Bioscience, Biotechnology, and Biochemistry

ISSN: 0916-8451 (Print) 1347-6947 (Online) Journal homepage: https://www.tandfonline.com/loi/tbbb20

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To cite this article: Kyoji YOSHINO, Yuko MIYAUCHI, Takashi KANETAKA, Yasutaka TAKAGI & Kunimasa KOGA (2009) Anti-Diabetic Activity of a Leaf Extract Prepared from *Salacia reticulata* in Mice, Bioscience, Biotechnology, and Biochemistry, 73:5, 1096-1104, DOI: 10.1271/bbb.80854

To link to this article: https://doi.org/10.1271/bbb.80854

Published online: 22 May 2014.

Biosci. Biotechnol. Biochem., **73** (5), 1096–1104, 2009

Anti-Diabetic Activity of a Leaf Extract Prepared from *Salacia reticulata* in Mice

Kyoji YOSHINO,[1] Yuko MIYAUCHI,[1] Takashi KANETAKA,[2] Yasutaka TAKAGI,[3] and Kunimasa KOGA[3,†]

[1]*Department of Chemistry and Biochemistry, Numazu College of Technology, 3600 Ooka, Numazu, Shizuoka 410-8501, Japan*
[2]*Seiko Co., Ltd., 50-6 Higashino, Nagaizumi-cho, Shizuoka 411-0931, Japan*
[3]*School of High Technology for Human Welfare, Tokai University, 317 Nishino, Numazu, Shizuoka 410-0395, Japan*

Received December 3, 2008; Accepted January 25, 2009; Online Publication, May 7, 2009
[doi:10.1271/bbb.80854]

The effects of a water extract prepared from the leaves of *Salacia reticulata* on the absorption of sugars in normal and type 1 diabetic mice were investigated. The simultaneous oral administration of the extract at a dose of 1.0 mg/mouse with maltose or sucrose inhibited the postprandial elevation of the plasma glucose and insulin levels and intestinal α-glucosidase activities in mice. In addition, the supply of a 0.01% solution of the extract as drinking water prevented the elevation of the plasma glucose level and intestinal α-glucosidase activities in type 1 diabetic mice. This treatment also prevented the elevation of the plasma, pancreatic, and kidney lipid peroxide levels, lowering of the plasma insulin level, and elevation of the kidney aldose reductase activities in diabetic mice. These results suggest that the water extract of the leaves of *S. reticulata* could be a beneficial food material for the prevention of diabetes and obesity because of its multiple effects.

Key words: *Salacia reticulata*; diabetes; α-glucosidase; aldose reductase; mouse

The frequency of both diabetes and obesity in the worldwide population is high and rising. Diabetes is a complex metabolic disorder caused by insulin insufficiency and/or insulin dysfunction[1)] characterized by aberrant blood glucose and insulin levels, especially after food intake. Furthermore, it is also characterized by polydipsia, polyphagia, glycosuria, frequent urination and blurred vision, and acetone breath resulting from an abnormal increase in the amount of ketone bodies in the blood is also known as a symptom in diabetic patients. Diabetes is classified into two types: type 1 (insulin-dependent) and type 2 (insulin-independent). Type 1 diabetes is caused by insulin insufficiency due to the lack of functional β-cells in the pancreas. Type 2 diabetes includes all cases of diabetes except those that are insulin-dependent. The causes of type 2 diabetes are complex, although one of the main causes is insulin dysfunction. Type 2 diabetes is the more common form of diabetes, accounting for 90% of the diabetic population.

An effective method for controling these carbohydrate-dependent diseases would be to restrict intestinal carbohydrate digestion. Starch and sucrose account for 80–90% of our daily intake of carbohydrates. Digestive enzymes convert starch to maltose and isomaltose. Together with sucrose, these disaccharides are converted to monosaccharides (glucose and fructose) by small intestinal α-glucosidases (AGc, EC3.2.1.20) and absorbed. Thus, intestinal AGcs such as maltase and sucrase play an important role in carbohydrate digestion and absorption. An inhibitor of intestinal AGc is useful to prevent diabetes and obesity by retarding carbohydrate digestion and absorption. Potent AGc inhibitors such as acarbose[2)] and voglibose[3)] have been applied clinically in the treatment of diabetic and obese patients. Prevention of these diseases has resulted in great deal of research interest in the physiological functions of food components. The AGc inhibitory effects of extracts from such plants as ezoshige,[4)] tochu-cha,[5)] welsh onion[6)] and clove,[7)] and the effects of such natural products as D-xylose[8)] and tea polyphenols[9)] have been reported *in vitro* and/or *in vivo*. We have previously reported that a 50% ethanol extract prepared from rosemary (*Rosmarinus officinalis*) inhibited rat intestinal AGc (maltase and sucrase) activity and significantly suppressed the postprandial elevation of the blood glucose level in mice after the administration of maltose or sucrose, and increased the blood glucose level in streptozotocin (STZ)-induced diabetic mice.[10)] STZ is widely used to induce type 1 diabetes.[11)] The dietary prevention of diabetes and obesity make it preferable to identify additional food materials with AGc inhibitory effects.

Water extracts of the stems or roots of some plants of *Salacia* sp. (family Hippocastanaceae) have been used for the herbal therapy of diabetes in one of the principles of traditional Indian medicine, Ayurveda, in India and Sri Lanka. In particular, the anti-diabetic effects of *Salacia reticulata* and *S. oblonga*, and their AGc inhibitory effects have been reported.[12)] In Sri Lanka,

† To whom correspondence should be addressed. Fax: +81-55-968-1156; E-mail: koga@wing.ncc.u-tokai.ac.jp

Abbreviations: AGc, α-glucosidase; AR, aldose reductase; MDA, malondialdehyde; STZ, streptozotocin; TBARS, thiobarbituric acid reactive substance

the plants of *Salacia* sp. are called "Kothala himbutu" in Sinhalese. The preventive effects of extracts of their stems or roots on the postprandial elevation of the blood glucose level have also been demonstrated in diabetic patients[13–15] and diabetic or obese rats.[16,17] Furthermore, some constituents of the plants of *Salacia* sp. are also known to be inhibitors of aldose reductase (AR, EC1.1.1.21).[18–20] AR is the first and rate-limiting enzyme on the polyol pathway,[21] and AR-dependent excess synthesis of polyols, mainly sorbitol converted from glucose, may be one of the mechanisms leading to such diabetic complications[22] as cataracts,[23] neuropathy,[24] nephropathy,[25] and retinopathy.[26] Therefore, extracts of the stems or roots of the plants of *Salacia* sp. could be expected to effectively prevent diabetes and these pathogenic complications.

