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Antioxidant and anti-glycation activities correlates with phenolic composition of tropical medicinal herbs

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ABSTRACT

Objective: To determine the contribution of total phenolic content (TPC) in glycation inhibitory activity of common tropical medicinal food and spices with potential antioxidative properties. **Methods:** *In vitro* glucose-bovine serum albumin (BSA) assay was used. Ethanolic extracts of ten common household condiments/herbs (*Allium sativum*, *Zingiber officinale*, *Thymus vulgaris*, *Petroselinum crispum*, *Murraya koenigii* Spreng, *Mentha piperita* L., *Curcuma longa* L., *Allium cepa* L., *Allium fistulosum* and *Coriandrum sativum* L.) were evaluated for antioxidative activity by 2,2-diphenyl-2-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP) and the TPC, flavonoid and tannins content were determined. **Results:** Findings showed good correlation between TPC/DPPH (r= 0.8), TPC/FRAP (r= 0.8), TPC/Anti-glycation (r=0.9), DPPH/Anti-glycation (r= 0.6), FRAP/Anti-glycation (r = 0.9), Flavonoid/Anti-glycation (r= 0.7) and Tannins/Anti-glycation (r = 0.8) and relatively fair correlation for TPC/Flavonoids (r = 0.5) and TPC/Tannins (r = 0.5). Results imply that these plants are potential sources of natural antioxidants which have free radical scavenging activity and might be used for reducing oxidative stress. **Conclusions:** The positive glycation inhibitory and antioxidative activities of these tropical herbs suggest a possible role in targeting ageing, diabetic complications and oxidative stress related diseases.

1. Introduction

Diabetes mellitus is a very common chronic disease which is associated with oxidative stress and non-enzymatic protein glycation[1]. The formation of advanced glycation endproducts (AGEs) is accelerated in hyperglycaemic conditions, which alter the structure and function of long-lived proteins[2]. The relevance of AGEs in pathogenesis of diabetic complications warrants an understanding of the factors interfering with AGEs formation[3]. Reports have suggested that oxidation reactions play a major role in accelerating the rate of AGEs formation[4]. Also, AGEs have the propensity to generate reactive oxygen species (ROS) in addition to auto-oxidation reactions yielding radicals and other reactive intermediates[5]. Moreover, reduced antioxidant activity associated with type 2 diabetes may be a primary factor in the vascular disease that diabetics often develop[6]. Antioxidants protect against glycation-derived free radicals and have been proposed as therapeutic agents[7]. Diabetes can be treated more effectively by the synergistic effect of compounds offering antioxidant and anti-glycation properties than targeting each individually[8]. In view of the growing role of AGEs in diabetes and age-associated pathologies, it has been suggested that inhibition of the formation of AGEs may prevent the progression of diabetic complications and slow down the ageing process[9]. Thus, on-going screening and development of novel compounds that offer combined antioxidant and anti-glycation properties will benefit to the treatment of diabetes mellitus[10]. It is well known that plants which possess antioxidative and pharmacological properties are related to the presence of phenolic compounds, especially phenolic acids and flavonoids[11].

Phenolic compounds are secondary plant metabolites comprising a wide variety of molecules that have a polyphenol structure (i.e. several hydroxyl groups on

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aromatic rings), but also molecules with one phenol ring, such as phenolic acids and phenolic alcohols[12]. Phenolic compounds are a much diversified group of phytochemicals that are widely distributed in plants, such as fruits, vegetables, tea, olive oil, and tobacco[13]. Recently, the identification and development of phenolic compounds or extracts from different plants considered powerful antioxidants *in vitro* has become a major area of health- and medical-related research[11]. Nowadays, there is a growing interest in substances exhibiting antioxidant properties, which are supplied to human organisms as food components or as specific preventive pharmaceuticals[14]. Many researchers have suggested that polyphenols may play an important role in preventing obesity[15], coronary heart disease[16], colon cancer[17], gastrointestinal disorders[18] and can also reduce the risk of diabetes[12]. Therefore, the process of AGEs formation may be retarded by antioxidative agents by preventing further oxidation of Amadori product and metal-catalyzed glucose oxidation. In this regard, several natural compounds known to possess antioxidative property, such as curcumin, and flavonoid-rich extracts, have been shown to prevent AGEs formation in vitro and in vivo[19]. Plant-based foods can improve glucose metabolism as well as enhance the overall health of diabetic patients[20]. In addition to the increasingly well-studied benefits of fruits and vegetables, many common household spices can make an important contribution to prevent or attenuate AGEs formation[6]. Food-derived AGEs are considerable risk factor for diabetic complications[3]. Although some studies highlighted the anti-glycating potential of a few natural sources, namely garlic[21], green tea[22] and tomato[23], adequate work has not yet been done to relate antioxidant properties of extracts to the anti-glycating potentials.

It has been established beyond question that antioxidants perform a crucial biochemical function in preventing reactive oxygen damage. However, antioxidants cannot protect proteins against every form of carbonylation. Antioxidants protect proteins against oxidative damage caused by free radicals, but not against equally damaging sugars. Glycation and oxidation reinforce each other in a vicious circle. Glycation has been described as amplifiers and integrators of oxidative damage[24]. Consequently, it is necessary to suppress all of these interrelated factors to protect the body's proteins. While antioxidants combat oxidation, they are defeated by glycation. Antioxidants are simply not enough to block the many biochemical pathways that damage proteins. Indeed, research showed that oxidation is not necessary for protein glycation and cross-linking[25], which leads to conclude therapeutic strategies relying solely on antioxidant activity to inhibit the Maillard reaction may have limited efficacy.

