Glycative Stress Research

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Original article

Cleaving effect of pomegranate (*Punica granatum*) extract on crosslink derived from advanced glycation endproducts

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Abstract

***Objectives*:** The accumulation of advanced glycation endproducts (AGEs) is a risk factor of glycation stress for age-related deterioration and diseases. AGE degeneration and excretion is a method reducing glycation stress. The purpose of the present study is to elucidate whether pomegranate (*Punica granatum*) can be a functional food which ruduces glycation stress. The AGE-derived and collagen-derived crosslink cleaving reactions were evaluated in pomegranate extracts and ellagitannins isolated and purified from pomegranates.

***Methods*:** For evaluation of AGE-derived and collagen-derived crosslink cleaving activity, 22 pomegranate-related samples were used and compared with *N*-phenacylthiazolium bromide (PTB) as a positive control and 6 hydroxybenzene compounds. To evaluate the AGE crosslink activity, by HPLC analysis we measured the amount of benzoic acid which was formed as a result of C-C binding breaking of α-diketone structure in 1-phenyl-1,2-propanegione (PPD), a model compound for AGE degeneration. For evaluation of collagen crosslink cleaving activity, using collagen-coated 96-well microplates, AGE-formed bovine serum albumin (AGE-BSA) was added to the wells and reacted with collagen, thus forming collagen crosslinks, followed by measuring residual AGE-BSA amount by ELISA. AGE-derived and collagen-derived crosslink cleaving ratio was calculated as a relative ratio with the ratio of PTB assumed to be 100.

***Results*:** AGE crosslink cleaving activity of 6 pomegranate extracts was higher than that of 0.4 mmol/L PTB. The activity ratio was highest in gallic acid (26.02%) and lowest in ellagic acid (3.24%) among the 16 pomegranate-derived compounds. Regarding the hydroxybenzene compounds, the activity ratio in trihydroxybenzene was higher in hydroxyquinol (51.62%) and pyrogallol (42.13%) than in phloroglucinol (6.06%), and the activity ratio in dihydroxybenzene was higher in hydroquinone (15.73%) and pyrocatechol (14.70%) than in resorcinol (6.88%).

Collagen crosslink cleaving activity was noted in 4 pomegranate extracts from pomegranate juice powder (78.50%), condensed and dried polyphenols (I) (24.99%), ellagitannins (74.75%), and leaf (62.44%). Also, 12 of 16 pomegranate-derived compounds showed collagen crosslink cleaving activity which was significantly correlated with AGE crosslink cleaving activity. The activity was not noted in delphinidin chloride, delphinidin 3,5-diglucoside chloride, gallic acid, and cyanidin 3-glucoside.

All of the samples of pomegranate extracts and related compounds showed AGE crosslink cleaving activity, and some of them showed collagen crosslink cleaving activity. A significantly positive correlation was noted between two actions ($r = 0.635$, $p = 0.034$). These findings indicate that the trihydroxybenzene structure of ellagitannins may be involved in the AGE crosslink cleaving activity of pomegranate.

***Conclusions*:** Pomegranate extracts and related compounds are confirmed to show AGE crosslink cleaving activity in addition to anti-oxidation, α-glucosidase inhibition and the prevention of AGE formation as previously reported, indicating that they can reduce glycation stress in various ways.

***KEY WORDS*:** advanced glycation endproducts (AGEs), pomegranate (*Punica granatum*), ellagitannins, AGE-derived crosslink cleaving reaction, collagen crosslink cleaving reaction

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Introduction

High glycation stress conditions cause formation and accumulation of advanced glycation endproducts (AGEs) in the body, and thus play a role as a risk factor in the pathogenesis of diseases such as diabetic complications, atheroscrerosis, osteoporosis, infertility, or Alzheimer's disease, and age-related conditions like elasticity reduction and loss of clearness in the skin [1]. AGE formation causes a yellow-brownish change of tissue and physical deterioration, especially in the collagen tissue of the skin, bone and cartilage, thus it is a topic in the field of aesthetics, cosmetics and health promotion. The methods for reducing glycation stress include prevention of postprandial hyperglucosemia, inhibition of AGE formation, and promotion of AGE regeneration and excretion [2].

