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# α-Glucosidase Inhibitory Activity of Some Sri Lanka Plant Extracts, One of Which, *Cassia auriculata*, Exerts a Strong Antihyperglycemic Effect in Rats Comparable to the Therapeutic Drug Acarbose

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Some Sri Lanka plant stuffs were examined regarding in vitro and in vivo α-glucosidase (AGH) inhibitory actions. According to the results, water extracts and methanol extracts of dried fruits of Nelli (*Phylanthus embelica*), methanol extracts of dried flowers of Ranawara (*Cassia auriculata*), and water extracts of latex of Gammalu (*Pterocarpus marsupium*) were found to have a potential AGH inhibitory activity. In particular, Ranawara methanol extract showed the strongest AGH inhibitory activity in vitro preferably on maltase giving an $IC_{50}$ value of 0.023 mg/mL and inhibited the maltase activity competitively. As a result of single oral administration of Ranawara (*C. auriculata*) methanol extract in Sprague−Dawley rats, a significant and potent lowering of blood glycemic response toward maltose ingestion was observed at 30 min after dosing of 5 mg/kg, thus, concurrently suppressed insulin activity. The $ED_{50}$ of the extract (4.9 mg/kg) clearly indicated that the antihyperglycemic effect was as potent as that of therapeutic drug, acarbose ($ED_{50}$ 3.1 mg/kg).

**KEYWORDS: α-Glucosidase; antidiabetic plants; diabetes mellitus control; *Phylanthus embelica*; *Cassia auriculata*; *Pterocarpus marsupium***

## INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by a congenital (type I insulin-dependent diabetes mellitus/IDDM) or acquired (type II noninsulin-dependent diabetes mellitus/NIDDM) inability to transport glucose from the bloodstream into cells. Type I is usually diagnosed in childhood, and the body makes little or no insulin. Type II diabetes is an insulin resistance condition that occurs in adulthood, and it afflicts approximately 90% of all diabetics. The most beneficial therapy for type II is said to be one that achieves optimal blood glucose control after a meal.

Carbohydrates are the major constituents of the human diet, and the main components of carbohydrates are starch and sucrose. Starch in meals is first decomposed into oligosaccharides by the enzyme α-amylase of saliva and pancreatic juice. AGH (EC 3.2.1.20), which is a membrane-bound enzyme located at the epithelium of the small intestine, catalyzes the cleavage of glucose from disaccharides and oligosaccharides. Hence, AGH inhibition is one of the effective treatments of diabetes mellitus. AGH inhibitors that retard digestion of both sucrose and starch are regarded as superior to α-amylase inhibitors (*1*); therefore, AGH inhibitors have received considerable attention in the past two decades as they are potential therapeutic agents for the treatment of diabetes. In addition, the STOP-NIDDM trial study by using therapeutic drug acarbose (*2*) has provided clinical evidence that the AGH inhibition is efficient in preventing borderline diabetes. The evidence strongly led us to study alternative medicinal foods. In the course of our studies on antidiabetic compounds from natural resources, we have successfully isolated and identified several AGH inhibitors (*3*−*7*). As a continuing part of our screening for AGH inhibitors, some Sri Lanka plant stuffs were investigated to clarify the suppression of the glucose production from dietary sugars through the inhibition of AGH activity. Among the plants that we studied, some are traditionally recommended for diabetic treatment and several studies have been conducted to determine the antihyperglycemic effect (*8*−*15*), whereas no attention has been directed to AGH inhibition by these plant stuffs.

## MATERIALS AND METHODS

**Materials.** AGH from rat intestinal acetone powder was purchased from Sigma-Aldrich Fine Chemicals (CMO, U.S.A.). Papain (14 units/mg of solid, from papaya latex, EC 3.4.22.2) was the product of Nacalai Tesque (Kyoto, Japan). CNBr activated Sepharose 4B was purchased from Pharmacia Biotech AB (Uppsala, Sweden). Other reagents were of analytical grade and used without further purification. Plant stuffs that were used in this study (**Table 1**) were purchased from herbal drug stores in the form in which they are used by native people.