The present study was conducted to determine whether anti-glycation properties could be attributed to phytochemical characteristics relating to antioxidant activity. Previous reports on relationship between the total phenol content (TPC), antioxidant capacity and anti-glycation potential demonstrated both a linear correlation and also no correlation in other cases[26]. The main purpose of this study was to compare the anti-glycation abilities to the TPC and antioxidant properties of ten common food plants of the Mauritian diet. This work is an attempt to find a link between common household spices and type 2 diabetes. Common household spices including *Allium sativum*, *Zingiber officinale*, *Thymus vulgaris*, *Petroselinum crispum*, *Murraya koenigii* Spreng, *Mentha piperita* L., *Curcuma longa* L., *Allium cepa* L., *Allium fistulosum* and *Coriandrum sativum* L. were researched so that any link found could lead to practical home-based recommendations for dietary modifications as strategic therapeutic treatment for diabetes.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA; Fraction V, fatty acid free, low endotoxin), D-glucose, sodium azide, phosphate buffered saline, aminoguanidine, urea, trichloroacetic acid (TCA), Folin-Ciocalteu's phenol reagent, 2,2-diphenyl-1-picryhydrazyl (DPPH), ascorbic acid, gallic acid, anhydrous sodium carbonate, ethanol, methanol 100%, iron(III) chloride 6-hydrate, potassium ferricyanide, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, $NaNO_2$, $AlCl_3$, 1 M NaOH, vanillin methanolic solution, concentrated hydrochloric acid (HCl) and catechin were purchased from Sigma-Aldrich (St. Louis, USA).

2.2. Plant materials and preparation of extracts

Plant materials were purchased at a local market. Ten commercially available food plants were tested namely garlic (*Allium sativum*), ginger (*Zingiber officinale*), thyme (*Thymus vulgaris*), parsley (*Petroselinum crispum*), curry leaves (*Murraya koenigii* Spreng), peppermint (*Mentha piperita* L.), turmeric (*Curcuma longa* L.), onion (*Allium cepa* L.), green onion scallion and coriander (*Coriandrum sativum* L.). Fresh plant materials were grounded into a paste and 5g of the latter were extracted with ethanol (50%) at a ratio of 10 mL per gram at room temperature (28±2 ℃) for 1 week. The extracts were centrifuged at 1 000 g for 10 minutes to remove precipitate.

2.3. In vitro glycation

Albumin glycation was determined using fluorometry as described by Matsuura *et al*[27]. Briefly, 1mg/ml of fatty acid-free BSA was incubated with D-glucose (200-400 mM)±100 μL of extracts in 0.2 M potassium phosphate buffered saline (PBS, pH 7.4 containing 0.01% sodium azide) at 37 ℃ for defined time periods. Aliquots of the reaction mixture were removed at weekly intervals and fluorescent AGEs were assessed by their emission at 440 nm following excitation at

370 nm using a spectrofluorimeter (F-7000 FL) as described previously[28]. The reactions were stopped by adding 10 μL of 100% (w/v) TCA and after 10 minutes the mixture was centrifuged at 10 000 g. The precipitate was re-dissolved in alkaline PBS and quantified for the relative amount of fluorescent AGEs as described above. Complete inhibition of fluorescent AGEs was assumed to occur when fluorescence was inhibited to that of albumin in the absence of glucose; which was used as negative control. Aminoguanidine (20 mM) was included as a positive control.

2.4. Measurement of anti-glycation activity

The fluorescence index due to glycation produced in presence of BSA and glucose was represented as 100% glycation which is the same as 0% inhibition in absence of tested extracts and controls. Any sample giving fluorescence equal to the fluorescence of BSA/glucose implied that there was no inhibition of glycation, whereas, any sample giving fluorescence lower to that of BSA/glucose indicated that there was inhibition of glycation by the extract present. As described by Chen *et al*[29], the percentage inhibition of glycation of each of the control and plant extract has been calculated as follows:

$$\frac{\text{Fluorescence of specimen - fluorescence of BSA/Glucose}}{\text{Fluorescence of BSA/Glucose}} \times 100$$

2.5. Effect of different concentrations of extract on formation of fluorescent AGEs

The inhibitory activity of the extracts on fluorescent AGEs was further confirmed in the concentration-dependent studies. In these experiments, extracts of curry leaves, peppermint, scallion and coriander various concentrations (15 mg/mL, 10 mg/mL, 7.5 mg/mL, 5.0 mg/mL and 2.5 mg/mL) were incubated with BSA and glucose at 37 ℃ for two weeks. Thereafter, the reaction was stopped as described above.

2.6. Determination of total phenolic content

The TPC in extracts was determined using the Folin-Ciocalteu's colorimetric method according to Katalinic *et al*[30]. Samples of extracts (0.1 mL) and 0.5 mL of Folin-Ciocalteu's phenol reagent (diluted 10 times) were mixed with 0.4 mL of 7.5% sodium carbonate for 1 hour and absorbance was measured at 765 nm using a Perkin-Elmer spectrophotometer. Standards of gallic acid were used to calibrate the method and results expressed as milligrams of gallic acid equivalents per milliliter (mg GAE/mL). Each assay was performed in triplicate.

