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# Original article Polyphenol content of various vegetables: Relationship to antiglycation activity

Yoshika Ishioka, Masayuki Yagi, Mari Ogura, Yoshikazu Yonei

Anti-Aging Medical Research Center and Glycation Stress Research Center, Graduate School of Life and Medical Sciences, Doshisha University, Kyoto, Japan

## Abstract

**Purpose:** Using various commercial vegetables in dried, powdered form, we investigated polyphenol content comparatively and in relation to antiglycation activity as reported previously.

*Method*: Using the same 187 commercial vegetables studied in a previous report, we produced dried, powdered samples and measured their polyphenol content by the Folin-Ciocalteu (FC) method. The results were calculated as catechin equivalents (mg catechin Eq) using (+)-catechin as a standard substance. The relationship of the results to inhibition of fluorescent advanced glycation end product (AGE) formation (as reported previously) was then analyzed in an *in vitro* glucose/human serum albumin (HSA) glycation model.

**Results:** In decreasing order, polyphenol content in the 187 vegetable samples (units: mg catechin Eq/mg solid content) was highest in the following samples: Chestnut, soft layer (*Castanea crenata*) 0.509; chestnut, outer skin (*Castanea crenata*) 0.444; water chestnut, seed coat (*Trapa japonica*) 0.274; lemon balm (*Melissa officinalis*) 0.125; spearmint (*Mentha spicata*) 0.124; rosemary (*Rosmarinus officinalis*) 0.115; bay leaves (*Laurus nobilis*) 0.096; lady's thumb (*Polygonum hydropiper*) 0.890; thyme (*Thymus*) 0.080; and mugwort, powder (*Artemisia indica* var. *maximowiczii*) 0.077. By vegetable type, polyphenol content (units: mg catechin Eq/mg solid content) was as follows: Nuts (0.105), herbs (0.063), leaf vegetables (0.032), stem vegetables (0.025), fruit vegetables (0.025), beans (0.024), grains (0.019), mushrooms (0.018), and root vegetables (0.017). By plant taxonomic family, polyphenol content was highest in samples of Fagaceae, Lamiaceae, Polygonaceae, Asteraceae, and Convolvulaceae in that order. No significant correlations were observed between polyphenol content and antiglycation activity (50% inhibitory concentration [IC50] values) in the vegetable samples.

*Conclusion:* Though various vegetables have substantial polyphenol content, various polyphenol-based or non-polyphenol-based components may contribute to antiglycation effect in different vegetable species.

KEY WORDS: glycation stress, advanced glycation end products (AGEs), vegetables, polyphenols

## Introduction

Glucose and other reducing sugars are an essential nutrient for sustenance of life. However, fructose, glucose, and other sugars bind to proteins non-enzymatically *in vivo* and alter the structure and function of proteins. This phenomenon is known as a glycation reaction and leads to formation and accumulation of advanced glycation end products (AGEs). In turn, binding of AGEs to receptors (receptors for AGEs, "RAGE") causes degradation of functional proteins and damage to tissues. Accumulation of AGEs also contributes to the onset and progression of diabetic complications, osteoporosis, and lifestyle diseases such as arteriosclerosis. *In vivo* glycation represents a risk factor for

Contact Address: Professor Yoshikazu Yonei, MD, PhD Anti-Aging Medical Research Center, Graduate School of Life and Medical Sciences, Doshisha University 1-3 Tataramiyakodani, Kyotanabe-shi, Kyoto 610-0394 Japan Phone/Fax: +81-77-465-6394 E-mail: yyonei@mail.doshisha.ac.jp Co-authors: Ishioka Y, dmn2005@mail4.doshisha.ac.jp; Yagi M, yagi@yonei-labo.com; Ogura M, mogura@dwc.doshisha.ac.jp accelerated aging and is known as glycation stress, a topic of recent interest <sup>1)</sup>. Mitigation of glycation stress to inhibit accumulation of AGEs is believed to play a role in prevention of lifestyle and other such diseases.

Familiar vegetables are rich in components such as vitamins and polyphenols<sup>2)</sup>. "Polyphenol" is a general term for substances found in molecules which have large multiples of phenolic hydroxy groups. More than 5000 types exist, such as catechin, contained in tea; rutin, contained in soba; and tannin, an astringent component of tea<sup>3)</sup>. Polyphenols are a component of bitter flavors and, for example, pigments which are biosynthesized to protect the body from active oxygen, which proliferates under ultraviolet light when plants are

exposed to light and grow. The antioxidation effect <sup>4-7</sup> and antiglycation effect <sup>8-10</sup> of polyphenols have attracted recent attention, and polyphenols have been shown to be an effective factor <sup>11-17</sup> in prevention of lifestyle diseases. Previous research has also been pursued among plants to determine whether vegetables which may be consumed in the form of familiar foods have previously unknown functional characteristics. Using an *in vitro*, glucose/human serum albumin (HSA) model <sup>2</sup>) to measure inhibitory effect on glycation reactions, such research has shown that many vegetables contain components with antiglycation activity <sup>18</sup>). In our research, we measured the polyphenol content of such vegetables to investigate its relationship to antiglycation activity.

