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Original Article

Effect of proteins, sugars and extraction methods on the anti-glycation activity of spices.

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Abstract

***Objective*:** The aim of this study was to determine the effect of different proteins, sugars, and extraction methods on the anti-glycation activity of water-and ethanol-extracted spices and herbs.

***Methods*:** Five *in vitro* glycation models were used: human serum albumin-glucose (HSA-Glucose), human serum albumin-fructose (HSA-Fructose), bovine serum albumin-glucose (BSA-Glucose), bovine serum albumin-fructose (BSA-Fructose), and bovine skin collagen Type-I-glucose (Collagen-Glucose). Water and ethanolic extracts of 40 spices were evaluated for their anti-glycation activity using these models.

***Results*:** Correlation was poor between HSA-Glu/Collagen-Glu ($R^2 = 0.073$, $p > 0.05$), HSA-Glu/BSA-Glu ($R^2 = 0.273$, $p = .015$), BSA-Glu/Collagen-Glu ($R^2 = 0.030$, $p > 0.05$), and HSA-Fru/BSA-Fru ($R^2 = 0.041$, $p > 0.05$) with water extraction. Correlation was also poor between HSA-Glu/Collagen-Glu ($R^2 = 0.156$, $p = 0.028$), HSA-Glu/BSA-Glu ($R^2 = 0.172$, $p = 0.044$), BSA-Glu/Collagen-Glu ($R^2 = 0.068$, $p > 0.05$), and HSA-Fru/BSA-Fru ($R^2 = 0.117$, $p > 0.05$) with ethanol extraction. These findings imply that anti-glycation activity is primarily determined by the protein used in glycation models. Correlation was good between HSA-Glu/HSA-Fru ($R^2 = 0.753$, $p < 0.001$), and BSA-Glu/BSA-Fru ($R^2 = 0.870$, $p < 0.001$) with water extraction, as well as between HSA-Glu/HSA-Fru ($R^2 = 0.967$, $p < 0.001$), and BSA-Glu/BSA-Fru ($R^2 = 0.642$, $p < 0.001$) with ethanol extraction. These findings demonstrate that type of sugar (glucose/fructose) has no marked effect on anti-glycation activity. Correlation was good between HSA-Glu-Water/HSA-Glu-Ethanol ($R^2 = 0.837$, $p < 0.001$), HSA-Fru-Water/HSA-Fru-Ethanol ($R^2 = 0.916$, $p < 0.001$), BSA-Fru-Water/BSA-Fru-Ethanol ($R^2 = 0.691$, $p < 0.001$), and Collagen-Glu-Water/Collagen-Glu-Ethanol ($R^2 = 0.725$, $p < 0.001$). However, correlation was poor between BSA-Glu-Water/BSA-Glu-Ethanol ($R^2 = 0.373$, $p < 0.001$), suggesting that extraction methods have a case-by-case effect on the degree of anti-glycation activity.

***Conclusion*:** The results emphasized the importance of protein selection in anti-glycation activity determination.

***KEY WORDS*:** Advanced Glycation End-products; Spices; Albumin; Collagen; Sugars

Introduction

Glycation is the non-enzymatic reaction between a protein and a reducing sugar, such as glucose and fructose. The addition of reducing sugars to amino groups in protein leads to the formation of a Schiff base, which rearranges to form a more stable Amadori product. Advanced glycation end products (AGEs) are formed after a series of complex reactions (*e.g.* oxidation, phosphorylation). The accumulation of such reaction products of protein glycation in living organisms leads to structural and functional modifications of tissue proteins [1)], and evidence has been presented that glycation leads to chemical modification of proteins, and other macromolecules, thereby contributing to the pathogenesis of diabetic complications [2)]. While AGEs progressively formed with normal age, even in the absence of disease formation is accelerated under diabetic complications. AGEs are not only markers but also important causative factors for the pathogenesis of diabetes, cataracts, atherosclerosis, diabetic nephropathy, and neurodegenerative diseases, including Alzheimer's disease. The design and discovery of inhibitors of AGE formation can therefore present a promising therapeutic approach to the prevention of diabetic or other pathogenic complications.

Aminoguanidine, a small, hydrazine-like small molecule, was the first AGE inhibitor explored in clinical trials. However, the drug was ultimately not approved for commercial

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production due to side effects observed in phase III clinical trials in patients with diabetes, perhaps related to the sequestration of pyridoxal, resulting in vitamin B6 deficiency[3]. Subsequent effort has therefore been directed at identifying phytochemical compounds in plants, fruits, spices, and herbal medicines effective against protein glycation.

Spices are common food adjuncts that have been used as flavoring, seasoning, coloring agents, and even preservatives throughout the world for thousands of years, particularly in India, China, and many other southern Asian countries. Spices rich in phytochemicals with anti-oxidant, anti-inflammatory, anti-carcinogenic, anti-microbial, hypolipidemic, and other beneficial physiological properties are used extensively in folk medicines to treat a range of chronic diseases[4]. Spice plants belong several main botanical families, such as Labiatae (also called Lamiaceae; (*e.g.* rosemary, oregano, thyme, basil, marjoram, savory, lemon balm, peppermint and spearmint), Apiaceae (*e.g.* anise, caraway, cumin, coriander), Lauraceae (*e.g.* bay leaf, cinnamon), Zingiberaceae (*e.g.* ginger, cardamom, turmeric), and Myrtaceae (*e.g.* clove), with typical distribution in tropical and temperature areas[5]. However, despite a number of intensive studies being conducted on the anti-glycation activity of spices data regarding such activity for different glycation models are insufficient and incomplete. In particular, no data have yet been gathered on the effect of proteins, sugars, and extraction methods on glycation.

Here, to investigate the extent to which different proteins, sugars, and extraction methods affect the anti-glycation activity of spices, we examined activity using five different *in vitro* glycation models: human serum albumin-glucose (HSA-Glucose), human serum albumin-fructose (HSA-Fructose), bovine serum albumin-glucose (BSA-Glucose), bovine serum albumin-fructose (BSA-Fructose), and bovine skin collagen type-I-glucose (Collagen-Glucose) with both water and ethanol extraction.

Methods

In vitro HSA-Glucose, BSA-Glucose, HSA-Fructose, BSA-Fructose and Collagen-Glucose models of glycation, were used to test the inhibition of AGE formation by spices.