**Sample Preparation.** Ten grams of the sample homogenized by a blender was primarily subjected to a 100 mL water extraction for 1 h

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**Table 1.** Studied Plants

| local name | scientific name | family | used part[a] | yield (mg/100 mg)[b] WF | MF |
|---|---|---|---|---|---|
| Beli | *Aegle marmelos* | Rutaceae | fruit | 31.4 | 6.0 |
| Polpala | *A. lanata* | Amaranthaceae | dried whole part | 3.4 | 1.0 |
| Kos | *A. heterophyllus* | Maraceae | leaves | 3.1 | 1.0 |
| Ranawara | *C. auriculata* | Leguminosae | dried flowers | 4.2 | 4.3 |
| Divul | *Limonia acidissima* | Rutaceae | fruit | 13.6 | 6.0 |
| Karawila | *M. charantia* | Cucurbitaceae | fruit | 3.1 | 1.0 |
| Passion | *Passioflora incarnata* | Passifloraceae | leaves | 4.1 | 1.7 |
| Nelli | *P. embelica* | Euphorbiaceae | dried fruits | 34.2 | 9.6 |
| Gammalu | *P. marsupium* | Leguminoceae | latex | 29.0 | 5.8 |
| Amberralla | *S. pinnata* | Anacardiaceae | fruit | 7.4 | 2.3 |
| Rasakida | *Tinospora cordifolia* | Menispermaceae | stem | 3.8 | 1.7 |
| Uluhal | *T. foenum* | Leguminosae | dried seeds | 12.5 | 5.6 |

[a] Parts were subjected to water and subsequent methanol extractions. [b] Yields of extract from 100 mg of plant parts were used.

at ambient temperature while stirring. After the extraction, the sample was centrifuged at 19 000*g* for 15 min and the obtained supernatant was then lyophilized. The remaining precipitate was subjected to a 100 mL methanol extraction for 1 h at ambient temperature while stirring. After the extraction, the sample was again centrifuged at 19 000*g* for 15 min, and the supernatant was taken evaporated in vacuo. The obtained water and methanol extracts (denoted as WF and MF, respectively) were stored −20 °C until use.

**Preparation of Immobilized AGH.** Immobilization of AGH partially purified from rat intestinal acetone powder by papain onto CNBr activated Sepharose 4B was performed according to our previous report (*16*). At first, the support (50 mg of dry gel) was immersed in 2 mL of 1 mM HCl for 15 min. After rinsing with 1 mM HCl and coupling buffer (0.1 M borate buffer containing 0.5 M NaCl), 1 mL of the prepared AGH (2 mg) was added to the support. After incubation at 20 °C for 2 h, the support was washed with the coupling buffer, followed by the addition of 1 mL of 0.1 M $\beta$-alanine as a blocking reagent. The preparations were kept at 4 °C in a model of intestinal fluid (0.1 M phosphate buffer, pH 6.8) described in the Japanese Pharmacopoeia (JP XIII).

**Immobilized AGH Inhibitory Assay.** Immobilized AGH activity was assayed according to the method (*16*) by adding 100 $\mu$L of inhibitor solution and 900 $\mu$L of 10 mM maltose or 45 mM sucrose solution to the immobilized AGH support (10 mg of wet gel). After incubation at 37 °C for 30 min, the reaction was stopped by the filtration. AGH inhibitory activity was estimated by the difference of liberated glucose with or without inhibitor. Determination of glucose was performed with F-kit Glucose (Roche Diagnostics Co., Tokyo, Japan).

$$\text{inhibitory ratio (\%)} = (A_C - A_{CB}) - (A_S - A_{SB})/(A_C - A_{CB}) \times 100$$

where $A_C$, $A_S$, $A_B$, $A_{CB}$, and $A_{SB}$ represent the absorbance of the control, sample, blank, control blank, and sample blank, respectively. The concentration of AGH inhibitor required to inhibit 50% of the AGH activity under the assay condition is defined as the $IC_{50}$ value.

**Kinetic Study.** Lineweaver−Burk plots for AGH inhibitory extracts were done by the reported assay (*17*), but the reaction time was 10 min. The substrate was maltose, and the final concentration of the maltose was changed (0.5, 1.0, 2.5, and 5.0 mM) while keeping the inhibitor concentration constant (0.01 or 0.02 mg/mL).