If water extracts prepared from the leaves of *Salacia* sp. can prevent diabetes and obesity similarly to extracts of the stems or roots, it would be economical with regard to cost, drying facilities, and speed of production. We investigated in the present study the inhibitory effects of water extracts from the leaves and stems of *S. reticulata* and from the leaves of *S. oblonga* on AGc and AR activities *in vitro*. Furthermore, their preventive effects on the postprandial elevation of plasma glucose level in mice and the anti-diabetic effect on an STZ-induced mouse model were also examined.

Materials and Methods

Preparation of various extracts of S. reticulata *and* S. oblonga. Dried leaves and stems of *S. reticulata* grown in Sri Lanka and leaves of *S. oblonga* grown in India (Ruta Co., Osaka, Japan) were powdered with a multi-bead disintegrator (Yasui Kikai, Osaka, Japan) at 2,000 rpm for 10 s. Powdered samples of 10 g were each extracted with 90 ml of water for 2 h at 50 °C with continuous shaking (120 rpm), before centrifugation at 5,000 rpm for 20 min at 10 °C. Each supernatant was lyophilized as its water extract. The precipitate after water extraction of the leaves of *S. reticulata* or *S. oblonga* was further extracted with 90 ml of 60% ethanol and centrifuged. The supernatant was lyophilized as the 60% ethanol extract. The resulting precipitate was extracted with 90 ml of 99.8% ethanol and centrifuged. The supernatant was lyophilized as the 99.8% ethanol extract. The amounts of water, protein, lipid, ash, carbohydrates, fiber, and polyphenols in each water extract from the leaves and stems of *S. reticulata* were determined by Japan Food Research Lab. (Tokyo, Japan). All chemicals used in this study were of reagent grade.

Determination of the amounts of various polyphenol compounds in the water extract from the leaves of S. reticulata. The amount of mangiferin in the water extract from the leaves of *S. reticulata* was determined by the high-performance liquid chromatographic (HPLC) method of Yoshikawa *et al.*[19] with a slight modification. The water extract was analyzed by HPLC (HP2000Plus; Jasco Co., Tokyo, Japan), using a Develosil ODS-SR column (2.0 × 250 mm, 5 µm; Nomura Chemical Co., Aichi, Japan). The mobile phase, consisting of a methanol–1% acetic acid solution (30:70), was pumped at 0.6 ml/min. Mangiferin was detected spectrophotometrically at 360 nm. The amounts of (−)-epicatechin (EC) and (−)-epigallocatechin (EGC) in the water extract from the leaves of *S. reticulata* were determined by the HPLC method.[27] The water extract was analyzed by HPLC (LC-10AD; Shimadzu Co., Kyoto, Japan), using a TSKgel ODS 80Ts column (4.6 × 250 mm, 5 µm; Tosoh Co., Tokyo, Japan) held at 30 °C. The mobile phase, consisting of a 0.1 M NaH_2PO_4 buffer (pH 2.5)–acetonitrile (87:13) containing 0.1 mM EDTA2Na, was pumped at 1.0 ml/min. The catechins were detected electrochemically at an applied potential of 0.6 V.

Determination of the in vitro *AGc activity in rat intestinal powder with addition of the extracts of* S. reticulata *and* S. oblonga. The *in vitro* AGc inhibitory test was performed by using a crude AGc solution prepared from rat intestinal acetone powder (Sigma Chemical Co., St. Louis, MO, USA). The inhibitory effect on the rat intestinal AGc activity was determined by using a slight modification of the method of Asano *et al.*[8] Briefly, 0.5 g of rat intestinal acetone powder was suspended in 15 ml of a 0.1 M phosphate buffer (pH 6.5) before sonication (3 times for 1.0 min). After centrifugation (at 3,000 rpm for 30 min), the resulting supernatant was used as the enzyme solution in the assay. A solution of 2% maltose (Wako Pure Chemicals Ind., Osaka, Japan) or 4.0% sucrose (Wako Pure Chemicals Ind.) in a 0.1 M phosphate buffer was used as the substrate solution. First, 0.8 ml of the substrate solution, 0.1 ml of the enzyme solution, and 0.1 ml of 0–400 µg/ml of the sample solution were mixed well and incubated at 37 °C for 30 min. The extracts of *S. reticulata* and *S. oblonga* were used as samples. After stopping the reaction by adding 0.1 ml of a 0.05 N NaOH solution, the amount of glucose produced in the reaction mixture was determined by the glucose oxidase method, using a TGO reagent.[28] The TGO reagent was prepared by mixing 1.0 mg of glucose oxidase (200 U/mg; Wako Pure Chemicals Ind.) and 0.3 mg of horseradish peroxidase (100 U/mg; Wako Pure Chemicals Ind.) in 0.5 ml of a 0.5 M Tris buffer (pH 7.0) with 0.5 ml of a 3,3′-dimethoxybenzidine solution (50 mg 3,3′-dimethoxybenzidine/5.0 ml of ethanol) and 1.0 ml of a Triton X-100 solution (1.0 ml of Triton X-100/4.0 ml of ethanol) made up to 100 ml with a 0.5 M Tris buffer (pH 7.0). Then, 0.1 ml of the enzymatic reaction mixture was added to 3.0 ml of the TGO reagent and incubated at 37 °C for 30 min. After incubation, 0.1 ml of a 4 N HCl solution was added, and the optical density at 420 nm was determined. The mean of three independent experiments was calculated.

Animals. Four-week-old male ddY mice were purchased from Japan SLC (Hamamatsu, Japan). The body weights of the mice used in this study were 25–28 g. The animals were housed in a room at 24 ± 1 °C with a 12-h light-dark cycle. Throughout the experiment, the animals were handled in accordance with the Guide for Animal Experiments in Numazu National College of Technology.

