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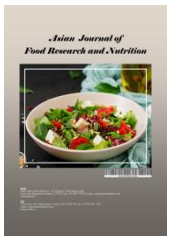

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Dietary Isosaponarin is Intestinally Metabolized to Isovitexin, Most of Which are Excreted in Feces without Being Absorbed

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TH and JL contributed equally to this study. Author TH designed the study and wrote the first draft of the manuscript. Author JL managed the analyses of the study, performed the statistical analysis and wrote the protocol. Author KK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Objective: The metabolism of isosaponarin was investigated using a Caco-2 intestinal epithelial model and animal experiment.
Background: Isosaponarin is a flavonoid in wasabi (*Wasabia japonica*) leaves and has unique structure, in which two glucose molecules bind to apigenin through *O*-glycosidic and *C*-glycosidic bonds.
Materials and Methods: The absorption and metabolism of isosaponarin was investigated by a Caco-2 intestinal epithelial model *in vitro* and a single oral administration to mice *in vivo*.
Results: These experiments showed that isosaponarin was hardly absorbed into the body. However, isosaponarin was metabolized to isovitexin (apigenin-6-*C*-glucoside) by hydrolysis of *O*-

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glycosidic bond. This hydrolysis was mainly caused at small intestine, and the gastric acid in the stomach might partially contribute to the hydrolysis. Both Caco-2 intestinal epithelial model and animal experiment indicated that isovitexin was also not absorbed into the body, and that half of the administered isosaponarin was excreted as isovitexin in feces.

Conclusion: Half of the administered isosaponarin was metabolized to isovitexin in the intestinal tract and then excreted, and the rest was probably degraded by intestinal microflora. Therefore, it was suggested that the bioavailability of dietary isosaponarin is very low.

Keywords: Isosaponarin; isovitexin; wasabi; Wasabia japonica; Caco-2 cells; ICR mice.

1. INTRODUCTION

Wasabi (*Wasabia japonica*) is a Japanese indigenous plant belonging to the Brassicaceae, and the beneficial effects including the appetite improvement and the antimicrobial activity have been well known from ancient times as reviewed by Chadwick et al. [1] and Hashimoto et al. [2]. The whole plant of wasabi including rhizomes, lateral roots, stalks, leaves, and flowers can be used as foodstuffs in Japan, *e.g.* the grated fresh wasabi rhizome is used as a popular condiment in Japanese cuisine such as *sushi*, *sashimi*, and *soba*. The stalks and leaves are used as the ingredients of wasabi paste condiment, and the stalks, leaves and flowers are also used for *tsukemono* (Japanese pickles) and *tempura*. On the other hand, 6-methylsulfinylhexyl isothiocyanate in wasabi has been reported to possess several health promoting activities related with cell cycle progression [3,4], and drug-metabolizing enzymes [5]. Furthermore, Nagai et al. [6] reported that isosaponarin (apigenin-6-*C*-glucosyl-4'-*O*-glucoside) (Fig. 1) derived from wasabi leaves promotes the production of type I collagen in human fibroblasts, and this compound is blended in cosmetics nowadays. Lu et al. [7] reported the inhibitory effect of isosaponarin on glutamate release in rat synaptosomes. According to these benefitable reports, isosaponarin is also expected to use as functional food materials. However, there is no information on the absorption and metabolism of dietary isosaponarin.

In general, flavonoid glycosides, particularly the mono-glucosides are absorbed into the body through two major pathways of the small intestine [8]. The first pathway is mediated by sodium-dependent glucose transporter-1 (SGLT-1) on the intestinal cellular surface, and flavonoid glycosides are absorbed through SGLT-1 as their glycoside forms [9]. Following the absorption, aglycones are released from the glycosides by intracellular β-glucosidases [10]. In the second pathway, lactase phlorizin hydrolase (LPH) on the intestinal cellular membrane hydrolyzed flavonoid glycosides to the aglycone and sugar moiety followed by the absorption of aglycones into the intestinal cells by simple diffusion [11]. Most of intracellular flavonoid aglycones incorporated into intestine are conjugated with sulfate and/or glucuronic acid in the intestinal cells, followed by entering into the blood and/or lymph [12]. Many studies have been reported that absorption and metabolism of flavonoids depend on their chemical structures [13,14].

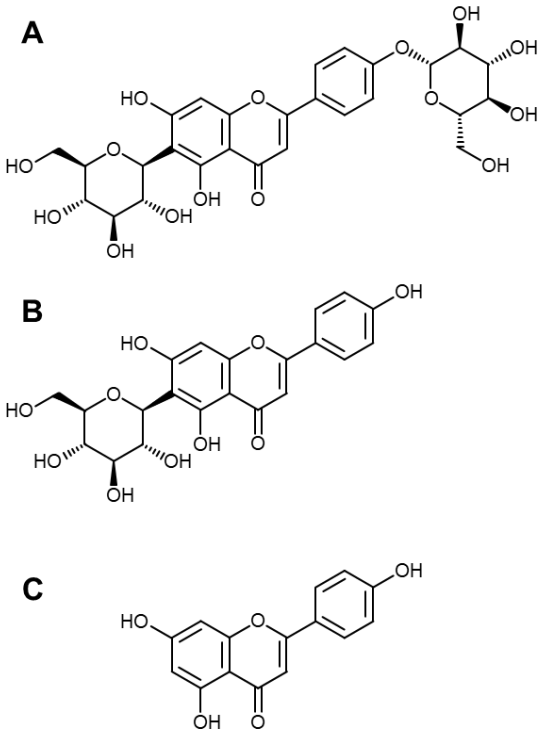

Fig. 1. Chemical structures of isosaponarin (A), isovitexin (B) and apigenin (C)

Thus, absorption and metabolism of flavonoid *O*-glycosides have been well studied. On the other hand, several flavonoid *C*-glycosides also naturally occur in plants [15-18]. It is likely that flavonoid *C*-glycosides is hardly metabolized and

absorbed into the body from small intestine [19]. Isosaponarin have two glucose units; *i.e.,* one is *O*-glycoside, and another is *C*-glycoside, as shown in Fig. 1. In the present study, the absorption and metabolism of isosaponarin was investigated by a Caco-2 intestinal epithelial model *in vitro* and a single oral administration to mice *in vivo*, on the basis of the prospect that this compound would be hydrolyzed to isovitexin (apigenin-6-*C*-glucoside) and/or apigenin in the gastrointestine.