2.7. Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) was assessed according to the procedure of Benzie and Strain[31]. Briefly, 0.5 mL of extracts was added to 1 mL of 0.2 M phosphate buffer, pH 6.6 and 1 mL potassium ferricyanide (1%). The mixtures were incubated at 50 ℃ for 20 minutes, after which 1 mL of 10% TCA was added. A 1 mL aliquot of the mixture was taken and mixed with 1 mL water and 0.5 mL of 1% $FeCl_3$. The absorbance at 578 nm was measured after 30 minutes. Ascorbic acid was used as positive control and results were expressed in milligrams of ascorbic acid equivalents per milliliter (mg AAE/mL).

2.8. Determination of the radical scavenging ability using the 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) assay

Radical scavenging activity of the extracts against stable 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) was determined spectrophotometrically using the method of Brand-Williams *et al*[32]. This activity was measured by the bleaching rate of a stable free radical. In its radical form, DPPH• absorbs at 515 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The changes in colour (from deep - violet to light - yellow) were measured on a UV/visible light Perkin-Elmer spectrophotometer. Briefly, different plant extracts were placed in cuvettes with 0.1 mM methanolic solution of DPPH. Different concentrations of the extracts were added to the DPPH and ascorbic acid was used as positive control. The decrease in absorbance at 515 nm was determined after 30 minutes. The absorbance of the DPPH radical without an antioxidant, i.e., negative control, was also measured. The ability to scavenge the DPPH• radical was calculated using the following equation: DPPH• scavenging effect (%) = [(AControl - ASample/AControl) ×100]; where AControl is the absorbance of the negative control reaction and a sample is the absorbance in the presence of extracts or standard positive control (ascorbic acid). The experiments were carried out in triplicate and the results expressed as a percentage of the control.

2.9. Determination of total flavonoid content

A colorimetric methodology was applied to measure total flavonoid content[22]. Briefly, 1.25 mL extracts, 0.075 mL 5% $NaNO_2$, 0.075 mL 10% $AlCl_3$, and 0.5 mL 1 M NaOH were pipetted into tubes, in that order. After the solution volume had been increased to 2.5 mL by adding double-distilled water, the solution absorbance at 510 nm was determined. Flavonoid content was estimated using catechin as a standard; analytical results are expressed in milligrams of catechin equivalents (CEs) per milliliter.

2.10. Determination of total condensed tannin content

The total amount of condensed tannin was determined spectrophotometrcally[22]. Extracts and the standard (5 μL)

were reacted with 4% vanillin methanolic solution (150 μL) and concentrated hydrochloric acid (HCl) (75 μL) for 20 minutes. Solution absorbance at 500 nm was detected. The amount of condensed tannins was estimated using catechin as a standard; analytical results were expressed in milligrams of CEs per milliliter.

2.11. Statistical analysis

Results were presented as mean±SD of experiments. Difference between groups and percentage inhibition of fluorescent AGEs was compared using unpaired t-test with one-tailed test. Correlations between variables were quantified by the correlation factor "r". Correlation and linear regression analysis was performed using Microsoft Excel 2007. In each analysis $P < 0.05$ was considered statistically significant.

3. Results

3.1. In vitro anti-glycation activity of extracts

The positive control aminoguanidine inhibited formation of fluorescent AGEs ($P<0.05$) by 75.9 % as shown in Figure 1. In vitro glycation assays demonstrated that the ten extracts (garlic, ginger, thyme, parsley, curry leaves, pepper mint, turmeric, onion, green onion scallion and coriander) exerted marked inhibition of fluorescent AGEs formation as depicted in Figure 1. When glycation was monitored over 2 weeks the percentage inhibition was found to be: garlic (26.1%), ginger (25.7%), thyme (42.3%), parsley (41.2%), curry leaves (40.9%), pepper mint (39.8%), turmeric (39.3%), onion (11.9%), scallion (27.9%) and coriander (38.8%).

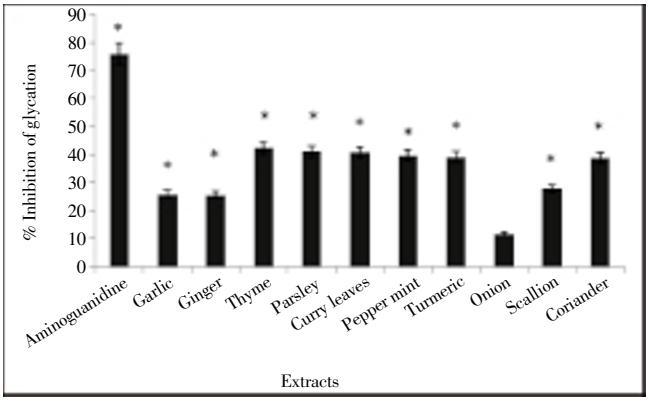

Figure 1. Anti-glycation activity of the ten food plants.
Percentage inhibition of fluorescent AGEs following glycation of BSA with 200 mM glucose in the presence of ten extracts in 0.2 M PBS at 37 ℃. Fluorescent AGEs were measured after 2 weeks. Results are presented as means±S.D (n=3). *Values significantly different ($P<0.05$) from negative control.