Extract preparation

A total of 40 spice samples (30 Japanese spices and 10 commonly used Bangladeshi spices) were collected for use in the present study. The Japanese spices were obtained from market, while the Bangladeshi spices were collected from a Bangladeshi shop in Tokyo, Japan. The samples were dried at 65°C for 72 hours, then ground and extracted with distilled water at 80°C in a water bath for 1 hour. The concentration of each sample was estimated from the weight difference before and after incubation of 5 mL sub-samples, dried in aluminum trays at 120°C for 1.5 hours.

Glycation models

The HSA model was prepared by incubating HSA with and without glucose at 60°C for 40 hours as previously reported[6]. The glucose (+) reaction solution contained 0.1M phosphate buffer (pH 7.4), 40 mg/mL HSA (Sigma-Aldrich Chemical Ltd, St. Louis, MO, USA), 2.0 M glucose solution, and distilled water at a 5:2:1:1 volume ratio. The glucose (-) reaction solution contained 0.1M phosphate buffer (pH 7.4), 40 mg/mL HSA, and distilled water at a 5:2:2 volume ratios. Similar methods were also reported at 37°C for 3-14 days[7-8] and the amount of AGEs generated were approximately as same as the amount generated after incubation at 60°C for 40 hours.[9]

A 100 µL aliquot of each test sample (spice samples of 1-hour extract, or water [control] was added to 900 µL of glucose (+) or glucose (-) HSA solution. After 40-hour incubation, the sample solution (200 µL), distilled water (200 µL), and 5 µg/mL quinine sulfate (200 µL) were dispensed into a black micro-plate. Fluorescence (excitation 370 nm/ detection 440 nm) was then measured using a SpectraMax® Paradigm® Multi-Mode Detection Platform (Molecular Devices, Lagerhausstrasse, Austria).

The inhibitory activity of each sample was calculated using the following equation:

Inhibitory activity against fluorescence AGEs (%) =
(1- (Glucose (+) sample - Glucose (-) sample) /
(Glucose (+) control - Glucose (-) control)) x 100.

The 50% inhibitory concentration (IC_{50}) against fluorescence AGEs was calculated from a regression curve of the inhibitory activity at three concentrations for each sample (n = 3). The HSA-Fructose, BSA-Glucose and BSA-Fructose models were prepared in the same manner as the HSA-Glucose model.

The glycation of bovine skin collagen type-I was modeled by incubating collagen with (+) and without (-) glucose at 60°C for 10 days. The glucose (+) reaction solution contained 0.1 M phosphate buffer (pH 7.4), 3 mg/mL bovine skin collagen type-I (Nippi, Tokyo, Japan), and 2.0 M glucose solution at a 5:2:2 volume ratio. The glucose (-) reaction solution contained 0.1 M phosphate buffer (pH 7.4), 3 mg/mL bovine skin collagen type-I (Nippi), and distilled water at a 5:2:2 volume ratio.

The activity of the extracts in the collagen model was measured using the same formula as with the HSA model. After calculation, activities of extracts were compared with that of aminoguanidine[10].

Statistical Analysis

Linear regression analysis was performed using Microsoft Excel 2010, Microsoft, Redmond, USA. Correlations between variables were quantified as Pearson correlation (r), which was calculated using SPSS Statistics 22, (IBM, Somers, NY) with two tailed significance ($p < 0.05$ and $p < 0.001$).

Results

Fluorescent AGE inhibition activity of spice samples

Monitoring the production of fluorescent products at excitation and emission maxima of 370 and 440 nm, respectively, allowed for assessment of total AGE formation. On evaluation of inhibitory activity on protein glycation of 40 spices and herbs with both water and ethanol extraction (***Tables 1*** and ***2***), the fluorescence of AGEs was shown to be

markedly inhibited by several extracts comparing with the aminoguanidine (AG).

Anti-glycation activity of the spice extracts varied widely across in vitro glycation models (***Tables 1*** and ***2***). The IC_{50} of water-derived extracts in different glycation models decreased as follows; in HSA-Glucose model: cloves (IC_{50}: 0.009 mg/mL) > marjoram (IC_{50}: 0.015 mg/mL) > lemongrass, ginger (IC_{50}: 0.02 mg/mL) > bay leaf (IC_{50}: 0.022 mg/mL) > spearmint (IC_{50}: 0.024 mg/mL). Garlic and dill did showed no activity in the HSA-Glucose model. In the HSA-Fructose model: cloves (IC_{50}: 0.018 mg/mL) > star anise (IC_{50}: 0.026 mg/mL) > savory (IC_{50}: 0.033 mg/mL) > allspice (IC_{50}: 0.036 mg/mL) > rosemary (IC_{50}: 0.04 mg/mL); In the BSA-Glucose model: lemon balm (IC_{50}: 0.056 mg/mL) > oregano (IC_{50}: 0.066 mg/mL) > cloves (IC_{50}: 0.098 mg/mL) > spearmint (IC_{50}: 0.105 mg/mL) > allspice (IC_{50}: 0.114 mg/mL). Green chili showed no activity in the BSA-Glucose model. In the BSA-Fructose model: cinnamon (IC_{50}: 0.017 mg/mL) > spearmint (IC_{50}: 0.056 mg/mL) > lemon balm (IC_{50}: 0.059 mg/mL) > rosemary (IC_{50}: 0.061 mg/mL) > savory (IC_{50}: 0.083 mg/mL). Of note, cinnamon had the strongest activity of all spices in the BSA-Fructose model, whereas its activity was quite low (IC_{50}: 60 mg/mL) in the HSA-Glucose model. Fenugreek, ginger, garlic, paprika, onion and cardamom did not show any activity in the BSA-Fructose model. However, the IC_{50} of ginger in the HSA-Glucose model was quite high (0.02 mg/mL). In the Collagen-Glucose model: cloves (IC_{50}: 0.001 mg/mL) > tarragon, savory, cinnamon, spearmint, star anise (IC_{50}: 0.003 mg/mL) > lemon balm (IC_{50}: 0.004 mg/mL) > bay leaf (IC_{50}: 0.006 mg/mL) > hibiscus, rosemary (IC_{50}: 0.008 mg/mL). Fenugreek, garlic and onion did not show any activity in the Collagen-Glucose model (***Table 1***).

