Lemongrass (Cymbopogon citratus) Ethanolic Extract Exhibited Activities That Inhibit ▯-glucosidase Enzymes and Postprandial Blood Glucose Elevation

Maria Dewi Puspitasari Tirtaningtyas Gunawan Puteri
Department of Food Technology, Faculty of Life Sciences and Technology, Swiss German University, Tangerang 15143, Indonesia, maria.gunawanputeri@sgu.ac.id

Felicia Melissa Tjiptadi
Department of Food Technology, Faculty of Life Sciences and Technology, Swiss German University, Tangerang 15143, Indonesia

Phebe Hendra
Faculty of Pharmacy, Universitas Sanata Dharma, Yogyakarta, 55282, Indonesia

Filiana Santoso
Department of Food Technology, Faculty of Life Sciences and Technology, Swiss German University, Tangerang 15143, Indonesia

Zalinar Udin
Research Center for Chemistry, Indonesian Institut of Science (LIPI-Kimia), Banten 15314, Indonesia

See next page for additional authors

Lemongrass (Cymbopogon citratus) Ethanolic Extract Exhibited Activities That Inhibit ⊠-glucosidase Enzymes and Postprandial Blood Glucose Elevation

Authors

Maria Dewi Puspitasari Tirtaningtyas Gunawan Puteri, Felicia Melissa Tjiptadi, Phebe Hendra, Filiana Santoso, Zalinar Udin, Nina Artanti, and Florence Ignatia Florence Ignatia

Lemongrass (*Cymbopogon citratus*) Ethanolic Extract Exhibited Activities That Inhibit α-glucosidase Enzymes and Postprandial Blood Glucose Elevation

Maria Dewi Puspitasari Tirtaningtyas Gunawan-Puteri[1*], Felicia Melissa Tjiptadi[1], Phebe Hendra[2], Filiana Santoso[1], Zalinar Udin[3], Nina Artanti[3], and Florence Ignatia[1]

1. Department of Food Technology, Faculty of Life Sciences and Technology, Swiss German University, Tangerang 15143, Indonesia
2. Faculty of Pharmacy, Universitas Sanata Dharma, Yogyakarta, 55282, Indonesia
3. Research Center for Chemistry, Indonesian Institut of Science (LIPI-Kimia), Banten 15314, Indonesia

*E-mail: maria.gunawanputeri@sgu.ac.id

Abstract

Lemongrass is a common ingredient in Indonesian traditional herbal medicine that potently inhibits carbohydrate hydrolysis. In this study, the in vitro α-glucosidase inhibitory (AGI) activity of lemongrass was compared with its in vivo activity to retard postprandial blood glucose elevation, and the bioactive compounds responsible for these activities were observed. Both water and ethanolic extracts of lemongrass (WLG and ELG, respectively) were tested in vitro for its inhibition of the sucrose and maltose hydrolyzing activities of rat intestinal glucosidase. ELG was observed to exert higher inhibitory activities (Sucrase IC_{50} = 8.74 mg/mL; Maltase IC_{50} = 18.93 mg/mL) than WLG. ELG was evaluated for its in vivo activity to retard blood glucose elevation in mice after sucrose, maltose, and glucose consumption. ELG was also fractionated using activity-guided chromatography, followed by liquid chromatography–mass spectrometry and high-performance liquid chromatography (HPLC). In vivo sugar tolerance test confirmed the AGI activity in a non-dose-dependent manner and showed potential additional mechanisms that may prevent postprandial hyperglycemia. The active principles were acquired in methanol-soluble fraction and purified using preparative thin-layer chromatography. HPLC analysis with commercial standards identified caffeic acid and kaempferol as the compounds responsible for the bioactivity of ELG. Results showed that lemongrass has the potential as herbal medicine ingredient in the management of diabetes.

Keywords: α-glucosidase inhibitor, Cymbopogon citratus, diabetes, Indonesian traditional medicine, lemongrass

Introduction

The treatment of diabetes mellitus has been an increasing concern in Indonesia, as it became the fifth country with the highest prevalence of diabetes after China, India, Brazil, and the US [1]. The α-glucosidase inhibitor (AGI) inhibits the hydrolysis of polysaccharides and disaccharides in the small intestine, thereby delaying the spike of postprandial blood glucose after meals [2–5]. AGIs can be a reasonable option as the first-line drug in the treatment of patients with diabetes mellitus type 2 as it specifically targets postprandial hyperglycemia. AGIs are also expected to cause no hypoglycemic events or other life-threatening events, even at overdoses, and no weight gain [6].