Determination of the plasma glucose and insulin levels, and small intestinal AGc activities in mice after an oral administration of disaccharide (maltose or sucrose) or monosaccharide (glucose) and a water extract of S. reticulata *or* S. oblonga. The effect of a water extract from the leaves or stems of *S. reticulata* or the leaves of *S. oblonga* on the elevation of the plasma glucose level in mice after an oral administration of maltose or sucrose was examined according to a modification of the method of Asano *et al.*[8] Briefly, 4-week-old male ddY mice were starved for 24 h, but given tap water *ad libitum*. The mice were assigned to 5 groups, each being given one of the following sample: (i) 0.6 ml of water (normal group), (ii) 160 mg of maltose in 0.6 ml of water, (iii) 160 mg of maltose and 1.0 mg of the water extract from the leaves of *S. reticulata* in 0.6 ml of water, (iv) 160 mg of maltose and 1.0 mg of the water extract from the leaves of *S. oblonga* in 0.6 ml of water, and (v) 160 mg of maltose and 1.0 mg of the water extract from the stems of *S. reticulata* in 0.6 ml of water. There were 6 mice in each group. The mice were sacrificed under diethyl ether anesthesia 30 min after administration of these solutions, and blood was obtained by heart puncture with a heparinized syringe. Plasma was prepared by centrifugation at 1,500 rpm for 5 min, and the plasma glucose level was determined with a Glucose C2 test kit (Wako Pure Chemicals Ind.; the mutarotase-glucose oxidase method). The plasma insulin level in the mice administered with the extract from the leaves or stems of *S. reticulata* was determined by using a Lebis® Insulin-Mouse (T type) ELISA kit (Shibayagi Co., Gunma, Japan). The mouse small intestine was also obtained after the animal experiments and homogenized in 19 volumes of saline to determine the AGc (maltase and sucrase) activities. The reaction mixture consisting of the homogenate diluted 3.3 times and 18 mg/ml of maltose or sucrose in a 0.1 M citrate buffer (pH 6.3) was incubated at 37 °C for 1 h. The amount of glucose produced in the reaction mixture was determined by using a Glucose C2 test kit. The same experiment was also performed by using sucrose or glucose instead of maltose.

Determination of the plasma glucose and insulin levels, and small intestinal AGc activities in STZ-induced diabetic mice administered with the water extracts of S. reticulata *and* S. oblonga. The effect of the water extract from the leaves or stems of *S. reticulata* or the leaves of *S. oblonga* on the elevation of the plasma glucose level of STZ-induced diabetic mice was examined according to a modification of the methods used in previous studies.[29–32] STZ was purchased from Wako Pure Chemicals Ind., and used as the inducer for an insulin-dependent diabetic model. Briefly, 4-week-old male ddY mice were divided into 5 groups, each receiving one of the following treatments: (i) intraperitoneal injection of 0.2 ml of water and a supply of drinking water (normal group), (ii) injection of a 2.5% STZ solution and a supply of drinking water, (iii) injection of STZ and a supply of a 0.01% aqueous solution of the water extract from the leaves of *S. reticulata* as drinking water, (iv) injection of STZ and a supply of a 0.01% aqueous solution of the water extract from the leaves of *S. oblonga* as drinking water, (v) injection of STZ and a supply of a 0.01% aqueous solution of the water extract from the stems of *S. reticulata* as drinking water. All the mice were fed with commercial laboratory feed (Rodent Lab Diet EQ 5L37; PMI Nutrition International, St. Louis, MO, USA), with drinking water or the extract solution of *S. reticulata* or *S. oblonga ad libitum* during the experimental period. There were 6 mice in each group. The mice were sacrificed under diethyl ether anesthesia 0, 1, and 4 d after injecting STZ, and blood was obtained. Mouse plasma was prepared as already described, and the plasma glucose level was determined by using a commercial kit. The mouse small intestine, pancreas, and kidney were obtained 4 d after injecting STZ. The plasma insulin level 4 d after injecting STZ was also determined by using a commercial kit. The AGc activities in the mouse small intestine were determined as already described.

Determination of lipid peroxide levels in the plasma, pancreas, and kidney of STZ-induced diabetic mice administered with a water extract of S. reticulata *or* S. oblonga. The mouse pancreas and kidney removed in the foregoing experiment were homogenized in 9 volumes of a 40 mM phosphate buffer (pH 7.4) to determine lipid peroxides as thiobarbituric acid reactive substances (TBARS). TBARS in mouse plasma and these organs were determined by the fluorometric method of Yagi[33] and the colorimetric method of Masugi and Nakamura,[34] respectively. The lipid peroxide level is expressed in terms of malondialdehyde (MDA).

Determination of AR activity with the addition of an extract of S. reticulata *or* S. oblonga in vitro. The *in vitro* AR inhibitory test was performed by using a commercial AR preparation (human recombinant; Wako Pure Chemicals Ind.). The inhibitory effect on AR activity was determined by using a slight modification of the method of Nishimura *et al.*[35] Briefly, 0.1 ml of a 0.03 U/ml AR solution, 0.8 ml of a substrate solution, 0.1 ml of 1.5 mM NADPH, 0.1 ml of 100 mM DL-glyceraldehyde, 0.6 ml of a 1.5 mM phosphate buffer (pH 6.2), and 0.1 ml of a 0–100 μg/ml sample solution were mixed well and incubated at 30 °C for 1 h. The water extracts of *S. reticulata* and *S. oblonga* were used as samples. The enzymatic reaction was stopped by cooling in iced water. The optical density at 340 nm was determined, and the consumption of NADPH in the reaction mixture was estimated. The mean of three independent experiments was calculated.

Determination of AR activities in the kidney of STZ-induced diabetic mice administered with a water extract of S. reticulata *or* S. oblonga. The kidneys removed from STZ-induced diabetic mice were homogenized in 9 volumes of a 5 mM phosphate buffer (pH 7.4) containing 10 mM 2-mercaptoethanol to determine the AR activities in accordance with the method of Iwata *et al.*[36] Briefly, the homogenate was centrifuged for 40 min at 8,000 × *g*, and the supernatant was prepared as the crude enzyme solution. The reaction mixture consisted of 20 μl of the enzyme solution, 10 μl of 75 μM NADPH, 10 μl of 10 mM DL-glyceraldehyde, and 60 μl of a 0.1 M phosphate buffer (pH 6.2), and was incubated at 25 °C for 3 min. The enzyme activities were estimated spectrophotometrically by calculating NADPH oxidation from the decrease of absorbance at 340 nm.

