Effects of hot-water extracts from 26 herbs on α-glucosidase activity

HIDETOMO KIKUCHI*, NANA TOYODA*, SATOKO EZAWA, SHIORI YOSHIDA, YASUHIDE HIBINO and KATSUYOSHI SUNAGA

Laboratory of Pharmacotherapy, Clinical Dietetics and Human Nutrition, Master's Program, Josai University Graduate School of Pharmaceutical Sciences, Sakado, Saitama 350-0295, Japan

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Abstract. α-glucosidase is a key enzyme that plays a role in glucose absorption in the gastrointestinal tract, and the inhibition of its activity induces the prevention of postprandial hyperglycemia. Several α-glucosidase inhibitors have been used as medicines for type 2 diabetes, but a similar effect is observed in natural resources, including traditional herbs and their phytochemicals. To identify the presence of the α-glucosidase inhibitory activity in herbs, in which various functional effects have been known to occur, the present study investigated the effects of hot-water extracts of 26 types of herbs on α-glucosidase activity in an *in vitro* assay. The results indicated significant increases in the inhibition of α-glucosidase activity in 1,000 μg/ml olive ($P<0.01$), white willow ($P<0.01$) and red rooibos hot-water extracts. Furthermore, ≥50% inhibition of α-glucosidase activity was determined to be significant in 1,000 μg/ml coltsfoot, green tea and bearberry hot-water extracts. In addition, the effects of bearberry, green tea and coltsfoot hot-water extracts on α-glucosidase activity *in vivo* were evaluated according to the blood glucose levels (BGLs) in maltose and glucose load model rats. It was indicated that the administration of these three herb extracts significantly reduced the increasing BGLs after maltose loading until 0.5 h compared with the control group. However, only coltsfoot extract significantly reduced the increasing BGLs after glucose loading until 0.5 h compared with the control group. Thus, the present results may facilitate the understanding of a novel functionality in traditional herbs, which could be useful for the prevention of disease onset and progression, such as in hyperglycemia and type 2 diabetes.

Correspondence to: Dr Hidetomo Kikuchi or Dr Katsuyoshi Sunaga, Laboratory of Pharmacotherapy, Clinical Dietetics and Human Nutrition, Master's Program, Josai University Graduate School of Pharmaceutical Sciences, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan
E-mail: hkikuchi@josai.ac.jp
E-mail: ksunaga@josai.ac.jp

*Contributed equally

Abbreviations: BGL, blood glucose level; AUC, area under the curve

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Introduction

Glucose is an essential nutrient in the human body, and its uptake via food results in energy production that is used by various tissues, having been delivered via the general circulation after it is absorbed in the intestine (1). The peak of the blood glucose level (BGL) after a meal, as opposed to that after fasting, is usually limited to 30-50 mg/dl, and this phenomenon is based on the net balance between the rate of carbohydrate being absorbed from the gastrointestinal tract and the rate at which it is taken up by the liver and peripheral tissues (1). Moreover, the disturbance of the net balance results in uncontrolled glucose regulation and induces hyperglycemia, which is widely recognized as a causal link between diabetes and diabetes-related complications (2).

α-glucosidase is a key enzyme that plays a role in glucose absorption in the gastrointestinal tract, and inhibition of its activity induces the prevention of hyperglycemia (3). In addition, α-glucosidase inhibitors such as acarbose, voglibose and miglitol have been used as medicines for type 2 diabetes (1,4). Furthermore, a similar effect is observed in natural resources. For instance, vegetables in the daily diet potentially inhibit α-glucosidase activity (5,6). Traditional natural products including herbs and their phytochemicals are also known for their α-glucosidase-inhibiting activities, and some of these products are already sold as functional foods (7,8). Our previous studies have focused on the biological activity and safety of natural products, including food, traditional herbs, kampo and their phytochemicals (9-12).

To identify the presence of an α-glucosidase inhibitory effect in herbs, in which various functional effects have been reported, the present study investigated the effects of hot-water extracts of 26 types of herbs on α-glucosidase activity in an *in vitro* assay. In addition, the effects of bearberry, green tea and coltsfoot hot-water extracts on α-glucosidase activity *in vivo* were evaluated according to the BGLs in maltose- and glucose-load model rats.

Materials and methods

Materials. Acarbose, maltose, glycerol and Glucose CII-test Wako kit were purchased from Wako Pure Chemical Industries, Ltd., unless otherwise stated. All herbs were purchased from various companies (Table I). Intestinal acetone powders from rat were purchased from Sigma-Aldrich (Merck KGaA).

Preparation of hot-water extract from herbs. The extracts were prepared from herbs based on a previously described method (9). Briefly, 1 g each herb was decocted with 20 ml Milli-Q water at 100˚C for 30 min. After the extracts were cooled and filtered, they were used in animal experiments as described below. Furthermore, to assess α-glucosidase activity, the extracts were evaporated using a freeze dryer, after which the dried samples were weighed and dissolved or suspended at a concentration of 100 mg/ml in Milli-Q water and stored at -20˚C until further use.

