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Original article

Anti-glycative effect of vegetable and fruit extracts on multiple glycation models

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Abstract

***Aim*:** Locomotive syndrome is one of the age-related symptoms based on weakness of the locomotive organs. As bone tissue is glycated by excess reducing sugar in the blood and synovial fluid, advanced glycation end products (AGEs) are formed. These AGEs mediate declining bone stiffness and elasticity, leading to bone fracture, osteoporosis and osteoarthritis. In the present study, we investigated the effect of 73 kinds of vegetable and fruit extracts on glycation using 3 different proteins, serum albumin: most abundant proteins in blood, type I and type II collagen: major structural protein in bone and soft tissue, respectively.

***Methods*:** To investigate the effect of plant extracts, 1 mg/mL solid content of extracts were used for three different glycation models such as human serum albumin (HSA) with glucose (glc-HSA), type I and type II collagens with fructose (fru-collagen I and fru-collagen II). Fluorescent AGEs were measured by their typical fluorescence of 370/440 nm. Intermediates of AGEs: 3-deoxyglucosone (3-DG), glyoxal (GO) and methylglyoxal (MGO) were determined using HPLC-UV analyses.

***Results*:** Among 73 kinds of plant extracts, 9 kinds of samples showed strongly inhibited fluorescent AGEs formation in all 3 different glycation models. Lady's thumb and the soft layer of chestnut were especially effective against not only fluorescent AGEs, but also the intermediates of AGEs such as 3-DG, GO and MGO.

***Conclusions*:** Among the 73 kinds of plant extracts, we demonstrated that lady's thumb and the soft layer of chestnut have potent anti-glycation activity against HSA and collagens.

***KEY WORDS*:** glycation, locomotive syndrome, collagen, plant extract, advanced glycation end products (AGEs)

Introduction

Locomotive organs such as muscular, joint and bone decline with age. In 2007, Japanese Orthopedic Association proposed the concept in which the comprehensive symptoms due to the failure of locomotive organs as locomotive syndrome [1,2]. Locomotive syndrome reduce people's mobility and increases the risk of falls. It may cause bone fracture, leading to further muscle weakness due to mobility limitation. Now a days, the percentage of people aged over 65 years old reached over 25% in the Japanese population. Thus, the prevention of age-related diseases, including locomotive syndrome, is beneficial for the protection of elderly people's health and quality of life (QOL).

Collagen is one of the most abundant proteins in the body and 28 types of collagen have been identified in vertebrates [3]. Type I collagen is abundant in bone, cornea, dermis and tendon and type II collagen is component protein of hyaline cartilage in joint and vitreous body. The amount of collagens declines with age [4]. Thus, protecting the quality of collagens is one of the important methods to prevent bone and joint diseases.

Advanced glycation end products (AGEs) are formed by glycation, non-enzymatic reaction between proteins and reducing sugars and accumulated with age. Not only do AGEs bound proteins possibly lose their functions, but the AGEs themselves induce inflammation in organs. Several lines of evidence indicated that the accumulation of AGEs is associated with age-related diseases such as cancer [5], diabetes mellitus [6], Alzheimer's disease [7,8] and cardiovascular disease [9]. Moreover, the accumulation of AGEs, loss of bone

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stiffness and elasticity due to formation of unnecessary cross-link in bone collagens. It may cause of osteoporosis [10] and osteoarthritis [11]. To protect against the accumulation of AGEs in organs, several chemical inhibitors have been established, however, none were approved in Japan due to serious side effects.

Over a couple of years we evaluated over 500 kinds of food materials against fluorescent AGE formation in human serum albumin (HSA) with glucose reaction model and we listed their IC_{50} [12-16]. However, we also demonstrated that the pattern of formed AGEs were different in each protein [15], thus anti-glycative effect of food materials might be different in each protein. In this study, we evaluates food materials against the formation of AGEs and its intermediates in not only HSA but also both type I and type II collagens.