# Method

## Sample preparation

The samples used were the same 187 varieties of commercial vegetables as in a previous report <sup>18</sup>. As reported previously <sup>18</sup>, dried powders were prepared by drying vegetables for 20 hours, slicing into 5 mm thicknesses, pulverizing, and sieving (approximately 120-mesh) the material.

#### Measurement of polyphenols

The polyphenol content in vegetable extract solutions was measured using the Folin-Ciocalteu (FC) method in conjunction with the "Commercial Product Test Results for Polyphenol-Containing Foods" produced by the National Consumer Affairs Center of Japan (NCAC)<sup>19,20)</sup>. Specifically, 100  $\mu$ L of various dried vegetable powder solutions was added to 50 µL of a 2-fold dilution of FC reagent (Wako Pure Chemical Industries; Chuo-ku, Osaka) and 500 µL of 0.4 M aqueous Na<sub>2</sub>CO<sub>3</sub> to make up a total volume of 650  $\mu$ L, the material was left to stand at ambient temperature for 30 minutes, and absorbency at 660 nm was then measured. A (+)-catechin solution (Wako Pure Chemical Industries) was used as a standard. Multiple (+)-catechin solutions were prepared in 11 steps as 1.0, 0.5, 0.25, 0.1, 0.05, 0.025, 0.0125, 0.01, 0.00625, 0.001, and 0 mg/mL concentrations;  $100 \,\mu$ L of each concentration of (+)-catechin solution was added to 50  $\mu$ L of the 2-fold diluted FC reagent and 500  $\mu$ L of 0.4 M aqueous Na<sub>2</sub>CO<sub>3</sub> to make up a total volume of 650  $\mu$ L, the material was left to stand at ambient temperature for 30 minutes, and absorbency at 660 nm was then measured. Using the (+)-catechin calibration curve prepared, total polyphenol content per solid unit of each vegetable extract solution was calculated as a catechin equivalent (mg catechin Eq/mg solid content). Measurement was repeated three times for each sample.

# **Results**

*Table 1* presents the results for measurement of polyphenol content (catechin equivalents) for the 187 vegetable samples. In decreasing order, polyphenol content was highest in chestnut, soft layer (*Castanea crenata*) 0.509; chestnut, outer skin (*Castanea crenata*) 0.444; water chestnut, seed coat (*Trapa japonica*) 0.274; lemon balm (*Melissa officinalis*) 0.125; spearmint (*Mentha spicata*) 0.124; rosemary (*Rosmarinus officinalis*) 0.115; bay leaves (*Laurus nobilis*) 0.096; lady's thumb (*Polygonum hydropiper*) 0.890; thyme (*Thymus*) 0.080; and mugwort, powder (*Artemisia indica* var. *maximowiczii*) 0.077 (*Fig. 1*).

Comparison of polyphenol content by vegetable category showed that polyphenol content (units: mg catechin Eq/mg solid content) was highest in nuts (0.105), herbs (0.063  $\pm$  0.038), leaf vegetables (0.032  $\pm$  0.017), stem vegetables (0.025  $\pm$  0.018), fruit vegetables (0.025  $\pm$  0.013), beans (0.024  $\pm$  0.006), grains (0.019  $\pm$  0.009), mushrooms (0.018  $\pm$  0.004), and root vegetables 0.017  $\pm$  0.011 in that order (*Fig. 2*). Polyphenol content was significantly higher in nuts than in all categories other than herbs (p < 0.05).

*Fig.3* presents polyphenol content (units: mg catechin Eq/ mg solid content) by taxonomic family for vegetable samples classified by plant taxonomy. Polyphenol content was highest in species of the Fagaceae ( $0.321 \pm 0.271$ ), Lamiaceae ( $0.074 \pm 0.040$ ), Polygonaceae ( $0.046 \pm 0.033$ ), Asteraceae ( $0.036 \pm 0.023$ ), and Convolvulaceae ( $0.033 \pm 0.002$ ) families in that order. In decreasing order, polyphenol content was lowest in species of the Cucurbitaceae ( $0.019 \pm 0.009$ ), Amaryllidaceae ( $0.017 \pm 0.008$ ), Poaceae (Gramineae ( $0.017 \pm 0.008$ )), Alliaceae ( $0.012 \pm 0.005$ ), and Araceae ( $0.010 \pm 0.005$ ) families. Polyphenol content was also significantly higher in Fagaceae than in the other 17 families and significantly higher in Lamiaceae than in Poaceae (Gramineae) (p < 0.05).