N-phenacylthiazolium bromide (PTB) is known as a cleaving material against AGE-derived crosslink. PTB recognizes crosslink proteins and α-diketone structure in AGEs and degenerates C-C bonds. PTB is reported to contribute to the prevention of AGE deposit and the treatment of diabetic complications in the vascular vessels [3]. Extracts of mugwort (*Artemisia indica*) and rooibos tea leaves (*Aspalathus linearis*) are known to possess a same action as PTB [4]. The extracts from these plants are confirmed to show anti-glycation effect and AGEs formation prevention in the *in vitro* glucose/albumin reaction [5].

Pomegranates (*Punica granatum*) are rich in ellagitannins. Ellagic acid, a degeneration product of ellagitannins, is reported to have anti-oxidative effects [6] and tyrosinase inhibition activity [7]. Recently, pomegranate extract and ellagitannins have been confirmed to prevent AGE formation in the *in vitro* glucose/albumin reaction [8,9].

The present study was conducted to elucidate the effect of pomegranate extract and ellagitannin, isolated and purified from pomegranate, on the AGE-derived crosslink cleaving reaction and the collagen crosslink breaking reaction, in order to evaluate the potential action of pomegranate as a functional food which reduces glycation stress.

Methods

Materials

In total, we used 22 samples of pomegranate extract and isolates and purified materials from the extract in the present study as follows; 6 pomegranate extracts were pomegranate juice residue extract, pomegranate juice powder, condensed and dried product of pomegranate polyphenols (I and II), pomegranate ellagitannins, and pomegranate leaf extract. The 16 purified components were gallic acid, punicalagin, eucalbanin B, pomegraniin A, punicalin, delphinidin 3, 5-diglucoside chloride, delphinidin chloride, eucarpanin T_1, oenothein B, penta-*O*-galloylglucouse, granatin B, granatin A, cyanidin chloride, cyanidin 3-glucoside chloride, pomegralignan, and ellagic acid (***Fig.1***).

These pomegranate samples were provided from Morishita Jintan Co., Ltd. (Chuo-ku, Osaka, Japan), except for pomegranate ellagitannins which were provided from Professor Hideyuki Ito, Okayama Prefectural University (Soja, Okayama, Japan).

For the comparison of AGE-derived crosslink cleaving actions, 6 hydroxybenzene compounds were used as follows; hydroxyquinol, pyrogallol, phloroglucinol, hydroquinone, pyrocatechol, and resorcinol. PTB was used as a positive control for AGE-derived crosslink cleaving actions. Samples were dissolved in distilled water, 100% or 50% ethanol. PTB was used as a positive control for evaluation of AGE-derived crosslink cleaving activity and collagen crosslink cleaving activity.

Measurement of AGE-derived crosslink cleaving activity

The AGE-derived crosslink cleaving activity was evaluated by the modified method of Vasan et al. [3]. Briefly, 1-phenyl-1,2-propane dione (PPD), dissolved in 50% acetonitrile, was used as a reactive substrate in the AGE crosslink model. For the measurement of AGE-derived crosslink cleaving activity, the samples (500 μL) were mixed with 10 mmol/L PPD 100 μL and 0.2 mol/L phosphate buffered saline (PBS) 400 μL, and then incubated at 37°C for 8 hours. The reaction was stopped by adding 2 mol/L hydrochloric acid (HC1) 200 μL, followed by centrifugation at 10,000 rpm (9,170 g) for 2 minutes. The benzoic acid amount in the supernatant was measured by high performance liquid chromatography (HPLC).

HPLC (LC-10A; Shimadzu, Nakagyo-ku, Kyoto, Japan) equipped with Cadenza CD-C18 75 x 4.6 mmID (Imtakt, Shimogyo-ku, Kyoto, Japan) was used. Analysis conditions were as follows: eluate, 0.2% acetic acid/acetonitrile (70/30 : volum/volum) containing 2 mmol/L ethylenediamine-*N, N, N', N'*-tetraacetic acid - disodium salt (EDTA-2Na) – dehydrate; flow rate, 1.0 mL/min; column temperature, 40°C; detection wave length, UV at 270 nm; injection volume, 50 μL.