Materials and Methods

Materials

HSA was purchased from Sigma-Aldrich (St. Louis, MO), type I collagen was obtained from Nippi (Tokyo, Japan) and type II collagen was provided from Elastin Products Company (Owensville, MO). All other chemicals were obtained from Wako (Osaka, Japan) or Dojindo (Kumamoto, Japan) for analytical grade.

Preparation of plants extract

The samples used were 38 varieties of vegetables and 35 varieties of fruit which are previously described for their inhibitory effect against fluorescent AGE formation in HSA with glucose model [13,14]. Samples were dried and ground, and then, 2 g of the powdered samples were mixed with 40 mL of distilled water. After incubation at 80 °C for 75 minutes, the extracted samples were centrifuged at 2,300 x g for 10 minutes and filtered using filter paper. Five mL of the plant extracts were used for measurement of solid content by evaporation, and then, leftover samples were adjusted at 10 mg/mL solid content using distilled water.

Preparation of glycated proteins

Three glycation models, i) HSA with glucose (glc-HSA), ii) type I collagen with fructose (fru-collagen I) and iii) type II collagen with fructose (fru-collagen II) were used. Briefly, i) 8 mg/mL HSA was mixed with 0.2 mol/L glucose in 50 mmol/L phosphate buffer (PB, pH 7.4) and incubated at 60 °C for 40 hours, ii) and iii) 0.6 mg/mL collagen with 0.4 mol/L fructose in 50 mmol/L PB (pH 7.4) were incubated at 60 °C for 24 hours (named "solution A"). To determine the effects of vegetables or fruit extract on glycation, 1 mg/mL solid content of the plant extracts were used instead of the same volume of distilled water (solution B). As a positive control, 0.1 mg/mL aminoguanidine (AG) was used.

Measurement of AGEs-derived fluorescence

AGEs-derived fluorescence was measured as previously described [17]. Briefly, 200 µL of the reaction mixture was used to measure fluorescence at an excitation wavelength of 370 nm and an emission wavelength of 440 nm by a Varioscan® Flash (Thermo Scientific, Waltham, MA) microplate reader. The value was calculated using the equation below.

Inhibition of AGEs-derived fluorescence [%]
= [1 - {fluorescence of (solution B) /fluorescence of (solution A)}] x 100

Measurement of intermediates of AGEs

Three kinds of AGE intermediates, 3-deoxyglucosone (3-DG), glyoxal (GO) and methylglyoxal (MGO), were measured using a Shimadzu high-performance liquid chromatography ultraviolet(HPLC-UV) system (Shimadzu Corporation, Kyoto, Japan). Samples were prepared as previously described [17]. Briefly, reaction mixtures were deproteinized using 6% perchrolic acid. After centrifugation, the supernatant was immediately neutralized by excess amounts of sodium bicarbonate. Then, 3-DG, GO and MGO were labeled with 2,3-diaminonaphthalene for 24 hours at 4 °C. The HPLC conditions were as follows; Column, UnisonUK - Phenyl, 75 mm x 3 mm I.D. column (Imtakt Corp, Kyoto, Japan); eluent, 50 mmol/L phosphoric acid and acetonitrile = 89:11. The flow rate and detection wavelengths were 1.0 mL/minute and 268 nm.

Statistics

Data were expressed as mean ± SD of at least three independent experiments. The statistical analyses were performed by an analysis of variance (ANOVA) using Dunnett's test for multiple comparisons between each of the samples and the control group. Differences were considered significant at p values less than 0.05.

Results

Effect of plant extract on fluorescent AGEs formation in HSA with glucose model.

First, we investigated that the effect of vegetable and fruit extracts on fluorescent AGEs formation in glc-HSA model at 1 mg/mL solid content of plant extracts. After 40 hours of incubation at 60 °C, fluorescent AGEs were measured at 370/440 nm, the characteristic wavelength of those. All 73 plant extracts significantly inhibited fluorescent AGE formation (***Table 1***). Eighteen kinds of plants inhibited fluorescent AGEs formation over 40 % and 64 kinds of those inhibited over 20 %

Effect of plant extract on fluorescent AGE formation in collagen with fructose models.