*Fig. 4* presents the relationship between polyphenol content and antiglycation activity in vegetable samples. The results showed that there were no significant correlations between polyphenol content and antiglycation activity. In the following 12 species, antiglycation activity was potent (50%) inhibitory concentration  $[IC_{50}] < 0.15 \text{ mg/mL}$ , but polyphenol content was low: Nalta jute (Corchorus olitorius); new ginger (Zingiber officinale); red leaf lettuce (Lactuca sativa); scallion (Allium fistulosum); spinach (Spinacia oleracea); rice eggplant, no peel (Solanum melongena); Boston lettuce (Lactuca sativa); green perilla, leaf (Perilla frutescens); edible chrysanthemum, petal (Chrysanthemum morifolium); ashitaba (Angelica keiskei); bell pepper (Capsicum annuum); and green leaf lettuce (Lactuca sativa). In the following 7 species, polyphenol content was high ( $\geq 0.05$  mg/mL), but antiglycation activity was weak (IC  $_{50} \ge 0.15$  mg/mL): Walnut (Juglans), ostrich fern (Matteuccia struthiopteris), thyme (Thymus), sweet leaf (Stevia rebaudiana), peppermint (Mentha x piperita), radish sprouts (Raphanus sativus), and pistachio (Pistacia vera).

#### Polyphenol (mg catechin/mL) ID Common Name Japanese name Scientific Name Vesicaria Family 0.015 Alliaceae 1 Green spring onion Ao-negi Allium fistulosum Leaf vegetable 0.018 2 Avocado Abogado Persea americana Fruit vegetable Lauraceae 3 Amanaga Chili pepper Fruit vegetable Solanaceae 0.032 Amanaga-tougarashi Amanaga capsicum 0.020 4 Edamame Eda-mame Clycine max Bean Fabaceae 5 Pumpkin Kabocha Cucurbita Fruit vegetable Cucurbitaceae 0.040 Root vegetables 6 Sweet potato Satsuma-imo Lpomoea batatas Convolvulaceae 0.035 7 Root vegetables 0.015 Eddoe Sato-imo Colocasia esculenta Araceae 0.014 Kidney bean Fruit vegetable Fabaceae 8 Saya-ingen Phaseolus vulgaris 0.023 9 Potato Jaga-imo Solanum tuberosum Root vegetables Solanaceae 10 Jumbo kidney bean Jumbo-ingen Phaseolus vulgaris Fruit vegetable Fabaceae 0.012 0.032 11Ginger Shouga Zingiber officinale Root vegetables Zingiberaceae 12 Welsh onion Allium fistulosum Leaf vegetable Alliaceae 0.014 Shiro-negi 13 Zucchini Zucchini Cucurbita pepo Fruit vegetable Cucurbitaceae 0.017 14 Broad bean Sora-mame Vicia faba Bean Fabaceae 0.026 15 Daikon (Japanese radish) Daikon Raphanus sativus Root vegetables Vesicaria 0.010 Root vegetables Amaryllidaceae 0.010 16 Onion Tama-negi Allium cepa 17 Winter melon Tougan Benincasa hispida Fruit vegetable Cucurbitaceae 0.011 18 Corn Toumorokoshi Zea mays Grain Poaceae (Gramineae) 0.010 Root vegetables 19 Dioscoreaceae 0.010 Chinese yam Naga-imo Dioscorea batatas Leaf vegetable 20 Nira Allium tuberosum 0.026 Chinese chive Amaryllidaceae 0.007 21 Garlic Ninniku Allium sativum Root vegetables Amaryllidaceae 0.014 22 Broccoli (Stem) Broccoli Brassica oleracea Fruit vegetable Vesicaria 23 Broccoli (Flower bud) Broccoli Brassica oleracea Fruit vegetable Vesicaria 0.027 24 Lotus roots Renkon Nelumbo nucifera Root vegetables Nelumbonaceae 0.021 25 Okra Okura Abelmoschus esculentus Fruit vegetable Malvaceae 0.035 0.010 Carrot Ninjin Daucus carota Root vegetables Apiaceae 26 0.019 27 Cherry tomato Mini-tomato Lycopersicon esculentum Mill Fruit vegetable Solanaceae 0.017 28 Tomato Tomato Solanum lycopersicum Fruit vegetable Solanaceae 29 Red onion Red onion Allium cepa Root vegetables Amaryllidaceae 0.015 30 Eggplant Nasu Solanaceae Fruit vegetable Solanaceae 0.024 31 Great burdock Gobou Arctium lappa Root vegetables Asteraceae 0.012 Cucumber Kyuri Cucumis sativus Fruit vegetable Cucurbitaceae 0.018 32 0.021 33 Bitter melon Gouya Momordica charantia Fruit vegetable Cucurbitaceae 34 Celery (Stem) Serori Apium graveolens Stem vegetables Apiaceae 0.018 0.056 35 Celery (Leaf) Serori Apium graveolens Stem vegetables Apiaceae 36 Cabbage Kyabetsu Brassica oleracea Leaf vegetable Vesicaria 0.016 Liliaceae 0.004 37 Lily bulb Yurine Lilium Root vegetables Shiitake mushroom 38 Shii-take Lentinula edodes Mushroom Tricholomataceae 0.020 (Pileus) Shiitake mushroom 39 Shii-take Lentinula edodes Mushroom Tricholomataceae 0.021 (The foundation) 40 Hen of the woods Mai-take Grifola frondosa Mushroom Meripilaceae 0.026 Mushroom 0.016 41 King oyster Eringi Pleurotus eryngii Pleurotaceae 42 Buna-shimeji Buna-shimeji Hypsizygus marmoreus Mushroom Lyophyllaceae 0.015 43 Pea Kinusaya Pisum sativum Fruit vegetable Fabaceae 0.025 44 Enoki-dake Flammulina velutipes Mushroom Tricholomataceae 0.019 Enoki mushroom 0.032 45 Midorimame-moyashi Vigna radiate Leaf vegetable Fabaceae Bean sprout 0.017 46 Garlic shoots Ninniku-no-me Allium sativum Stem vegetables Amaryllidaceae 47 Bell pepper Piman Capsicum annuum Fruit vegetable Solanaceae 0.027 Vesicaria 0.026 48 Pak choy Chingen-sai Brassica rapa Leaf vegetable 49 Mioga ginger Myouga Zingiber mioga Fruit vegetable Zingiberaceae 0.015 Liliaceae 50 Asparagus Asuparagasu Asparagus officinalis Stem vegetables 0.024 Brassica oleracea Vesicaria 0.052 51 Broccoli sprouts Hatsuga-broccoli Leaf vegetable 0.051 52 Super-Hatsuga-broccoli Brassica oleracea Leaf vegetable Vesicaria Broccoli super sprouts 0.065 53 Radish sprouts Kaiware Raphanus sativus Leaf vegetable Vesicaria

