions of culinary herbs and spices have effective inhibitory effect on glucose- and fructose-mediated BSA-glycation *in vitro*. The presence of phytochemicals tends to suggest potential for pharmacological and other biological relevance of the culinary herbs and spices in drug discovery. The present results suggest that the extracts may act as antioxidants with suppressing effect on the formation of AGEs. Findings from this study indicate that some culinary herbs and spices with both high anti-glycation and high antioxidant activities may offer potential therapeutic treatment prospects for interfering in the glycation pathway and against oxidative stress-related diseases either as dietary interventions or glycation inhibitors. However, further studies are required to isolate the active principle from the crude extract for proper drug development.

Conflict of interest statement

We declare that we have no conflict of interest.

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