**Animal Experiments in SD Rat.** Four male 7 week old Sprague−Dawley (SD) rats (SPF/VAF Crj: SD, Charles River Japan, Kanagawa, Japan) in each rat experiment were fed a laboratory diet (MF, Oriental Yeast, Tokyo, Japan) and given water ad libitum. All rats were housed for 1 week at 21 ± 1 °C and 55.5 ± 5% humidity under controlled lighting from 8:30 a.m. to 8:30 p.m. Each rat was unfed for 16 h before a single oral administration of the sample by gavage. At 5 min after the sample administration, 1 mL of a 2 g/kg substrate (maltose, sucrose, or glucose) solution was administered to each rat. Control rats were administered the same volume of substrate solution without sample. At 0, 30, 60, and 120 min, blood samples were collected from the tail vein and immediately subjected to a blood glucose level (BGL)

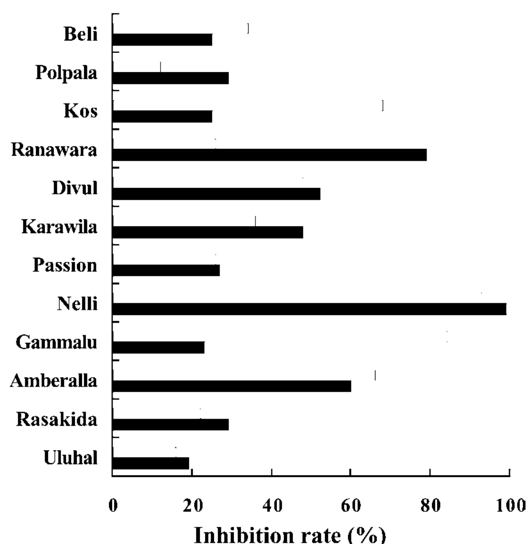


**Figure 1.** AGH inhibition by water (□) and methanol (■) extracts of Sri Lanka plant stuffs. AGH activity was assayed by immobilized AGH assay using maltose as a substrate at 37 °C for 30 min. All extracts were in the final concentration of 1 mg/mL.

measurement by a disposable glucose sensor (Glutest Pro, Sanwa Chemical Research Co., Tokyo, Japan). The remaining serum was subjected to an insulin assay (Shibayagi's Insulin Assay Kit, Shibayagi Co., Gunma, Japan). All of the measurements were done at four replicates.

**Data Analysis.** Each result for the administration study is expressed as the mean of BGL (mg/dL of plasma) ± SD. Statistical differences of BGL between control and sample groups at each administration time were evaluated by using a two factor analysis of variance followed by Dunnett's *t*-test for posthoc analysis. The significant difference in insulin levels between vehicle and sample administration groups was done by unpaired Student' *t*-test. $P < 0.05$ was considered to be statistically significant. The analysis was performed with Stat View J5.0 (SAS Institute Inc., Cary, NC).

## RESULTS AND DISCUSSION

**AGH Inhibition.** On the basis of the prevalence that a delay or inhibition of carbohydrates digestion (e.g., digestion of maltose to glucose) would contribute to optimize a postprandial BGL (*1*−*3*, *18*), our first attempt to screen AGH inhibitory plant stuffs was done with respect to maltase inhibition. **Figure 1** summarizes maltase inhibitory activity of 12 plant stuffs screened in this experiment. All extracts that were used for the screening were in the final concentration of 1.0 mg/mL. As a result of this preliminary screening, all of the extracts showed

**Table 2.** AGH Inhibitory Activity of Some Active Extracts

| inhibitor[b] | IC50 value (mg/mL) | |
|---|---|---|
| | maltose[a] | sucrose[a] |
| Ranawara-MF | 0.023 | 0.398 |
| Nelli-WF | 1.020 | *c* |
| Nelli-MF | 0.484 | 0.196 |
| Gammalu-WF | 0.412 | 0.196 |

[a] Ten millimolar maltose or 45 mM sucrose was used as a substrate. [b] One mg/mL of sample was used to evaluate AGH inhibitory activity. [c] Not determined.