#### Table 1. Polyphenol content in 187 vegetable samples.

ID	Common Name	Japanese name	Scientific Name	Vesicaria	Family	Polyphenol (mg catechin/mL)
54	Water morning glory (Sprout)	Kushin-sai	Ipomoea aquatic	Leaf vegetable	Convolvulaceae	0.032
55	Lettuce	Retasu	Lactuca sativa	Leaf vegetable	Asteraceae	0.011
56	Red leaf lettuce	Sunny-retasu	Lactuca sativa	Leaf vegetable	Asteraceae	0.047
57	Leaf lettuce	Green leaf	Lactuca sativa	Leaf vegetable	Asteraceae	0.025
58	Napa cabbage	Haku-sai	Brassica rapa	Leaf vegetable	Vesicaria	0.014
59	Paprika (Red color)	Papurika	Capsicum annuum	Fruit vegetable	Solanaceae	0.015
60	Paprika (Yellow color)	Papurika	Capsicum annuum	Fruit vegetable	Solanaceae	0.017
61	Paprika (Orange color)	Papurika	Capsicum annuum	Fruit vegetable	Solanaceae	0.017
62	Chicory	Chikori	Cichorium introbus	I auf vegetable	Asterocene	0.024
62	Criticol y	Kilw ze	Clebionia cononania	Leaf vegetable	Asteraceae	0.024
05	Dedich	KIKU-IIA	Giebionis coronaria	Leaf vegetable	Asteraceae	0.013
64	Radish	Radish	Raphanus sativus	Root vegetables	Vesicaria	0.038
65	Scallion	Wakegi	Allium fistulosum	Leaf vegetable	Amaryllidaceae	0.024
66	Parsley	Paseri	Petroselinum crispum	Herb	Apiaceae	0.030
67	Spinach	Horenso	Spinacia oleracea	Leaf vegetable	Chenopodiaceae	0.024
68	Nalta jute	Moroheiya	Corchorus olitorius	Leaf vegetable	Tiliaceae Juss.	0.046
69	Mizuna (Potherb mustard)	Mizu-na	Brassica rapa	Leaf vegetable	Vesicaria	0.024
70	Mibuna	Mibu-na	Brassica rapa	Leaf vegetable	Vesicaria	0.031
71	Komatsuna (Japanese mustard spinach)	Komatsu-na	Brassica rapa	Leaf vegetable	Vesicaria	0.021
72	Red cabbage	Aka-kyabetu	Brassica oleracea	Leaf vegetable	Vesicaria	0.037
73	Carrot greens (Leaf)	Ninjin-sai	Daucus carota	Leaf vegetable	Apiaceae	0.022
74	Japanese honeywort	Mitsuba	Cryptotaenia canadensis	Leaf vegetable	Apiaceae	0.018
75	Green perilla (Leaf)	Oba	Perilla frutescens	Leaf vegetable	Lamiaceae	0.037
76	Japanese parsley	Seri	Oenanthe javanica	Leaf vegetable	Apiaceae	0.030
77	Boston lettuce	Sarada-na	Lactuca sativah	Leaf vegetable	Asteraceae	0.027
78	Japanese horseradish	Wasabi	Wasahia janonica	Root vegetables	Vesicaria	0.005
79	White mushroom	White mushroom	Agaricus hisporus	Mushroom	Pleurotaceae	0.017
80	Green pepper	Shishitou	Cansicum annuum	Fruit vegetable	Solanaceae	0.026
81	Malabar spinach	Teurumuraeaki	Basella alba	I eaf vegetable	Basellaceae	0.026
82	Malabar spinach (Leof)	Tsurumurasaki	Basella alba		Basellaceae	0.042
82	Malabar spinach (Stam)	Taurumuraaaki	Pasalla alba	Leaf vegetable	Basellaceae	0.042
0.0	Shimmer Chimmer (Stein)		Basella alba	Leaf vegetable	Dasenaceae	0.018
84	Shirona Chinese cabbage	Sniro-na	Brassica rapa	Leaf vegetable	Vesicaria	0.024
85	Garden pea sprouts (Sprout)	Mame-nae	Pisum sativm	Leaf vegetable	Fabaceae	0.030
86	Water morning glory	En-sai (Enkushin)	Ipomoea aquatic	Leaf vegetable	Convolvulaceae	0.032
87	Red giant elephant ear (Petiole)	Beni-zuiki	Colocasia gigantean	Stem vegetables	Araceae	0.008
88	Ginkgo	Ginnan	Ginkgo biloba	Nut	Ginkgoaceae	0.029
89	Chrysanthemum (Petal)	Shokuyo-kiku	Chrysanthemum morifolium	Fruit vegetable	Asteraceae	0.067
90	Jew's ear fungs	Kikurage	Auricularia auricular	Mushroom	Auricularaceae	0.014
91	Chinese yam	Yamato-imo	Dioscorea batatas	Root vegetables	Dioscoreaceae	0.007
92	Horseradish	Seiyou-wasabi	Armoracia rusticana	Root vegetables	Vesicaria	0.011
93	New ginger	Shin-shouga	Zingiber officinale	Root vegetables	Zingiberaceae	0.019
94	Saltwort	Wakame-okahiiiki	Salsola komarovii	Leaf vegetable	Chenopodiaceae	0.022
95	Common ice plant	Barafu	Mesembryanthemum crystallinum	Leaf vegetable	Aizoaceae	0.013
96	Wasabi leaves	Wasabi-na	Brassica juncea	Leaf vegetable	Vesicaria	0.027
97	Perilla (Shiso)	Hojiso	Perilla frutescens	Fruit vegetable	Lamiaceae	0.068
98	Giant elephant ear	Hasu-imo	Colocasia oigantea	Stem vegetables	Araceae	0.000
00	Red rhubarbe	Aka ruhahu	Rhoum rhaharhatum	Stem vegetables	Polygopacasa	0.007
100	Dod shuberba (Marcarel)	Also mikely	Dhoum shah arb atom	Stom vegetables	Dolygonaceae	0.020
100	Red mubarbe (None peel)	Aka-rubabu	RHEUM FRADARDAIUM	Stem vegetables	Polygonaceae	0.015
101	Ked rhubarbe (Peel)	Ked-rubaubu	Kneum rhabarbatum	Stem vegetables	Polygonaceae	0.053
102	Snallot	Esharotto	Autum oschaninii	Koot vegetables	Alliaceae	0.005
103	Cauliflower (Flower bud)	Karihurawa	Brassica oleracea	Fruit vegetable	Vesicaria	0.019
104	Cauliflower (Stem)	Karifurawa	Brassica oleracea	Fruit vegetable	Vesicaria	0.020