Measurement of α-glucosidase activity in vitro. α-glucosidase activity was measured according to a previously described method with modifications (13). Briefly, 10 µl of 72.5 mg/ml intestinal acetone powder suspension in 50 mM Tris-HCl (pH 7.8) containing 20% glycerol or Milli-Q water (blank), 5 µl of 5.17 µg/ml (8 µM) acarbose (final concentration: 0.52 µg/ml, as a positive control), 26 types of herb extracts (Table I) [concentrations: 2.5, 5.0, 7.5 and 10 mg/ml (final concentrations: 250, 500, 750 and 1,000 µg/ml, respectively)] and Milli-Q water (control or blank) and 27 µl Milli-Q water were mixed in 1.5 ml tubes, and then pre-incubated in a heat block at 37˚C for 10 min. Subsequently, 8 µl of 500 mM maltose monohydrate solution in Milli-Q water (final concentration: 80 mM) was added and incubated in a heat block at 37˚C for 30 min. The tubes were incubated at 100˚C for 2 min to terminate the reaction. To assess the amount of glucose production, a Glucose CII-test Wako kit was used according to the manufacturer's protocol with modifications. Samples were centrifuged at 20,400 x g for 5 min, and 2 µl supernatants and 100 µl chromogenic solution were mixed and incubated in a 96-well plate at 37˚C for 5 min. Subsequently, the glucose levels of the samples were determined by measuring the absorbance at 505 nm using a microtiter plate reader, SpectraMax Pro M5e (Molecular Devices, LLC). The relative α-glucosidase inhibitory activity (% of inhibition) was calculated using the following equation:

$$\% \text{ of inhibition}=[(A_{505\ control}-A_{505\ blank})-(A_{505\ sample}-A_{505\ blank})]/(A_{505\ control}-A_{505\ blank}),$$

where $A_{505\ sample}$ denotes the positive control or herb extract samples.

The IC_{50} values were calculated from the relative α-glucosidase inhibitory activity curve.

Animals. All experiments and the care and handling of animals were approved by the Josai University Institutional Animal Care and Use Committee. In total, 48 male Wistar rats (age, 5 weeks; weight, 140-160 g), were obtained from CLEA Japan, Inc. The rats were housed in 16 cases, with three rats per cage. Animals were housed in a 12-h light/dark cycle and maintained at a constant temperature of 22±2˚C and humidity of 55±10%. The rats were allowed 1 week to adapt to the laboratory environment before the experiments and fed laboratory pellet chow (CE-2; CLEA Japan, Inc.) and water *ad libitum*. After the experiments had been completed, all rats were euthanized via the intraperitoneal injection of sodium pentobarbital (150 mg/kg).

To investigate the antihyperglycemic effects based on α-glucosidase inhibition in rats, an oral glucose tolerance test (OGTT) was performed based on a previously published method with modifications (14). The access to pellet chow supplied to the rats was denied the night before the experiment. The rats were then randomly divided into five groups: Control (n=6); acarbose (24 mg/kg, n=6); bearberry (~13.7 mg/ml, n=6); green tea (~8.8 mg/ml, n=6); and coltsfoot (~7.2 mg/ml, n=6). All groups were orally administrated with distilled water (control), each of three herb extracts or acarbose (5 ml/kg, respectively) 0.5 h before oral administration of 2 g/kg maltose or glucose. A diagram of the timeline of the experiment is presented in Fig. 1.

Measurement of BGLs. Blood samples (~10 µl) were collected from all groups into sensor chips (Breeze2 sensor; Panasonic Corporation) sequentially at 0.083, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 h via a small incision in the tail vein using a razor blade. Then, the blood samples were measured using a glucometer (Breeze2; Bayer Thai Co., Ltd.). The area under the curve (AUC) was calculated using GraphPad Prism Ver 8.1.2 (GraphPad Software, Inc.) for subsequent analysis.

Statistical analysis. Statistical analysis was performed with GraphPad Prism Ver 8.1.2 (GraphPad Software, Inc.) using a one-way ANOVA (*in vitro* studies) followed by Dunnett's test for multiple comparisons and a repeated-measures one-way ANOVA or a repeated-measures two-way ANOVA (*in vivo* studies) followed by Bonferroni's multiple comparison test. $P<0.05$ was considered to indicate a statistically significant difference. Data from the animal experiments are presented as the mean ± SEM or mean ± 95% CI (n=6, respectively), and the other data as the mean ± SD of three separate experiments.