Lemongrass (*Cymbopogon citratus*) is widely cultivated in tropical and subtropical countries. Lemongrass is a popular spice and flavoring agent in various traditional beverage and food recipes that are also known to have various health benefits [7, 8]. Because of its strong roots that hold the soil, lemongrass plantations in Indonesia have also been known to prevent soil erosion in terrace farming. Commercialization of lemongrass as scientific-based herbal medicine, known as *obat herbal terstandar* (OHT) in Indonesia, is expected to increase both the value and the plantation of this plant [9]. The National Agency of Drug and Food Control of the Republic of Indonesia stated that herbal medicine registration as OHT must include the identification of active compounds as biomarkers, use of biomarker content for product quality control, and in vivo confirmatory tests of its activity and toxicity [10].

Ethnopharmacological and pharmacological investigations of lemongrass summarized the uses and potential functions of the essential oil and water extract of lemongrass (WLG) for various health benefits [8], in-

cluding the hypoglycemic effect of its fresh leaf aqueous extract [11] and in vitro antidiabetic activity of its essential oil [12]. Previous research on the exploration of Indonesian plant activities also reported the AGI activity of aqueous and methanolic extracts of lemongrass [13–15]. However, in vivo activity confirmation and evaluation of active compounds responsible for the AGI activity have not been conducted.

Common preparation methods for medicinal herbs in the traditional industry include water extraction and/or juice expression to produce ready-to-drink concoctions. Meanwhile, large herbal companies in Indonesia employ ethanolic extraction, followed by the drying process to produce an herbal powder with a long shelf life [16]. This indicates the need to evaluate the activities of both WLG and ethanolic extract of lemongrass (ELG). In this study, the in vitro AGI activities of both WLG and ELG were compared, and the extract with higher activity was further evaluated for its in vivo activity and subjected to a series of activity-guided fractionations to isolate and identify the active compound(s). Therefore, this study is expected to provide valuable insights into Indonesian herbal industries and the use of lemongrass as an herbal ingredient with AGI activity for the treatment of diabetes.

Materials and Methods

Materials. Dried lemongrass was purchased from a plantation in Karang Anyar Lereng Lawu, Indonesia. Distilled water and ethanol 96% technical grade were obtained from Sumber Abadi, Serpong, Indonesia. During the evaluation of in vitro AGI activity, the following chemicals were used: sucrose and maltose (Merck, Germany) analytical grade, potassium phosphate (Merck, Germany), potassium dihydrogen phosphate (Sinopharm Chemical Reagent Co., Shanghai), dimethyl sulfoxide (DMSO; Merck, Germany), rat intestinal acetone powder (Sigma-Aldrich, Germany), ethylenediaminetetraacetic acid (EDTA; Merck, Germany), aluminum oxide 60 (Merck, Germany), tris(hydroxymethyl)aminomethane (Merck, Germany), hydrochloric acid (HCl; PT. Smart Lab, Indonesia), and glucose C-II test kit solution (Wako Pure Chemical Co., Japan).

The materials used for in vivo evaluation include rat intestinal acetone powder (Sigma-Aldrich, Germany), sodium carboxymethyl cellulose (Brataco Chemika, Indonesia), blood glucose test strip (glucometer; GlucoDr® Auto, All Medicus Co., Ltd.), and mice feed (pellet BR2). All other materials were analytical grade and supplied by Merck, Germany. Adult male Swiss mice weighing 20–30 g were procured from the Imono Laboratory, Sanata Dharma University, Indonesia. The mice were housed in standard cages under standard laboratory conditions of 22 ± 2 °C and 12:12 h light/dark cycle. Standard pellet diet and water were given ad libitum. All procedures described were reviewed and approved with approval number KE/FK/1013/EC/2019 by the Medical and Health Research Ethics Committee, Faculty of Medicine, Gadjah Mada University–Dr. Sardjito General Hospital, Yogyakarta, Indonesia.

The chemicals used for the isolation stage were *n*-hexane analytical grade (Merck, Germany), methanol analytical grade (Merck, Germany), ethyl acetate analytical grade (Merck, Germany), thin layer chromatography (TLC) silica gel 60 F_{254} (Merck, Germany), and preparative thin-layer chromatography (PLC) silica gel 60 F_{254} (Merck, Germany).

Lemongrass extraction. Dried lemongrass was macerated using water and ethanol 96% for 24 h. The dried lemongrass-to-solvent ratio was 3:5 (v/v). Crude extracts were filtered using vacuum filtration and were evaporated using a vacuum rotary evaporator at 50°C. Then, both WLG and ELG were stored in a dark glass bottle at 4 °C.