ID	Common Name	Japanese name	Scientific Name	Vesicaria	Family	Polyphenol (mg catechin/mL)
105	Cauliflower (Leaf)	Karifurawa	Brassica oleracea	Fruit vegetable	Vesicaria	0.025
106	Turnip (Root)	Ko-kabura	Brassica rapa	Root vegetables	Vesicaria	0.017
107	Turnip	Ko-kabura	Brassica rapa	Root vegetables	Vesicaria	0.020
108	Green cucumber	Ao-uri	Cucumis sativus	Fruit vegetable	Cucurbitaceae	0.011
109	Kagahuto cucumber	Kagahuto-kyuri	Cucumis sativus	Fruit vegetable	Cucurbitaceae	0.013
110	Rice eggplant (None peel)	Bei-nasu	Solanum melongena	Fruit vegetable	Solanaceae	0.023
111	Rice eggplant (Peel)	Bei-nasu	Solanum melongena	Fruit vegetable	Solanaceae	0.066
112	Kintoki carrot* (Root)	Kintoki-ninjin	Daucus carota	Root vegetables	Apiaceae	0.008
113	Kintoki carrot* (Leaf)	Kintoki-ninjin	Daucus carota	Root vegetables	Apiaceae	0.023
114	Red bell pepper	Aka-piman	Capsicum annuum	Fruit vegetable	Solanaceae	0.020
115	Leaf ginger (Root)	Ha-shouga	Zingiber officinale	Root vegetables	Zingiberaceae	0.023
116	Leaf ginger (Leaf)	Ha-shouga	Zingiber officinale	Root vegetables	Zingiberaceae	0.017
117	Mugwort (Powder)	Yomogi	Artemisia indica	Leaf vegetable	Asteraceae	0.077
118	Azuki bean	Azuki	Vigna angularis	Bean	Fabaceae	0.027
119	Red kidnev beans	Kintoki-mame	Phaseolus vulgaris	Bean	Fabaceae	0.026
120	Black-eved pea	Sasage	Vigna unguiculata	Bean	Fabaceae	0.029
121	Black soybeen	Kuro-mame	Glycine max	Bean	Fabaceae	0.023
122	Sovheen	Daizu	Glycine max	Bean	Fabaceae	0.019
122	Black sesame	Kuro-goma	Sesamun indicum	Nut	Pedaliaceae	0.033
123	White sesame	Shiro-goma	Sesamun indicum	Nut	Pedaliaceae	0.017
124	White onium poppy coods	Shiro-gonia	Sesaman matcam	Nut	Tedamaceae	0.017
125	(Seed)	Shirokeshi-no-mi	Papaver somniferum	Nut	Papaveraceae	0.012
126	Almond	Almond	Amygdalus communis	Nut	Rosaceae	0.009
127	Cahew nuts	Kashu-nattsu	Anacardium occidele	Nut	Anacardiaceae	0.021
128	Walnut	Kurumi	Juglans	Nut	Juglandaceae	0.059
129	Pumpkin seeds (Seed)	Kawanashi-kabocha- no-tane	Cucurbita sp.	Nut	Cucurbitaceae	0.019
130	Peanut	Rakkasei	Arachis hypogaea	Nut	Fabaceae	0.036
131	Polished rice A	Haku-mai A	Oryza sativa	Grain	Poaceae (Gramineae)	0.016
132	Red-kerneled rice	Aka-mai	Oryza sativa	Grain	Poaceae (Gramineae)	0.024
133	Reddish black rice	Kuro-mai	Oryza sativa	Grain	Poaceae (Gramineae)	0.017
134	Rolled barley	Oshi-mugi	Hordeum vulgare	Grain	Poaceae (Gramineae)	0.011
135	Pearl barley	Hato-mugi	Coix lacryma-jobi	Grain	Poaceae (Gramineae)	0.010
136	Pistachio	Pisutachio	Pistacia vera	Nut	Anacardiaceae	0.058
137	Foxtail millet	Awa	Setaria italic	Grain	Poaceae (Gramineae)	0.010
138	Japanese millet	Hie	Echinochloa esculenta	Grain	Poaceae (Gramineae)	0.033
139	Proso millet	Kibi	Panicum miliaceum	Grain	Poaceae (Gramineae)	0.008
140	Amaranth	Amaransasu	Amaranthus	Grain	Amaranthaceae	0.030
141	Quínua	Kinusu	Chenopodium quinoa	Grain	Chenopodiaceae	0.032
142	Pecan nuts	Pecan	Carya illinoinensis	Nut	Juglandaceae	0.059
143	Lady's thumb	Tade	Polygonum hydropiper	Leaf vegetable	Polygonaceae	0.089
144	Polished rice B	Haku-mai B	Orvza sativa	Grain	Poaceae (Gramineae)	0.010
145	Unpolished rice B	Gen-mai	Oryza sativa	Grain	Poaceae (Gramineae)	0.012
146	Rice B bran	Nuka	Oryza sativa	Grain	Poaceae (Gramineae)	0.029
147	Garbanzo	Garbanzo	Cicer arietinum	Bean	Fabaceae	0.015
148	Lentil	Renzu-mame	Lens culinaris	Bean	Fabaceae	0.027
140	Bay leaves	Ray leaf	Laurus nobilis	Herb	Lauraceae	0.027
150	Water caltron (Seed)	Hishi	Trana janonica	Nut	Tranaceae	0.014
151	Water caltron (Seed cost)	Hishi	Trapa japonica	Nut	Trapaceae	0.014
152	Butterscotch muchroom	Nameko	Pholiota microspore	Mushroom	Strophariaceae	0.274
152	Chestnut (Seed)	Kuri	Castanga cregata	Nut	Бадасеае	0.012
153	Chestnut (Outer skin)	Kuri	Castanaa cronata	Nut	Fagaceae	0.444
154	Chestnut (Outer Skill)	Kuri	Castanea crenata	Nut	Fagaceac	0.444
133	Chestnut (Soit layer)	KUII	Channed Crenald	INUL	ragaceae	0.309
156	(Receptacle)	Shokuyo-kiku	Chrysanthemum morifolium	Fruit vegetable	Asteraceae	0.024