Calculation of AGE crosslink cleaving activity ratio

When 1 mol of PPD breaks down, 1 mol of benzoic acid is formed [3]. The AGE crosslink cleaving ratio was defined as a value when the benzoic acid amount in the reaction solution was divided by the PPD amount added to the reaction solution which was measured by HPLC. The benzoic acid amount in each sample, measured similarly, was pre-excluded from the value of benzoic acid amount in the reaction solution. The AGE crosslink cleaving activity was expressed by calculation as a relative ratio with the activity by 0.4 mmol/L PTB assumed to be 100.

Preparation of AGE-BSA

AGEs combined with bovine serum albumin (AGE-BSA) were prepared as a crosslink model between collagen and proteins. Briefly, 40 mg/mL BSA 4 mL were mixed with 0.1 mol/L PBS (pH 7.4) 10 mL, 2 mol/L glucose 2 mL and distilled water 4 mL, and then incubated at 60°C for 40 hours. After the reaction, AGE-BSA solutions were purified using a disposable column (PD-10; GE Healthcare Japan, Hino, Tokyo, Japan), followed by adjusting of the protein concentrations to attain 10 μg/mL.

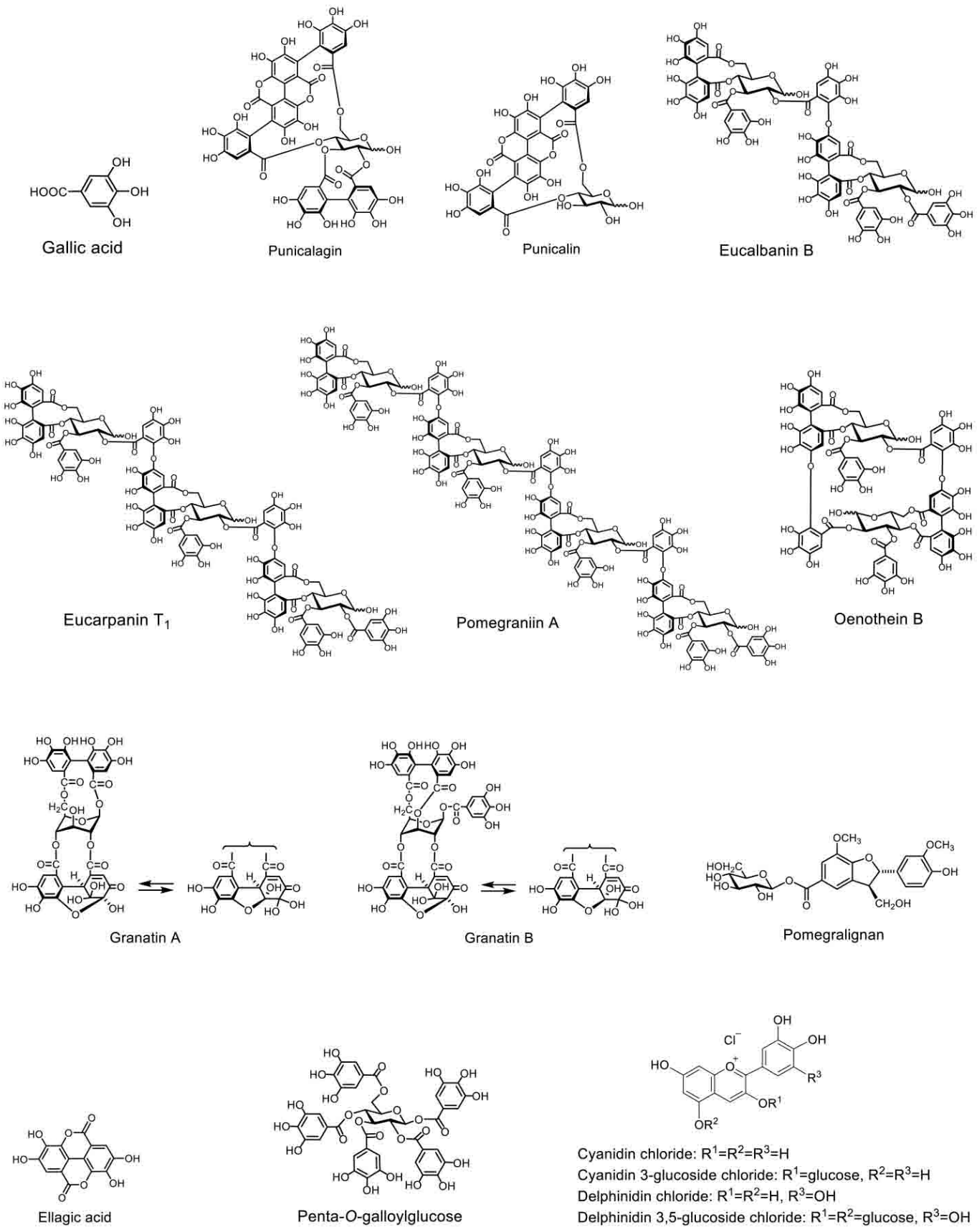


Fig 1. **Chemical structure of compounds and purified materials in pomegranate.**

Measurement of collagen crosslink cleaving activity

Collagen crosslink cleaving activity was evaluated by the modified method of Vasan et al.[3]. Prepared AGE-BSA was added to each well of a 96-well microplate coated with collagen (BioCoat Collagen I 96-well plate; Corning International, Minato-ku, Tokyo, Japan) and incubated at 37°C for 4 hours, followed by 3 minutes of shaking after adding 0.05% polyethylene (20) sorbitan monolaurate (Tween20)/ phosphate buffered saline (PBS) (-). Wells were washed 3 times by filling and wasting PBS. Then, each sample 100 μL was added to a well and incubated at 37°C for 18 hours. After washing wells, the primary antibody (anti-albumin bovine serum rabbit – polyclonal, 1/20,000 dilution) (Anti-Albumin Bovine Serum Rabbit-Poly; Rockland, Limerick, PA, USA) 100 μg/well was added and reacted at room temperature for 30 minutes. After washing the wells, the secondary antibody (Goat anti-rabbit IgG horseradish peroxidase conjugate; Bio-Rad, Shinagawa-ku, Tokyo, Japan) 100 μg/well was added and reacted at room temperature for 30 minutes.