In vitro rat intestinal glucosidase inhibitory activity. Glucosidase inhibitory activity analysis was conducted using enzymatic reactions as previously described [17] with some modifications. To produce the crude rat intestinal glucosidase, 1 g rat intestinal acetone powder was crushed until homogenous and mixed with 20 mL of 0.1 M potassium phosphate buffer containing 5 mM EDTA in a cold mortar on an ice bath to prevent enzyme degradation. The mixture was placed in prechilled centrifuge tubes and centrifuged for 60 min (11,000 rpm, 4 °C). Any precipitation from centrifugation was solubilized and centrifuged for a second time in the same manner to increase efficiency.

The inhibitory activity against sucrose hydrolysis was measured using the following procedures: Two test tubes, as sample and control, containing 0.2 mL sucrose solution (56 mM) in potassium phosphate buffer (0.1 M, pH 7) and 2 test tubes, as sample and control blanks, containing 0.4 mL potassium phosphate buffer (0.1 M, pH 7) were preincubated at 37 °C for 5 min. The control and control blank were defined as 100% and 0% enzyme activity, respectively. Lemongrass extract diluted in 50% DMSO (0.1 mL) was added to the sample and sample blank test tubes; whereas 50% DMSO (0.1 mL) was added to the control and control blank test tubes. Then, crude rat intestinal glucosidase (0.2 mL) was added only to the test tubes containing the sucrose solution. The reaction was conducted at 37 °C for 15 min and terminated by adding Tris–HCl buffer (2 M, pH 6.3, 0.75 mL). Then, the reaction mixtures were passed through a short column of basic alumina (30 mm × 5 mm) to remove phenolics, which may interfere with the subsequent glucose quantification. The mixture

(0.05 mL) was incubated with glucose C-II test kit solution (0.2 mL) in 96-well microplate at 37 ℃ for 30 min. The optical density of the wells was measured at 492 nm. The inhibitory activity was calculated using the following formula (Equation 1):

$$\% \; Inhibition \; \frac{(c-cb)-(s-sb)}{(c-cb)} \times 100\% \qquad (1)$$

Equation 1. Formula for AGI activity calculation, where *C* is the absorbance value for the control, *Cb* is the absorbance value for the control blank, *S* is the absorbance value for the sample, and *Sb* is the absorbance value for the sample blank

The procedures for inhibitory activity assay against maltose hydrolysis were the same as above, except for replacing sucrose solution (56 mM, 0.2 mL) with maltose solution (3.5 mM, 0.35 mL) and reducing the amount of enzyme solution from 0.2 mL to 0.05 mL. The experiments were conducted in two replications, and the results were presented as half-maximum inhibitory concentration (IC_{50}). The slope test was employed to determine IC_{50}. Analysis of variance (ANOVA) and Tukey's HSD were used (α value = 0.05) to determine the highest inhibitory activity between WLG and ELG. The extract with the highest AGI activity was selected for further tests.

In vivo sugar tolerance test. On the day of the experiment, 80 mice were fasted overnight and randomly divided into 16 groups, each consisting of 5 mice. One group was assigned as normal control (Group C), five groups (SC, SA, S1, S2, and S3) were assigned to the oral sucrose tolerance test (OSTT), five groups (MC, MA, M1, M2, and M3) were assigned to the oral maltose tolerance test (OMTT), and five groups (GC, GA, G1, G2, and G3) were assigned to the oral glucose tolerance test (OGTT).

Groups SC, MC, and GC served as sucrose control, whereas Groups SA, MA, and GA received 0.08 g acarbose/kg BW treatment. In OSTT, Groups S1, S2, and S3 received 0.22, 0.67, and 2.00 g ELG/kg BW treatments, respectively. Groups SC, SA, S1, S2, and S3 received 4 g sucrose/kg BW sugar induction (OSTT) 30 min after ELG or acarbose treatment. OMTT and OGTT were conducted in the same manner as above, except for replacing 4 g sucrose/kg BW with 3 g maltose/kg BW for OMTT and 2 g glucose/kg BW for OGTT (Table 1).