3.2. Effect of different concentrations of extracts on fluorescent AGE formation

Extracts of curry leaves, scallion, pepper mint and coriander at different concentrations were found to inhibit the formation of fluorescent AGEs (Figure 2). At 200 mM glucose the IC_{50} values were 8.6 mg/mL for curry leaves, 24.1 mg/mL for scallion, 7.7 mg/mL for pepper mint and 17.2 mg/mL for coriander. The correlation coefficient for curry leaves was r = 0.991, scallion r = 0.978, pepper mint r = 0.966 and coriander r = 0.938.

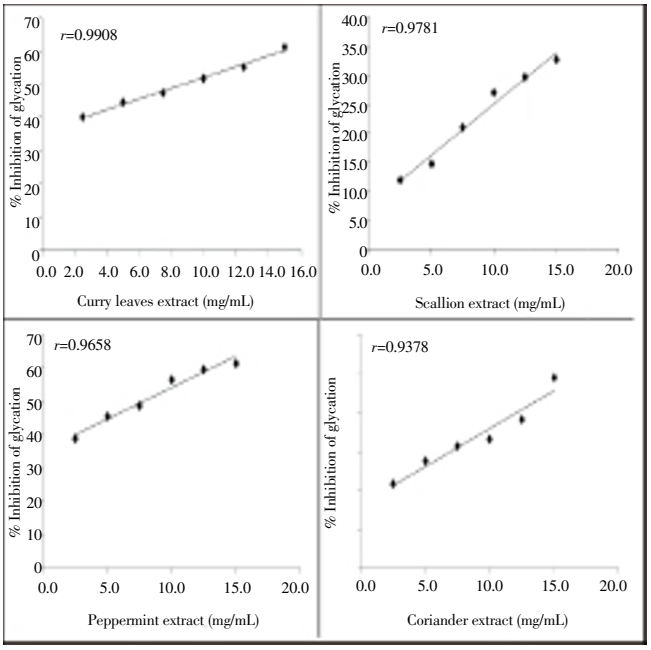

Figure 2. Inhibition of fluorescent AGEs by graded concentrations of curry leaves, scallion, pepper mint and coriander.
Fluorescent AGEs formed by glycation of BSA (1 mg/mL) by 200 mM glucose in the presence of 2.5-15.0 mg/mL extract at 37 ℃ for two weeks. Results are presented as mean±SD (n=3).

3.3. DPPH-radical-scavenging capacity of extracts of different herbs and spices

Free radical scavenging capacity of the extracts was detected by DPPH and compared to the positive control ascorbic acid (Figure 3). The SC_{50} is the amount of antioxidant necessary to decrease the initial DPPH absorbance by 50%. The results were expressed as milligrams of ascorbic acid equivalents per milliliter (mg AAE/mL). The absorbance of all graphs decreased as the amount of extract increased. Thus, the SC_{50} were in the order of thyme (0.09 mg AAE/mL), aminoguanidine (0.125 mg AAE/mL), garlic (8.0 mg AAE/mL), turmeric (14.4 mg AAE/mL), curry leaves (26.7 mg AAE/mL), ginger (39.0 mg AAE/mL), onion (62.2 mg AAE/mL), pepper mint (99.2 mg AAE/mL), scallion (110.0 mg AAE/mL), coriander (891.0 mg AAE/mL), parsley (1606.0 mg AAE/mL).