Based on the efficacy of plant extracts in the glc-HSA glycation model, we selected the top 20 plant extracts and further evaluated the effects of those on fru-collagen I and fru-collagen II glycation models. All 20 plant extracts significantly inhibited fluorescent AGEs for both fru-collagen I and fru-collagen II glycation models (***Table 2, 3***). Notably, 9 kinds of plant extracts, Chinese quince, chestnut (soft layer), chrysanthemum (petal), raspberry, reddish black rice, lady's thumb and peel of 3 kinds of apples (san-jyonagold, kogyoku and toki), decreased the glycation-derived fluorescent AGEs by over 80 % in both types of collagens at 1 mg/mL of solid content.

Table 1. Inhibitory effect of plant compounds on HSA-derived fluorescent AGE formation

Ranking	English name	Part	Japanese name	Scientific name	Inhibition of fluorescent AGEs [%]
	Aminoguanigine 0.1 mg/ml				53.10 ± 1.46
1	Chestnut	soft layer	Kuri	*Castanea crenata*	79.73 ± 0.05
2	Chestnut	outer skin	Kuri	*Castanea crenata*	75.20 ± 0.15
3	Lady's thumb		Tade	*Polygonum hydropiper*	73.61 ± 0.29
4	Pomegranate	peel	Zakuro	*Punica granatum*	62.87 ± 0.16
5	Chrysanthemum (yellow)	petal	Shokuyo-kiku	*Chrysanthemum morifolium*	62.14 ± 0.09
6	Water chestnut	outer skin	To-Bishi	*Trapa bicornis*	50.69 ± 0.29
7	Chinese quince		Karin	*Pseudocydonia sinensis*	49.87 ± 0.26
8	Belvedere fruit		Tonburi	*Bassia scoparia*	49.32 ± 0.10
9	Malabar spinach		Tsurumurasaki	*Basella alba*	48.97 ± 0.06
10	Apple : toki	peel	Ringo: Toki	*Malus domestica*	46.44 ± 0.30
11	Rosemary		Rosemary	*Rosmarinus officinalis*	45.20 ± 0.36
12	Citrus sudachi	peel	Sudachi	*Citrus sudachi*	44.69 ± 0.41
13	Apple : san-jyonagold	peel	Ringo: San-jyonagold	*Malus domestica*	44.34 ± 0.20
14	Citrus sudachi	pulp	Sudachi	*Citrus sudachi*	43.89 ± 0.39
15	Nalta jute		Moroheiya	*Corchorus olitorius*	42.86 ± 0.49
16	Apple : kogyoku	peel	Ringo: Kogyoku	*Malus domestica*	41.59 ± 0.37
17	Lemon		Lemon	*Citrus x limonium*	41.56 ± 0.41
18	Reddish black rice		Kuro-mai	*Oryza sativa*	40.14 ± 0.36
19	Raspberry		Raspberry	*Rubus idaeus*	39.99 ± 0.71
20	Red-kernelled rice		Aka-mai	*Oryza sativa*	39.66 ± 0.13
21	Ostrich fern		Kogomi	*Matteuccia struthiopteris*	37.81 ± 0.34
22	Rucola		Rukkora	*Eruca vesicaria*	37.06 ± 0.52
23	Apple : hokuto	peel	Ringo: Hokuto	*Malus domestica*	37.03 ± 0.50
24	Red rhubarb		Aka-rubabu	*Rheum rhabarbatum*	36.81 ± 0.41
25	Lime		Lime	*Citrus aurantifolia*	35.72 ± 1.94
26	Shirona Chinese cabbage		Shiro-na	*Brassica rapa*	35.62 ± 0.37
27	Apple : jyonagold	peel	Ringo: Jyonagold	*Malus domestica*	35.55 ± 0.60
28	Butterbur scape	peel	Fukinotou	*Patasites Japonicus*	35.49 ± 0.23
29	Pea	pod	Endou-mame	*Pisum sativum*	35.47 ± 1.09
30	Red giant elephant ear		Beni-zuiki	*Colocasia gigantean*	34.84 ± 0.36
31	Apple : yoko	peel	Ringo: Youkou	*Malus domestica*	34.56 ± 0.59
32	Japanese staunton-vine		Mube	*Stauntonia hexaphylla*	34.55 ± 0.30
33	Apple : san-fuji	peel	Ringo: San-fuji	*Malus domestica*	34.02 ± 0.26
34	Variety of wild mustard		Mibu-na	*Brassica rapa*	34.01 ± 0.16
35	Black-eyed pea		Sasage	*Vigna unguiculata*	33.91 ± 0.55
36	Black soybean		Kuro-mame	*Glycine max*	33.46 ± 0.79