Table 1. Treatments of Mice Groups

Mice Group	Sugar Challenge	Sample Treatment	Group Name
C	None	None	Normal Control
SC		None	Sucrose Control
SA		0.08 g acarbose/kg BW	Sucrose + Acarbose
S1	4 g sucrose/kg BW	0.22 g ELG/kg BW	Sucrose + ELG 0.22
S2		0.67 g ELG/kg BW	Sucrose + ELG 0.67
S3		2.00 g ELG/kg BW	Sucrose + ELG 2.00
MC		None	Maltose Control
MA		0.08 g acarbose/kg BW	Maltose + Acarbose
M1	3 g maltose/kg BW	0.22 g ELG/kg BW	Maltose + ELG 0.22
M2		0.67 g ELG/kg BW	Maltose + ELG 0.67
M3		2.00 g ELG/kg BW	Maltose + ELG 2.00
GC		None	Glucose Control
GA		0.08 g acarbose/kg BW	Glucose + Acarbose
G1	2 g glucose/kg BW	0.22 g ELG/kg BW	Glucose + ELG 0.22
G2		0.67 g ELG/kg BW	Glucose + ELG 0.67
G3		2.00 g ELG/kg BW	Glucose + ELG 2.00

Blood was collected from the tail vein of the mice at 0 min before sugar induction and at 15, 30, 60, 90, and 120 min after sugar induction. Postprandial glucose (PPG) levels were determined using a glucometer [18,19].

The results are expressed as the mean ± standard deviation. The trapezoidal rule was used to determine the area under the curve (AUC) blood glucose [20,21,22]. Data were analyzed using one-way ANOVA, followed by post hoc Scheffe tests using SPSS 22. A p value of <0.05 was considered statistically significant. AUC was estimated using the following formula for the trapezoidal rule (Equation 2):

$$AUC = \sum_{i=0}^{n-1}(t_{i+1} - t_i)(C_{i+1} - C_i)/2 \qquad (2)$$

Equation 2. Formula for AUC calculation of blood glucose slope after sugar consumption, where *t* is the time and *C* is the concentration of glucose

Isolation of α-glucosidase inhibiting principles. ELG was gradually extracted using *n*-hexane, ethyl acetate, and methanol. The resulting solutions were separately evaporated and subjected to rat intestinal glucosidase inhibitory activity assay. The fraction with the highest AGI activity was further fractionated using reversed-phase PLC (RP-PLC) (Supelco silica gel 60 RP-18 F_{254}, 20 cm × 20 cm, 1 mm, Merck KGaA, Darmstadt, Germany; mobile phase: ethyl acetate–methanol 4:1; detection: UV 254 and 356 nm). This particular fraction with the highest AGI activity was fractionated again using RP-PLC (Supelco silica gel 60 RP-18 F_{254}, 20 cm × 20 cm, 1 mm, Merck KGaA, Darmstadt, Germany; mobile phase: ethyl acetate–methanol 2:3; detection: UV 254 and 356 nm) to isolate the bioactive compound.

The bioactive compound was analyzed using liquid chromatography–mass spectrometry (LC-MS) (UPLC

HSS C18, 3.5 µm, 2.1 mm × 100 mm column at a temperature of 40 ℃ and gradient eluent of methanol–water 10:90 to 90:10 for 30 min, ES (positive) scanning). Comparison with the pure standard was also conducted using high-performance liquid chromatography (HPLC; Agilent Technology, 1220 Infinity II LC; Column: Zorbax Eclipse Plus 2.6 mm × 150 mm, 5 µm; mobile phase: acetonitrile–glacial acetic acid–water (15:0.5:85, v/v) with pH adjusted to 4.5, isocratic elution at a flow rate of 1.0 mL/min; detection: UV 321 nm).

Results and Discussion

In vitro evaluation of α-glucosidase inhibitory activity. AGIs are expected to effectively inhibit postprandial hyperglycemia by suppressing carbohydrate digestion (Figure 2). In this study, AGI activity against the digestion of sucrose and maltose was observed. In conjunction with solvents commonly used in Indonesian herbal industries, lemongrass was extracted using ethanol 96% (ELG) and water (WLG). The inhibitory activities of both extracts against the hydrolysis of sucrose and maltose by rat intestinal α-glucosidase were evaluated. The activities were expressed as IC_{50} to show the concentration required to inhibit 50% of enzymatic activity. ELG showed higher inhibitory activities (Sucrase IC_{50} = 8.74 mg/mL; Maltase IC_{50} = 18.93 mg/mL) than WLG (Sucrase IC_{50} = 132.89 mg/mL; Maltase IC_{50} = 302.27 mg/mL). Both extracts tend to have high inhibitory activities against sucrose hydrolysis.