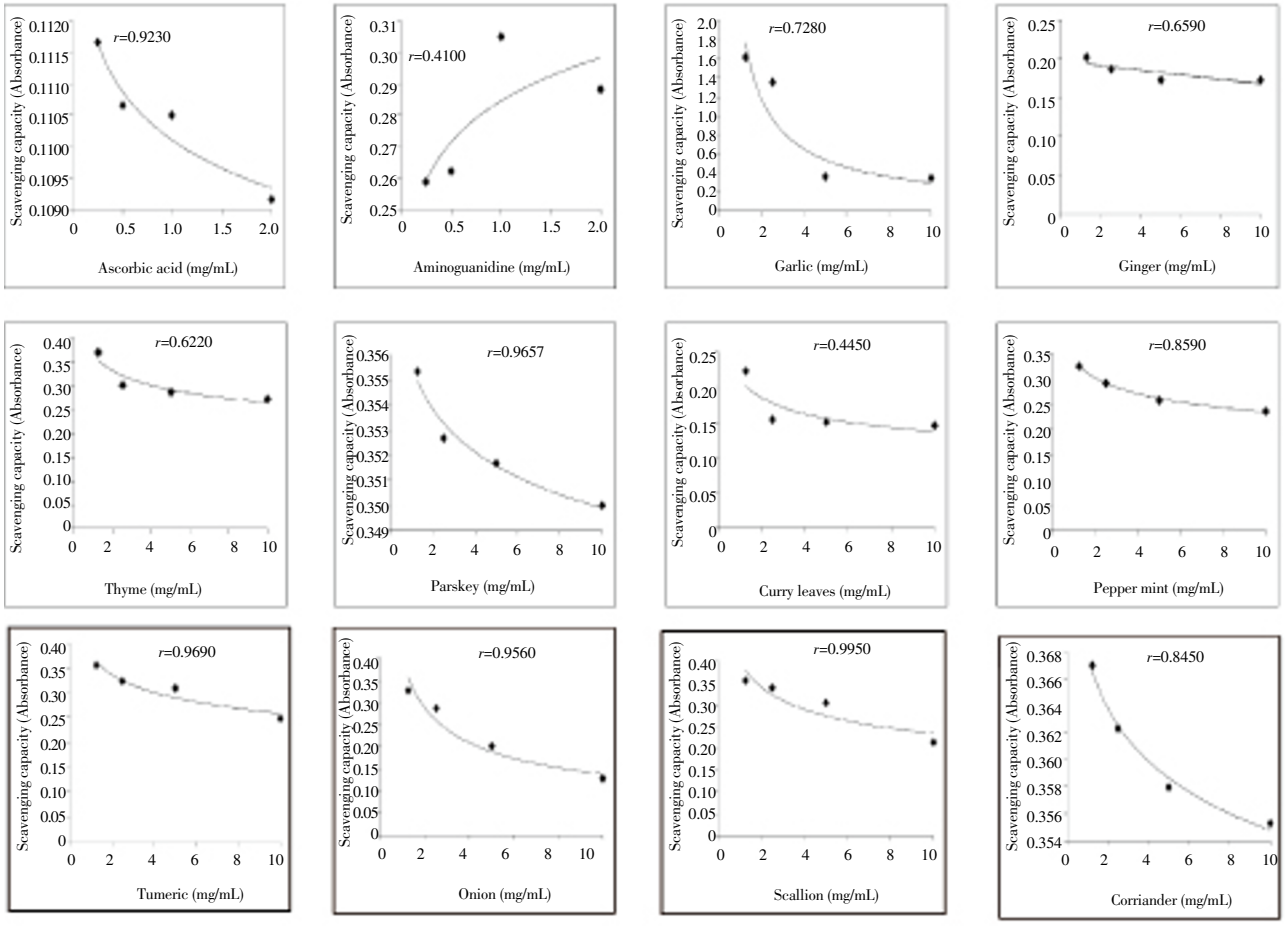

Figure 3. DPPH scavenging activity of ten food plant extracts.

Ascorbic acid, aminoguanidine, garlic, ginger, thyme, parsley, curry leaves, pepper mint, turmeric, onion, scallion and coriander. Results are presented as mean±SD (n=3).

3.4. Correlation between TPC, DPPH, FRAP and anti-glycation ability of different extracts

In the correlation analysis, the properties namely TPC, DPPH, FRAP and anti-glycation potencies of the extracts were compared with each other. The results obtained are depicted in Table 1. The comparative evaluation depicted that several groups could be revealed based on the differential properties. In this respect, when TPC and DPPH were compared, coriander, turmeric, scallion, pepper mint, onion, parsley, ginger and curry leaves showed a positive correlation of r = 0.8 (Figure 4A and B).

According to the TPC and FRAP properties observed, the ten extracts could be categorized into four different groups. The first group (Figure 4C) consisted of scallion, pepper mint, onion and parsley having increasing FRAP potential (0.812 mg AAE/ml, 0.942 mg AAE/mL, 0.986 mg AAE/mL, 0.942 mg AAE/mL respectively) with increasing TPC (1.303 mg GAE/mL, 1.451 mg GAE/mL, 1.515 mg GAE/mL, 1.583 mg GAE/mL respectively). The second group included ginger, curry leaves, thyme and garlic where TPC (2.261 mg GAE/mL, 2.580 mg GAE/mL, 2.708 mg GAE/mL, 3.068 mg GAE/mL respectively) was inversely related to FRAP potential (2.986 mg AAE/mL, 2.116 mg AAE/mL, 1.942 mg AAE/mL, 0.957 mg AAE/mL). The stand alone groups were coriander exhibiting low TPC (0.443 mg GAE/mL) and low FRAP potential (0.319 mg AAE/mL) whereas turmeric revealed low TPC (1.019 mg GAE/mL) but high FRAP potential (2.043 mg AAE/mL).

When TPC and percentage inhibition of glycation were compared, pepper mint, onion and parsley exhibited a positive correlation of r = 0.9 (Figure 4D). On the other hand coriander and scallion showed low TPC with relatively high anti-glycation capacity and turmeric showed low TPC with low anti-glycation activity. In contrast, ginger, curry leaves, thyme and garlic demonstrated high TPC with relatively high anti-glycation property.

Comparison of FRAP to anti-glycation capacity revealed a positive correlation of r = 0.9 for coriander, scallion, parsley and thyme (Figure 4E) while onion, curry leaves and garlic displayed low anti-glycation capacity with average FRAP. Pepper mint showed both low FRAP and anti-glycation capacities where turmeric depicted high FRAP with low anti-glycation property and ginger high FRAP with average anti-glycation activity.

The link between DPPH and anti-glycation expressed a positive correlation of r = 0.6 for turmeric, onion and curry leaves (Figure 4F) whilst garlic, ginger, coriander, parsley and thyme showed high anti-glycation activity with low DPPH scavenging capacity. Scallion indicated high anti-