Table 1. Inhibitory effect of plant compounds on HSA-derived fluorescent AGE formation (continued)

Ranking	English name	Part	Japanese name	Scientific name	Inhibition of fluorescent AGEs [%]
37	Apple : fuji	peel	Ringo: Fuji	*Malus domestica*	33.36 ± 0.53
38	Pak choy		Chingen-sai	*Brassica rapa*	33.30 ± 0.45
39	Apple : akibae	peel	Ringo: Akibae	*Malus domestica*	33.26 ± 0.27
40	Apple : alps-otome	peel	Ringo: Alpus-otome	*Malus domestica*	32.74 ± 0.52
41	Apple : mutsu	peel	Ringo: Mutsu	*Malus domestica*	32.60 ± 0.49
42	Saltwort		Wakame-okahijiki	*Salsola komarovii*	32.24 ± 0.32
43	blueberry		blueberry	*Vaccinium corybosum*	31.56 ± 0.37
44	Chinese yam		Yamato-imo	*Dioscorea batatas*	31.41 ± 0.65
45	Azuki bean		Azuki	*Vigna angularis*	30.19 ± 0.39
46	Apple : orin	peel	Ringo: Ourin	*Malus domestica*	30.18 ± 0.44
47	Red cabbage		Aka-kyabetu	*Brassica oleracea*	29.95 ± 1.02
48	Apple : sekaiichi	peel	Ringo: Sekaiichi	*Malus domestica*	28.75 ± 0.63
49	Passion fruit		Passion fruit	*Passiflora edulis*	28.62 ± 1.14
50	Strawberries		Ichigo	*Fragaria x ananassa*	28.11 ± 0.82
51	Scallion		Wakegi	*Allium fistulosum*	27.57 ± 0.72
52	Citrus hassaku	peel	Hassaku	*Citrus haisaku*	27.17 ± 0.25
53	Zabon (pomelo)	peel	Zabon	*Citrus maxima*	26.72 ± 0.96
54	Peach		Momo	*Prunus persica*	26.49 ± 0.81
55	Wasabi leaves		Wasabi-na	*Brassica juncea*	26.44 ± 0.50
56	Water chestnut	nut	To-Bishi	*Trapa bicornis*	25.75 ± 1.30
57	Plum		Sumomo	*Prunus domestica*	25.30 ± 1.39
58	Red kidney beans		Kintoki-mame	*Phaseolus vulgaris*	25.24 ± 1.80
59	Mizuna (potherb mustard)		Mizu-na	*Brassica rapa*	24.97 ± 0.46
60	Chestnut	nut	Kuri	*Castanea crenata*	23.48 ± 0.58
61	Pecan nuts		Pecan	*Carya illinoinensis*	22.91 ± 0.68
62	Pomegranate	pulp	Zakuro	*Punica granatum*	22.83 ± 1.39
63	White mushroom		White mushroom	*Agaricus bisporus*	22.61 ± 2.03
64	Citrus buntan	peel	Buntan	*Citrus grandis*	21.11 ± 0.82
65	Yuzu	peel	Yuzu	*Citrus junos*	19.60 ± 0.84
66	Mangosteen	pulp	Mangosteen	*Garcinia mangostana*	19.16 ± 1.29
67	Pineapple		Pineapple	*Ananas comosus*	18.70 ± 1.65
68	King oyster		Eringi	*Pleurotus eryngii*	14.15 ± 1.33
69	Grapefruit (red)		Red grapefruit	*Citrus x paradisi*	13.69 ± 0.56
70	Spinach		Horenso	*Spinacia oleracea*	13.00 ± 0.59
71	Mango		Mango	*Mangifera indica*	12.16 ± 1.57
72	Horseradish		Seiyou-wasabi	*Armoracia rusticana*	11.28 ± 0.98
73	Soybean		Daizu	*Glycine max*	9.23 ± 1.67

The results are expressed as mean ± SD of 3 experiments. HSA, human serum albumin; AGEs, advanced glycation end products; SD, standard deviation.