2. MATERIALS AND METHODS

2.1 Cell Culture

Human colon carcinoma Caco-2 cells (passage number 45) were obtained from the Riken Cell Bank (Ibaraki, Japan). Cells were maintained in a complete Dulbecco's Modified Eagle's Medium with 4,500 mg/ml glucose (DMEM; Sigma-Aldrich, St. Louis, MO) supplemented 50,000 U/L penicillin G, 50,000 μg/L streptomycin and 10% fetal bovine serum (FBS; BioWest, Nuaille, France), in a humidified atmosphere of 5% CO_2 - 95% air at 37˚C. Cells were sub-cultured at 80 - 90% confluency.

2.2 Caco-2 Intestinal Epithelial Model

Caco-2 cells at passage of 50 - 60 were used for an intestinal epithelial model, which was performed with the BD BioCoat™ HTS Caco-2 Assay System (BD Bioscience, Bedford, MA) according to the manufacture's protocol. Caco-2 monolayer was estimated by the transepithelial electrical resistance (TEER) values and the lucifer yellow permeability assay. The TEER value was routinely measured with the Millicell®-ERS system (Millipore) according to the method described previously [20]. When the TEER value of Caco-2 monolayer, which was calculated as [(the TEER value of an insert seeded cells) – (the TEER value of a blank insert without cells)] × growth area (0.3 cm^2), was more than 350 Ω•cm^2, the Caco-2 monolayer was judged to complete the formation of the intestinal epithelial model.

The lucifer yellow permeability assay was performed according to the protocol "Lucifer Yellow Permeability Assay Using BD Falcon™ HTS 96-Multiwell Insert Systems" provided by BD Bioscience with some modifications to adjust for 24-multiwell inserts. In a complete Caco-2 intestinal epithelial model, the amount of lucifer yellow in basolateral compartment was less than 1% of lucifer yellow added to the apical compartment.

2.3 Permeability Assay of Isosaponarin and Isovitexin in a Caco-2 Intestinal Epithelial Model

The medium was removed from both apical and basolateral compartments, and then the Caco-2 monolayer in the insert were gently washed with HBSS (pH 7.4) for 30 min in an incubator. The inserts were set on an assay plate, the Enhanced Recovery Plate (BD Bioscience). The test compound, isosaponarin or quercetin, dissolved in DMSO at 10 mM was diluted in HBSS at 10 μM, and the solution (500 μl) was gently added to the apical compartment. Isosaponarin was kindly provided from Kinjirushi (Aichi, Japan), and quercetin was purchased from Extrasynthese (Genay, France). The basolateral compartment was immediately added 1,000 μl of HBSS. After 0.5, 1, or 2 h of incubation at 37°C, HBSS in the apical and basolateral compartments were separately collected as apical and basolateral solutions, respectively. The apical and basolateral compartments were added 200 μL and 800 μL of methanol, respectively, and shaken for 30 min on a reciprocal shaker at 37°C. The sum of methanol was collected as a cellular extract. The apical and basolateral solutions and cellular extract were added 5 μl of 100 μM flavone (Nacalai Tesque; Kyoto, Japan) as an internal standard. The basolateral solution and cellular extract were divided into two aliquots, and these aliquots and the apical solution were evaporated with a centrifugal concentrator. The dried residues were stored at -80°C until HPLC analysis. To determine the concentration of conjugates, one of the aliquots from the basorateral solution and cellular extract was dissolved in 50 μl of distilled water and incubated at 37°C for 45 min with 50 μl of 0.2 M acetate buffer (pH 5.0) containing 20 units of sulfatase/β-glucuronidase (sulfatase (≥ 10,000 unit/g solid) type H-1 from *Helix pomatia* containing β-glucuronidase (≥ 300 unit/mg solid at pH 5.0); Sigma-Aldrich). The mixture was added same volume (100 μl) of methanol and centrifuged at 11,000 × *g* for 10 min, and the supernatant was subjected to HPLC analysis. To determine the concentration of aglycones, the dried residues from another aliquot and the apical solution were dissolved in 100 μl of 50% methanol (v/v) filtered through a 0.2-μm membrane filter (Millex-LG, Millipore), and subjected to a HPLC analysis.

2.4 HPLC Analysis

Isosaponarin, isovitexin and apigenin were quantitatively analyzed with the Hitachi HPLC system (Tokyo, Japan) equipped with a pump (L-7100), a column oven (L-7300), an UV-VIS detector (L-7420), and a D-7000 chromatography data station software. These flavonoids were monitored with a wavelength at 340nm, and the column used was a Capcell pak C18 UG120 column (250 mm × 4.6 mm i.d., Shiseido, Tokyo, Japan) maintained at 35°C and joined with a guard column (10 mm × 4.0 mm i.d., Shiseido). The mobile phase consisted of (A) 50 mM sodium phophate adjusted to pH 3.3 with phosphoric acid, methanol (9:1, v/v) and (B) sodium phophate (pH 3.3), methanol (3:7, v/v). The gradient program started at 1.0 mL/min at 30% B, 30 - 50% B in 10 min, 50 - 80% B in 10 min, 80 - 100% B in 20 min and then 100% B for 10 min. In all analyses, the column was re-equilibrated at 30% B for 8 min. The injection volume was 10 µl.

2.5 Animal Experiments

The animal treatment was approved by the institutional Animal Care and Use Committee (permission number 20-05-11) and carried out according to the Guidelines on Animal Experimentation of Kobe University. Female ICR mice (6 weeks old; Japan SLC, Shizuoka, Japan) were maintained with standard diet Labdiet® 5L37 (Japan SLC) in a temperature-controlled room at 22 - 25°C with 12-h light/dark cycles, and acclimated for 1 week before animal experiments. Mice were fasted overnight but allowed free access to drinking water. Mice were then administered isosaponarin (50 mg/kg body weight) dissolved in distilled water by gavage. Twelve mice were housed in metabolic cages to collect the feces and sacrificed 8, 12, 24 and 48 h after the administration by collecting blood from the heart under anesthesia, while nine mice were housed in plastic cages and sacrificed 1, 2, and 4 h after the administration in the same way. Mice were physically normal without decrease in body weight and diarrhea throughout the experiment, and the food and water intake were not different from control mice. The mice were anesthetized with pentobarbital sodium, and the blood was collected from the heart with a heparinized syringe at corresponding time points. The stomach, small intestine, and large intestine were carefully removed, and the gastrointestinal remnants were separately collected by perfusion with 3 ml of ice-cold PBS and transferred to conical tubes. These tissues and remnants were immediately frozen by liquid nitrogen and stored at -80°C until HPLC analysis. The plasma was prepared from the blood by centrifugation at 450 × *g* for 15 min at 4°C. The 0-h control mice were administered nothing.