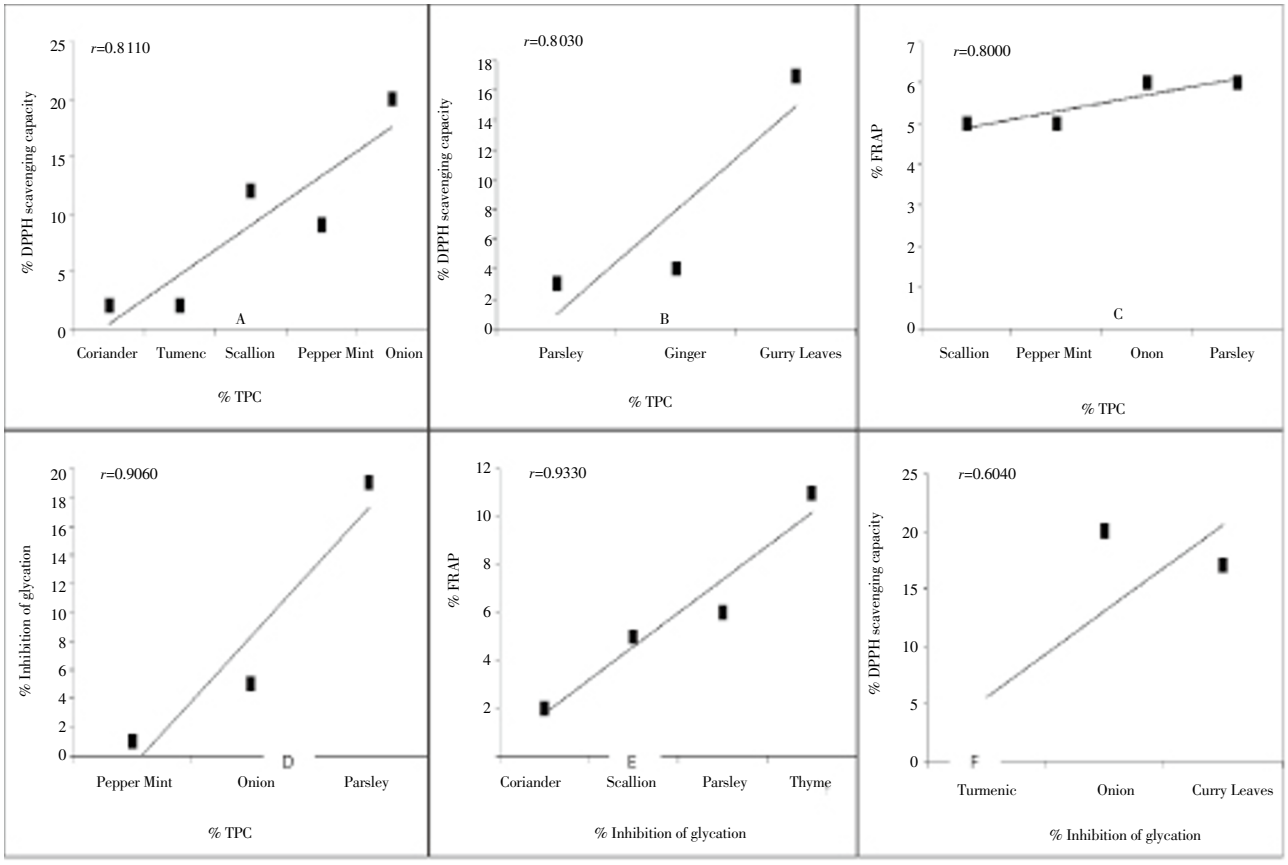

Figure 4. Correlation between TPC, DPPH, FRAP and % inhibition of glycation of food plant extracts.
(A) and (B) Percentage TPC v/s percentage inhibition of glycation between coriander, turmeric, scallion, pepper mint, onion, parsley, ginger and curry leaves. (C) Percentage TPC v/s percentage FRAP between scallion, pepper mint, onion and parsley. (D) Percentage TPC v/s percentage inhibition of glycation between pepper mint, onion and parsley. (E) Percentage inhibition of glycation v/s percentage FRAP between coriander, scallion, parsley and thyme. (F) Percentage inhibition of glycation v/s percentage DPPH scavenging capacity between turmeric, onion and curry leaves.

Table 1
TPC, FRAP, DPPH, flavonoid, and tannins properties of ten common food plant extracts.

Herbs	TPC mg GAE/mL	FRAP mg AAE/mL	DPPH mg AAE/mL	Flavonoids mg CE/mL	Tannins mg CE/mL
Garlic	3.068±0.008	0.957±0.140	0.027±0.095	0.710±0.131	3.167±0.004
Ginger	2.261±0.032	2.986±0.113	0.365±0.001	0.754±0.019	3.048±0.004
Thyme	2.708±0.025	1.942±0.181	0.209±0.007	1.074±0.043	8.214±0.008
Parsley	1.583±0.085	0.942±0.046	0.343±0.005	0.091±0.006	1.167±0.002
Curry Leaves	2.580±0.016	2.116±0.008	1.670±0.002	0.315±0.027	1.381±0.002
Pepper Mint	1.451±0.008	0.942±0.0335	0.918±0.071	0.217±0.002	1.500±0.000
Turmeric	1.019±0.013	2.043±0.189	0.206±0.173	0.658±0.010	2.048±0.001
Onion	1.515±0.007	0.986±0.064	1.990±0.003	0.068±0.016	1.286±0.003
Scallion	1.303±0.006	0.812±0.038	1.220±0.021	0.171±0.028	1.381±0.001
Coriander	0.443±0.002	0.319±0.021	1.920±0.004	0.083±0.002	1.286±0.002

glycation with high DPPH scavenging capacity and pepper mint low anti-glycation with high DPPH scavenging capacity.