Results

Dose-dependent inhibitory effects of acarbose on α-glucosidase activity. To assess the effects of acarbose on α-glucosidase inhibitory activity and to calculate the IC_{50} value of acarbose, α-glucosidase activity was assessed *in vitro*. It was demonstrated that α-glucosidase inhibitory activity significantly increased in a dose-dependent manner (Fig. 2A). Furthermore, the IC_{50} value was 0.52 µg/ml (95% CI, 0.4356-0.6047; R^2=0.9528).

Inhibitory effect of herb extracts on α-glucosidase activity. To investigate the α-glucosidase inhibitory effect of the 26 types of 1,000 µg/ml herb extracts, α-glucosidase activity was assessed *in vitro* (Fig. 2B). Overall, >50% inhibition of α-glucosidase activity was significantly demonstrated in 0.52 µg/ml acarbose, which served as the positive control, and in 1,000 µg/ml coltsfoot, green tea and bearberry extracts. Moreover, significant increases in the inhibition of α-glucosidase were observed in

Table I. List of herb information.

Common name	Scientific name	Family name	Site	Production areas	Sales company	Yield (%)
Garlic	*Allium sativum*	*Liliaceae*	Bulb	Unknown	Mizkan Nakanos Co., Ltd.	59.0
Burdock[a]	*Arctium lappa*	*Asteraceae*	Root	China	Connect Co., Ltd. (https://www.enherb.jp/)	50.3
Bearberry	*Arctostaphylos uva-ursi*	*Ericaceae*	Leaf	Spain	Mono Co., Ltd.	27.3
Red rooibos	*Aspalathus linearis*	*Leguminosae*	Leaf	South Africa	Nasukogen HERB's Co., Ltd. (http://www.n-park.jp/)	10.7
Green Tea	*Camellia Sinensis*	*Theaceae*	Leaf	Japan	Yamamoto nouen	17.5
Hemp[a]	*Cannabis sativa*	*Moraceae*	Seed	China	Ohtsuyashop Co., Ltd. (http://ohtsuya.com/)	9.2
Cayenne pepper	*Capsicum annuum*	*Solanaceae*	Fruit	China	Ohtsuyashop Co., Ltd. (http://ohtsuya.com/)	23.2
Cinnamon	*Cinnamomum verum*	*Lauraceae*	Bark	China	SB foods Co., Ltd. (https://www.sbfoods.co.jp/)	11.2
Bitter orange peel[a]	*Citrus aurantium*	*Rutaceae*	Hull	Paraguay	Yuwn, Inc. (http://company.yuwn.com/)	31.4
Lemon grass[a]	*Cymbopogon citratus*	*Poaceae*	Leaf	India	Tree of life Co., Ltd. (https://www.treeoflife.co.jp/)	8.5
Heath	*Erica vulgaris*	*Ericaceae*	Leaf & flower	Poland	Nasukogen HERB's Co., Ltd. (http://www.n-park.jp/)	6.7
Eucalyptus	*Eucalyptus globulus*	*Myrtaceae*	Leaf	Australia	Hyakka-Saen. Co., Ltd. (http://www.hyakka-saen.com/)	18.2
Bladderwrack	*Fucus vesiculosus*	*Fucus*	Thallus	Canada	NATURAL LIFE Co., Ltd.	27.0
Soybean	*Glycine max*	*Leguminosae*	Seed	Japan	Mitake Food Manufacturing Co., Ltd. (http://official.mitake-shokuhin.co.jp/)	29.5
Maitake	*Grifola frondosa*	*Polyporaceae*	Fruiting body	Japan	Natural health Co., Ltd.	24.3
Maca	*Lepidium meyenii*	*Brassicaceae*	Tuber	Peru	Marukai Corporation Co., Ltd. (http://www.marukai.co.jp/)	28.1
Melilot	*Melilotus officinalis*	*Leguminosae*	Terrestrial	Bulgaria	Connect Co., Ltd. (https://www.enherb.jp/)	18.1
Arhat Fruit	*Momordica grosvenorii*	*Cucurbitaceae*	Fruit	China	NATURAL LIFE Co., Ltd.	31.6
Olive	Olea europaea	Oleaceae	Leaf	Japan	Hyakka-Saen. Co., Ltd. (http://www.hyakka-saen.com/)	24.1
American ginseng[a]	Panax quinquefolius	Araliaceae	Root	America	Kouyou International Commercial Co., Ltd. (https://www.rakuten.co.jp/yanwo/)	26.1
Psyllium[a]	Plantago Ovata	Plantaginaceae	Seed	Thailand	RST spices Shop (https://rstspices.com/)	1.8
Blackcurrant	Ribes nigrum	Grossulariaceae	Leaf	France	Purpurea (http://www.purpurea.jp/)	23.4
White willow	Salix alba	Salicaceae	Bark	Poland	Connect Co., Ltd. (https://www.enherb.jp/)	11.5
Pau d'arco	Tabebuia avellanedae	Bignoniaceae	Bark	Brazil	TFI-Yokohama F1 Co., Ltd.	10.7
Coltsfoota	Tussilago farfara	Asteraceae	Leaf	France	Aromafrance Co., Ltd. (https://www.aromafrance.net/)	14.4
Corn tea	Zea mays	Poaceae	Fruit/seed	Japan	Honjien Co., Ltd. (https://www.honjien.co.jp/)	8.6

[a]Ground herbs.