Statistical analyses. All data are presented as the mean and/or mean ± SD. Statistical analyses in this experiment were performed by using Student's *t*-test or Cochran-Cox's modified *t*-test to determine the significance of differences between the appropriate groups, with $P < 0.05$ considered to indicate statistical significance.

Results and Discussion

Yields of various extracts from the leaves and stems of S. reticulata *and the leaves of* S. oblonga

The yields of the water extracts from the leaves and stems of *S. reticulata* and the leaves of *S. oblonga* were 33.0%, 23.5%, and 15.4%, respectively. The respective yields of the 60% ethanol extracts from the leaves of *S. reticulata* and *S. oblonga* were 6.0% and 2.6%, and those of the 99.8% ethanol extracts were 0.4% and 7.7%, respectively. In both cases of the leaves of *S. reticulata* and *S. oblonga*, the yields of the water extracts were much higher than those of the other fractions. The yield of the water extract from the leaves of *S. reticulata* was higher than that of *S. oblonga*. The total yield of the extracts from the leaves of *S. reticulata* (39.4%) was also higher than that of *S. oblonga* (25.7%).

Inhibitory effects of various extracts from the leaves and stems of S. reticulata *and the leaves of* S. oblonga *on the rat intestinal AGc activity*

We investigated the inhibitory effects of the various extracts of the leaves of *S. reticulata* and *S. oblonga* on the rat intestinal AGc activity *in vitro*. As shown in Fig. 1, almost all the extracts assayed in this experiment, with the exception of the 99.8% extract of *S. oblonga*, exhibited an inhibitory effect on the rat intestinal maltose activity at 400 μg/ml. A strong inhibitory effect on the rat intestinal AGc activity was found in the water extracts from the leaves of *S. reticulata* and *S. oblonga*, with respective inhibition ratios of 78.5% and 44.6%. The water extract from the leaves of *S. reticulata* was especially effective. We then compared the inhibitory effect on rat intestinal AGc activity of the water extract from the leaves of *S. reticulata* to that from the stems of *S. reticulata* which is known to act as an AGc inhibitor.[37] The 50% inhibitory concentrations (IC_{50} values) of the water extracts from the leaves and stems of *S. reticulata* were 220 and 31 μg/ml for the maltose substrate, and 110 and 13 μg/ml for the sucrose substrate, as shown in Fig. 2. We have previously reported a natural potent AGc inhibitor, the 50% ethanol

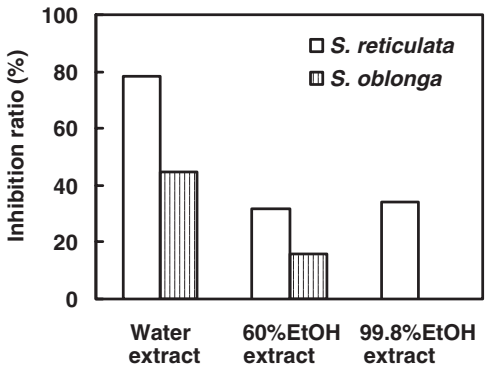

Fig. 1. Inhibitory Effects of the Extracts of the Leaves of *S. reticulata* and *S. oblonga* on the Rat Intestinal AGc Activity.

Each value is the mean of three independent experiments. Sample concentration, 400 μg/ml; substrate, 2.0% maltose.

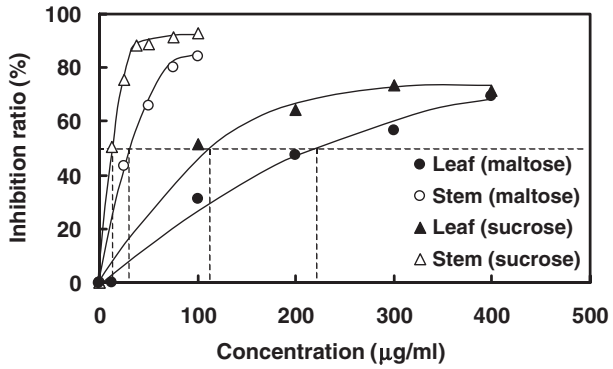

Fig. 2. Inhibitory Effects of Water Extracts Prepared from the Leaves and Stems of *S. reticulata* on the Rat Intestinal AGc Activity.

Each value is the mean of three independent experiments. Substrate, 2.0% maltose or 4.0% sucrose.

Table 1. Main Components of the Water Extracts Prepared from the Leaves and Stems of *S. reticulata*

Component (%, w/w)	Leaf	Stem
Water	6.1	5.2
Protein	8.7	2.8
Lipid	7.5	2.4
Ash	4.5	2.9
Carbohydrates	27.8	10.4
Fiber	45.4	76.3
Polyphenols (as gallic acid)[a]	3.9	0.9

[a]Folin-Denis method

extract of rosemary (*Rosemarinus officinalis*), with respective IC_{50} values of 711 μg/ml and 683 μg/ml for the maltose and sucrose substrates.[10] Even luteolin, one of the main active components in the extract of rosemary, showed inhibitory effects on the AGc activity with IC_{50} values of 173 μg/ml and 126 μg/ml for the maltose and sucrose substrates, respectively. These results suggested that an inhibitory action of the water extract from the leaves of *S. reticulata* on the rat intestinal AGc activity was present, although the activity was weaker than that of the stems. The compositions of water, protein, lipid, ash, carbohydrates, fiber, and polyphenols in the leaves and stems of *S. reticulata* are shown in Table 1. The amounts of almost all components, including polyphenols, determined in this experiment in the leaves of *S. reticulata* tended to be higher than those in the stems. In contrast, only the amount of fiber in the stems of *S. reticulata* was higher than that of the leaves. As AGc inhibitors in the stems or roots of some plants of *Salacia* sp., kotalagenin 16-acetate,[18] kotalanol,[38] mangiferin,[19] and salacinol[39] have been identified. Kotalagenin 16-acetate is a friedelane-type triterpene, and mangiferin is a polyphenol compound. Kotalanol and salacinol both consist of a thiosugar sulfonium sulfate structure. As other polyphenol compounds in the roots of *S. reticulata*, such catechins as EC and EGC have been detected.[19] We determined the contents of mangiferin and these catechins in the water extract from the leaves of *S. reticulata* in this study. The amount of mangiferin was about 0.62% in the leaf extract and was equivalent to 16% of the amount of polyphenols in the leaf extract. The amounts of EC and EGC were about 0.0054% and 0.46% in the leaf extract, respectively. The total amount of these catechins was equivalent to 12% of the amount of polyphenols in the leaf extract. Mangiferin may be one of the active components involved in the AGc inhibitory effect of the leaves of *S. reticulata*, because the amount of polyphenols in the leaves was higher than that in the stems, as shown in Table 1, and the inhibitory activities of EC and EGC on AGc were not as strong as those of the other gallated catechins.[9] However, the specific activity of kotaranol and salacinol is known to be much higher than that of mangiferin.[38,39] Further investigations are necessary to identify the active components in the leaves of *S. reticulata* and *S. oblonga*.