ID	Common Name	Japanese name	Scientific Name	Vesicaria	Family	Polyphenol (mg catechin/mL)
157	Pea (Pod)	Endou-mame	Pisum sativum	Bean	Fabaceae	0.020
158	Pea (Bean)	Endou-mame	Pisum sativum	Bean	Fabaceae	0.022
159	Turnip rape	Nanohana	Brassica rapa	Fruit vegetable	Vesicaria	0.033
160	Horikawa gobo*	Horikawa-gobou	Arctium lappa	Root vegetables	Asteraceae	0.049
161	Shogoin turnip*	Seigoin-kabura	Brassica rapa	Root vegetables	Vesicaria	0.007
162	Ashitaba	Ashitaba	Angelica keiskei	Leaf vegetable	Apiaceae	0.041
163	Kyoto eggplant*	Nasubi "Kyo-shizuku"	Solanum melongena	Fruit vegetable	Solanaceae	0.030
164	Ostrich fern	Kogomi	Matteuccia struthiopteris	Leaf vegetable	Athyriaceae	0.060
165	Kamo eggplant*	Kamo-nasu	Solanum melongena	Fruit vegetable	Solanaceae	0.019
166	Manganzi Chile pepper* (Red)	Mannganji-tougarashi	Capsicum annuum	Fruit vegetable	Solanaceae	0.024
167	Manganzi Chile pepper* (Green)	Manganji-tougarashi	Capsicum annuum	Fruit vegetable	Solanaceae	0.025
168	Manganzi Chile pepper* (Mix)	Manganji-tougarashi Mix	Capsicum annuum	Fruit vegetable	Solanaceae	0.040
169	Takagamine Chile pepper*	Takagamine-tougarashi	Capsicum annuum	Fruit vegetable	Solanaceae	0.024
170	Husimiamanaga Chile pepper* (Red)	Fushimi-amanaga- tougarashi	Capsicum annuum	Fruit vegetable	Solanaceae	0.028
171	Husimiamanaga Chile pepper* (Green)	Fushimi-amanaga- tougarashi	Capsicum annuum	Fruit vegetable	Solanaceae	0.029
172	Super Yuga tomato*	Super Yuga tomato	Solanum lycopersicum	Fruit vegetable	Solanaceae	0.020
173	Renaissance tomato*	Runessansu tomato	Solanum lycopersicum	Fruit vegetable	Solanaceae	0.013
174	Kyo-akane tomato*	Kyo-akane tomato	Solanum lycopersicum	Fruit vegetable	Solanaceae	0.019
175	Kyo-temari tomato*	Kyo-temari tomato	Solanum lycopersicum	Fruit vegetable	Solanaceae	0.019
176	Rucola	Rukkora	Eruca vesicaria	Herb	Vesicaria	0.017
177	Sweet basil	Sweet bajiru	Ocimum basilicum	Herb	Lamiaceae	0.037
178	Common sage	Seiji	Salvia officinalis	Herb	Lamiaceae	0.026
179	Dill	Diru	Anethum graveolens	Herb	Apiaceae	0.048
180	Italian parsley	Italian-pasure	Petroselinum neapolitanum	Herb	Apiaceae	0.024
181	Chervil	Chabiru	Anthriscus cerefolium	Herb	Apiaceae	0.045
182	Thyme	Taimu	Thymus sp.	Herb	Lamiaceae	0.059
183	Sweet leaf	Sutebia	Stevia rebaudiana	Herb	Asteraceae	0.080
184	Lemon balm	Lemon baumu	Melissa officinalis	Herb	Lamiaceae	0.125
185	Peppermint	Peppermint	Mentha x piperita	Herb	Lamiaceae	0.051
186	Spearmint	Spearmint	Mentha spicata	Herb	Lamiaceae	0.124
187	Rosemary	Rosemary	Rosmarinus officinalis	Herb	Lamiaceae	0.115