The IC_{50} of water-derived extracts in different glycation models decreased as follows. HSA-Glucose model: spearmint (IC_{50}: 0.0003 mg/mL) > black pepper (IC_{50}: 0.011 mg/mL) > turmeric (IC_{50}: 0.018 mg/mL) > cloves (IC_{50}: 0.022 mg/mL) > lemon balm (IC_{50}: 0.037 mg/mL). Onion, garlic and black cumin did not show any activity in the HSA-Glucose model. HSA-Fructose model: cloves (IC_{50}: 0.02 mg/mL) > allspice (IC_{50}: 0.028 mg/mL) > rosemary (IC_{50}: 0.032 mg/mL) > bay leaf (IC_{50}: 0.035 mg/mL) > lemon balm (IC_{50}: 0.037 mg/mL). Onion, garlic, green chili, red chili, fenugreek, black cumin did not show any activity in the HSA-Fructose model. BSA-Glucose model: lemon balm (IC_{50}: 0.009 mg/mL) > allspice (IC_{50}: 0.012 mg/mL) > marjoram (IC_{50}: 0.021 mg/mL) > black pepper (IC_{50}: 0.026 mg/mL) > turmeric (IC_{50}: 0.042 mg/mL). Coriander, green chili, onion, and garlic did not show any activity in the BSA-Glucose model. BSA-Fructose model: celery (IC_{50}: 0.018 mg/mL) > black pepper, rosemary (IC_{50}: 0.021 mg/mL) > thyme (IC_{50}: 0.032 mg/mL) > turmeric (IC_{50}: 0.039 mg/mL) > lemon balm (IC_{50}: 0.043 mg/mL). Fenugreek and onion show any activity in the BSA-Fructose model. Collagen-Glucose model: hibiscus, cinnamon (IC_{50}: 0.004 mg/mL) > thyme, rosemary (IC_{50}:0.006mg/mL) > spearmint (IC_{50}: 0.007mg/mL) > tarragon, peppermint, allspice (IC_{50}: 0.01 mg/mL). Fenugreek did not show any activity in the Colagen-Glucose model (***Table 2***). These data suggest that the IC_{50} values of spice and herbs varied across different glycation models with both water and ethanol extraction. The wide variations in anti-glycation activity of spices and herbs across different glycation models were likely due to the different proteins, sugars, and extraction methods involved.

Effect of different proteins on glycation

To investigate the effect of different proteins on glycation, we analyzed correlation of IC_{50} values of spices between HSA-Glucose/Collagen-Glucose, HSA-Glucose/BSA-Glucose, BSA-Glucose/Collagen-Glucose, and HSA-Fructose/BSA-Fructose models, with both water and ethanol extraction. Linear correlation was poor with water extraction between HSA-Glucose/Collagen-Glucose ($R^2 = 0.073$), HSA-Glucose/BSA-Glucose ($R^2 = 0.273$), BSA-Glucose/Collagen-Glucose ($R^2 = 0.0301$), and HSA-Fructose/BSA-Fructose ($R^2 = 0.041$) (***Fig. 1a***). Correlation was similarly poor with ethanol extraction between HSA-Glucose/Collagen-Glucose ($R^2 = 0.028$), HSA-Glucose/BSA-Glucose ($R^2 = 0.172$), BSA-Glucose/Collagen-Glucose ($R^2 = 0.068$), and HSA-Fructose/BSA-Fructose ($R^2 = 0.117$) (***Fig. 1b***). Such low R^2 values both with water and ethanol extraction suggest that the anti-glycation activity of spices is primarily determined by the protein type used in glycation models. These findings emphasize the importance of protein selection in anti-glycation activity determination.

Effect of different sugars on glycation

To investigate the effect of different sugars on glycation, we analyzed correlation of IC_{50} values of spices between HSA-Glucose/HSA-Fructose, and BSA-Glucose/BSA-Fructose, with both water and ethanol extraction. Linear correlation was highly significant with water extraction between HSA-Glucose/HSA-Fructose ($R^2 = 0.753$) and BSA-Glucose/BSA-Fructose ($R^2 = 0.870$) (***Fig. 2a***). Correlation was similarly high with ethanol extraction between HSA-Glucose/HSA-Fructose ($R^2 = 0.967$) and BSA-Glucose/BSA-Fructose ($R^2 = 0.642$) (***Fig. 2b***). Such high R^2 values suggest a lack of any significant change in anti-glycation activity based on glucose or fructose usage in a given model.

Effect of different extraction methods on glycation

To investigate the effect of different extraction methods on glycation, we anayzed correlation of IC_{50} values of spices between HSA-Glucose-Water/HSA-Glucose-Ethanol, HSA-Fructose-Water/HSA-Fructose-Ethanol, BSA-Glucose-Water/BSA-Glucose-Ethanol, BSA-Fructose-Water/BSA-Fructose-Ethanol and Collagen-Glucose-Water/Collagen-Glucose-Ethanol (***Fig. 3***). Linear correlation was high for HSA-Glucose-Water/HSA-Glucose-Ethanol ($R^2 = 0.837$), HSA-Fructose-Water/HSA-Fructose-Ethanol ($R^2 = 0.916$), BSA-Fructose-Water/BSA-Fructose-Ethanol ($R^2 = 0.6907$), and Collagen-Glucose-Water/Collagen-Glucose-Ethanol ($R^2 = 0.850$). However, correlation was comparatively poor for BSA-Glucose-Water/BSA-Glucose-Ethanol ($R^2 = 0.373$) (***Fig. 3***). These findings suggest that the extraction method has a case-by-case effect on anti-glycation activity.

Table 1. Effect of water extracted spices on glycation induced by 5 in vitro glycation models