After washing each well, 100 μg/well of TMB (3, 3', 5, 5'-tetramethylbenzidine) One Component HRP (horseradish peroxidase) Microwell Substrate (SurModics, Eden Prairie, MN, USA) and 50 μL/well of 1.5 mg/mL polyethylene glycol 6000 (PEG6000) were added and reacted for 20 minutes in a dark condition. After the reaction, 1 N HCl 100 μL/well, stop solution, was added to each well, followed by the measurement of absorbance in each well by using a microplate reader (SpectraMax Paradigm Multi-Mode Microplate Reader; Molecular Devise, Sunnyvale, CA, USA) at dual wavelengths of 450 nm (main) / 630 nm (sub).

The AGE-BSA amount in each well was calculated by using the AGE-BSA standard curve in which reaction was conducted without adding samples. The AGE-BSA collagen crosslink cleaving ratio (%) was calculated by using the formula below. The relative ratio was also calculated with the measured value by 10 mmol/L PTB assumed to be 100.

Cleaving ratio (%) = { (added AGE-BSA amount – remaining AGE-BSA amount) / added AGE-BSA amount} × 100

Statistical analyses

Results are expressed as mean ± standard deviation (SD) after 3 times repeated measurement (n = 3). Pearson's correlation coefficient test was used to compare relationships between AGE-derived crosslink cleaving action and collagen crosslink cleaving action.

The significance level was set at less than 5%. All analyses were performed using SPSS II software (IBM Japan, Chuo-ku, Tokyo, Japan).

Results

AGE crosslink cleaving activity

When the AGE crosslink cleaving activity in 6 kinds of pomegranate extracts was compared, the activity ratio of pomegranate ellagitannins (31.39%) and pomegranate leaf extract (31.22%) was higher than in the 4 kinds of pomegranate fruit extracts (***Table 1***).