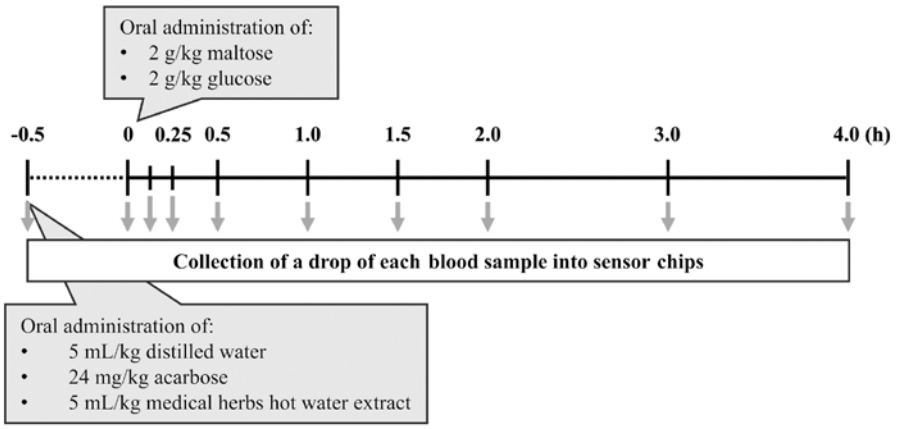

Figure 1. Diagram representing the timeline of treatment with hot-water extracts from herbs, maltose and glucose in rats.

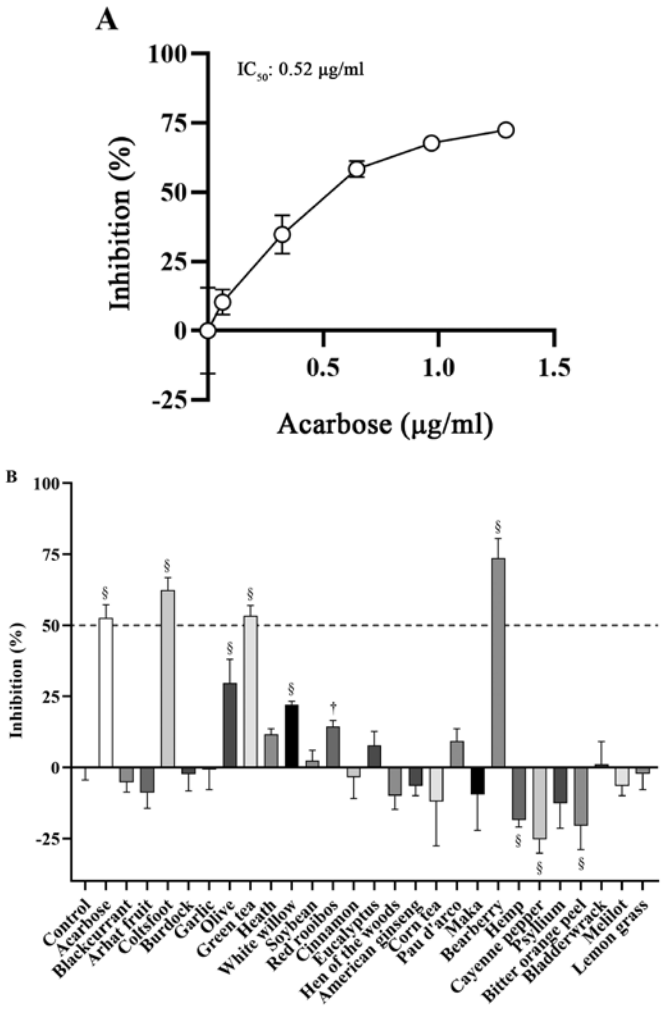

Figure 2. Inhibitory effect of herb extracts on α-glucosidase activity. Dose-dependent inhibitory activity of (A) acarbose and (B) 1,000 μg/ml various herb extracts on α-glucosidase activity. Data are presented as the mean ± SD of three separate experiments. Data were analyzed using an ordinary one-way ANOVA followed by Dunnett's test for multiple comparisons. †P<0.05 and §P<0.01 vs. the control.

1,000 μg/ml olive (P<0.01), white willow (P<0.01) and red rooibos extracts (P<0.05). However, significant decreases were observed in 1,000 μg/ml hemp, cayenne pepper and bitter orange peel extracts (P<0.01; Fig. 2B).