In a previous study, lemongrass had been extracted using methanol, water, chloroform, and acetone. Evaluation of AGI activities of the extracts showed that methanol extract has the highest activity [15]. Due to the proximity of methanol and ethanol polarities, the
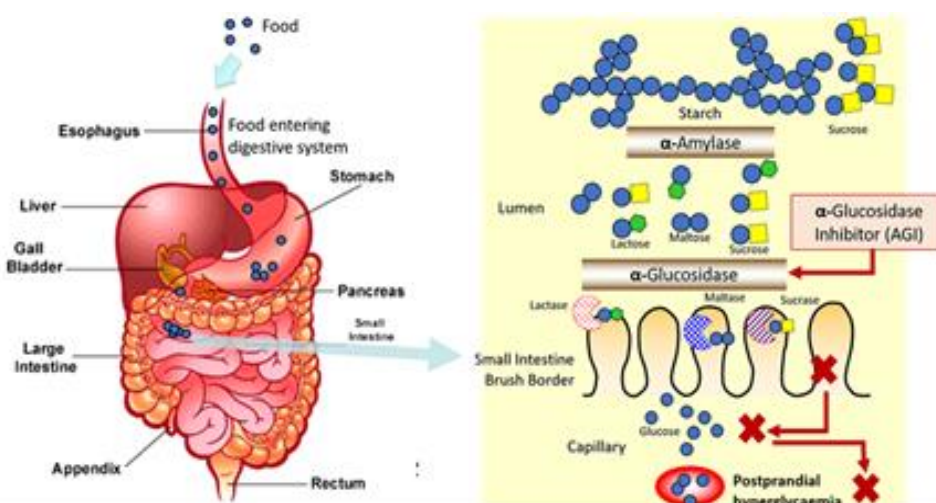

Figure 2.   Schematic Mechanism of the Retardation of PPG Elevation Due to the Inhibition of the Hydrolysis of Disaccharides to Glucose by AGI

previous study was in agreement with the better activity of ethanolic extraction in this study. This study confirmed the lemongrass activities to inhibit the hydrolysis of sucrose and maltose by intestinal mammalian glucosidase enzyme with higher activity extracted in ethanol as solvent. Due to its higher AGI activity, ELG was selected for further tests.

In vivo evaluation of antihyperglycemic activity. Sugar treatment increased the PPG level in mice 15 min after administration. The AUC of the PPG level 120 min after administration was compared between groups of mice to evaluate the significant changes caused by the treatments. Sugar treatments (SC, MC, and GC) significantly increased the total PPG level in comparison to the normal control group with no sugar treatment (C). Before glucose was transported to the cells and stored as a source of energy, glucose existed in the bloodstream [23]. Acarbose is a common antihyperglycemic agent that is used as a positive control in this study. Groups of mice treated with acarbose (SA, MA, and GA) before sugar administration have significantly lower total PPG levels in comparison to those that are only receiving sugar treatments (SC, MC, and GC), although the values are still higher than C. Acarbose was shown to suppress and decrease the spike of blood glucose as it inhibits the breakdown of carbohydrate [24] and the absorption of monosaccharides [25].

In OSTT, the total PPG elevation of mice treated with acarbose and ELG (SA, S1, S2, and S3) was significantly lower than that of mice in the sucrose control group (SC) (Figure 3), confirming its antihyperglycemic activity that inhibits the breakdown of carbohydrate [26]. At the concentrations used in this study (i.e., 0.22, 0.67, and 2.00 g/kg BW), ELG activity was significantly lower than acarbose activity and did not show a dose-dependent manner. Therefore, the optimum effective dose in this study was 0.22 g/kg BW
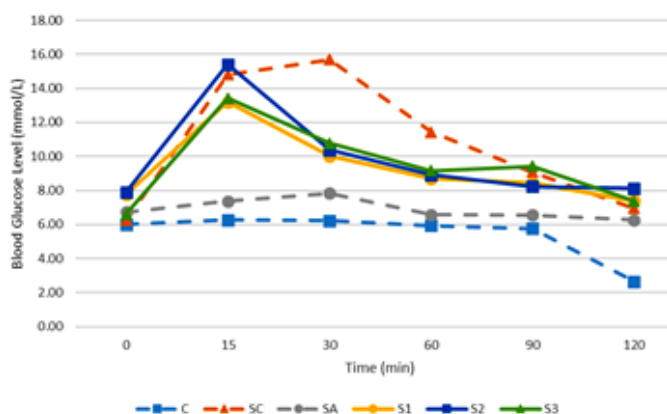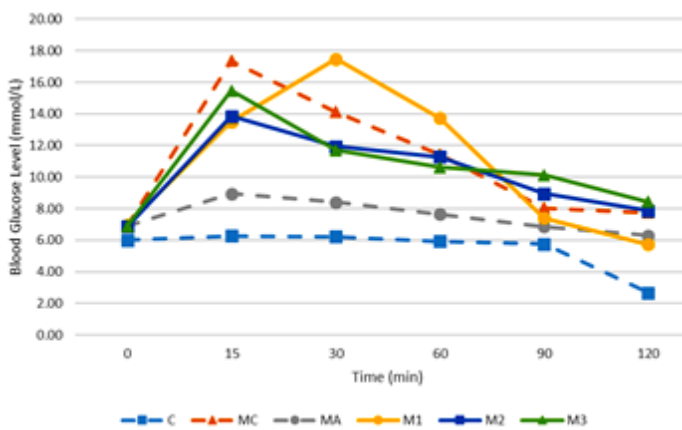
ELG (S1) treatment as it has a significantly lower total PPG level compared with SC and a similar effect to higher ELG doses (S2 and S3).