Table 2. Inhibitory effect of plant compounds on type I collagen-derived fluorescent AGE formation

Ranking	English name	Part	Japanese name	Scientific name	Inhibition of fluorescent AGEs [%]
	Aminoguanigine 0.1 mg/ml				56.69 ± 0.58
1	Chinese quince		Karin	*Pseudocydonia sinensis*	92.71 ± 0.20
2	Apple : san-jyonagold	peel	Ringo: San-jyonagold	*Malus domestica*	91.65 ± 0.18
3	Apple : kogyoku	peel	Ringo: Kogyoku	*Malus domestica*	89.77 ± 0.29
4	Apple : toki	peel	Ringo: Toki	*Malus domestica*	89.48 ± 0.09
5	Chestnut	soft layer	Kuri	*Castanea crenata*	87.86 ± 0.12
6	Chrysanthemum (yellow)	petal	Shokuyo-kiku	*Chrysanthemum morifolium*	85.55 ± 0.23
7	Raspberry		Raspberry	*Rubus idaeus*	83.42 ± 0.29
8	Reddish black rice		Kuro-mai	*Oryza sativa*	82.45 ± 0.38
9	Lady's thumb		Tade	*Polygonum hydropiper*	81.40 ± 0.09
10	Chestnut	outer skin	Kuri	*Castanea crenata*	77.13 ± 0.80
11	Pomegranate	peel	Zakuro	*Punica granatum*	74.79 ± 0.28
12	Rosemary		Rosemary	*Rosmarinus officinalis*	70.79 ± 0.22
13	Nalta jute		Moroheiya	*Corchorus olitorius*	70.41 ± 0.24
14	Belvedere fruit		Tonburi	*Bassia scoparia*	69.32 ± 0.29
15	Water chestnut	outer skin	To-Bishi	*Trapa bicornis*	63.44 ± 0.54
16	Lemon		Lemon	*Citrus x limonium*	60.69 ± 0.95
17	Citrus sudachi	peel	Sudachi	*Citrus sudachi*	57.07 ± 0.83
18	Red-kernelled rice		Aka-mai	*Oryza sativa*	47.22 ± 0.82
19	Malabar spinach		Tsurumurasaki	*Basella alba*	25.96 ± 1.21
20	Citrus sudachi	pulp	Sudachi	*Citrus sudachi*	25.86 ± 0.72

The results are expressed as mean ± SD of 3 experiments. AGEs, advanced glycation end products; SD, standard deviation.

Table 3. Inhibitory effect of plant compounds on type II collagen-derived fluorescent AGEs formation