2.6 Extraction of Isosaponarin, Isovitexin and Apigenin from Plasma

Forty micro-liter of plasma was transferred into a microtube, and incubated at 37°C for 45 min with 40 µl of 0.1 M acetate buffer (pH 5.0) with or without 20 units of sulfatase/β-glucuronidase. The mixture was added 5 µl of 100 µM flavone as an internal standard and 450 µl of methanol, and agitated with a vortex mixer. After centrifugation at 2,000 × *g* for 15 sec, the 400 µl of supernatant was transferred to a new microtube. The residue was added 400 µl of methanol again and centrifuged at 2,000 × *g* for 15 sec to obtain methanol extract. This extraction process was repeated twice, and the sum of supernatant was evaporated with a centrifugal concentrator. The dried residue was dissolved in 100 µl of 50% (v/v) methanol and filtered through a 0.2-µm membrane filter, and analyzed on HPLC.

2.7 Extraction of Isosaponarin, Isovitexin and Apigenin from Gastrointestinal Tissues, its Remnants and Feces

The extraction of isosaponarin, isovitexin and apigenin from the tissues, gastrointestinal remnants and feces was performed according to the method described previously [21] with some modifications. In brief, the tissues; stomach, small intestine, and large intestine, were minced with surgical scissors, added 4 ml of 50% methanol (v/v) and 5 µl of 100 µM flavone as an internal standard, and homogenized thrice at 2,500 rpm for 7 sec by a cell disruptor, Multi-beads shocker. On the other hand, the gastrointestinal remnants and feces were homogenized with 6 volumes of 50% methanol (v/v) by the same method. The homogenates were agitated with a vortex mixer, sonicated for 15 min and centrifuged at 1,780 × *g* for 10 min. The supernatants were collected, agitated with a vortex mixer and filtered through a 0.2-µm membrane filter. The filtrate was subjected to a HPLC analysis.

3. RESULTS

3.1 Assembly of Caco-2 Intestinal Epithelial Model by 5-day Method

To investigate the intestinal absorption of isosaponarin, a Caco-2 intestinal epithelial model assembled by a 5-day method was used for isosaponarin permeability assay. The Caco-2 intestinal epithelial model was estimated by a TEER value and a lucifer yellow permeability assay. To compare a 5-day method used in the present study and a 21-day method used in the previous study [20], 3 nmol of quercetin or quercetin glucoside was subjected to the model assembled by a 5-day method in prior to the isosaponarin permeability assay. The amount of quercetin in the apical compartment was decreased in a time-dependent manner, while that of quercetin glucoside was hardly changed at 0.5, 1 and 2 h after the addition (Fig. 2). These results were consistent with the results of the previous report [20]. Therefore, the Caco-2 monolayer assembled by a 5-day method used in the present study was able to be used as an intestinal epithelial model for isosaponarin permeability assay as well as that by a 21-day method.

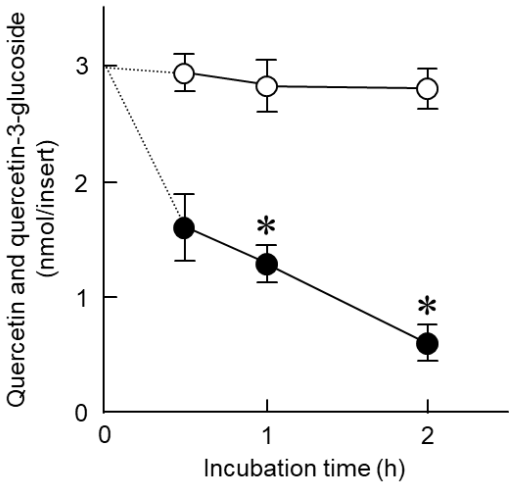

Fig. 2. Amounts of quercetin and quercetin glucoside in apical solutions

*Quercetin (solid circle) decreased rapidly after addition to the apical compartment, whereas quercetin glycosides (open circle) hardly decreased little after addition. Data are expressed as means ±SD (n=6). *p < 0.05 compared with the corresponding values at 0.5 h*

3.2 Isosaponarin Permeability Assay

Five hundred micro-liter of 10 μM isosaponarin (5 nmol) was added to the apical compartment of the Caco-2 intestinal epithelial model, and the amounts of isosaponarin and the prospected metabolites, isovitexin and apigenin, were measured by HPLC analysis in the apical solution, cellular extract and basolateral solution 0.5, 1 and 2 h after the addition (Table 1). Isosaponarin in the apical compartment slightly decreased to 4.9 nmol at 0.5 and 1 h, and 4.8 nmol at 2 h, while isosaponarin in the cellular extract and isovitexin in the apical compartment slightly increased. However, it is unlikely that these metabolism and absorption was due to the aggressive capacity of cells, because these changes were very small. Apigenin was undetected in any fractions. Thus, isosaponarin was hardly received metabolism and absorption in the Caco-2 intestinal epithelial model. In addition to isosaponarin, isovitexin was also subjected to the permeability assay with the Caco-2 intestinal epithelial model. More than 95% of isovitexin added to the apical compartment remained in the apical compartment 0.5 and 1 h after the addition. Apigenin were undetected in both apical and basolateral compartments (Table 2). These results indicated the isovitexin were also not received deglycosidation and absorption at the small intestine.