more or less inhibition against maltase. Among these, WF and/or MF of five plant stuffs (Amberralla, *Spondias pinnata*; Gammalu, *Pterocarpus marsupium*; Nelli, *Phyllanthus embelica*; Ranawara, *Cassia auriculata*; and Kos, *Artocarpus heterophyllus*) showed a relatively high maltase inhibitory ratio of larger than 60%. In particular, WF and/or MF of Gammalu (*P. marsupium*), Nelli (*P. embelica*), and Ranawara (*C. auriculata*) were found to be the most powerful AGH inhibitors with an inhibitory ratio of >75%. Thus, these three plant stuffs were selected and used for further experiments. Nelli (*P. embelica*), which is used as an indigenous medicine for various illnesses in Sri Lanka, has already been reported to possess diverse bioactivities such as antioxidant (*19*) and antiinflammatory (*20*) activities. However, there were no reports on the antihyperglycemic effect of Nelli (*P. embelica*) so far. In addition, although an aqueous leaf extract of Ranawara (*C. auriculata*) was found to lower the serum glucose level in normal rats (*21*) and Gammalu (*P. marsupium*) was reported to have $\beta$-cell protective and regenerative properties (*22*), an insulin-like activity (*23*), there was no study on AGH inhibitory activity. Thus, this was the first finding that Nelli (*P. embelica*), Gammalu (*P. marsupium*), and Ranawara (*C. auriculata*) have a potential ability to exert an antihyperglycemic effect via the retardation of AGH (maltase) action. In contrast, Karawila (*Momodica charantia*) (*13*), Uluhal (*Trigonella foenum-graceum*) (*14*), and Rasakida (*Aerva lanata*) (*15*) that have already proven antihyperglycemic effects showed only a poor AGH inhibitory activity as shown in **Figure 1**.

**Measurement of $IC_{50}$ Value.** On the basis of the result of the preliminary screening experiment (**Figure 1**), Ranawara-MF, Nelli-WF and -MF, and Gammalu-WF with higher maltase inhibitory ratios were subjected to an AGH inhibitory assay by using the immobilized AGH assay system. Maltase inhibitory activity as well as sucrase was examined to ensure the practical inhibitory preference toward each hydrolylase. As summarized in **Table 2**, all of the extracts showed a potent AGH inhibitory activity with an $IC_{50}$ value of less than 1 mM except Nelli-WF, which was slightly higher than 1 mM. Nelli-WF, Nelli-MF, and Gammalu-WF that showed a similar AGH inhibitory potential were found to inhibit favorably sucrase activity ($IC_{50}$, 0.196 mg/mL) rather than maltase activity. In contrast, Ranawara-MF showed a higher maltase inhibitory activity ($IC_{50}$, 0.023 mg/mL) by a factor of >20 as compared with other extracts such as Nelli-MF ($IC_{50}$, 0.484 mg/mL). The AGH inhibitory action of Ranawara-MF was also quite specific for maltase, because sucrase inhibitory activity was much weaker than that of maltase inhibitory activity that showed an $IC_{50}$ value of 0.398 mg/mL.

To our knowledge, all of these plant extracts shown in **Table 2** were one of the strongest natural maltase inhibitors as compared to other reported natural inhibitors such as green tea ($IC_{50}$, 0.735 mg/mL) (*17*) and oolong tea ($IC_{50}$, 1.34 mg/mL) (*17*). In particular, the maltase inhibitory activity of Ranawara-MF was superior to that of anthocyanin extract of purple sweet potato ($IC_{50}$, 0.26 mg/mL) (*5*), by which a glycemic rise was suppressed by 15.6% after the administration (400 mg/kg dose) in SD rats (*7*). Yoshikawa et al. (*24*) have revealed that the water soluble fraction from the roots and stems of *Salacia reticulata* strongly inhibited rat intestinal maltase activity ($IC_{50}$, 0.035 mg/mL). In addition, they identified a potent inhibitor of salacinol responsible for the AGH inhibition of the fraction, in which the maltase inhibitory potency of salacinol was only one-third lower than that of the therapeutic drug of acarbose. This indicates that potent AGH inhibitory natural compounds such as salacinol should be present in Ranawara-MF having a strong maltase inhibitory activity as in the *S. reticulata* fraction. According to the findings, only the MF of Ranawara gives a maltase inhibitory activity (**Figure 1**), which indicates that slightly hydrophobic compounds such polyphenolics would be responsible for its potent maltase inhibition.