No	Common Name	Scientific Name	Family Name	HSA-G	HSA-F	BSA-G	BSA-F	Colla-G
1	Anise	*Pimpinella anisum*	Apiaceae	0.162	0.205	0.212	0.269	0.126
2	Oregano	*Oreganum vulgare*	Lamiaceae	0.084	0.070	0.066	0.168	0.015
3	Basil	*Ocimum basilicum*	Lamiaceae	0.082	0.087	0.135	0.133	0.024
4	Caraway	*Carum carvi*	Apiaceae	0.074	0.073	0.431	0.269	0.032
5	Juniper berries	*Juniperus communis*	Cupressaceae	0.226	0.337	0.765	1.117	0.151
6	Peppermint	*Mentha pierita*	Lamiaceae	0.062	0.074	0.244	0.246	5.197
7	Fenugreek	*Trigonella foenum-graecum*	Fabaceae	0.837	>200	>200	None	None
8	Fennel	*Foeniculum vulgare*	Apiaceae	0.046	0.194	2.690	1.456	0.180
9	Tarragon	*Artemisia dracunculus*	Asteraceae	0.132	0.102	0.195	0.148	0.003
10	Laurel	*Litsea glutinosa*	Lauraceae	0.150	0.052	0.486	0.182	0.041
11	Savory	*Satureja montana*	Lamiaceae	0.050	0.033	0.124	0.083	0.003
12	Cumin	*Cuminum cyminum*	Apiaceae	0.159	0.139	1.150	0.258	0.164
13	Hibiscus	*Hibiscus rosa-sinensis*	Malvaceae	0.171	0.311	0.502	0.317	0.008
14	Japanese Pepper	*Zanthoxylum piperitum*	Rutaceae	0.053	0.062	0.808	0.181	0.028
15	Allspice	*Pimenta dioica*	Myrtaceae	0.120	0.036	0.114	0.111	0.027
16	Marjoram	*Origanum majorana*	Lamiaceae	0.015	0.064	0.233	0.089	0.025
17	Lemon balm	*Melissa officinalis*	Apiaceae	0.034	0.036	0.056	0.059	0.006
18	Ajwain	*Trachyspermum ammi*	Apiaceae	0.039	0.208	2.668	0.521	0.085
19	Lemongrass	*Cymbopogon citratus*	Poaceae	0.020	0.134	1.747	0.307	0.026
20	Common mallow	*Malva sylvestris*	Malvaceae	0.026	0.178	0.200	0.349	0.211
21	Coriander	*Coriandrum sativum*	Apiaceae	0.329	0.101	>200	0.8	0.095
22	Star anise	*Illicium verum*	Schisandraceae	17.676	0.026	0.640	0.243	0.003
23	Ginger	*Zingiber officinale*	Zingiberaceae	0.02	0.190	>200	None	0.227
24	Paprika	*Capsicum annuum*	Solanoideae	0.361	2.781	11.155	None	0.017
25	Thyme	*Thymus vulgaris*	Lamiaceae	4.305	0.058	0.130	2.750	0.016
26	Dill	*Anethum graveolens*	Apiaceae	None	0.124	2.097	0.823	0.071
27	Celery	*Apium graveolens*	Apiaceae	0.178	0.119	0.622	0.458	0.072
28	Cardamom	*Elettaria cardamomum*	Zingiberaceae	0.245	0.461	1.577	None	0.018
29	Rosemary	*Rosmarinus officinalis*	Lamiaceae	0.054	0.040	0.127	0.061	0.008
30	Spearmint	*Mentha spicata*	Lamiaceae	0.024	0.057	0.105	0.056	0.003
31	Red chili*	*Capsicum frutescens*	Solanaceae	0.18	1.320	6.802	9.293	0.158
32	Green chili*	*Capsicum pubescens*	Solanaceae	0.564	0.683	None	2.062	0.492
33	Onion*	*Allium cepa*	Liliaceae	2.454	>200	>200	None	None
34	Cloves*	*Syzygium aromaticum*	Myrtaceae	0.009	0.018	0.098	0.147	0.001
35	Garlic*	*Allium sativum*	Amaryllidaceae	None	>200	>200	None	None
36	Black pepper*	*Piper nigrum*	Piperaceae	0.028	0.049	4.555	0.150	0.017
37	Black cumin*	*Nigella sativa*	Ranunculaceae	0.529	0.450	13.808	1.023	0.189
38	Turmeric*	*Curcuma longa*	Zingiberaceae	0.094	0.102	0.783	1.626	0.019
39	Bay leaf*	*Cinnamomum tamala*	Lauraceae	0.022	0.036	0.185	0.189	0.004
40	Cinnamon*	*Cinnamomum verum*	Lauraceae	60.995	0.383	4.722	0.017	0.003
41	Aminoguanidine	*Positive Control*	–	0.056	–	–	–	0.138

* Bangladeshi spices; HSA-G, human serum albumin – glucose model; HSA-F, human serum albumin – fructose model; BSA-G, bovine serum albumin – glucose model; BSA-F, bovine serum albumin – fructose Model; Collagen-G, bovine skin Collagen type-I – glucose model; (-), Data not available. Aminoguanidine IC_{50} was measured only with water extraction.

Table 2. Effect of ethanol extracted spices on glycation induced by 5 in vitro glycation models