3.3 Oral Administration of Isosaponarin in Mice

To confirm the results from the Caco-2 intestinal epithelial model under the *in vivo* condition, mice were orally administered 2.5 μmol isosaponarin (approximately 50 mg/kg body weight) dissolved in distilled water, and the concentration of isosaponarin and the prospected metabolites, isovitexin and apigenin, in the plasma was determined by HPLC analysis. The recovery percentage of flavones, an internal standard, was more than 98.5%. The neither isosaponarin nor prospected metabolites were undetected in the intact plasma and sulfatase-treated plasma within 48 h under the HPLC condition used in the present study (Fig. 3). On the other hand, isovitexin was slightly detected in the homogenates of gastrointestinal tissues, *i.e.*, stomach (0.31% of equivalent amounts of isosaponarin administered mice), small intestine (4.9%), and large intestine (2.5%) (Table 3). Since these amounts were exceedingly small, isovitexin might be adsorbed on the surface of the tissues, *i.e.* isovitexin was not absorbed into the tissues. In addition, isosaponarin and apigenin were undetectable in these tissues (data not shown). These results indicate that

isosaponarin and/or the prospected metabolites are not absorbed into the body *in vivo*, and are consistent with the results from the Caco-2 intestinal epithelial model.

Table 1. The isosaponarin permeability assay with Caco-2 intestinal epithelial model

Incubation time (h)	Fractions	Isosaponarin (nmol/insert)[1]	Isovitexin (nmol/insert)	Apigenin (nmol/insert)
0.5	Apical solution	4.92 ± 0.06 (98.4 ± 1.2) [2]	0.083 ± 0.002 (1.65 ± 0.06) [3]	N.D.
	Cellular extract	0.13 ± 0.00 (2.55 ± 0.04)	N.D.	N.D.
	Basolateral solution	N.D.	N.D.	N.D.
1	Apical solution	4.88 ± 0.04 (97.6 ± 0.8)	0.085 ± 0.001 (1.70 ± 0.02)	N.D.
	Cellular extract	0.15 ± 0.01 (2.95 ± 0.01)	N.D.	N.D.
	Basolateral solution	N.D.	N.D.	N.D.
2	Apical solution	4.80 ± 0.07 (96.0 ± 1.4)	0.22 ± 0.02 (4.35 ± 0.40)	N.D.
	Cellular extract	0.17 ± 0.00 (2.95 ± 0.01)	N.D.	N.D.
	Basolateral solution	N.D.	N.D.	N.D.

Data are expressed as means±SD (n=6). N.D., not detected.
[1] The concentration of flavonoids without conjugation
[2] Parentheses show the recovery % of amounts of isosaponarin added to the insert (5 nmol/insert).
[3] Parentheses show the recovery % of equivalent amounts of isosaponarin added to the insert (5 nmol/insert).

Table 2. The isovitexin permeability assay with Caco-2 intestinal epithelial model

Incubation time (h)	Fractions	Isovitexin (nmol/insert)	Apigenin (nmol/insert)
0.5	Apical solution	4.77 ± 0.07 [1] (95.3 ± 1.3) [2]	N.D.
	Cellular extract	N.D.	N.D.
	Basolateral solution	N.D.	N.D.
1	Apical solution	4.87 ± 0.11 (97.3 ± 2.1)	N.D.
	Cellular extract	N.D.	N.D.
	Basolateral solution	N.D.	N.D.

Data are expressed as means ± SD (n=6). N.D., not detected
[1] Concentration of flavonoids without conjugation
[2] Parentheses show the recovery % of equivalent amounts of isosaponarin added to the insert (5 nmol/insert).

Table 3. Isovitexin in the gastrointestines of isosaponarin-administered mice

Time (h)	Stomach (μmol)	Small intestine (μmol)	Large intestine (μmol)
1	0.0078 ± 0.0025 [1] (0.31 ± 0.10) [2]	0.12 ± 0.05 (4.9 ± 1.9)	0.061 ± 0.031 (2.5 ± 1.2)
2	0.0023 ± 0.0015 (0.09 ± 0.06)	0.012 ± 0.003 (0.47 ± 0.12)	0.059 ± 0.003 (2.4 ± 0.1)
4	0.0023 ± 0.0018 (0.09 ± 0.07)	0.0045 ± 0.0035 (0.18 ± 0.14)	0.013 ± 0.003 (0.50 ± 0.12)
8	0.0025 ± 0.0018 (0.10 ± 0.07)	N.D.	N.D.

Data are expressed as means ± SD (n=3). N.D., not detected.
[1] Amounts of isovitexin without conjugation
[2] Parentheses show the recovery % of equivalent amounts of isosaponarin administered mice (2.5 μmol).