These values of AGE crosslink cleaving activity were higher than that of 0.4 mmol/L PTB. The added concentration of pomegranate juice extract was even 10 times higher, however, it showed less activity than those of pomegranate ellagitannins and pomegranate leaf extract (***Table 2***).

Table 1. AGE crosslink cleaving activity of pomegranate extracts.

Sample	Concentration	AGE crosslink cleaving ratio (%)
Pomegranate juice residue extract [1]	10 mg/mL	29.92 ± 1.02
Condensed and dried pomegranate polyphenols (II) [2]	10 mg/mL	27.88 ± 3.29
Condensed and dried pomegranate polyphenols (I) [2]	10 mg/mL	25.05 ± 1.04
Pomegranate juice powder [3]	10 mg/mL	11.77 ± 0.53
Pomegranate ellagitannins	1 mg/mL	31.39 ± 0.02
Pomegranate leaf extract	1 mg/mL	31.22 ± 0.40

1) Polyphenols in hot water extract from squeezed pomegranate residue are condensed and dried.
2) Pomegranate juice polyphenols are filtrated and condensed using resin columns, and the dried.
3) Pomegranate juice is dried and powdered.
AGE, advanced glycation endproduct. Results are expressed as means ± standard deviation (n = 3).

Table 2. AGE crosslink cleaving activity of pomegranate derived compounds.

Sample	AGE crosslink cleaving ratio (%)	Relative ratio [1)]
Gallic acid	26.02 ± 0.07	409
Punicalagin	15.32 ± 2.57	241
Eucalbanin B	13.93 ± 0.31	219
Pomegraniin A	13.51 ± 0.08	212
Punicalin	12.68 ± 2.02	199
Delphinidin 3,5-diglucoside chloride	12.66 ± 0.32	199
Delphinidin chloride	12.38 ± 0.77	194
Eucarpanin T_1	12.32 ± 0.24	194
Oenothein B	11.35 ± 0.06	178
Penta-*O*-galloylglucose	9.49 ± 0.04	149
Granatin B	8.07 ± 0.18	127
Granatin A	7.66 ± 0.38	120
Cyanidin chloride	7.36 ± 4.85	116
Cyanidin 3-glucoside chloride	3.85 ± 0.04	60
Pomegralignan	3.48 ± 0.20	55
Ellagic acid	3.24 ± 0.10	51
PTB (positive control)	6.37 ± 2.71	100

1) Relative ratio of AGE crosslink cleaving activity when the ratio of 0.4 mmol/L PTB is assumed to be 100.
Each sample concentration: 0.4 mmol/L. AGE, advanced glycation endproduct; PTB, *N*-phenacylthiazolium bromide. Results are expressed as means ± standard deviation (n = 3).

Among the 16 types of pomegranate-derived compounds, AGE crosslink cleaving activity was highest in gallic acid (26.02%), and lowest in ellagic acid (3.24%), the difference was approximately 8-fold (***Table 3***).

AGE crosslink cleaving activity was compared among hydroxybenzene compounds. Regarding trihydroxybenzene, the activity was higher in hydroxyquinol (51.62%) and pyrogallol (42.13%) than in phloroglucinol (6.06%) (***Fig. 2***). For dihydroxybenzene, the activity was higher in hydroquinone (15.73%) and pyrocatechol (14.70%) than in resorcinol (6.88%).

The AGE crosslink cleaving activity of two compounds, hydroxyquinol and pyrogallol, were higher than that of PTB.

Collagen crosslink cleaving activity

Results of collagen crosslink cleaving activity in 6 kinds of pomegranate extracts were as follows. Activity was high in 4 extracts; pomegranate juice powder (78.50%), condensed and dried pomegranate polyphenol (I) (24.99%), pomegranate ellagitannins (74.75%), and pomegranate leaf extract (62.44%) (***Table 4***). Pomegranate ellagitannins and pomegranate leaf extract showed high activity although their sample concentrations were lower than that of the rest.

Results of the collagen crosslink cleaving activity in 13 kinds of pomegranate derived compounds showed that the activity was generally noted except for in 4 compounds, delphinidin chloride, delphinidin 3,5-diglucoside chloride, gallic acid, and cyanidin 3-glucoside. The activity ratio was highest in eucalbanin B, that of which was 3.8 times higher than that of punicalagin with the lowest activity.