SI	Common Name	Scientific Name	Family Name	HSA-G	HSA-F	BSA-G	BSA-F	Colla-G
1	Anise	*Pimpinella anisum*	Apiaceae	0.426	0.161	1.480	0.287	0.086
2	Oregano	*Oreganum vulgare*	Lamiaceae	0.124	0.045	0.146	0.088	0.020
3	Basil	*Ocimum basilicum*	Lamiaceae	0.093	0.095	0.161	0.079	0.024
4	Caraway	*Carum carvi*	Apiaceae	0.158	0.107	>200	0.223	0.024
5	Juniper berries	*Juniperus communis*	Cupressaceae	0.580	0.399	2.339	0.630	0.053
6	Peppermint	*Mentha pierita*	Lamiaceae	0.089	0.063	0.241	0.120	0.010
7	Fenugreek	*Trigonella foenum-graecum*	Fabaceae	0.620	None	121.125	None	None
8	Fennel	*Foeniculum vulgare*	Apiaceae	0.615	0.235	1.884	0.596	0.099
9	Tarragon	*Artemisia dracunculus*	Asteraceae	0.096	0.061	0.084	0.151	0.010
10	Laurel	*Litsea glutinosa*	Lauraceae	0.169	0.056	0.164	0.101	0.021
11	Savory	*Satureja montana*	Lamiaceae	0.0383	0.051	0.047	0.075	0.015
12	Cumin	*Cuminum cyminum*	Apiaceae	0.422	0.151	0.541	0.301	0.072
13	Hibiscus	*Hibiscus rosa-sinensis*	Malvaceae	0.058	0.300	0.270	0.322	0.004
14	Japanese Pepper	*Zanthoxylum piperitum*	Rutaceae	0.059	0.061	0.135	0.134	0.020
15	Allspice	*Pimenta dioica*	Myrtaceae	0.043	0.028	0.012	0.193	0.010
16	Marjoram	*Origanum majorana*	Lamiaceae	0.077	0.083	0.021	0.120	0.02
17	Lemon balm	*Melissa officinalis*	Apiaceae	0.0373	0.037	0.009	0.043	0.015
18	Ajwain	*Trachyspermum ammi*	Apiaceae	0.125	0.215	0.080	90.342	0.041
19	Lemongrass	*Cymbopogon citratus*	Poaceae	0.192	0.144	0.223	0.460	0.028
20	Common mallow	*Malva sylvestris*	Malvaceae	0.301	0.135	0.178	0.340	0.180
21	Coriander	*Coriandrum sativum*	Apiaceae	4.42	4.900	None	0.140	0.057
22	Star anise	*Illicium verum*	Schisandraceae	0.076	0.039	0.232	0.065	0.129
23	Ginger	*Zingiber officinale*	Zingiberaceae	2.620	1.261	>200	0.530	0.032
24	Paprika	*Capsicum annuum*	Solanoideae	1.022	2.858	6.608	0.804	0.476
25	Thyme	*Thymus vulgaris*	Lamiaceae	0.178	0.062	0.083	0.032	0.006
26	Dill	*Anethum graveolens*	Apiaceae	0.780	0.751	>200	0.059	0.040
27	Celery	*Apium graveolens*	Apiaceae	0.049	0.138	0.269	0.018	0.043
28	Cardamom	*Elettaria cardamomum*	Zingiberaceae	0.110	17.359	>200	3.790	0.055
29	Rosemary	*Rosmarinus officinalis*	Lamiaceae	0.045	0.032	0.053	0.021	0.006
30	Spearmint	*Mentha spicata*	Lamiaceae	0.0003	0.043	0.089	0.120	0.007
31	Red chili*	*Capsicum frutescens*	Solanaceae	4.990	None	>200	18.230	1.125
32	Green chili*	*Capsicum pubescens*	Solanaceae	2.980	None	None	>200	0.471
33	Onion*	*Allium cepa*	Liliaceae	None	None	None	None	11.227
34	Cloves*	*Syzygium aromaticum*	Myrtaceae	0.022	0.020	0.049	0.046	0.014
35	Garlic*	*Allium sativum*	Amaryllidaceae	None	None	None	>200	143.523
36	Black pepper*	*Piper nigrum*	Piperaceae	0.011	0.064	0.026	0.021	0.090
37	Black cumin*	*Nigella sativa*	Ranunculaceae	None	None	5.02	3.045	5.198
38	Turmeric*	*Curcuma longa*	Zingiberaceae	0.018	0.041	0.042	0.040	0.030
39	Bay leaf*	*Cinnamomum tamala*	Lauraceae	0.065	0.035	0.120	0.090	0.014
40	Cinnamon*	*Cinnamomum verum*	Lauraceae	0.172	0.039	0.245	0.350	0.004
41	Aminoguanidine	*Positive Control*	–	0.056	–	–	–	0.138

* Bangladeshi spices; HSA-G, human serum albumin – glucose model; HSA-F, human serum albumin – fructose model; BSA-G, bovine serum albumin – glucose model; BSA-F, bovine serum albumin – fructose Model; Collagen-G, bovine skin Collagen type-I – glucose model; (-), Data not available. Aminoguanidine IC_{50} was measured only with water extraction.

a)

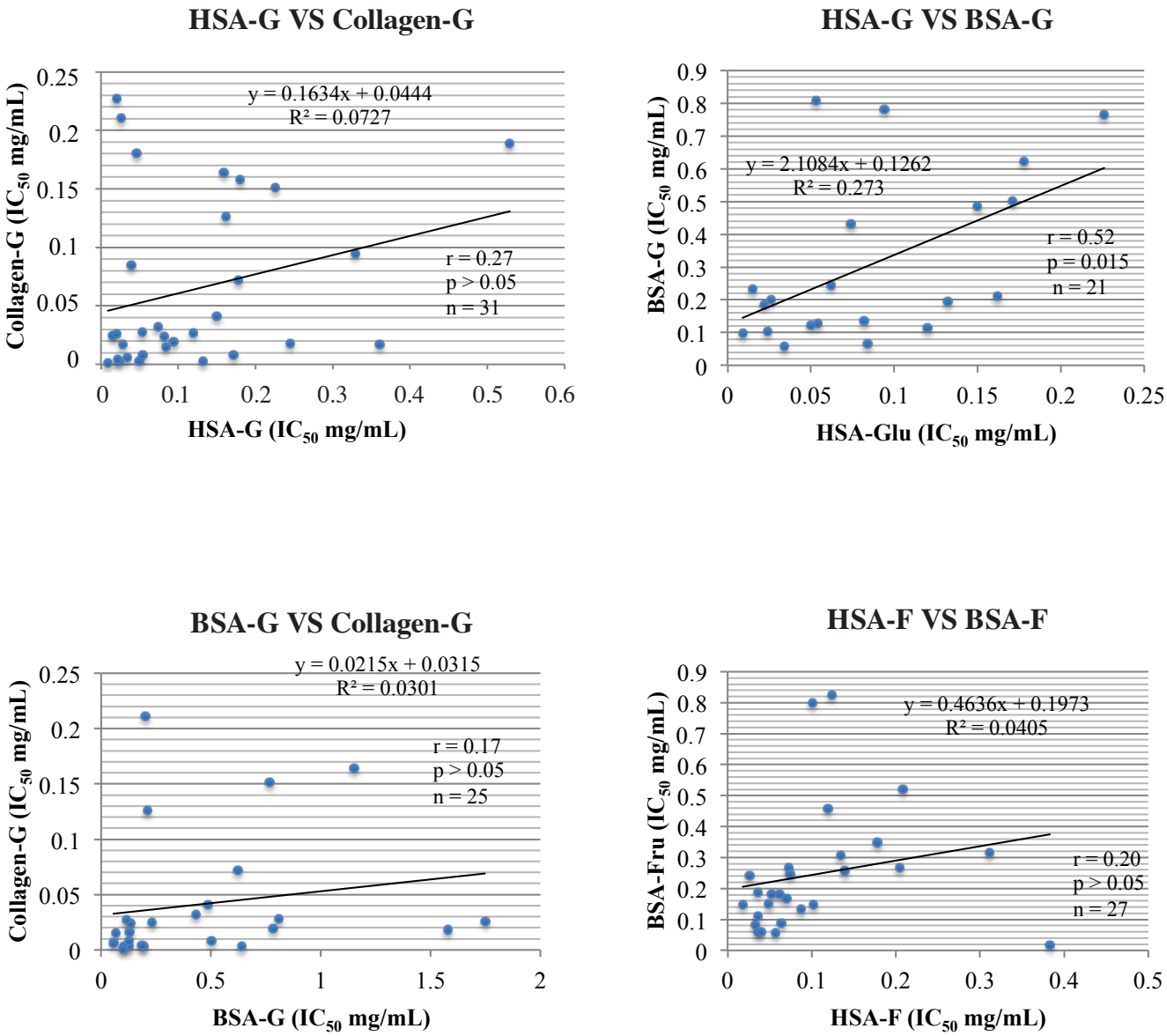


Fig 1.(a) **Correlation between HSA-Glucose/Collagen-Glucose, HSA-Glucose/BSA-Glucose, BSA-Glucose/Collagen-Glucose, and HSA-Fructose/BSA-Fructose in water extraction.**

b)