Ranking	English name	Part	Japanese name	Scientific name	Inhibition of fluorescent AGEs [%]
	Aminoguanigine 0.1 mg/ml				60.33 ± 1.06
1	Chinese quince		Karin	*Pseudocydonia sinensis*	92.47 ± 0.10
2	Apple : toki	peel	Ringo: Toki	*Malus domestica*	92.26 ± 0.07
3	Apple : san-jyonagold	peel	Ringo: San-jyonagold	*Malus domestica*	92.21 ± 0.10
4	Apple : kogyoku	peel	Ringo: Kogyoku	*Malus domestica*	91.07 ± 0.14
5	Chrysanthemum (yellow)	petal	Shokuyo-kiku	*Chrysanthemum morifolium*	88.99 ± 0.13
6	Chestnut	soft layer	Kuri	*Castanea crenata*	87.84 ± 0.12
7	Raspberry		Raspberry	*Rubus idaeus*	87.19 ± 0.23
8	Lady's thumb		Tade	*Polygonum hydropiper*	86.09 ± 0.06
9	Reddish black rice		Kuro-mai	*Oryza sativa*	82.21 ± 0.44
10	Pomegranate	peel	Zakuro	*Punica granatum*	80.18 ± 0.44
11	Citrus sudachi	pulp	Sudachi	*Citrus sudachi*	79.50 ± 0.24
12	Lemon		Lemon	*Citrus x limonium*	78.29 ± 0.54
13	Chestnut	outer skin	Kuri	*Castanea crenata*	76.20 ± 0.29
14	Rosemary		Rosemary	*Rosmarinus officinalis*	74.81 ± 0.27
15	Belvedere fruit		Tonburi	*Bassia scoparia*	72.83 ± 0.42
16	Water chestnut	outer skin	To-Bishi	*Trapa bicornis*	72.14 ± 0.62
17	Nalta jute		Moroheiya	*Corchorus olitorius*	71.98 ± 0.28
18	Citrus sudachi	peel	Sudachi	*Citrus sudachi*	63.97 ± 0.74
19	Red-kernelled rice		Aka-mai	*Oryza sativa*	32.03 ± 1.47
20	Malabar spinach		Tsurumurasaki	*Basella alba*	30.48 ± 1.02

The results are expressed as mean ± SD of 3 experiments. AGEs, advanced glycation end products; SD, standard deviation.

Effect of plant extract on formation of intermediate of AGEs in 3 different glycation models.

Through 3 types of proteins and sugar models, we demonstrated that 9 kinds of plant extracts were markedly effective against fluorescent AGE formation in HSA and collagens. Next, we further evaluated the efficacy of those 9 plant extracts against the formation of AGE intermediates. In the process of AGE formation, various kinds of intermediates are produced and accumulated in the body. Among a large number of AGE intermediates, we measured three different kinds of intermediates such as 3-DG, GO and MGO by HPLC-UV analyses. As shown in ***Fig. 1-A*** and ***1-B***, 3-DG and GO were significantly reduced by all 9 plant extracts across all 3 glycation models. In particular, GO was markedly abolished by plant extracts except for reddish black rice (***Fig. 1-B***). About MGO formation, plant extract was highly effective in the glc-HSA model, however, chrysanthemum (petal), raspberry and peel of toki apple did not affect MGO formation in fru-collagen I model (***Fig. 1-C***). Reddish black rice inhibited neither type I nor type II collagen-derived MGO formation, while it inhibited MGO formation in the glc-HSA model (***Fig. 1-C***). The overall results suggested that lady's thumb and the soft layer of chestnut were highly effective against the formation of AGE intermediates in all 3 glycation models.

Discussion

The average life span has become prolonged in the world, especially in Japan with the longest life expectancy country in 2016 at 83.7 years old [18]. However, a gap between total life span and healthy life years is around 9 years for males and 12 years for females [19]. Osteoarthritis (OA) and osteoporosis (OP) are known to be major painful health problems in the joints and bones of the elderly, impairing their activity of