Correlation between AGE crosslink cleaving activity and collagen crosslink cleaving activity

Two kinds of pomegranate extracts and 9 kinds of pomegranate-derived compounds showed both AGE crosslink cleaving activity and collagen crosslink cleaving activity. There was a significant positive correlation ($r = 0.635$, $p = 0.034$) among these samples (***Fig. 3***).

Discussion

AGE crosslink cleaving activity by pomegranate

From the results of the present experiment, AGE crosslink cleaving activity was higher in pomegranate extract, in which ellagitannins are rich, than in all four extracts from pomegranate juice. In the 12 compounds with higher AGE crosslink cleaving activity, the trihydroxybenzene structure was recognized as a common structure, but not noted in the remaining 4 compounds. These findings indicate that the

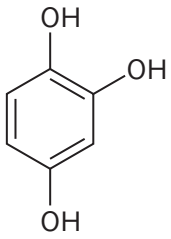
Table 3. Collagen crosslink cleaving activity of pomegranate extracts.

Sample	Concentration	Cleaving ratio (%)	Relative ratio [1]
Pomegranate juice powder [4]	5 mg/mL	78.50 ± 5.55	130
Condensed and dried pomegranate polyphenols (I) [2]	5 mg/mL	24.99 ± 7.90	41
Condensed and dried pomegranate polyphenols (II) [2]	5 mg/mL	- 14.84 ± 25.18	NE
Pomegranate juice residue extract [3]	5 mg/mL	- 78.44 ± 52.88	NE
Pomegranate ellagitannins	1 mg/mL	74.75 ± 0.21	124
Pomegranate leaf extract	1 mg/mL	62.44 ± 1.52	103
PTB (positive control)	10 mmol/L	60.4 ± 11.62	100

1) Relative ratio of collagen crosslink cleaving activity when the ratio of 10 mmol/L PTB is assumed to be 100.
2) Pomegranate juice polyphenols are filtrated and condensed using a resin column, and then dried.
3) Polephenols in hot water extract from squeezed pomegranate residue are condensed and dried.
4) Pomegranate juice is dried and powdered.
PTB, *N*-phenacylthiazolium bromide; NE, no effect. Results are expressed as means ± standard deviation (n = 3).

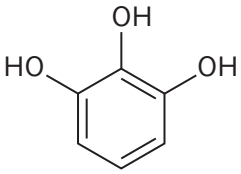
trihydroxybenzene

hydroxyquinol
(1,2,4-trihydroxybenzene)



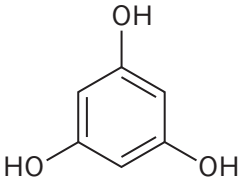
51.62 ± 0.02 %

pyrogallol
(1,2,3-trihydroxybenzene)



42.13 ± 0.25 %

phloroglucinol
(1,3,5-trihydroxybenzene)



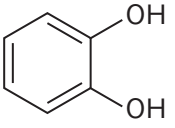
6.06 ± 0.06 %

dihydroxybenzene

hydroquinone
(1,4-dihydroxybenzene)

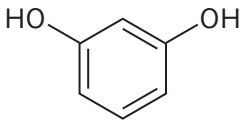
15.73 ± 0.18 %

pyrocatechol
(1,2-dihydroxybenzene)



14.7 ± 0.02 %

resorcinol
(1,3-dihydroxybenzene)



6.88 ± 0.03 %

Fig 2. **Chemical structure and AGE crosslink cleaving activity of hydroxybenzene compounds.**
Concentration of each sample; 10 mg/mL. Results are expressed as means ± standard deviation (n = 3).

Table 4. Collagen crosslink cleaving activity of pomegranate-derived compounds.