To calculate the IC_{50} values of the three herb extracts, the dose-dependent inhibition of α-glucosidase activity was assessed (Fig. 3). The IC_{50} values were 801.3 (bearberry; 95% CI, 751.9-854.0; R^2=0.9739; Fig. 3A), 744.6 (green tea; 95% CI, 696.7-795.1, R^2=0.9767; Fig. 3B) and 724.0 μg/ml (coltsfoot; 95% CI, 641.9-818.1; R^2=0.9538; Fig. 3C).

Effects of bearberry, green tea and coltsfoot extracts on BGLs in rats. All rats were healthy before the experiments, and adverse events were not observed during the experiments. It was revealed that treatment with 2 g/kg maltose caused temporary hyperglycemia in the rats (maltose control group), as indicated by a significant increase in BGLs at 0.25 (P<0.01), 0.5 (P<0.01) and 1.0 h (P<0.05; Fig. 4A-D). BGLs upon administration of 5 ml/kg bearberry extract were significantly lower compared with the levels in the maltose control group at 0.083 (P<0.05) and 0.5 h (P<0.01; Fig. 4A). Moreover, BGLs after administration of 5 ml/kg green tea extract were significantly lower compared with the levels in the maltose control group at 0.5 h (P<0.01; Fig. 4B). In addition, BGLs after administration of 5 ml/kg coltsfoot extract were significantly lower compared with the levels in the maltose control group at 0.25 (P<0.01) and 0.5 h (P<0.01; Fig. 4C). After administration of 5 ml/kg acarbose (24 mg/kg, positive control group) BGLs were significantly lower compared with the levels in the maltose control group at 0.25 (P<0.01), 0.5 (P<0.01) and 1.5 h (P<0.05; Fig. 4D). Furthermore, the AUC of the acarbose group, compared with the maltose control group, was significantly decreased (P<0.05), but no significant differences were observed in each of the three herb extracts (Fig. 4E).

It was demonstrated that treatment with 2 g/kg glucose caused temporary hyperglycemia in the rats serving as the glucose control group, as indicated by a significant increase in BGLs at 0.083 (P<0.05), 0.25 (P<0.01), 0.5 (P<0.01), 1.0 (P<0.01) and 1.5 h (P<0.01; Fig. 5A-D). BGLs after the administration of 5 ml/kg bearberry (Fig. 5A) and green tea extracts (Fig. 5B) were not significantly different compared with the levels of each time-point in the glucose control group. Moreover, BGLs upon administration of 5 ml/kg coltsfoot extract were significantly lower compared with the levels of each time-point in the glucose control group at 0.25 (P<0.05) and 0.5 h (P<0.05; Fig. 5C). It was indicated that the AUCs in

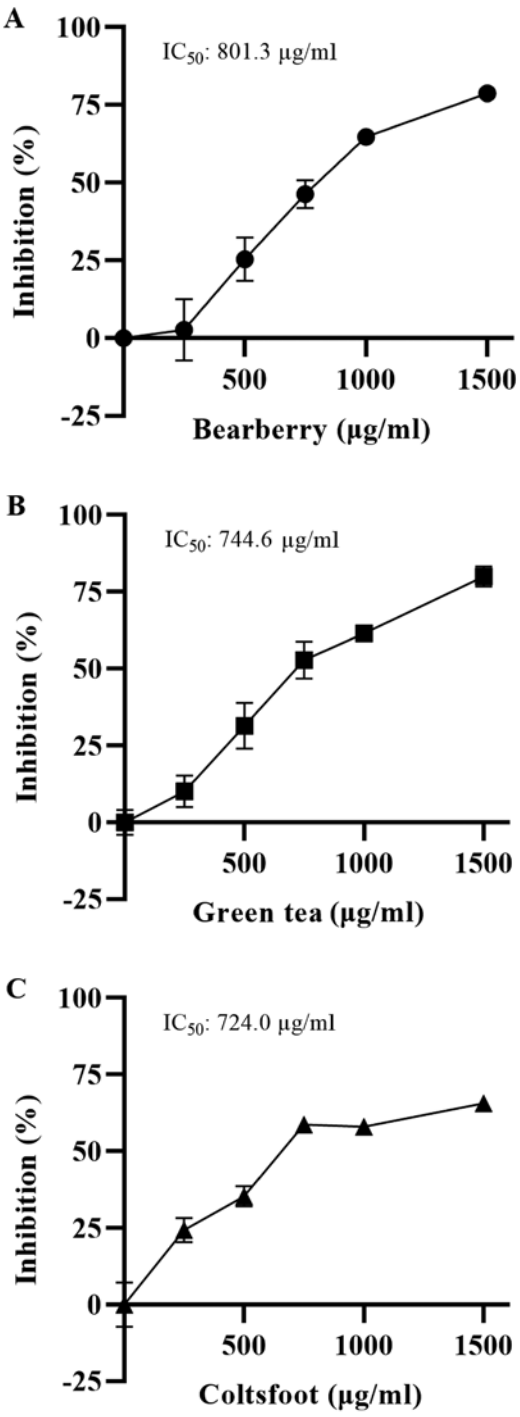

Figure 3. Inhibitory effect of herb extracts on α-glucosidase activity. Dose-dependent inhibitory activity of (A) bearberry, (B) green tea and (C) coltsfoot extracts on α-glucosidase activity. Data are presented as the mean ± SD of three separate experiments.