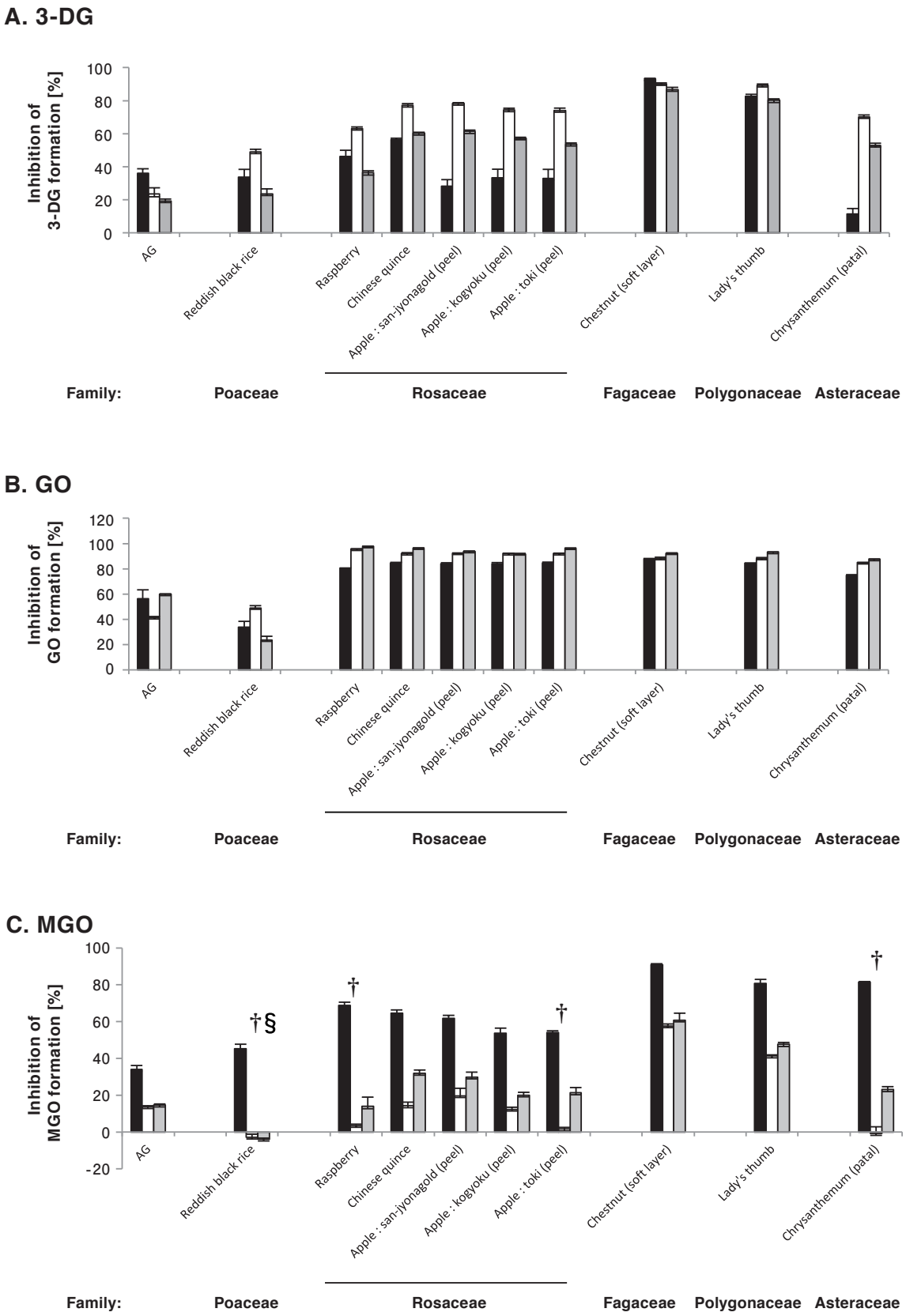

Fig. 1. **Effect of plant extracts on the intermediates of AGE formation in various glycation models.**

One mg/mL solid content of plant extracts were used to determine the inhibitory effect of plant extracts on the intermediates of AGE formation. HPLC-UV analyses were performed to detect (A) 3-DG (B) GO and (C) MGO. Aminoguanidine (0.1 mg/mL) was used as a positive control. Black bar; glc-HSA model, white bar; fru-collagen I model and grey bar; fru-collagen II model. All data were shown as the mean ± SD (n = 3) of the inhibition ratios against water. † no significance vs. water in fru-collagen I model, § no significance vs. water in fru-collagen II model and rest of those were $p < 0.01$ vs. water. AGE, advanced glycation end product; HPLC, high performance liquid chromatography; UV, ultraviolet; 3-DG, deoxyglucosone; GO, glyoxal; MGO, methylglyoxal; glc, glucose; HSA, human serum albumin; fru, fructose; SD, standard deviation.

daily life (ADL) and QOL. Based on the cohort study entitled research on osteoarthritis/osteoporosis against disability (ROAD) in 2005, Yoshimura *et al*. estimated that a total of 47 million people are affected by either OA or OP in Japan [20]. Thus, to protect people from the pain and disability coming from OA and OP, this is an important approach for the extension of a healthy life expectancy in an aging society.