The PPG levels of SC continued to increase up to 15.7 ± 3.1 mmol/L 30 min after sucrose administration. The PPG levels of SA, S1, S2, and S3 also increased but only up to 15 min after sucrose administration and returned to the basal level faster than SC. These results indicated that ELG might also facilitate glucose clearance from the blood vessel, adding another mechanism for its postprandial antihyperglycemic activity [25].

In OMTT, ELG treatments at the concentrations used in this study (M1, M2, and M3) were unable to reduce the total PPG elevation in comparison to those untreated maltose control (MC) (Figure 4). This result was consistent with the result of the in vitro evaluation, which showed that ELG has lower inhibitory activity against maltose hydrolysis than sucrose hydrolysis. Although ELG activity to inhibit maltose hydrolysis was not shown as the AUC of the total PPG elevation, M2 and M3 have significantly lower maximum PPG levels (13.85 ± 2.3 and 15.46 ± 1.9 mmol/L, respectively) than MC (17.4 ± 1.4 mmol/L) 15 min after maltose administration. Groups of mice treated with a low dose of ELG (M1) have a similar maximum PPG level (17.5 ± 2.9 mmol/L) to MC. However, M1 was able to delay the increase to 30 min after maltose administration in comparison to MC (15 min after maltose administration). ELG inhibits maltose digestion and retards the absorption of glucose, thereby preventing PPG spikes that are often associated with atherosclerosis [27] and other complications [4]. Although M1 was unable to reduce the total PPG elevation, the PPG level of mice treated with 0.67 and 2.00 g/kg BW (M2 and M3) returned to the basal state faster than MC, indicating its mild activity in facilitating glucose clearance from the blood vessel [25].

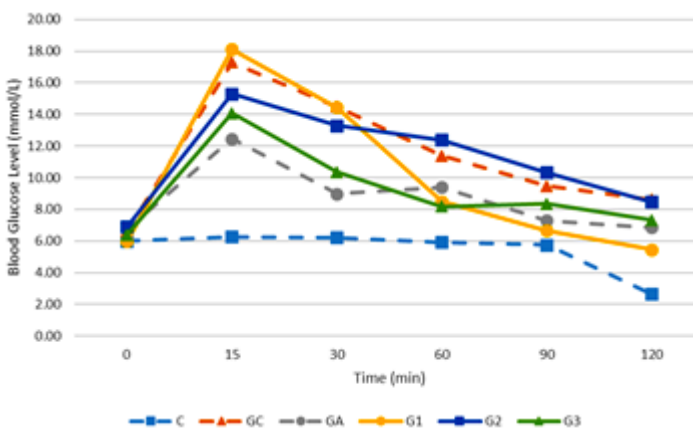| Mice Group	Average AUC (mmol.min /L)	%AUC reduction
C	687.6 ± 41.6 [b,c]	-
SC	1341.2 ± 56.1 [a,c]	-
SA	825.2 ± 46.1 [a,b]	38.47
S1	1103.4 ± 72.2 [a,b,c]	17.73
S2	1158.0 ± 91.2 [a,b,c]	13.66
S3	1161.5 ± 61.8 [a,b,c]	13.4

All treatments were done orally. The results are presented as the average $\pm$ SD; a: $p < 0.05$ vs normal; b: $p < 0.05$ vs sucrose; c: $p < 0.05$ vs acarbose.