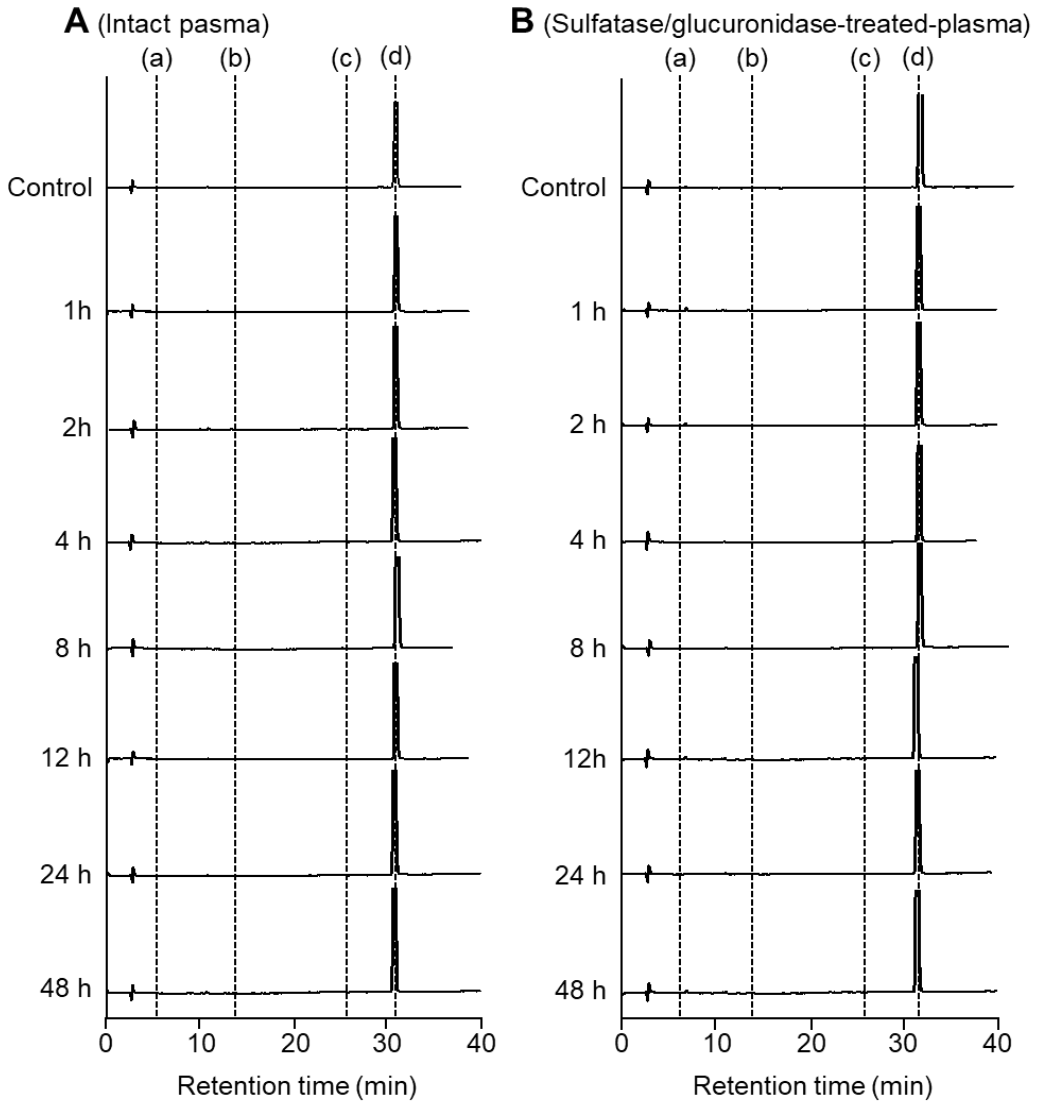

Fig. 3. Chromatogram of plasma after oral administration of isosaponarin

(a), (b), (c), and (d) indicated the retention times for isosaponarin (5.8 min), isovitexin (13.6 min), and apigenin (25.7 min), and (d) flavone (32.5 min) as an internal standard, respectively.

3.4 Isosaponarin and the Metabolites in the Gastrointestinal Remnants

Because isosaponarin and the prospected metabolites were unlikely to be absorbed into the body, the amounts in the gastrointestinal remnants and feces were determined by HPLC analysis. Thirty six percent of the original dose (2.5 μmol) remained in the gastric remnants 1 h after the administration, and isosaponarin gradually decreased to 1.7% at 12 h (Fig. 4A). Although isosaponarin was also detected in the small and large intestinal remnants, the maximum amounts were less than 1% of the original dose (Figs. 4B-C). Isovitexin equivalent to 8.6% of the administered isosaponarin was detected in the gastric remnants 1 h after the administration, and then the amount was gradually decreased to less than 1% at 12 h (Fig. 4A). The amounts of isovitexin in the small and large intestinal remnants were equivalents to 13% (Fig. 4B) and 28% (Fig. 4C), respectively, 1 h after the administration. The amounts of isovitexin in the small and large intestinal remnants were obviously higher than that of isosaponarin detected at the same time points.

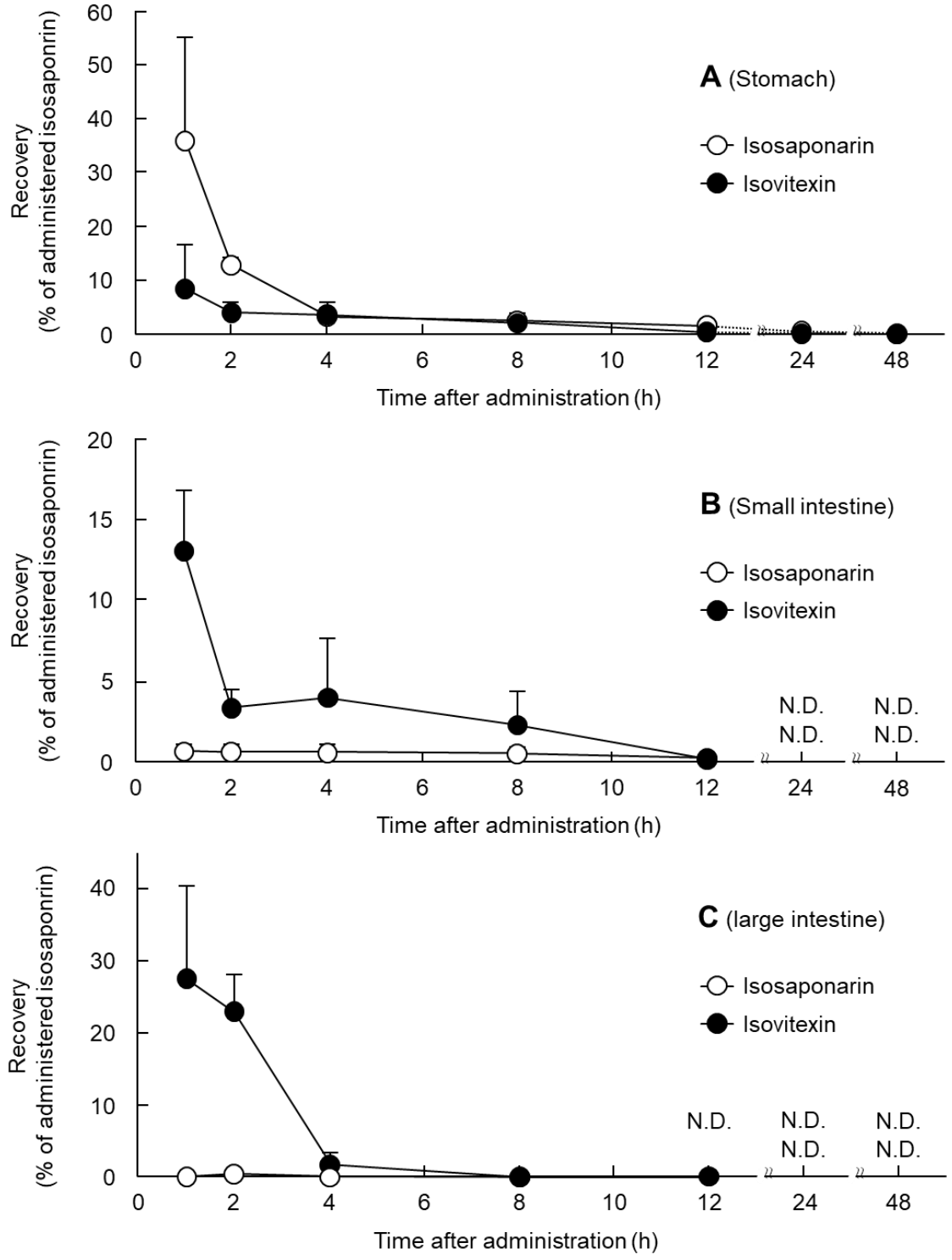

Fig. 4. The recovery of isosaponarin and isovitexin in the gastrointestinal remnants
Data are expressed as means ±SD (*n*=3). N.D., not detected.