The glycation of bone collagen is one of the contributing factors for the loss of bone stiffness and resilience. One of the cross-linking AGEs, pentosidine, is accumulated in the bone of OP patients [21,22]. Furthermore, in canine anterior cruciate ligament transection (ACLT) by which the experimental OA model is conducted, intraarticular injections of one of the reducing sugar, ribose accelerated OA with enhanced cartilage AGE levels [23]. Therefore, protecting bone collagens against glycation may contribute to the prevention of OP and OA.

We have evaluated over 500 kinds of food materials against fluorescent AGE formation in HSA [12-16]. In the present study, we initially choose 73 kinds of plants from these studies to evaluate their anti-glycative effect against collagens. HSA is one of the most abundant proteins in blood, it is frequently exposed to blood glucose consecutively. Vaculík *et al*. demonstrated that blood pentosidine levels correlate with bone pentosidine levels and it is higher in OA patients [24]. Also type 2 diabetes is considered as an additional risk factor for OA and bone fracture [25,26]. Thus, the aim of this study is to investigate plants which have an efficacy against glycation for not only bone collagens, but also HSA.

We evaluated the effect of 1 mg/mL plant extracts on fluorescent AGEs in HSA, type I collagen and type II collagen (***Table 1-3***). Chestnut, lady's thumb, pomegranate peel and chrysanthemum petal (yellow) strongly inhibited fluorescent AGE formation in all 3 glycation models. Chrysanthemum contains luteolin, which is the flavone already reported about its anti-glycative efficacy [27]. Polyphenols in pomegranate such as ellagitannin, punicalagin and urolithin are also known to have a potent anti-glycative effect [28,29]. On the other hand, Rosaceae family such as Chinese quince and apple peel (san-jyonagold, kogyoku and toki) showed a potent effect against fluorescent AGE formation in collagens, while weaker in HSA. Procyanidins are oligomeric flavonoids such as catechin and epi-cathechin in variety of plants including apple and Chinese quince. Procyanidin B2 is involved in AGE inhibition in soluble proteins from goat lens [30] and procyanidin oligomer inhibited the formation of pentosidine in collagen [31]. Although the reason why the *Rosaceae* family is more effective against collagen-glycation model is unclear, He *et al*. demonstrated that procyanidin stabilizes collagen structure and improve its thermal stability [32]. These findings indicate that procyanidine in the *Rosaceae* family may be partially involved in the anti-glycative effect due to the stabilization of collagen structure, but further study in necessary to clarify the exact mechanisms.

Intermediates of AGEs such as 3-DG, GO and MGO are highly reactive compounds and these intermediates contribute to form different AGEs through different pathways. We investigated that 9 kinds of plant extract inhibited fluorescent AGE formation in all three proteins (***Fig. 1***). Lady's thumb and soft layer of chestnut showed a notably potent efficacy against intermediate for mation. The soft layer of chestnut contains polyphenols as 71% of its carbohydrates and majority of polyphenols exist as tannin [33]. Lady's thumb belongs to the Polygonaceae family and, including carotenoid, lutein is known to be an antioxidant [34]. In the process of AGE formation, proteins and sugars first form a Schiff base, then rearrange Amadori products and the intermediates of AGEs [35]. Once their structures are cleaved by oxidation, various kinds of AGEs are formed. Therefore, antioxidants like polyphenols and lutein may contribute to inhibit the glycation pathway. However, in this study we used water to extract compounds from plants, and lutein has a low solubility in water. Our data indicate that lutein may contribute less against glycation in this condition, but further studies will be needed to determine the essential compounds in lady's thumb.

In conclusion, our study shows that lady's thumb and the soft layer of chestnut are potentially effective plants against glycation not only in serum albumin, but also collagen.

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Conflict of Interest Statement

The authors claim no conflict of interest in this study.

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