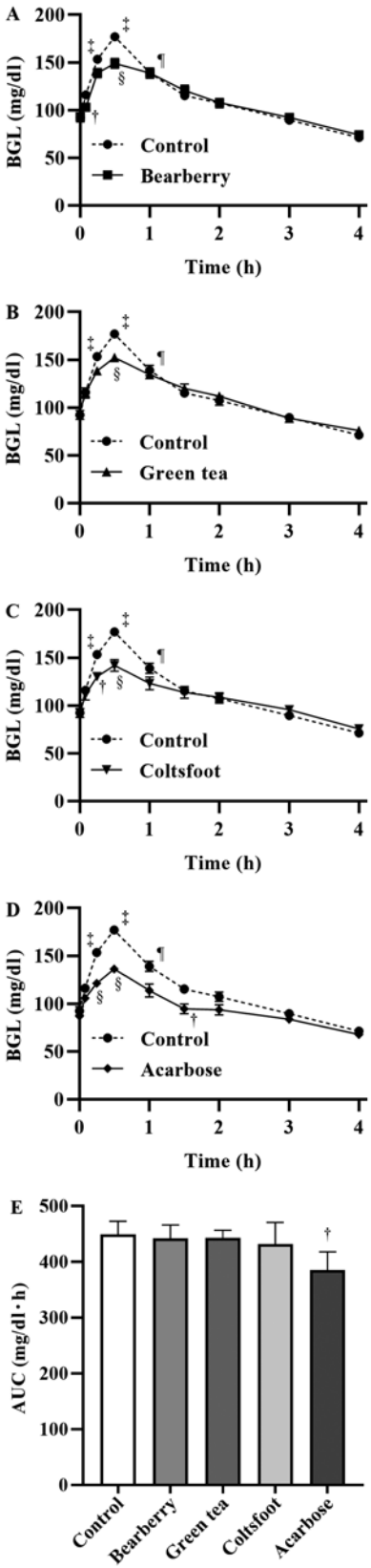

Figure 4. Effects of bearberry, green tea and coltsfoot extracts on BGLs. Blood glucose kinetics in maltose-treated rats after administration of (A) bearberry, (B) green tea, (C) coltsfoot and (D) acarbose extracts. (E) Comparison of the AUCs. Data are presented as (A-D) the mean ± SEM or (E) the mean ± 95% CI (n=6, respectively). Data were analyzed using (A-D) a repeated-measures two-way ANOVA and (E) a repeated-measures one-way ANOVA followed by Bonferroni's multiple comparison test. ¶P<0.05 and ‡P<0.01 vs. the BGL at time 0 in the maltose control group. †P<0.05 and §P<0.01 vs. the BGL at each time-point in the maltose control group. BGL, blood glucose level; AUC, area under the curve.

each of the three herb extracts were not significantly different compared with those in the glucose control group (Fig. 5D).

Discussion

In the present study, it was demonstrated that acarbose, an α-glucosidase inhibitor, significantly inhibited α-glucosidase activity *in vitro*, indicating that acarbose can be used as a positive control in the measurement of this activity *in vitro*. In