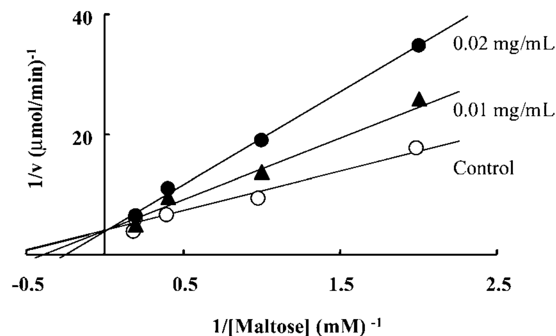


**Figure 2.** Lineweaver–Burk plots for kinetic analysis of AGH inhibition by Ranawara-MF. The enzyme reaction was performed incubating the mixture for 10 min at 37 °C with each designated concentration of maltose (0.5–5 mM) in the absence or presence of Ranawara-MF at two different concentrations: 0 (○); 0.01 (▲); and 0.02 (●) mg/mL of Ranawara-MF.

**Kinetic Studies.** To clarify the maltase inhibition mode of Ranawara-MF that showed the most potent maltase inhibitory activity, the Lineweaver−Burk plots were performed (**Figure 2**). The plots clearly demonstrated that the extract inhibited maltase action competitively giving a $K_i$ value of 0.023 mg/mL as in the case of salacinol (*24*) and acarbose (*1*). This result also indicated that the extract would be a group of competitive maltase inhibitors.

**Antihyperglycemic Effect of Ranawara-MF in SD Rats.** A single oral administration study of Ranawara-MF with potent maltase inhibitory activity was performed to clarify the anti-hypertensive effect in vivo. Rat experiments were done after administration of 5 mg/kg Ranawara-MF, followed by substrate (2 g/kg dose of maltose, sucrose, or glucose) in 8 week old SD rats. **Figure 3** shows the change in BGL during the 120 min protocol in maltose-loaded rats. The results of the present study clearly showed that a single oral administration of Ranawara-MF resulted in a significant ($P < 0.01$) BGL reduction up to 60 min ($BGL_{30min}$, 122.6 ± 8.1 mg/dL of plasma; $BGL_{60min}$, 106.6 ± 10.0 mg/dL of plasma) as compared with the control ($BGL_{30min}$, 182.4 ± 5.4 mg/dL of plasma; $BGL_{60min}$, 175.0 ± 7.0 mg/dL of plasma). Thereafter, the BGL returned to a baseline level at 120 min (89.6 ± 4.0 mg/dL of plasma). Suzuki et al. (*25*) have reported a suppression effect of postprandial glucose level of Banaba leaf extract with in vitro maltase inhibitory activity of 0.89 mg/mL, in which a transient reduction of BGL by 100 mg/kg dose of the extract was observed only at 15 min in soluble starch-loaded rats. Similarly, a significant BGL reduction of anthocyanin extract from purple sweet potato (400 mg/kg) was observed only at 30 min in maltose-loaded SD rats

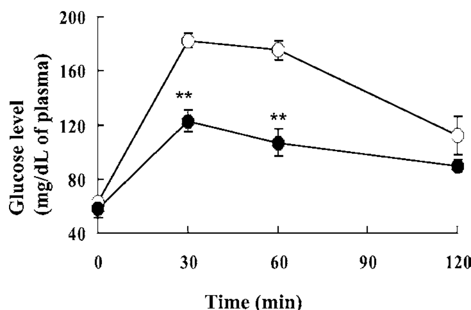


**Figure 3.** Effect of Ranawara-MF on BGLs after a single oral administration of 2 g/kg maltose in SD rats. One milliliter of 5 mg/kg Ranawara-MF (●) was dosed. After 5 min, 1 mL of a 2 g/kg maltose solution was administered to each rat. Control (○) was administered with the same volume of maltose solution without inhibitor. At 0, 30, 60, and 120 min, ∼20 $\mu$L of blood samples was collected and immediately subjected to BGL assay by a disposable glucose sensor. Data are the means (mg/dL of plasma) ± SD. Significant differences between sample and control groups were examined with Dunnett's *t*-test ($n = 4$, $^{**}P < 0.01$).