Furthermore, isovitexin was detected in the feces but isosaponarin was not. The isovitexin excreted into the feces for 8 h accounted for 51% equivalents of dosed isosaponarin (Fig. 5), and did not increase thereafter. This result suggests that most of isovitexin was excreted into the feces within 8 h after the administration while a little isovitexin and isosaponarin remained in the

gastrointestinal remnants. Apigenin were not detected in the gastrointestinal remnants and feces throughout the experiments. These results indicated that isosaponarin was mainly metabolized to isovitexin in the stomach or small intestine, and most isovitexin was not absorbed into body and was excreted with feces.

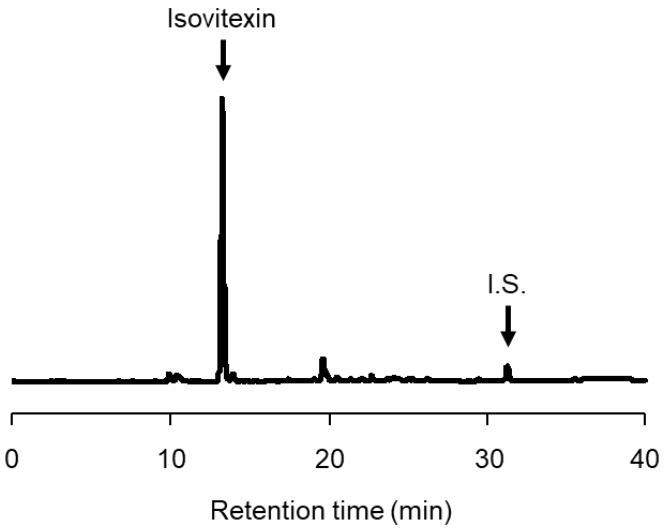

Fig. 5. Representative chromatogram of feces of mice

HPLC analysis detected isovitexin extracted from the feces, but not isosaponarin. Isovitexin and flavone as internal standard (I.S.) were detected at 13.6 min and 32.5 min, respectively

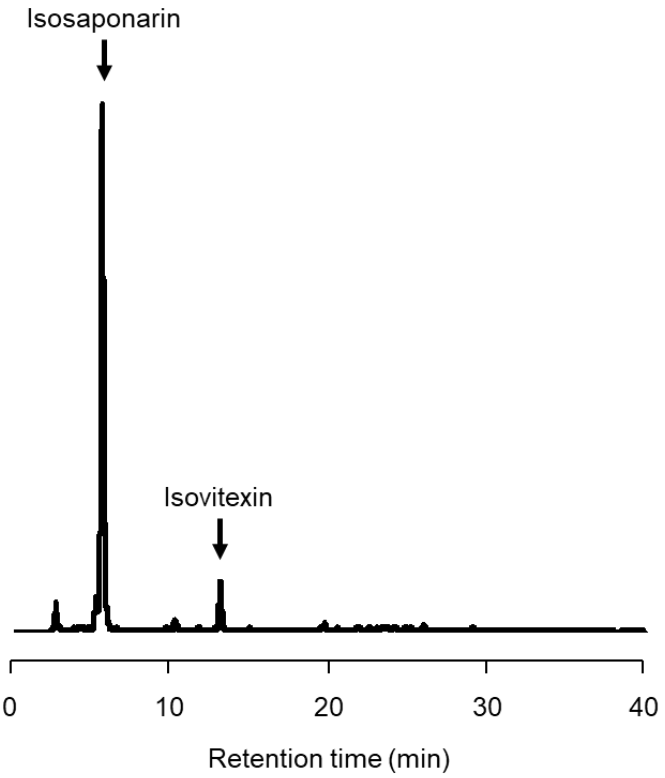

Fig. 6. Chromatogram of HCl-hydorolyzed isosaponarin

Isosaponarin and isovitexin were detected at 5.8 min and 13.6 min, respectively.

3.5 Hydrochloric Acid Hydrolysis of Isosaponarin *in vitro*

To investigate whether isosaponarin is metabolized to isovitexin by gastric acid in the stomach, 0.15 μmol of isosaponarin was incubated with 2N HCl at 37°C for 30 min. Isosaponarin very slightly decreased to 0.148 μmol, and isovitexin was slightly detected at 0.009 nmol (Fig. 6). Therefore, the acidic hydrolysis was minor deglycosidation of isosaponarin to isovitexin. This result suggests that isosaponarin is metabolized to isovitexin mainly in small intestinal tract, although a small amount of isosaponarin may be metabolized to isovitexin by gastric acid in stomach.

4. DISCUSSION

The aim of the present study was to investigate the absorption and metabolism of isosaponarin derived from wasabi (*Wasabia japonica*) leaves. The Caco-2 intestinal epithelial model and animal experiment indicated that isosaponarin and isovitexin were hardly absorbed into the body. The animal experiment suggested that isosaponarin was metabolized to isovitexin, and isovitexin equivalent to the approximately half of administered isosaponarin was excreted with feces within 8 h after the administration. Thus, the present study demonstrated that dietary isosaponarin was hardly absorbed into the body and preferably metabolized to isovitexin, and isovitexin was also hardly absorbed.