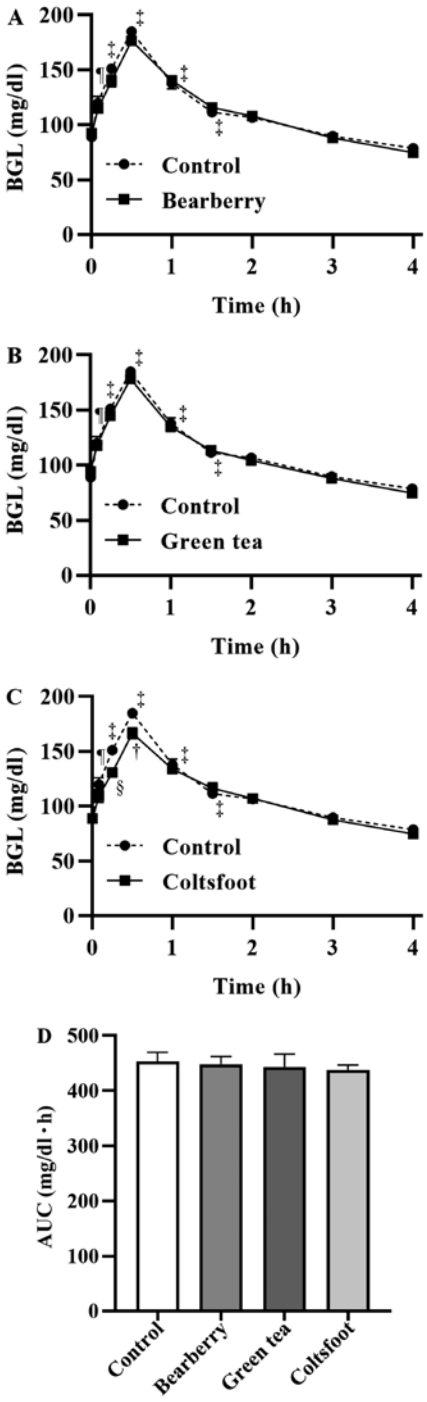

Figure 5. Effects of bearberry, green tea and coltsfoot extracts on BGLs. Blood glucose kinetics in glucose-treated rats after administration of (A) bearberry, (B) green tea and (C) coltsfoot extracts. (D) Comparison of the AUCs. Data are presented as (A-C) the mean ± SEM or (D) the mean ± 95% CI (n=6, respectively). Data were analyzed using (A-C) a repeated-measures two-way ANOVA and (D) a repeated-measures one-way ANOVA followed by Bonferroni's multiple comparison test. ¶P<0.05 and ‡P<0.01 vs. the BGL at time 0 in the glucose control group. †P<0.05 and §P<0.01 vs. the BGL at each time-point in the glucose control group. BGL, blood glucose level; AUC, area under the curve.

total, 6/26 herb extracts, olive, white willow, rooibos, bearberry, green tea and coltsfoot, significantly inhibited α-glucosidase activity *in vitro*. However, 3/26 herb extracts, hemp, cayenne pepper and bitter orange peel, induced significant α-glucosidase activation. Although the mechanisms underlying the effects of these herbs are unknown, it was speculated that the inherent values of the herb extracts in the measurement of absorbance had an effect on the measurement of α-glucosidase activity.

Olive leaf (*Olea europea*) is widely recognized as a natural resource with potential beneficial effects (15). Previous studies have reported that 80% aqueous ethanol extract of olive significantly inhibited BGLs in starch-intubated into fasting healthy or streptozotocin (STZ)-injected diabetic Sprague-Dawley rats, and treatment with olive extract was associated with a beneficial hypoglycemic effect in patients with diabetes (16). In addition, hot water and 98% ethanol extract was revealed to inhibit α-amylase activity, and olive powder and its phytochemicals reduced BGLs in GK/Jcl rats (known as type 2 diabetic rats) orally treated with starch (17). Furthermore, hydroxytyrosol and oleuropein, phytochemicals in olive extracts were revealed to significantly inhibit α-glucosidase activity (18). Thus, both these previous results and those of the present study indicate that olive extract may induce a decrease in BGLs via an α-glucosidase inhibitory effect.

Tea produced from rooibos (*Aspalathus lineari)* leaf is generally known as 'rooibos tea' or 'rooibos tisane' (19). Moreover, rooibos is classified as red or green depending on the presence or absence of fermentation treatment (19). Pharmacologically, rooibos is traditionally used in the treatment of asthma, colic, eczema, headache, nausea and mild depression; it has also been used as an antihypertensive, immune stimulant, laxative, sedative and spasmolytic agent, as well as for the treatment of atherosclerosis and diabetes (19). A previous *in vitro* study reported that 65, 75 or 85˚C aqueous extracts from red (fermented) rooibos significantly inhibited α-glucosidase activity (20). However, an *in vivo* study by Ulicná *et al* (21) revealed that hot aqueous extracts from fermented rooibos did not significantly affect plasma glucose, glycated hemoglobin and fructosamine levels in STZ-induced diabetic Wistar rats; similar results were also reported by Ayeleso *et al* (22). Therefore, these studies and the present results indicated that the hot-water extract of red rooibos may not affect BGLs via α-glucosidase inhibitory effects *in vivo*.

White willow, the white bark of *Salix alba*, is known to have an anti-inflammatory effect resulting from the suppression of prostaglandin synthesis by salicin, the main component of white willow (23). However, there are few experiments on the hyperglycemic and/or α-glucosidase inhibitory effects of the white willow. The present results indicated that the white willow extract inhibited α-glucosidase activity *in vitro*; however, more detailed *in vivo* experiments are required in the future.