Effects of an oral administration of the water extracts of S. reticulata *and* S. oblonga *on the plasma glucose and insulin levels, and on the intestinal AGc activity in mice administered with disaccharide or monosaccharide*

The plasma glucose levels in the mice administered with maltose or sucrose mixed with the water extracts from the leaves and stems of *S. reticulata* and the leaves of *S. oblonga* are shown in Fig. 3A and D. The plasma respective glucose levels in the mice administered with maltose and sucrose at an oral dose of 160 mg/mouse were 4.8 and 2.1 times higher than those in normal mice 30 min after the administration. Simultaneous oral administration of the water extracts from the leaves and stems of *S. reticulata* and *S. oblonga* at a dose of 1.0 mg/mouse with maltose or sucrose at a dose of 160 mg/mouse significantly inhibited this postprandial elevation of the plasma glucose level in mice. However, the plasma glucose level in mice administered with the water extracts of *S. reticulata* and *S. oblonga* were no lower than those in normal mice. The order of the suppressive activities of these extracts against the postprandial elevation of the plasma glucose level in mice was as follows: stems of *S. reticulata* > leaves of *S. reticulata* ≥ leaves of *S. oblonga*. As shown in Fig. 3B and E, the intestinal maltase or sucrase activity in the mice was significantly higher after an oral administration of maltose or sucrose. This postprandial elevation of intestinal maltase or sucrase activity in the mice was significantly suppressed by the extracts of *S. reticulata* and *S. oblonga*, except for the case of the sucrase activity in mice administered with the extract from the leaves of *S. oblonga*. The order of the suppressive activities of these extracts against the postprandial elevation of intestinal AGc activity in mice was similar to the order for the plasma glucose level as follows: stems of *S. reticulata* > leaves of *S. reticulata* > leaves of *S. oblonga*. The plasma insulin level in mice administered with sucrose at an oral dose of 160 mg/mouse was higher than that in normal mice 30 min after the administration, as shown in Fig. 3C.

We examined the effects of a simultaneous oral administration of the water extracts from the leaves and stems of *S. reticulata* at a dose of 1.0 mg/mouse each on the plasma glucose level in mice administered with glucose at a dose of 160 mg/mouse. As shown in Fig. 3G, the postprandial elevation of plasma glucose level in the mice was not suppressed by the extracts from the leaves and stems of *S. reticulata*. These results suggest that the preventive effects of the water ex-

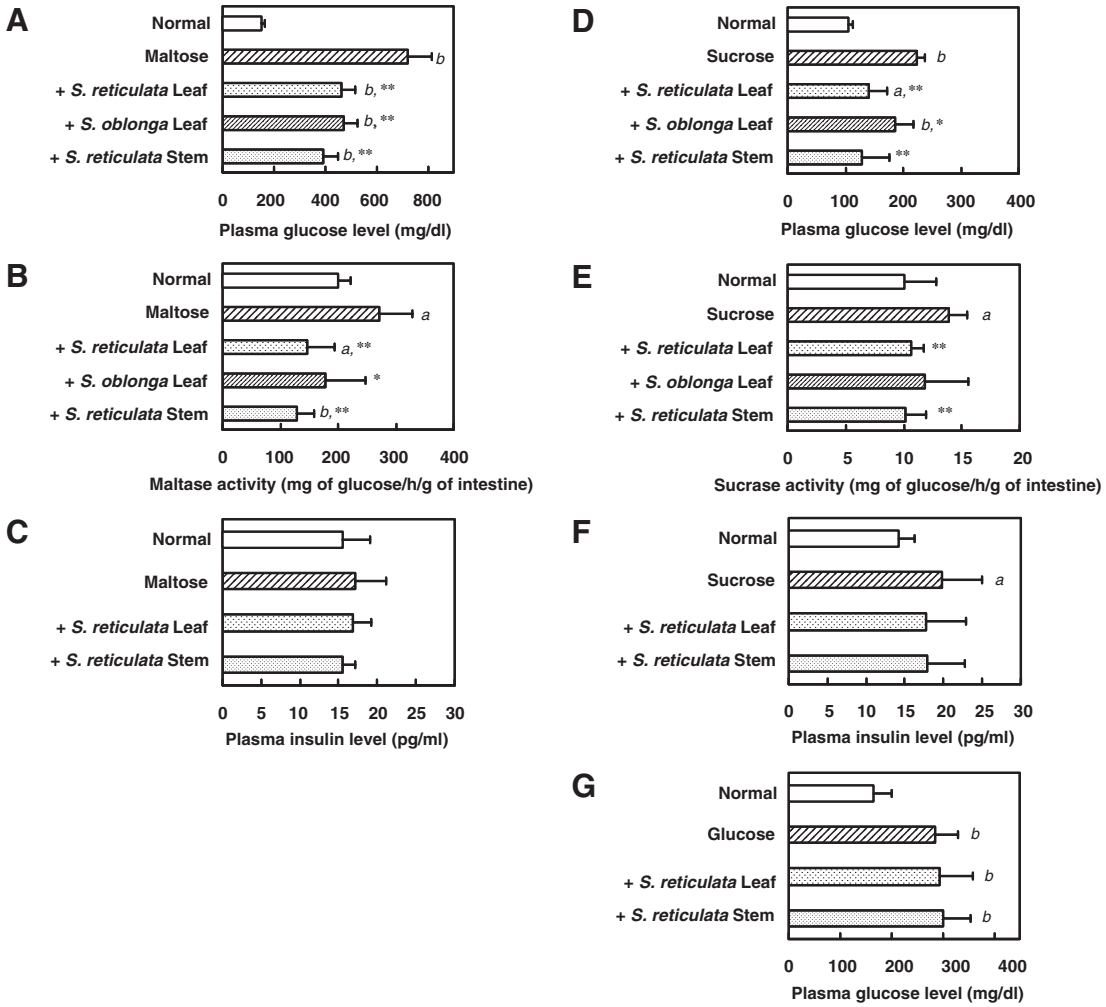

Fig. 3. Effects of Water Extracts Prepared from the Leaves and/or Stems of *S. reticulata* and *S. oblonga* on the Plasma Glucose Level, Intestinal AGc Activity, and Plasma Insulin Level in Mice Administered with Disaccharides (Maltose and Sucrose) or Monosaccharide (Glucose).