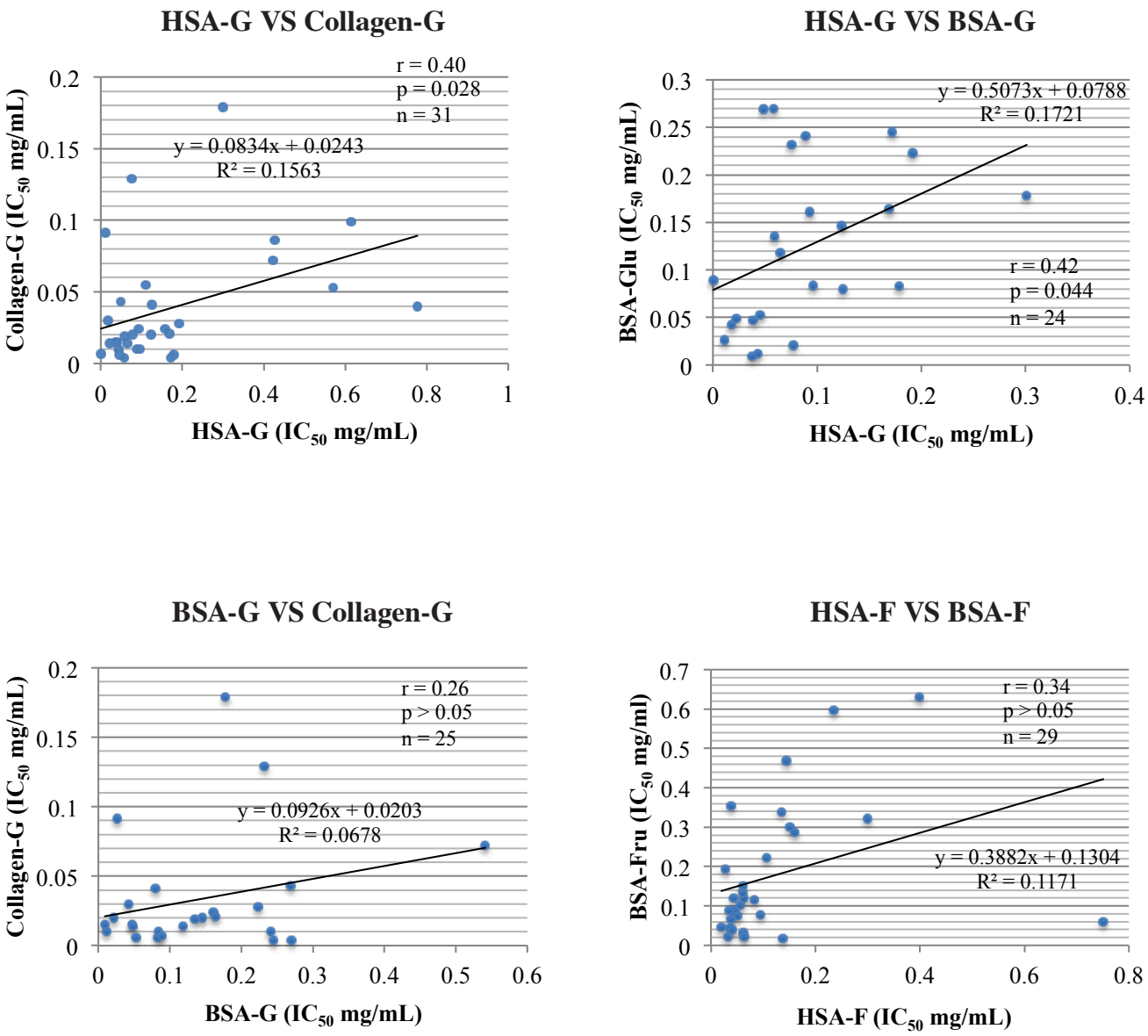
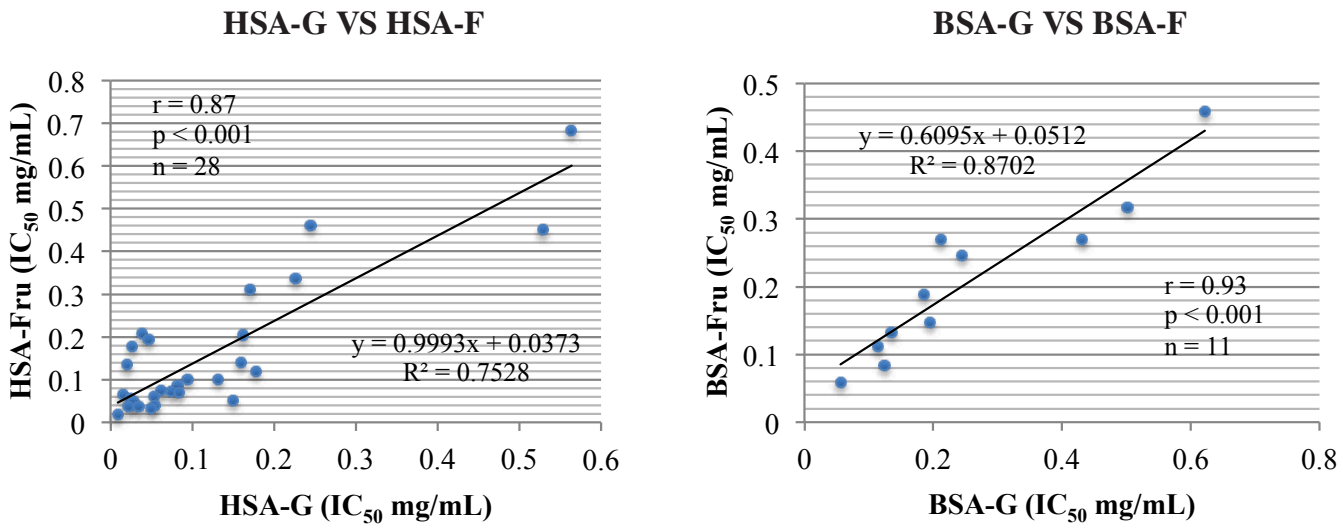