Sample	Cleaving ratio (%)
Eucalbanin B	78.49 ± 0.25
Cyanidin chloride	45.18 ± 11.43
Pomegralignan	45.09 ± 3.98
Punicalin	30.74 ± 7.39
Granatin B	29.23 ± 12.00
Penta-*O*-galloylglucose	26.71 ± 5.81
Granatin A	25.23 ± 4.73
Ellagic acid	21.06 ± 13.18
Punicalagin	20.67 ± 5.63
Delphinidin chloride	-3.18 ± 4.93
Delphinidin 3,5-diglucoside chloride	-8.76 ± 18.39
Gallic acid	-59.06 ± 64.99
Cyanidin 3-glucoside chloride	-175.54 ± 52.18

Concentration of each sample: 0.4 mmol/L. Results are expressed as means ± standard deviation (n = 3).

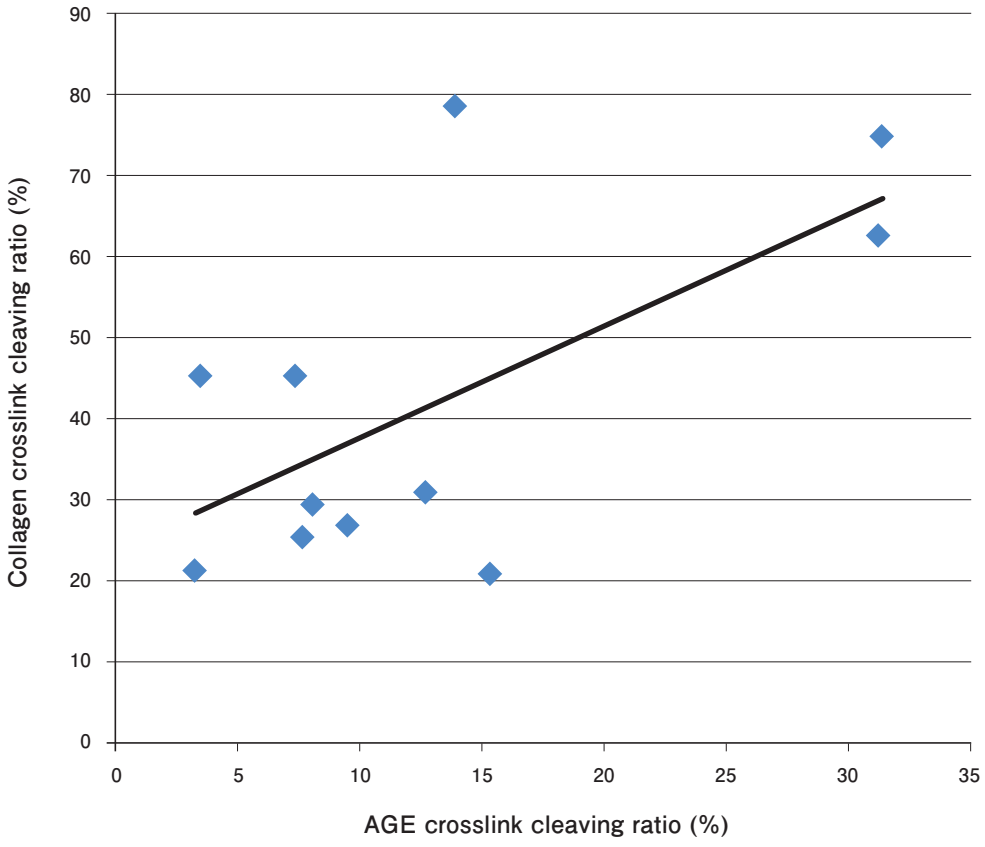


Fig 3. **Relationship between AGE crosslink cleaving activity and collagen crosslink cleaving activity**
Regression formula: y = 1.3824x + 23.704, r = 0.635, p = 0.036, n = 11, Pearson's regression analysis.

trihydroxybenzene structure in ellagitannins may be involved in the mechanism of AGE crosslink cleaving activity.

PTB, which was used as a positive control, recognizes the dicarbonyl structure in the Amadori-protein-ene-dion-derived protein crosslinks which are formed by glycation stress and break the protein-protein crosslink [3]. Regarding the crosslink cleaving activity in natural products, there is one report of terpinen-4-ol, one of monoterpene alcohols, contained in yuzu (*Citrus junos*), a species of Asian citrus fruits (Rutaceae) [10]. The mechanism of protein crosslink cleaving activity by terpinen-4-ol has been speculated as follows; First step is nucleophilic substitution caused by hydroperoxide, then carboxylate ester is formed by a similar reaction to Baeyer-Villiger oxidation, finally reaching the hydrolysis of carboxylate esters.