Bearberry (*Arctostaphylos uva-ursi*) is one of the most commonly used antimicrobial botanicals for the treatment of urinary tract infections (24); however, only a few studies have been conducted to investigate its hyperglycemic and/or α-glucosidase inhibitory effects. Huerta *et al* (25) reported that 80˚C aqueous extracts of bearberry leaves inhibited α-amylase and α-glucosidase activity *in vitro*. Moreover, the present results demonstrated that bearberry extracts had ≥50% inhibition of α-glucosidase activity (IC_{50}=801.3 μg/ml). However, Swanston-Flatt *et al* (26) revealed that the use of standard powdered diets containing powdered bearberry *ad libitum* did not induce a reduction of the increasing BGLs in STZ-injected adult male mice. In contrast, in the present

study, administration of bearberry extract reduced the increasing BGLs until 0.5 h in maltose-treated rats, but not in glucose-treated rats. Furthermore, the administration of bearberry extract in rats did not reveal any effect on AUCs, an indicator of glucose absorption. These differences may be because the present study used extracts different from those used by Swanston-Flatt *et al* (26). Therefore, the details of this result may become more evident in future studies using an animal model of diabetes. However, it can be speculated that bearberry can inhibit α-glucosidase activity, resulting in antihyperglycemic activity.

Camellia sinensis leaf is used to produce various types of teas, such as green tea, black tea and oolong tea; in particular, green tea is one of the most widely consumed beverages in the world (27). Previous studies on green tea, including those focusing on its antihyperglycemic, α-glucosidase inhibitory and antidiabetic activities, have been reported *in vitro*, *in vivo* and in clinical research (28-33). Furthermore, the present results demonstrated that each extract of green tea extract had ≥50% inhibition (IC_{50}=744.6 μg/ml), and administration of green tea extract reduced the increasing BGLs at 0.5 h in maltose-treated rats, but not in glucose-treated rats. However, the administration of green tea extract in rats had no effect on AUCs. Moreover, similar results have been revealed by Nishiumi *et al* (34); OGTT in mice when fed a control diet or high-fat diet with water, hot-water extracts of green tea or black tea demonstrated a in reduction the BGLs. Furthermore, the effect on AUCs was not found in control diet-fed mice, but was reduced in high-fat diet-fed mice (34). In addition, the intake of their extracts prevented the impairment of glucose transporter type 4-dependent glucose transport in muscle in high-fat diet-fed mice. Yang *et al* (35) revealed that weight reduction, alleviation of metabolic syndrome and risk reduction in diabetes were only observed in individuals who consume ≥3-4 cups of tea (600-900 mg tea catechins) daily. Thus, based on all these results it was speculated that green tea may inhibit α-glucosidase activity, resulting in antihyperglycemic activity. Furthermore, the various effects of green tea may reduce the risk of diabetes.

Numerous parts of coltsfoot (*Tussilago farfara*), including the flower buds, flowers, leaves and roots, have been traditionally used for the treatment of several conditions, such as colds, asthma, influenza, gastroenteritis diarrhea, metabolic stimulation and wounds in China, North Africa, Siberia and Europe (36). Moreover, Uysal *et al* (37) reported that methanolic extracts from the leaves of coltsfoot exhibited significant α-glucosidase activity. In addition, Gao *et al* (38) and Sun *et al* (39) revealed that methanolic extracts from the flower buds of coltsfoot exhibited maltase inhibitory activity, with maltose as a substrate. However, the effects of hot-water extract obtained from the leaves of coltsfoot remain unknown. The present results demonstrated that coltsfoot extract had ≥50% inhibition (IC_{50}=724.0 μg/ml), and administration of coltsfoot extract reduced the increasing BGLs until 0.5 h, not only in maltose-treated rats, but also in glucose-treated rats. However, the administration of coltsfoot extract in rats had no effect on AUCs. These results indicated that the reducing effect of these extracts on increasing BGLs results not only from the inhibition of α-glucosidase activity, but also from the absorption of dietary glucose. However, the effect of the absorption of dietary glucose requires further examination in future experiments, such as increasing BGLs without intestinal absorption.

In conclusion, the present results revealed that the hot-water extracts from olive, white willow, red rooibos, bearberry, green tea and coltsfoot inhibited α-glucosidase activity *in vitro*. Moreover, bearberry, green tea and coltsfoot extracts suppressed BGL increase following maltose administration, due to their α-glucosidase inhibitory effects. Furthermore, it was demonstrated that coltsfoot extract exerted inhibitory effects on glucose absorption, as well as α-glucosidase activity. Collectively, the present results may facilitate the understanding of the novel functionality in traditional herbs, which will aid in the prevention of disease onset and progression in hyperglycemia and type 2 diabetes, among other diseases. However, to assess the effective use of the present findings, the active substance(s) need to be identified and the improving effects of hyperglycemia should be examined using a diabetic animal model.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

KS conceived this study, and with HK had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the design of the study. NT and SE acquired data. HK, NT, SY and YH analyzed and interpreted the data. HK and NT wrote the draft of the manuscript, and KS and YH revised it critically for important intellectual content. All authors participated in the preparation of this manuscript, revised it critically for important intellectual content and approved the version submitted for publication.

Ethics approval and consent to participate

All experiments and the care and handling of animals were approved by the Josai University Institutional Animal Care and Use Committee.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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