Isosaponarin (apigenin-6-*C*-glucosyl-4'-*O*-glucoside) have two glucose units in the structure; one is bound to the OH group at 4'-position on the B-ring of apigenin aglycone by *O*-glycosidic bond, and another one is bound to the carbon at 6-position on the A-ring by *C*-glycosidic bond. In the present study, apigenin was not detected in the any experiments; the Caco-2 intestinal epithelial model (Table 1), animal experiment (Fig. 3), and HCl hydrolysis experiment *in vitro* (Fig. 6), although isovitexin (apigenin-6-*C*-glucoside) was detected as an intestinal metabolite (Table 3, Figs. 4B-C). These results were indicated that deglycosylation of *O*-glucoside occurred in the gastrointestineal tract but the deglycosylation of *C*-glucoside was not. Quercetin-4'-*O*-glucoside was reported to be hydrolyzed by LPH [20], suggesting that *O*-glucoside at 4'-position on flavonoids is likely to be hydrolyzed by LPH. In the present study, the animal experiment demonstrated that isovitexin increased at small intestine immediately after the administration (Fig. 4B). Furthermore, the HCl hydrolysis experiment showed that most isosaponarin was hardly hydrolyzed by gastric acids though it was slightly influenced. These results suggested that isosaponarin was mainly metabolized to isovitexin by LPH, which hydrolyzed *O*-glucoside of isosaponarin at small intestine, while the acidic hydrolysis by gastric acid is also considered as the minor metabolic pathway of isosaponarin.

Zhang et al. [19] demonstrated that flavone *C*-glucosides; orientin (luteolin-8-*C*-glucoside), homoorientin (luteolin-6-*C*-glucoside), vitexin (apigenin-8-*C*-glucoside) and isovitexin were poorly absorbed in the gastrointestinal tract, and 21% of *C*-glucosides were excreted in the feces at 24 h. Thus, most flavonoid *C*-glycosides are unlikely to be absorbed at gastrointestinal tracts and excreted in the original form. In the present study (Fig. 3), isosaponarin and isovitexin were undetected in the plasma at any time points in this study, suggesting that isovitexin and isosaponarin was not absorbed in the original forms at the intestine. On the other hand, certain flavonoid *C*-glycosides were reported to be absorbed in the original form. For example, puerarin (daidzein-8-*C*-glucoside) was rapidly absorbed from the intestine without metabolism, and mainly excreted in the urine as the hydroxylated derivatives [22]. And it was also reported that puerarin was partially hydrolyzed to aglycone in the body [23]. Further study is needed to clarify the differences between the unabsorbed and absorbable flavonoid *C*-glycosides.

Flavonoid *C*-glycosides have been considered to contribute a diverse range of biological activities including the antimicrobial activity [24], and antioxidative activity [25]. Isosaponarin have been reported to promote the biosynthesis of type I collagen in human fibroblasts *in vitro* [6] and to inhibit to release glutamate in rat synaptosomes [7], but there is no information on the biological activity of dietary isosaponarin *in vivo*. Since dietary isosaponarin is immediately metabolized to isovitexin in the present study (Fig. 4), some health beneficial effects of isovitexin are expected rather than that of isosaponarin following the intake of isosaponarin. A recent study demonstrated that oral administration of isovitexin (15 mg/kg) has an anti-hyperglycemic action in rats [26]. Huang et al. [27] demonstrated that isovitexin suppressed the release of tumor necrosis factor α (TNF-α), production of prostaglandin E2, and expression

of cyclooxygenase-2 in lipopolysaccharide-activated RAW264.7 macrophages. It is well known that TNF-α production is increased under chronic hyperglycemia, and TNF-α has harmful effects on insulin sensitivity [28]. The suppressive effects of isovitexin on TNF-α production in intestinal macrophages [27] may contribute to the anti-diabetic activity *in vivo* [26]. In addition to isovitexin, daily oral administration of luteolin-6-*C*-glucoside (isoorientin) was reported to show subacute hypoglycaemic effect on streptozotocin-induced diabetic rats [29]. Thus, the consumption of flavonoid *C*-glycosides showed anti-diabetic activity *in vivo*. Dietary isosaponarin may also contribute to the prevention and treatment of diabetes.

Although most of isovitexin metabolized from isosaponarin were excreted with feces (Fig. 5), isovitexin might be also considered to be catabolized by intestinal microflora. Zhang et al. [19] proposed the metabolic pathway of isovitexin degradation initiated by the intestinal microflora; *i.e.*, intestinal microflora hydrolyzes *C*-glucoside of isovitexin and cleaves C-ring, resulting in the production of phloroglucinol and phloretic acid from A-ring and B-ring of apigenin, respectively. In this study, recovery amounts in the feces were approximately half of the administered isosaponarin (Fig. 5), indicating that the other half was considered to be catabolized to the small phenolic molecules such as phloroglucinol and phloretic acid by intestinal microflora. These phenolic compounds are reported to have a several biological activities; *e.g.*, the protective effects of phloroglucinol on ionizing radiation-induced cell damage through inhibition of oxidative stress *in vitro* and *in vivo* [30] the protective effect of phloroglucinol on myocardial ischaemia-reperfusion injury [31], and the antibacterial activity of phloretic acid [32]. It may be necessary to examine the pharmacokinetics of catabolites such as phloroglucinol and phloretic acid after ingestion of isosaponarin.

5. CONCLUSION

The present study showed that dietary isosaponarin is hardly absorbed into the body and metabolized to isovitexin in the gastrointestinal tract. In other words, the bioavailability of isosaponarin is very low, suggesting that they cannot be expected to have beneficial functions as dietary isosaponarin. On the other hand, most of isovitexin is excreted with feces, but isovitexin and its catabolites by intestinal microflora may be beneficial to human health. Further study is needed to elucidate the health-promoting effects of isovitexin and its catabolites.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chadwick CI, Lumpkin TA, Elberson LR. The botany, uses and production of *Wasabia japonica* (Miq.) (Cruciferae) Matsum Econ. Bot. 1993;47(2):113-35.
2. Hashimoto T, Yamada T, Nagai M, Yamada K, Tanaka M, Shimoaki T, et al. Wasabi. In: Govil JN,Singh VK, editors. Recent progress in medicinal plants ethonomedicune: Source & Mechanism, Studium Press LLC (USA). 2010;30:65-84.
3. Kinae N, Masuda H, Shin IS, Furugori M, Shimoi K. Functional properties of wasabi and horseradish. BioFactors. 2000;13:265-9.
4. Uto T, Fujii M, Hou D-X. 6-(Methylsulfinyl) hexyl isothiocyanate suppresses inducible nitric oxide synthase expression through the inhibition of Janus kinase 2-mediated JNK pathway in lipopolysaccharide-activated murine macrophages. Biochem Pharmacal. 2005;70:1211-21.
5. Morimitsu Y, Nakagawa Y, Hayashi K, Fujii H, Kumagai T, Nakamura Y, et al. A sulforaphane analogue that potently activates the Nrf2-dependent detoxification pathway. J Biol Chem. 2002;5:3456-63.
6. Nagai M, Akita K, Yamada K, Okunishi I. The effect of isosaponarin isolated from wasabi leaf on collagen synthesis in human fibroblasts and its underlying mechanism. J Nat Med. 2010;64(3):305-12.
7. Lu CW, Yeh KC, Chiu KM, Lee MY, Lin TY, Wang SJ. The effect of isosaponarin derived from wasabi leaves on glutamate release in rat synaptosomes and its underlying mechanism. Int J Mol Sci. 2022;23(15):8752.
8. Spencer JP, Chowrimootoo G, Choudhury R, Debnam ES, Srai SK, Rice-Evans C. The small intestine can both absorb and glucuronidate luminal flavonoids. FEBS Lett. 1999;458(2):224-30.
9. Walgren RA, Lin J-T, Kinne RK-H, Walle T. Cellular uptake of dietary flavonoid quercetin 4'-β-glucoside by sodium-