A, Plasma glucose level (mg/dl) after the administration of maltose. B, Intestinal maltase activities (mg of glucose produced for 1 h/g of intestine) after the administration of maltose. C, Plasma insulin level (pg/ml) after the administration of maltose. D, Plasma glucose level (mg/dl) after the administration of sucrose. E, Intestinal sucrase activity (mg of glucose produced in 1 h/g of intestine) after the administration of sucrose. F, Plasma insulin level (pg/ml) after the administration of sucrose. G, Plasma glucose level (mg/dl) after the administration of glucose. Mean ± SD ($n = 6$). Dose, 1.0 mg/mouse. Significant differences from the value for the normal group, $^{a}P < 0.05$, $^{b}P < 0.01$; and from the value for the saccharide plus water-administered group, $^{*}P < 0.05$, $^{**}P < 0.01$.

tracts from the leaves and stems of *S. reticulata* and *S. oblonga* on the postprandial elevation of plasma glucose level in mice administered with disaccharides could have mainly been due to their inhibitory effects on intestinal AGc activity and suppression of the absorption of sugars by the mice. The effect of the water extract from the leaves of *S. reticulata* is thought to have been the same as that from the stems in controlling the postprandial elevation of the blood glucose level in the animals.

Effects of water extracts of S. reticulata *and* S. oblonga *on the plasma glucose and insulin levels and small intestinal AGc activity in STZ-induced diabetic mice*

The plasma glucose level in STZ-induced diabetic mice supplied with a 0.01% aqueous solution of the water extracts from the leaves and stems of *S. reticulata* and the leaves of *S. oblonga* as drinking water is shown in Fig. 4A. The plasma glucose levels 1 and 4 d after an intraperitoneal injection of 0.2 ml of a 2.5% aqueous solution of STZ were significantly higher than that in the normal mice. The supply of a 0.01% aqueous solution of the water extracts from the leaves and/or stems of *S. reticulata* and *S. oblonga* as drinking water significantly suppressed the elevation of plasma glucose level in STZ-induced diabetic mice 1 and 4 d after the injection of STZ.

As shown in Fig. 4B and C, the intestinal maltase and sucrase activities in the mice were significantly higher than those in normal mice 4 d after the injection of STZ. It has previously been reported that such intestinal AGc activities as those of maltase, sucrase, isomaltase, and trehalase were elevated in STZ-induced diabetic rats.[40,41] In those previous studies, the intestinal maltase and sucrase activities were found to be highly sensitive to the experimental rat diabetic model, and STZ-induced diabetic rats showed a rapid elevation of the activities of these enzymes. The AGc activities were increased in some rat diabetic models regardless of the type of diabetes. The increase in intestinal AGc activities may be one reason for the postprandial hyperglycemia seen in diabetes. The increase in intestinal AGc activities in rat models of diabetes mellitus could be due to hyper-

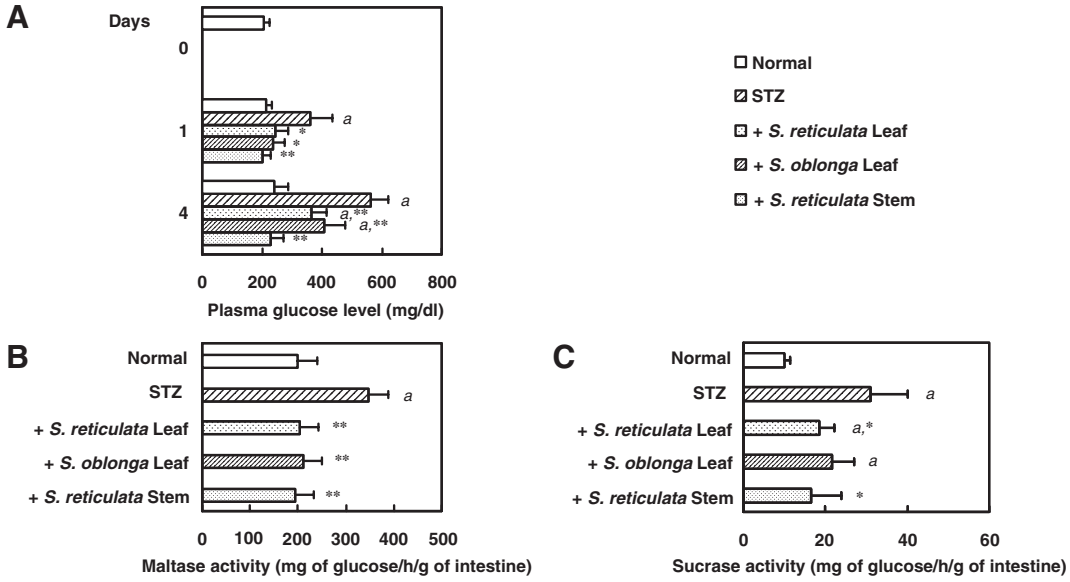

Fig. 4. Effects of Water Extracts Prepared from the Leaves and/or Stems of *S. reticulata* and *S. oblonga* on the Plasma Glucose Level and Small Intestinal AGc Activity in STZ-Induced Diabetic Mice.

A, Plasma glucose level (mg/dl). B, Intestinal maltase activity (mg of glucose produced in 1 h/g of intestine) 4 d after administration. C, Intestinal sucrase activity (mg of glucose produced in 1 h/g of intestine) 4 d after administration. Mean ± SD ($n = 6$). The sample solution was supplied as drinking water at a concentration of 0.01%. Significant differences from the value for the normal group, $^{a}P < 0.01$; and from the value for the STZ group, $^{*}P < 0.05$, $^{**}P < 0.01$.