IC<sub>50</sub>: 50% inhibitory concentration. Polyphenol content shown as catechin equivalents. Mean of three measurements for each sample shown. \*Kyoto regional vegetable



## Polyphenol (mg catechin Eq/mL)

Fig 1. Polyphenol content ranking of 187 vegetable samples (catechin equivalents, top 100)



## Polyphenol (mg catechin Eq/mg solid content)

#### Fig 2. Polyphenol content by vegetable category

Polyphenol content shown as catechin equivalents. Parentheses indicate number of samples included in vegetable category. Bars indicate standard deviation. No. of sample measurements n=3.



## Polyphenol (mg catechin Eq/mg solid content)

#### Fig 3. Polyphenol content by vegetable sample taxonomic family

Polyphenol content shown as catechin equivalents. Parentheses indicate number of samples included in vegetable family. Bars indicate standard deviation. No. of sample measurements n=3.



Fig 4. Relationship between polyphenol quantity and antiglycation activity in vegetable samples Antiglycation activity represents inhibitory activity for fluorescent AGE formation (IC50) in dried vegetable powder samples in a previously reported<sup>18</sup> in vitro glucose/HSA reaction model. Polyphenol content shown as catechin equivalents. Regression function: y = -3E-13x + 0.0338, R<sup>2</sup> = 0.0004, p > 0.05. HSA, human serum albumin; AGEs, advanced glycation end-products; IC50, 50% inhibitory concentration.

## Discussion

Many reports state that tea leaves <sup>9,21</sup>, herbs and spices<sup>22-24</sup> herbal remedies<sup>25</sup>, and other natural, plant-based materials contain substantial quantities of polyphenols and have an antiglycation effect in vitro or in animal experiments. Our results for measurement of polyphenol content in 187 vegetables showed that polyphenol content was highest in nuts, herbs, leaf vegetables, stem vegetables, fruit vegetables, and beans in that order. By plant taxonomic family, polyphenol content was highest in samples of Fagaceae, Lamiaceae, Polygonaceae, Asteraceae, and Convolvulaceae in that order. However, in a previous report 18), results for measurement of inhibitory activity for fluorescent AGE formation in an in vitro glucose/HSA model found high antiglycation activity among herbs, leaf vegetables, and stem vegetables in that order. By taxonomic family, high antiglycation activity was observed in species of the Asteraceae, Lamiaceae, Basellaceae, and Polygonaceae families. In all vegetable samples generally, no significant correlations were observed between polyphenol content and antiglycation activity, but in terms of vegetable type, a high

association between polyphenol content and antiglycation activity was anticipated for herbs, leaf vegetables, and stem vegetables. Likewise, by taxonomic family, a high association was anticipated for species of the Asteraceae, Lamiaceae, and Polygonaceae families. The Asteraceae family contains high levels of the polyphenols luteolin and anthrocyanidin<sup>8</sup>. Lamiaceae species contain copious rosmarinic acid <sup>26,27</sup>, and Polygonaceae species contain copious quercetin<sup>28</sup> and kaempferol<sup>29</sup>.