Figure 3. Blood Glucose Reduction of Sucrose-induced Mice Over Time and Average AUC and Percentage of AUC Reduction of ELG after Sucrose Oral Induction in Mice ($n = 5$) from the Groups of Normal Control (C), Sucrose Control (SC), Sucrose + Acarbose 0.08 g/kg BW (SA), Sucrose + ELG 0.22 g/kg BW (S1), Sucrose + ELG 0.67 g/kg BW (S2), and Sucrose + ELG 2.00 g/kg BW (S3)

Mice Group	Average AUC (mmol.min /L)	%AUC reduction
C	687.6 ± 41.6 [b,c]	-
MC	1340.7 ± 77.5 [a,c]	-
MA	904.1 ± 34.2 [a,b]	32.57
M1	1392.2 ± 84.5 [a,c]	-3.84
M2	1252.2 ± 137.5 [a,c]	6.6
M3	1312.1 ± 41.7 [a,c]	2.13

All treatments were done orally. The results are presented as the average ± SD; a: $p < 0.05$ vs normal; b: $p < 0.05$ vs maltose; c: $p < 0.05$ vs acarbose.

Figure 4. **Blood Glucose Reduction of Maltose-induced Mice Over Time and Average AUC and Percentage of AUC Reduction of ELG after Maltose Oral Induction in Mice ($n = 5$) from the Groups of Normal Control (C), Maltose Control (MC), Maltose + Acarbose 0.08 g/kg BW (MA), Maltose + ELG 0.22 g/kg BW (M1), Maltose + ELG 0.67 g/kg BW (M2), and Maltose + ELG 2.00 g/kg BW (M3)**

Mice Group	Average AUC (mmol.min /L)	%AUC reduction
C	687.6 ± 41.6 [b,c]	-
GC	1389.3 ± 66.2 [a,c]	-
GA	1043.4 ± 80.8 [a,b]	24.9
G1	1178.7 ± 135.1 [a,b]	15.16
G2	1388.9 ± 60.1 [a,c]	0.03
G3	1097.3 ± 59.5 [a,b]	21.02

All treatments were done orally. The results are presented as the average ± SD; a: $p < 0.05$ vs normal; b: $p < 0.05$ vs glucose; c: $p < 0.05$ vs acarbose.

Figure 5. **Blood Glucose Reduction of Glucose-induced Mice Over Time and Average AUC and Percentage of AUC Reduction of ELG after Glucose Oral Induction in Mice ($n = 5$) from the Groups of Normal Control (C), Glucose Control (GC), Glucose + Acarbose 0.08 g/kg BW (GA), Glucose + ELG 0.22 g/kg BW (G1), Glucose + ELG 0.67 g/kg BW (M2), and Glucose + ELG 2.00 g/kg BW (M3)**

ELG activities that prevent PPG spikes after sucrose and maltose consumption and total PPG elevation after sucrose consumption confirmed its in vivo AGI activity that inhibits digestive enzymes from breaking down disaccharides. The in vivo result was consistent with the in vitro result, indicating the stronger activity of ELG to inhibit sucrose hydrolysis than maltose hydrolysis (Figure 3). The non-dose-dependent manner of the activity showed that ELG activity is also correlated with glucose counterregulatory responses to hypoglycemia [26, 28]. The fast PPG level reduction to its basal state indicated that ELG might also have cell glucose uptake activity that is also preferable for the treatment of hyperglycemia [29–30]. Therefore, OGTT was further conducted to confirm this activity.

In OGTT, ELG at doses of 0.22 and 2.00 mg/kg BW (G1 and G3) were surprisingly able to reduce the total

PPG elevation in comparison to glucose control (GC) with a similar level to acarbose (GA), whereas ELG at a dose of 0.67 mg/kg BW (G2) did not (Figure 5). Again, the ELG activity did not show a dose-dependent manner, and the 0.22 mg/kg dose was once again shown to be a better treatment option because of its similar or even stronger effect on PPG level reduction compared with higher doses. Glucose counterregulatory responses to hypoglycemia at higher doses in healthy individuals might be responsible for the observed result in G2 mice [26,28]. Glucose, which is derived from the diet and synthesized by the body, needs a transporter, such as SGLT and GLUT, to be distributed to the bloodstream and cells [31]. Several mechanisms for the antihyperglycemic activity of lemongrass extract against glucose induction include the inhibition of the glucose transporter and the acceleration of the number of glucose transporters [32,33,34]. ELG activities that inhibit both PPG spike

and total PPG elevation in OGTT showed that ELG not only inhibits disaccharides from hydrolyzing enzymes but also reduces the glucose transporter that transports glucose from the digestive tract to the blood vessel and/or increases cell glucose uptake that facilitates glucose clearance from the blood vessel.