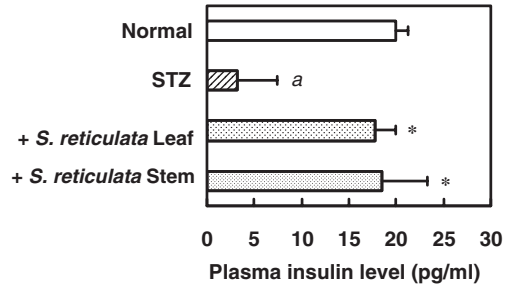

Fig. 5. Effects of Water Extracts Prepared from the Leaves and Stems of *S. reticulata* on Plasma Insulin Level in STZ-Induced Diabetic Mice 4 d after Administration.

Unit, pg/ml. Mean ± SD ($n = 6$). The sample solution was supplied as drinking water at a concentration of 0.01%. Significant differences from the value for the normal group, $^{a}P < 0.01$; and from the value for the STZ group, $^{*}P < 0.01$.

plasia[42] and thereby the resulting increase in the mucosal protein content of the intestines. In the present study, supplying a 0.01% aqueous solution of the extracts of *S. reticulata* and *S. oblonga* as drinking water significantly suppressed the elevation of intestinal AGc activities in mice, except for the case of sucrase activity in the mice administered with the extract from the leaves of *S. oblonga*. The order of the suppressive activities of these extracts against the plasma glucose level and intestinal AGc activity in STZ-induced diabetic mice was as follows: stems of *S. reticulata* > leaves of *S. reticulata* ≥ leaves of *S. oblonga*.

The plasma insulin level 4 d after an intraperitoneal injection of 0.2 ml of a 2.5% aqueous solution of STZ was significantly lower than the level in normal mice, as shown in Fig. 5. Supplying a 0.01% aqueous solution of the water extracts from the leaves and stems of *S. reticulata* as drinking water significantly suppressed this lowering of the plasma insulin level in STZ-induced diabetic mice 4 d after the injection of STZ. These results indicate that the water extract from the leaves of *S. reticulata* could be as effective as the extract of the stems in controlling the postprandial elevation of blood glucose level in type 1 diabetes.

Effects of the water extract of S. reticulata *on the lipid peroxide levels in the plasma, pancreas, and kidney of STZ-induced diabetic mice*

It has recently been reported that the oxidative stress induced by some abnormal conditions, including free-radical production, glycation reactions,[43] and the polyol pathway,[44] could cause some diabetic microvascular complications[45,46] in the kidneys, lens, and aorta.[47,48] STZ produces free radicals in animal bodies and destroys the β-cells in the pancreas with necrosis.[49] In STZ-induced diabetic animals, the levels of lipid peroxidation products have been shown to be elevated in the plasma[50,51] and in the liver, kidney, heart, and muscle.[52] Increased oxidative stress in STZ-induced diabetic rats and mice could result in nephropathy.[53–55]

The plasma, pancreatic, and kidney lipid peroxide levels in STZ-induced diabetic mice supplied with a 0.01% aqueous solution of the water extracts from the leaves and stems of *S. reticulata* as drinking water are shown in Fig. 6. The plasma, pancreatic, and kidney lipid peroxide levels 4 d after an intraperitoneal injection of 0.2 ml of a 2.5% aqueous solution of STZ were significantly higher than the levels in normal mice. The supply of a 0.01% aqueous solution of the water extracts from the leaves and stems of *S. reticulata* as drinking water significantly suppressed the elevation of the plasma, pancreatic, and kidney lipid peroxide levels in STZ-induced diabetic mice 4 d after the injection of STZ. The suppressive effect of the extract from the stems of *S. reticulata* against the plasma, pancreatic, and kidney lipid peroxide levels in STZ-induced diabetic mice tended to be higher than that of the extract from the

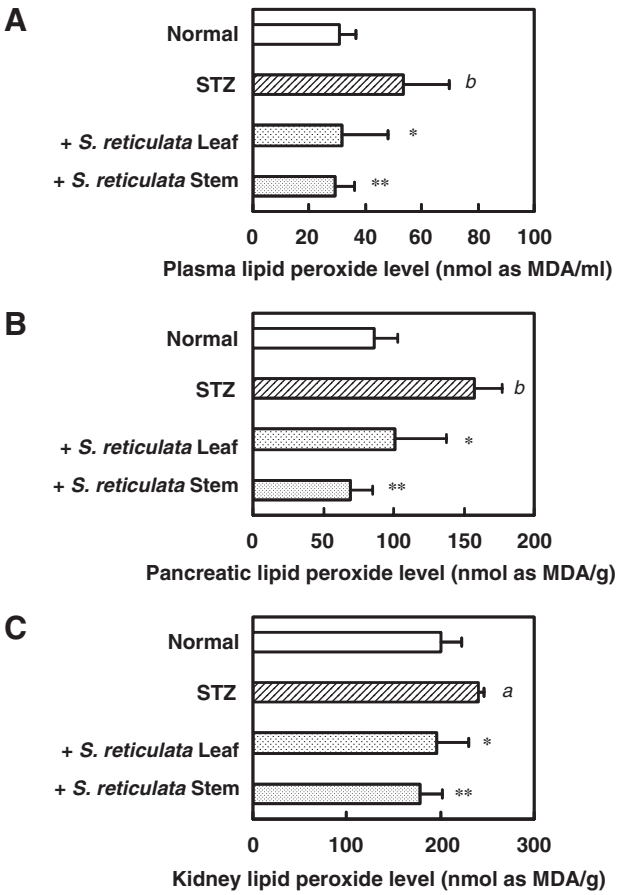

Fig. 6. Effects of Water Extracts Prepared from the Leaves and Stems of *S. reticulata* on Lipid Peroxide Levels in the Plasma, Pancreas, and Kidney in STZ-Induced Diabetic Mice 4 d after Administration.