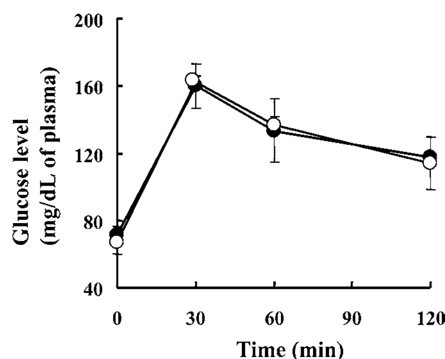


**Figure 4.** Effect of Ranawara-MF on BGL after a single oral administration of 2 g/kg sucrose in SD rats. One milliliter of 20 mg/kg Ranawara-MF (●) was dosed. After 5 min, 1 mL of a 2 g/kg sucrose solution was administered to each rat. Control (○) was administered with the same volume of sucrose solution without inhibitor. At 0, 30, 60, and 120 min, ∼20 mL of blood samples was collected and immediately subjected to BGL assay by a disposable glucose sensor. Data are the means (mg/dL of plasma) ± SD. Significant differences between sample and control groups were examined with Dunnett's *t*-test ($n = 4$, $P > 0.05$).

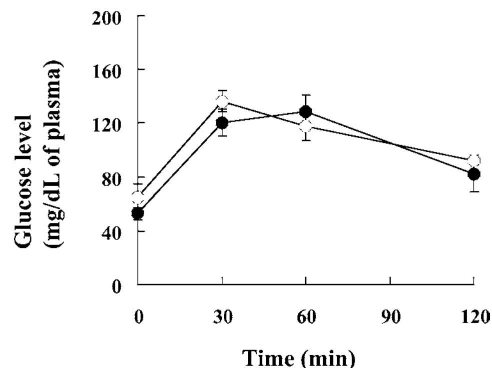


**Figure 5.** Effect of Ranawara-MF on BGL after a single oral administration of 2 g/kg glucose in SD rats. One milliliter of 20 mg/kg Ranawara-MF (●) was dosed. After 5 min, 1 mL of a 2 g/kg glucose solution was administered to each rat. Control (○) was administered with the same volume of glucose solution without inhibitor. At 0, 30, 60, and 120 min, ∼20 $\mu$L of blood samples was collected and immediately subjected to BGL assay by a disposable glucose sensor. Data are the means (mg/dL of plasma) ± SD. Significant differences between sample and control groups were examined with Dunnett's *t*-test ($n = 4$, $P > 0.05$).

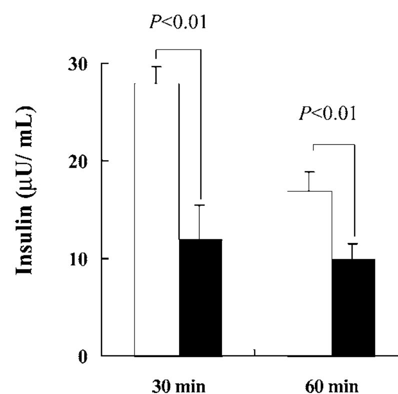


**Figure 6.** Effect of Ranawara-MF on the postprandial insulin response in maltose-loaded SD rats. Five mg/kg Ranawara-MF (■) was ingested to male 8 week old SD rats with 2 g/kg maltose. Control (□) was ingested maltose without Ranawara-MF. Data are expressed as means ± SD. Significant differences between sample and control groups at $P < 0.01$ were examined with Student's *t*-test ($n = 4$).

(*7*). Thus, the suppression effect of Ranawara-MF seemed to be longer acting rather than these maltase inhibitors.