Fig 1.(b) **Correlation between HSA-Glucose/Collagen-Glucose, HSA-Glucose/BSA-Glucose, BSA-Glucose/Collagen-Glucose, and HSA-Fructose/BSA-Fructose in ethanol extraction.**

a)



b)

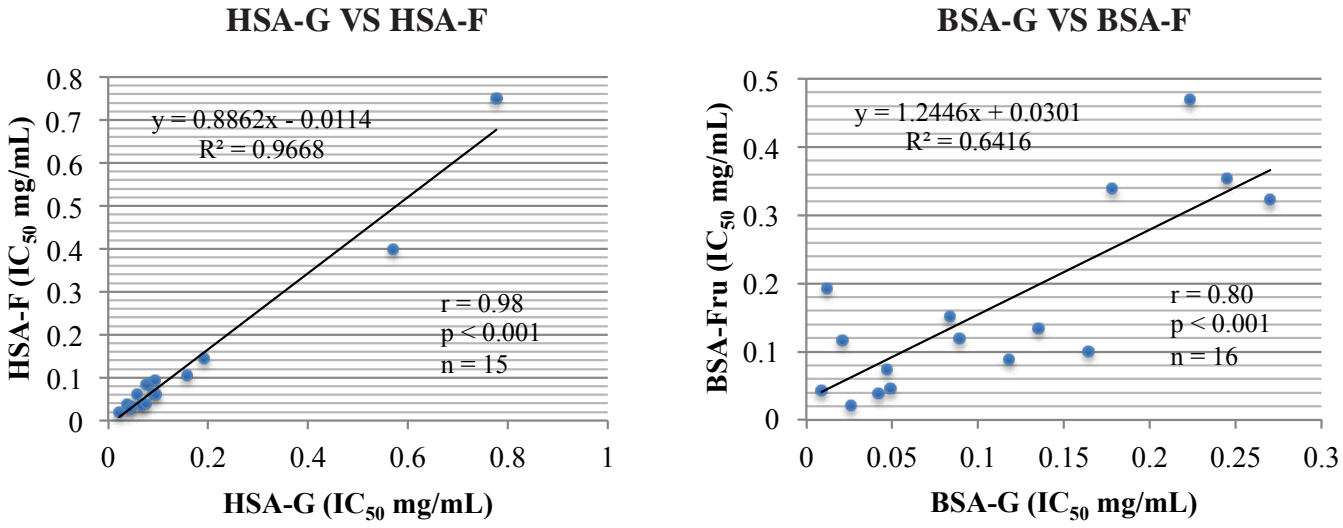
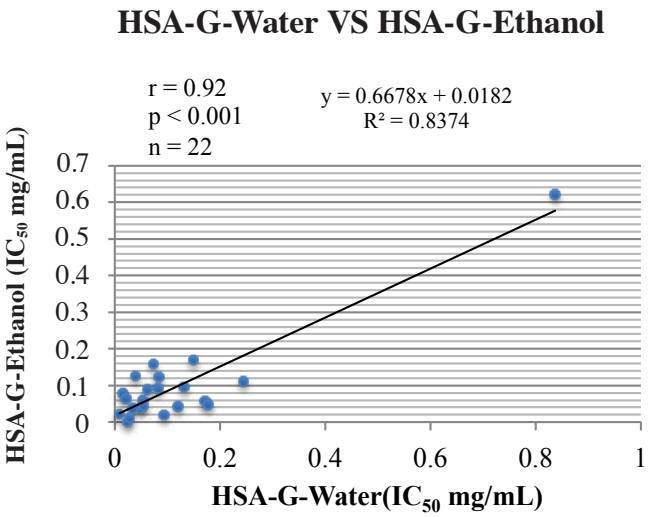
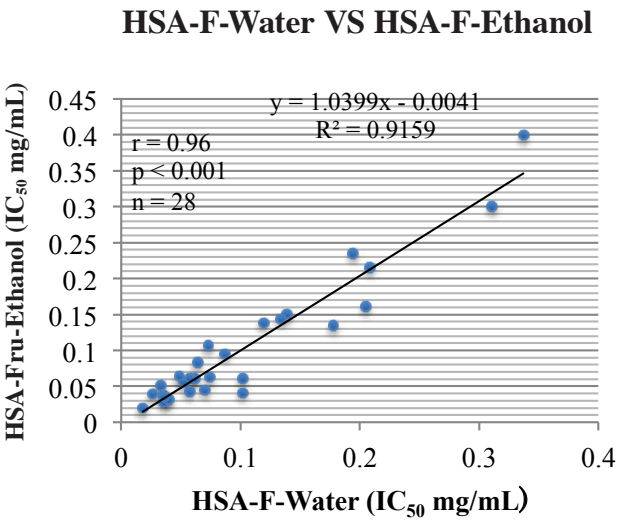
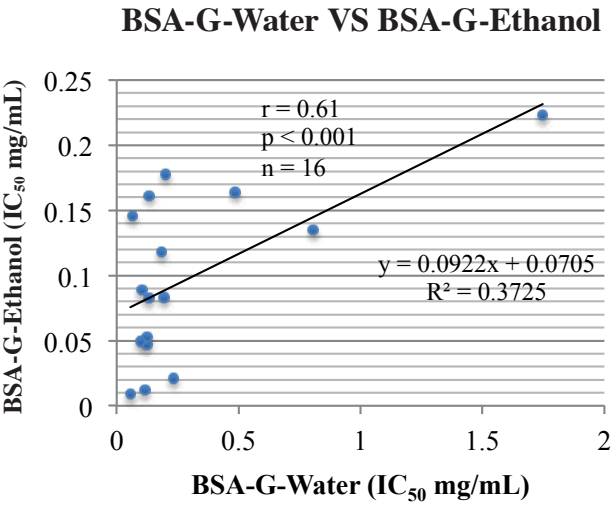
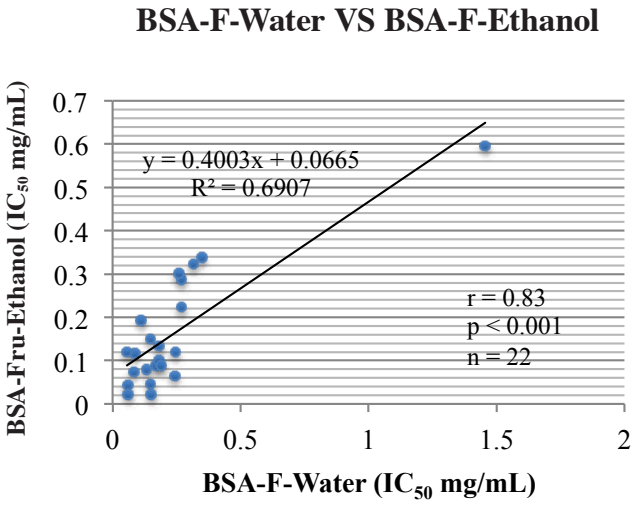