By individual vegetable, antiglycation activity was potent (IC<sub>50</sub> < 0.15 mg/mL) but polyphenol content low (< 0.05 mg catechin Eq/mg solid content) in 12 vegetables: Nalta jute, new ginger, red leaf lettuce, scallion, spinach, rice eggplant (no peel), Boston lettuce, green perilla, edible chrysanthemum (petal), ashitaba, bell pepper, and green leaf lettuce. Polyphenol content was also extremely low (< 0.0125 mg catechin Eq/mg solid content) but antiglycation activity slightly weaker (0.15 mg/mL  $\leq$  IC<sub>50</sub> < 0.5 mg/ mL) than that of aminoguanidine in four vegetables: Green cucumber (*Cucumis sativus*), great burdock (*Arctium lappa*), lettuce (*Lactuca sativa*), and red giant elephant ear (petiole, *Colocasia gigantea*). In these 16 vegetables, components other than polyphenols are believed to contribute to antiglycation effect. Polyphenol content was high ( $\geq 0.05$  mg catechin Eq/mg solid content) but antiglycation activity weak (IC<sub>50</sub>  $\geq 0.15$  mg/mL) in seven vegetables: Walnut, ostrich fern, thyme, sweetleaf, peppermint, radish sprouts, and pistachio. Polyphenols in these samples may contain components which inhibit antiglycation activity.

There are numerous reports on research concerning vegetables. Results for measurement of hydroxyl radical antioxidant capacity (H-ORAC) in 23 different Japanese vegetables showed a mean estimated H-ORAC value of 594.3  $\mu$ mol trolox-equivalent (TE/100 g)<sup>30</sup>. Consumption of 350 g of these vegetables per day would result in consumption of 2,080  $\mu$ mol TE/day of hydrophilic antioxidants.

Results for measurement of polyphenol content, lowdensity lipoprotein (LDL) oxidation and 1,1-diphenyl-2picrylhydrazyl (DPPH) radical reduction in 30 different Vietnamese vegetables by FC method showed that antioxidant activity was high in vegetables with high polyphenol content<sup>31</sup>). The mean daily intake of polyphenols by Vietnamese individuals was found to be 595 mg catechin Eq, with the highest contribution, 45%, from water spinach (*Ipomoea aquatica*), green vegetable.

Research measuring polyphenol content,  $\alpha$ -glucosidase inhibitory activity and antioxidant activity by FC method in extracts from 28 different Vietnamese vegetables showed a positive correlation between this activity and polyphenol content<sup>32</sup>. In particular, four species possessed  $\alpha$ -glucosidase inhibitory activity and antioxidant activity equivalent to or better than that of guava leaf extract: *Syzygium zeylanicum*, *Cleistocalyx operculatus*, *Horsfieldia amygdalina*, and *Careya aroborea*.

Our research and these reports  $^{31,32}$  show that the FC method used for polyphenol measurement produces a high correlation (r = 0.96) with total phenolic OH number and is an analytical method allowing evaluation of the total phenolic OH number in various polyphenol compounds  $^{33)}$ . Measurement sensitivity is comparatively higher than in other measurement methods. Though reactivity to compounds with differing chemical structures differs to some extent, the FC method tends to produce high values in compounds with an odiphenol structure.

On this basis, we believe that a commitment to consuming a target amount of 350 g/day of various vegetables, regarded as a preferable figure for required daily vegetable intake, may provide substantial antioxidant effect <sup>30</sup> and antiglycation activity <sup>18</sup> and may also go beyond inhibition of aging to play a role in preventing the onset and progression of lifestylerelated diseases.

## **Conclusion**

Though vegetables contain substantial quantities of polyphenols, it may be the case that various components, polyphenolic or non-polyphenolic, contribute to antiglycation effect in different vegetable species. By vegetable type, we found a high association between polyphenol quantity and antiglycation activity in herbs, leaf vegetables, and stem vegetables. By taxonomic family, we found a similar association in species of the Asteraceae, Lamiaceae, and Polygonaceae families. A contribution by non-polyphenolic substances was suggested in nalta jute, new ginger, red leaf lettuce, scallion, spinach, rice eggplant (no peel), Boston lettuce, green perilla, edible chrysanthemum (petal), ashitaba, bell pepper, green leaf lettuce, green cucumber, great burdock, lettuce, and red giant elephant ear (petiole). We believe that this information is useful for creating dietary regimens to counter glycation stress.

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# **Conflict of Interest Statement**

The authors state that performance of this study entailed no issues representing a conflict of interest.

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