A, Plasma lipid peroxide level (nmol as MDA/ml). B, Pancreas lipid peroxide level (nmol as MDA/g). C, Kidney lipid peroxide level (nmol as MDA/g). Mean ± SD ($n = 6$). The sample solution was supplied as drinking water at a concentration of 0.01%. Significant differences from the value for the normal group, $^{a}P < 0.05$, $^{b}P < 0.01$; and from the value for the STZ group, $^{*}P < 0.05$, $^{**}P < 0.01$.

leaves. This result suggests that the water extract from the leaves of *S. reticulata* could be as effective as the extract from the stems in preventing injury derived from oxidative stress in STZ-induced diabetic mice. In particular, the inhibitory effect on oxidative stress in the pancreas could contribute to the prevention of its β-cell destruction such antioxidative constituents as catechins, lignans, mangiferin, and triterpenesin in some plants of *Salacia* sp. have been reported in some previous studies.[56,57]

Suppressive effects of the water extracts of S. reticulata *and* S. oblonga *on AR activity*

The polyol pathway catalyzed by AR is thought to contribute to the development and progression of several diabetic complications similar to the formation of advanced glycation end products. AR could cause the production and accumulation of D-sorbitol from excess D-glucose, and convert NADPH to $NADP^{+}$.[58,59] A substance that can inhibit the activities of both AGc and AR could be extremely effective for preventing diabetes and the associated complications.

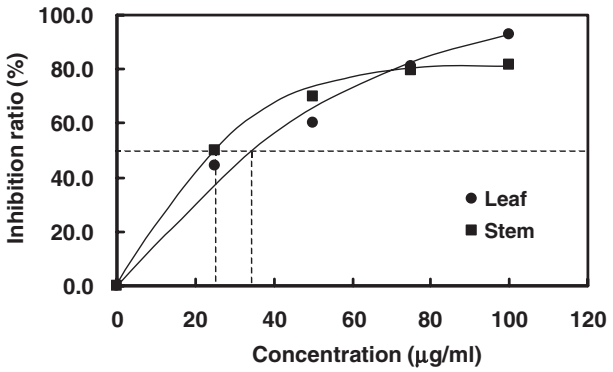

Fig. 7. Inhibitory Effects of Water Extracts Prepared from the Leaves and Stems of *S. reticulata* on the Recombinant Human AR Activity.

Each value is the mean of three independent experiments. Substrate, 100 mM DL-glyceraldehyde.

We investigated the inhibitory effects of water extracts of the leaves of *S. reticulata* and *S. oblonga* on the human recombinant AR activity in a series of *in vitro* experiments. Both the extracts of *S. reticulata* and *S. oblonga* exhibited an inhibitory effect on the AR activity at 60 μg/ml. The inhibition ratios were 75.0% and 29.1%, respectively. The water extract from the leaves of *S. reticulata* was effective. We then compared the inhibitory effect on AR activity of the water extract from the leaves of *S. reticulata* to that from the stems of *S. reticulata*. The IC_{50} values of the water extracts from the leaves and stems of *S. reticulata* were 33 and 25 μg/ml, respectively, as shown in Fig. 7. Unlike the case of the inhibitory effects on the AGc activity, the inhibitory effects of the leaves and stems of *S. reticulata* on the AR activity were almost the same. The IC_{50} value for the water extract of *Aralia elata* on the AR activity *in vitro* has been reported to be 11.3 μg/ml, although that of *Emblica officinalis* was 880 μg/ml.[60,61] The water extracts from the leaves and stems of *S. reticulata* can be expected to inhibit both AR and AGc activities. Kotalagenin 16-acetate,[18] 3β,22β-dihydroxyolean-12-en-29-oic acid, tingenone, tingenine B, regeol A, triptocalline A, and mangiferin[19,20] have been reported as possible AR inhibitors in the stems or roots of some plants of *Salacia* sp. Mangiferin is known to be a common inhibitor of AGc and AR activities. Thus, mangiferin would contribute to the inhibitory effect of the leaves of *S. reticulata* on the AR activity.

Effects of the water extracts of S. reticulata *and* S. oblonga *on the kidney AR activity in STZ-induced diabetic mice*

It has been reported that the lens AR activity and sorbitol formation were increased in STZ-induced diabetic rats.[62] Few studies about the kidney AR activity in STZ-induced diabetic animals have been reported, although nephropathy was observed in STZ-induced diabetic rats 8 weeks after an injection of STZ.[63] AR activity is known to be closely associated with nephropathy in diabetic patients,[44] and thus the kidneys of STZ-induced diabetic animals can be anticipated to show increased AR activity. The kidney AR activity in STZ-induced diabetic mice supplied with a 0.01% aqueous solution of the water extracts from the leaves and stems of *S. reticulata* as drinking water is

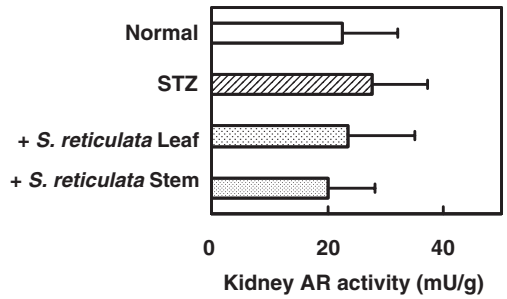

Fig. 8. Effects of Water Extracts Prepared from the Leaves and Stems of *S. reticulata* on the Kidney AR Activity in STZ-Induced Diabetic Mice 4 d after Administration.

Unit, mU/g. Mean ± SD ($n = 6$). The sample solution was supplied as drinking water at a concentration of 0.01%.

shown in Fig. 8. A slight lesion in the kidney of STZ-induced diabetic mice at the early stage was observed in this study. Supplying a 0.01% aqueous solution of the extracts of *S. reticulata* as drinking water tended to suppress the kidney AR activity in the mice.

The safety of the extracts from the plants of *Salacia* sp. has been confirmed in previous studies investigating subchronic toxicity, genotoxicity, *etc*.[64–67] The leaves of *Salacia* sp. are considered to be an attractive material as a foodstuff chiefly for two reasons. One reason is that the leaves of *Salacia* sp. are easier to harvest than the roots and stems. In particular, in the case of a thick stem or root harvest, it is necessary to cut the tree down. However, the leaf harvest may not injure the trees so much. The leaf harvest would have appeal for protecting the environment. Another reason is related to the food habits. We have the dietary habit of drinking a hot water extract of *Camellia sinensis* leaves as green tea or black tea. The leaves of *S. reticulata* would appeal to dietary habits for us. Consumers can confirm and enjoy the leaves of *S. reticulata*, that is, a raw material without any extraction processing. The hot water extract of *S. reticulata* leaves was slightly reddish and had a fresh aroma and a light taste. The leaves of *S. reticulata* retain strong antidiabetic activity; although weaker than the stem, the leaves are considered to be a beneficial food material.

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