3.5. Correlation between inhibition of glycation, total phenolic, flavonoid and tannins contents

Relationship between TPC, flavonoid and tannins content revealed a corelation coefficient of r = 0.5 (Figure 5A). The comparative analysis demonstrated that several groups could be revealed based on the phytochemical properties. Thus a positive correlation of r = 0.8 was found for the group comprising of pepper mint, turmeric and onion (Figure 5C) and a correlation coefficient of r = 0.7 for curry leaves, garlic and ginger (Figure 5D) where inhibition of glycation increased with increasing flavonoid content. Coriander, scallion and parsley showed relatively high glycation inhibitory effect with low flavonoid content whereas thyme

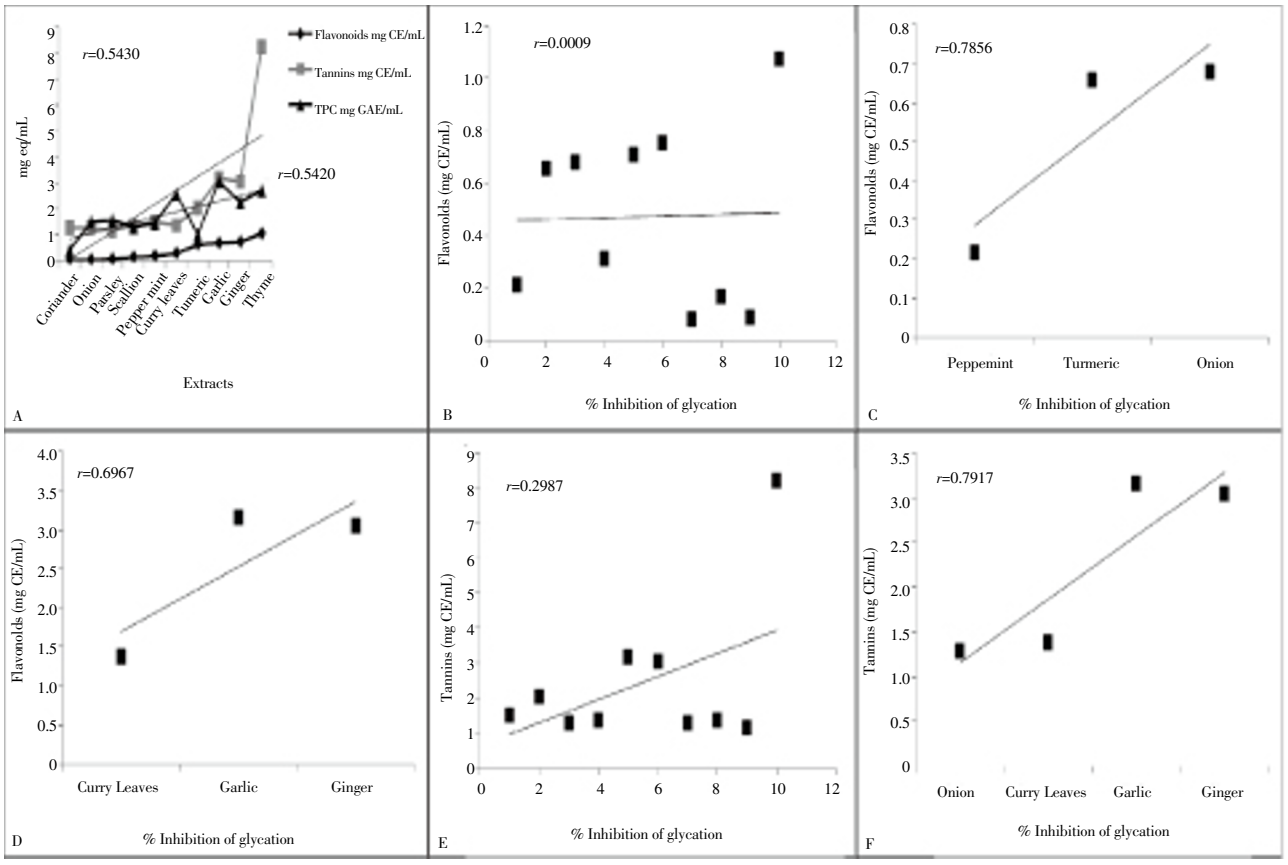

Figure 5. Correlation between inhibition of glycation total phenolic, flavonoid and tannins content. Results are presented as mean±SD (n=3).

had high glycation inhibition with high Flavonoid content (Figure 5B).

Comparison of tannins content to anti-glycation capacity revealed a positive correlation of r = 0.8 for onion, curry leaves, garlic and ginger (Figure 5E). Peppermint and turmeric showed low glycation inhibitory activity with low tannins content while coriander, scallion and parsley showed relatively high glycation inhibition with low tannins content and thyme showed relatively high inhibition of glycation with high tannins content (Figure 5D).