dependent glucose transporter SGLT1. J Pharmacol Exp Ther. 2000; 294(3):837-43.

10. Ioku K, Pongpiriyadacha Y, Konishi Y, Takei Y, Nakatani N, Terao J. β-Glucosidase activity in the rat small intestine toward quercetin monoglucosides. Biosci Biotechnol Biochem. 1998;62:1428-31.
11. Day AJ, Canada JF, Diaz JC, Kroon PA, Mclauchlan R, Faulds CB, et al. Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. FEBS Lett. 2000;468(2-3):166-70.
12. Rice-Evans C. Flavonoids and isoflavones: absorption, metabolism, and bioactivity. Free Radic Biol Med. 2004; 36,827-8.
13. Walle T. Absorption and metabolism of flavonoids. Free Radic Biol Med. 2004;36:829-37.
14. Graefe EU, Wittig J, Mueller S, Riethling AK, Uehleke B, Drewelow B, et al. Pharmacokinetics and bioavailability of quercetin glycosides in humans. J Clin Pharmacol. 2001;41:492-9.
15. Murota K, Shimizu S, Miyamoto S, Izumi T, Obata A, Kikuchi M, et al. Unique uptake and transport of isoflavone aglycones by human intestinal caco-2 cells: comparison of isoflavonoids and flavonoids. J Nutr. 2002;132(7):1956-61.
16. Abou-Zaid MM, Lombardo DA, Kite GC, Grayer RJ, Veitch NC. Acylated flavone C-glycosides from Cucumis sativus. Phytochemistry. 2001;58:167-72.
17. Krafczyk N, Glomb MA. Characterization of phenolic compounds in rooibos tea. J Agric Food Chem. 2008; 56,3368-76.
18. Joubert E. HPLC quantification of the dihydrochalcones, aspalathin and nothofagin in rooibos tea (*Aspalathus linearis*) as affected by processing. Food Chem. 1996;55:403-11.
19. Zhang Y, Tie X, Bao B, Wu X, Zhang Y. Metabolism of flavone C-glucosides and p-coumaric acid from antioxidant of bamboo leaves (AOB) in rats. Br J Nutr. 2007;97:484-94.
20. Murota K, Shimizu S, Chujo H, Moon J-H, Terao J. Efficiency of absorption and metabolic conversion of quercetin and its glucosides in human intestinal cell line Caco-2. Arch Biochem Biophys. 2000;384(2):391-7.
21. Walton MC, Hendriks WH, Broomfield AM, McGhie TK. Viscous food matrix influences absorption and excretion but not metabolism of blackcurrant anthocyanins in rats. J Food Sci. 2009;74:22-9.
22. Prasain JK, Jones K, Brissie N, Moore R, Wyss JM., Barnes S. Identification of puerarin and its metabolites in rats by liquid chromatography-tandem mass spectrometry. J Agric Food Chem. 2004;52(12):3708-12.
23. Yasuda T, Kano Y, Saito K, Ohsawa K. Urinary and biliary metabolites of puerarin in rats. Biol Pharm Bull. 1995;18:300-3.
24. Hultin PG. Bioactive C-glycosides from bacterial secondary metabolism. Curr Top Med Chem. 2005;5:1299-331.
25. Fanz G, Grun M. Chemistry, occurrence and biosynthesis of C-glycosyl compounds in plants. Planta Med. 1983;47:131-40.
26. Folador P, Cazarolli LH, Gazola AC, Reginatto FH, Schenkel EP, Silva FR. Potential insulin secretagogue effects of isovitexin and swertisin isolated from *Wilbrandia ebracteata* roots in non-diabetic rats. Fitoterapia. 2010;81:1180-7.
27. Huang ST, Chen CT, Chieng KT, Huang SH, Chiang BH, Wang LF, et al. Inhibitory effects of a rice hull constituent on tumor necrosis factor α, prostaglandin E2, and cyclooxygenase-2 production in lipopolysaccharide-activated mouse macrophages. Ann N Y Acad Sci. 2005;1042:387-95.
28. Cao XY, Wang XH, Ma SL, Yang XJ, Wang XQ, Ding H, et al. Study of relationship between stress hyperglycemia and insulin-resistance related factors. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue. 2006;12:751-4.
29. Sezik E, Aslan M, Yesilada E, Ito S. Hypoglycaemic activity of *Gentiana olivieri* and isolation of the active constituent through bioassay- directed fractionation techniques. Life Sci. 2005;76(11):1223-38.
30. Kang KA, Zhang R, Chae S, Lee SJ, Kim J, Kim J, et al. Phloroglucinol (1,3,5-trihydroxybenzene) protects against ionizing radiation-induced cell damage through inhibition of oxidative stress in vitro and in vivo. Chem Biol Interact. 2010;185:215-26.
31. Li TT, Zhang YS, He L, Li NS, Peng J, Li YJ. Protective effect of phloroglucinol against myocardial ischaemia-reperfusion injury is related to inhibition of myeloperoxidase activity and inflammatory

cell infiltration. Clin Exp Pharmacol Physiol. 2011;38:27-33.

32. Huberman L, Gollop N, Mumcuoglu KY, Breuer E, Bhusare SR, Shai Y, et al. Antibacterial substances of low molecular weight isolated from the blowfly, *Lucilia sericata*. Med Vet Entomol. 2007;21:127-31.

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