A single oral administration study of Ranawara-MF in sucrose-loaded rats was performed with a higher dose of 20 mg/kg Ranawara-MF as compared with maltose-loaded rats (**Figure 4**), because sucrase inhibitory activity ($IC_{50}$, 0.398 mg/mL) in vitro is weaker than that of maltase inhibitory activity as shown in **Table 2**. A glucose-loaded rat experiment was also performed at a dose of 20 mg/kg (**Figure 5**). As a result, neither sucrose nor glucose ingestion with Ranawara-MF in rats affected the postprandial BGL curve during the 120 min protocol regardless of their higher dosage. No BGL reduction of Ranawara-MF in sucrose-loaded rats (**Figure 4**) seems to be valid, because the in vitro sucrase inhibitory activity of the extract was ca. 1/15 times poorer than that of maltase inhibitory activity as shown in **Table 2**; at least 100 mg/kg dose of the extract would be needed to reduce a BGL in sucrose-loaded rats, according to the result obtained in maltose-loaded rats (**Figure 3**). In addition, no BGL reduction of Ranawara-MF in glucose-loaded rats clearly pointed out that the significant antihyperglycemic effect in maltose-loaded rats that resulted from intestinal maltase inhibition, not from intestinal glucose transporter (SGLT1) inhibition. In addition, significant ($P < 0.01$) decreases in serum insulin levels at 30 (12.2 ± 3.5 vs 27.3 ± 1.7 $\mu$U/mL for control) and 60 min (10.5 ± 1.6 vs 17.6 ± 1.8 $\mu$U/mL for control) demonstrated (**Figure 6**) that the BGL reduction by Ranawara-MF was achieved by the retardation of glucose production at the intestine, not by its promotion of insulin secretion at the $\beta$-Langerhans' islet.

**Dose Dependency on Antihyperglycemic Effect of Ranawara-MF.** Dose dependency of Ranawara-MF in maltose-loaded SD rats was performed to clarify the BGL reduction potency. Acarbose ingested experiments were also performed as a positive control. As shown in **Figure 7**, Ranawara-MF suppressed a BGL rise dose dependently as acarbose did. The $ED_{50}$, which is a dose of Ranawara-MF to suppress the increase in the relative area under the curve (AUC) to 50% of the control, was evaluated to be 4.9 mg/kg. The $ED_{50}$ value was much lower than that of natural maltase inhibitor, diacylated anthocyanin ($ED_{50}$, 220 mg/kg) (*7*). Surprisingly, the $ED_{50}$ of the extract (4.9 mg/kg) clearly indicated that the antihyperglycemic effect was as potent as that of therapeutic drug, acarbose ($ED_{50}$ 3.1 mg/kg). Even if the extract was a natural product, the strong BGL reduction power was thought to be sufficient in improving

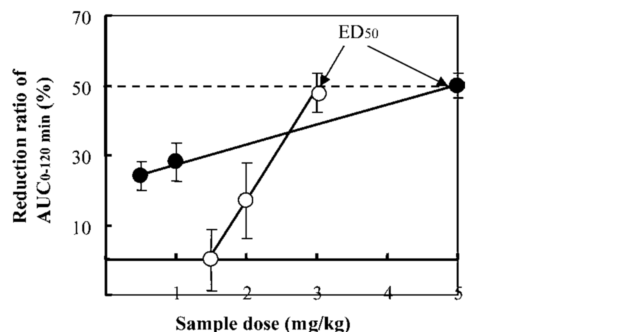


**Figure 7.** Dose dependency of Ranawara-MF and acarbose on the reduction of glycemic responses in maltose-loaded (2 g/kg) SD rats. $AUC_{0-120min}$ is the AUC of incremental BGLs up to 120 min. The reduction ratio of $AUC_{0-120min}$ of Ranawara-MF (●) or acarbose (○) against $AUC_{0-120min}$ of control was used for estimating either $ED_{50}$. Data are expressed as means ± SD ($n = 4$).

excess hyperglycemic levels. Further studies and interests will be focused on identification of compounds responsible for AGH inhibition from Ranawara-MF as well as other active plant stuffs as listed in **Table 2**.

In conclusion, the present study revealed that some Sri Lanka plant stuffs exerted a potent AGH inhibitory activity. In particular, the methanol extract of dried flowers of Ranawara (*C. auriculata*) was found to have a strong antihyperglycemic power in SD rats as potent as the therapeutic drug of acarbose.

## ABBREVIATIONS USED

AGH, α-glucosidase; WF, water fraction; MF, methanol fraction.

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