Fig 2.(a) **Correlation between HSA-Glucose/HSA-Fructose and BSA-Glucose/BSA-Fructose in water extraction.**
(b) **Correlation between HSA-Glucose/HSA-Fructose and BSA-Glucose/BSA-Fructose in ethanol extraction.**









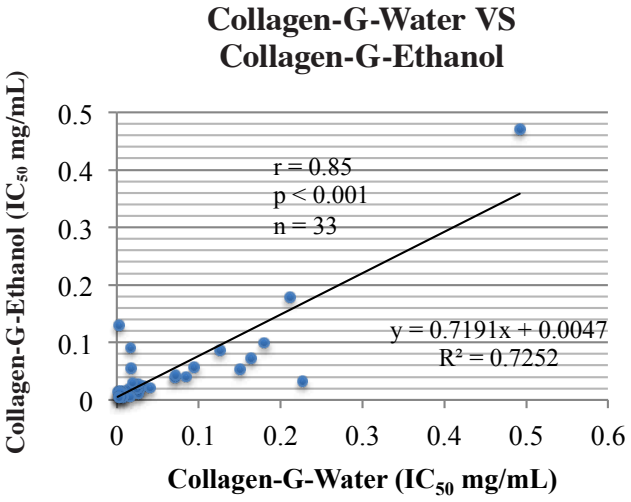


Fig 3. **Correlation between HSA-Glucose-Water/HSA-Glucose-Ethanol, HSA-Fructose-Water/HSA-Fructose-Ethanol, BSA-Glucose-Water/BSA-Glucose-Ethanol, BSA-Fructose-Water/BSA-Fructose-Ethanol, and Collagen-Glucose-Water/Collagen-Glucose-Ethanol.**

Discussion

Correlation was poor between HSA-Glu/Collagen-Glu, HSA-Glu/BSA-Glu, BSA-Glu/Collagen-Glu, and HSA-Fru/BSA-Fru with both water and ethanol extraction. These findings imply that anti-glycation activity is primarily determined by the protein used in glycation models. It gives emphasis to the protein selection in anti-glycation activity determination. We also found that type of sugar (glucose/fructose) has no marked effect, and extraction methods have a case-by-case effect on anti-glycation activity.

It has been reported that several plants showing anti-glycation activity in the BSA-Fructose model had high content of phenolic compounds. A literature search, subsequently revealed that many purified phenolic compounds (flavones, flavanones, flavanols, isoflavones, proanthocyanidins, and other phenolics) and phenolic-rich plant extracts exert strong inhibitory activity in this particular bioassay [11]. Our study showed that water extraction of cloves, oregano, cinnamon, basil, caraway, rosemary, savory showing anti-glycation activity in the HSA-Glucose model had high contents of polyphenols (data not shown). Evaluation of the polyphenol concentration of spices, showed that cloves had the highest concentration (data not shown) which was consistent with previous findings of extremely strong anti-oxidant activity and a high level of phenolics in cloves [12]. A previous study reported that the anti-glycation activity of the extracts was correlated with their anti-oxidant properties [13]; indeed, other compounds with anti-oxidant power have also been reported to exhibit anti-glycation activity [14]. The results support the hypothesis that anti-oxidant phenolic compounds contribute to the anti-glycation capacity of spices to an extent that depends strongly on their amount.

In the present study, we demonstrated that a compound's anti-glycation activity is primarily determined by the protein type used in the glycation model. Some researchers have hypothesized that a variety of AGEs are generated from different proteins, possibly due to differences in amino acid content between proteins. Contents of lysine and arginine are presented in ***Table-3*** with these amino acids being shown to be rich in BSA, HSA, and keratin among the six types of proteins examined. Lysine and arginine amount seems to be correlated with N^{ε}-carboxymethyllysine, but not 3-deoxyglucosone, glyoxal, or methylglyoxal [6].

Ethanol-derived extracts from thyme showed better anti-glycation activity both in HSA-Glucose (IC_{50}: 0.18) and BSA-Fructose (IC_{50}: 0.03) models than water-derived extracts on the same models (IC_{50}: 4.31 and 2.75, respectively) in the present study. Similar results were also noted for star anise, peppermint and, ajwain in HSA-Glucose, BSA-Glucose and, Collagen-Glucose models. We assume that the ingredients responsible for the anti-glycation activity of thyme, star anise, peppermint and, ajwain are hydrophobic in nature and water insoluble. Future studies should attempt to extract and purify those compounds likely to have the most potent anti-glycative activity.

Fructose is more reactive than glucose, and fluorescence AGE formation of fructose and collagen proceeded at a faster rate than that of glucose and collagen. At 60°C, proteins (collagen, HSA, and BSA) glycated faster in the presence of fructose than with glucose [6]. This finding suggests that fructose – but not glucose – accelerates the protein glycation reaction, and thereby AGE formation. However, as with other natural compounds, when evaluating the inhibitory activity of spices, fructose and glucose have no significant effect on measurements.

Conclusion

Here, we examined the effect of different proteins, sugars and extraction methods on the anti-glycation activity of spices in five different glycation models. Our finding revealed that anti-glycation activity of spices is determined largely by protein type, with sugars having little influence on activity. However, extraction methods did have a case-by-case effect on anti-glycation activity.

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Conflict of Interest Statement

The authors state no conflicts of interest.

Table 3. Percentage content of arginine and lysine in the protein tested.

Amino acid content (%)	BSA	Keratin	Elastin	Proteoglycan	Collagen	HSA
Arginine	4.28	4.82	0.89	5.90	4.78	4.43
Lysine	9.88	0.00	5.37	6.00	3.90	9.85
Arginine + Lysine	14.17	4.82	6.26	11.90	8.68	14.29

BSA, bovine serum albumin; HSA, human serum albumin.
Data referenced from National Center for Biotechnology Information, except proteoglycan, which was obtained from Biomatec Japan (Hokkaido, Japan).

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