In the present study comparing the AGE crosslink cleaving activity of hydroxybenzene compounds, the activity was higher in trihydroxybenzene than in dihydroxybenzene. Furthermore, the activity was higher in the compounds with larger deviation in substitute positions. For example, the activity was high in compounds with a hydroquinone structure in which hydroxyl groups are located at 1, 2, 4 moiety, in a benzene structure or with a pyrocatechol structure in which hydroxy groups are located at 1, 2, 3 moiety. These findings indicate the involvement of nucleophilicity in the trihydroxybenzene structure of ellagitannins with AGE crosslink cleaving activity caused by pomegranate.

Collagen crosslink cleaving activity by pomegranate

Vassan et al. used the collagen crosslink cleaving activity by PTB as an *in vitro* model of AGE crosslink cleaving activity [3]. In our experiment, all 6 samples of pomegranate extracts and 13 pomegranate-derived compounds showed AGE crosslink cleaving activity. However, 4 compounds, delphinidin chloride, delphinidin 3,5-diglucoside chloride, gallic acid and cyanidin 3-glucoside chloride, did not show the collagen crosslink cleaving effect. We cannot find any relationship between collagen crosslink cleaving activity and the features in material structures.

On the other hand, both the AGE crosslink cleaving activity and collagen crosslink cleaving activity were noted in 2 pomegranate extracts and 9 pomegranate-derived compounds with significant correlation between two activity markers. These findings support the possibility that the AGE-BSA collagen cleaving reaction can be an *in vitro* model for evaluating AGE crosslink cleaving activity in other samples.

Potential effect of glycation stress reduction by pomegranate-induced AGE crosslink cleaving

In the present study, PTB, namely called "AGE breaker", was used as a positive control for evaluating AGE crosslink cleaving activity. PTB was reported to inhibit AGE crosslink formation in collagen in diabetic rats to which 10 mg/kg PTB was orally administered for 4 weeks [3], and showed the AGE degeneration effect in blood vessels [11]. Furthermore, 3-phenacyl-4,5-dimethylthiazorium chloride (ALT-711), a more water-soluble compound than PTB, was confirmed to prevent atherosclerosis and to enhance AGE degeneration in blood vessels when administered to diabetic rats [12]. In the clinical trial in which ALT-711, 210 mg/day or 420 mg/day for 8 weeks, was orally administrated to men, improvement effects were noted in vascular stiffening and uncontrolled systolic blood pressure [13,14]. On the contrary, another report showed that PTB had no effect on AGE crosslink cleaving against skin and tail tendon collagen in diabetic rats [15].

In the open clinical trial in which pomegranate extract (100 mg/day) was administerd to healthy subjects for 12 weeks, it was noted that a reduction of HbA1c at 8 and 12 weeks, yielded glycated albumin, and 3-deoxyglucosone, an intermediate in the glycation reaction, at 8 week compared with pre-values [16].

Pomegranate extracts and ellagitannins are confirmed to have a preventive effect of AGE formation [8,9], inhibitory effect of α-amylase and α-glucosidase activity [17], and anti-oxidant action [6] in addition to the AGE crosslink cleaving action in this study. Thus pomegranate can be expected as a new functional food which reduces glycative stress through the various mechanisms.

Conclusions

The present study showed the AGE crosslink cleaving activity of pomegranate extracts and pomegranate-derived compounds. Trihydroxybenzene structure in ellagitannins may be involved with its mechanism. Some of these compounds also have collagen crosslink cleaving activity which was correlated with AGE crosslink cleaving activity.

Coupled with the finding that pomegranate extracts have AGE crosslink cleaving activity in addition to the previously reported activities of anti-oxidation, α-glucosidase inhibition and prevention of AGE formation, pomegranate can be expected as a functional food which can reduce glycation stress in various mechanical ways.

Acknowledgements

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Conflicts of interest statement

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