4. Discussion

In the present study we evaluated the anti-glycating effect of ethanolic extracts of various plant-based foods of the Mauritian diet. Nine out of the ten plants investigated showed significant inhibitory potential against *in vitro* protein glycation. Prominent among them were thyme, parsley, curry leaves, turmeric, pepper mint and coriander which inhibited in vitro fluorescent AGE formation to 38%-42% at 10 mg/mLconcentration. However, their respective anti-glycating activity was relatively less than that of aminoguanidine. The mechanism of anti-glycation activity of the extracts was not explored; however, the relationships between TPC, antioxidative properties and inhibition of glycation capacity were explored. Like other natural compounds that have been shown to possess anti-glycation activity[33], it is very likely that the extracts may exert their inhibitory effect on glycation by impeding further oxidation of glycated proteins and metal-catalyzed oxidation of glucose that leads to the formation of AGEs[34]. Our study demonstrated that the anti-glycation activity of the extracts was correlated with their antioxidant properties. The results suggest that the antioxidant and anti-glycation properties of food plants could be explained, at least in part, by the synergistic effect of phenolic compounds present in the extracts. Recently other compounds having antioxidant power have been reported to exhibit anti-glycation activity[1]. However, the anti-glycation potency seems to correlate only partially with the antioxidative property. Therefore, to investigate the antioxidant activity of compound(s), choosing an adequate assay based on the compound(s) of interest is critical[17]. These findings lead the authors to conclude that other mechanisms must be involved in the anti-glycation activity of these extracts. More specific studies are needed to address that question. Nonetheless, anti-glycation activities were positively correlated with TPC, DPPH radical scavenging activity and FRAP potential. Flavonoids, particularly the quercetin (as a flavonol) has been associated with a reduced incidence of heart disease in diabetes mellitus[22]. The scavenging of free radicals derived from glycation may play an important role in this phenomenon. This mechanism may help to provide a protective effect against hyperglycaemia mediated damage[13]. Results obtained in the present study show that TPC which indeed includes flavonols, have inhibitory effects

on glycation.

Together, these results provide evidence that antioxidant phenolic metabolites mediate to some extent the anti-glycation activity of our plant collection, a relationship that likely extends to other food plants. The finding that food plants possess anti-glycation and antioxidant activity implies that making these plants an integral part of daily consumption may retard the process of AGEs formation by preventing oxidation. In fact, inhibitors of AGEs that have antioxidant activity may act as preventive agents against diabetic complications. Whether incorporating food plants presenting anti-glycation capacity in people's diet will have the same effect *in vivo* remains to be studied in both animal and human.

This study showed that inhibition of protein glycation by dietary agents merit considerable attention. These herbs and spices are largely free of adverse effects as they are either consumed as dietary components routinely or used in traditional medicines[33]. Glycation and AGE-induced toxicity are known to be associated with increased free radical production. Benefits of using compounds with combined anti-glycation and antioxidant properties have been reported[35,36]. Free radical-mediated toxicity and AGE formation are prevented or reduced by these compounds[3]. In this context, most of the anti-glycating agents described in the present study have been shown to possess antioxidant potential. Furthermore, the low-molecular-weight compounds such as polyphenols, phenolic acids or flavonoids, which are known to have antioxidant activity[37-40], are likely to be present in our plant extracts. Dietary compounds that can reduce glycation may reasonably serve as valuable adjuvants, promoting the health of the aged and diabetics. Hence the cumulative effect of antioxidant and anti-glycating activities might contribute to effective action. The beneficial effects of dietary agents observed *in vivo*[3] provide an indication of their potential use for the management of diabetic complications. Nutritional intervention has been shown to have an important role in the management of diabetes and its complications. Current dietary strategies are centered on nutrients, energy restriction, and antioxidant and hypoglycaemic effects[33] but are not focused sufficiently on the anti-glycating activity by dietary components. Food-derived and exogenous AGEs have not received adequate recognition as risk factor for diabetic complications[41-44]. Therefore, effective restriction of those dietary components and/or modulation of food-derived AGE by blending those foods with the diet sources having anti-glycating potential should be considered for the optimal management of AGE-mediated pathologies[22], particularly for those who are at risk of developing diabetic complications.

Nonetheless, the major question that needs to be addressed is whether TPC and antioxidant potential of food plants are predictive of the ability to inhibit protein glycation. It is known that Flavonoids and other constituents in plants inhibit protein glycation. However, the top dietary sources of these compounds have not been screened systematically for potential effects on glycation. The results should be considered as representative, in as much as the quantities of phenolics observed depend upon the solvent used, as well as cultivars and conditions for plant growing, harvesting, drying, and storage. Some spices possess a higher TPC, but glycation is reduced to similar extents as presented in this study.

The results of the present study clearly demonstrate that several species of this set of medicinal herbs and spices also possess promising antidiabetic activity, thereby further confirming the soundness of our ethnobotanical approach and the potential of identifying efficacious treatments complementary to modern medication, such as biguanides, sulfonylureas, and thiazolidinedione. Studies will also be required to address the elucidation of active principles and their mechanisms of action. Such information will allow standardization of treatments based on content of active ingredients or on biological activity, rather than on content of nonspecific phytochemical markers.

In the present investigation, food plants with antioxidative and radical scavenging activity were found to possess an *in vitro* anti-glycation activity based on glucose-BSA assay. The mechanism of anti-glycation activity and application as a therapeutic agent need further investigation. Taken together, these data indicated that the ten food plants possessed *in vitro* anti-glycation activity which partially correlated with TPC, radical scavenging capacity and FRAP. These phytochemical profiles were determined to establish their contribution to antioxidant and anti-glycation properties. We conclude that the plants with both high anti-glycation and high antioxidant activities are potential bioactive compounds in the treatment of diabetes. Other perhaps unexpected natural products with anti-glycation properties need to be investigated. Antioxidant activity is certainly one common denominator among the spices. Spices have other health benefits, from improving arthritis conditions to functioning as anti-cancer agents. Moderately spicing our food can thus bring some beneficial health effects.

Conflict of interest statement

We declare that we have no conflict of interest.

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