Isolation of α-glucosidase inhibiting principles. Bioactive compounds responsible for the AGI activity were isolated using activity-guided fractionation (Figure 1). ELG (13.90 g) was fractionated using *n*-hexane, ethyl acetate, and methanol, yielding a 3.99 g *n*-hexane-soluble fraction (ELG-He), a 2.01 g ethyl-acetate-soluble fraction (ELG-Et), and a 5.57 g methanol-soluble fraction (ELG-Me). ELG-Me exhibited the highest AGI activity (60.67 ± 3.46%) at a concentration of 1 mg/mL, whereas ELG-He and ELG-Et only showed 40.22 ± 2.30% and 43.78 ± 1.57% inhibition, respectively.

The active fraction (ELG-Me) was subjected to PLC fractionation using ethyl acetate–methanol (4:1) as the mobile phase. Collected fractions were subjected to AGI activity assay. The active fractions with 42.39 ± 4.24%

AGI activity (ELG-Me-V, Rf = 0.03–0.25, 890 mg) was further subjected to LC-MS evaluation, and the resulting compound with the mass spectrum of m/z 182.80 was identified as caffeic acid ($C_9H_8O_4$) with the molecular mass of 180.16 and the mass spectrum of m/z 285.37 was identified as kaempferol ($C_{15}H_{10}O_6$) with the molecular mass of 286.23 (Figure 6). The RP-PLC purified fraction (ELG-Me-V-3) was further compared with the standard in HPLC to confirm the isolation of caffeic acid.

Kaempferol and caffeic acid were previously identified to be present in lemongrass [8]. Moreover, research conducted by Zang et al. in 2011 and Chandramohan et al. in 2015 showed that kaempferol exhibited an antidiabetic effect by increasing the insulin level and decreasing the plasma glucose of diabetic rats [35,36]. Another research showed that caffeic acid was also able to lower the blood glucose of diabetic rats [37]. The finding of this study indicated the potential use of ELG in the management of hyperglycemia.
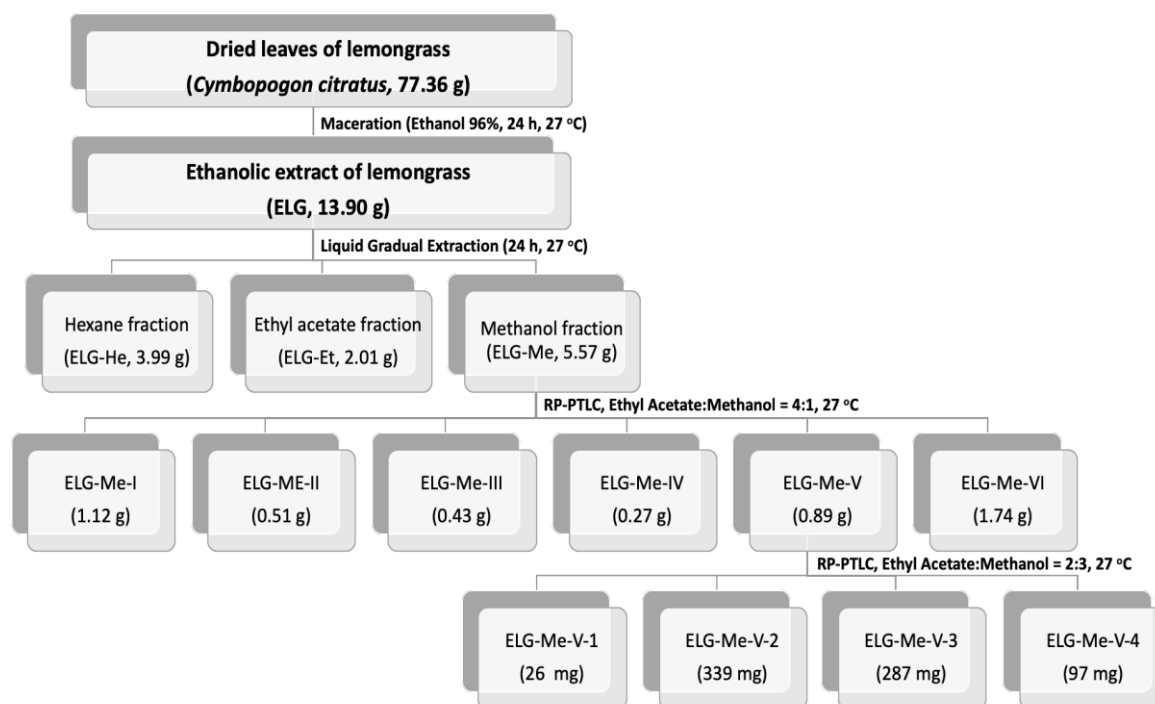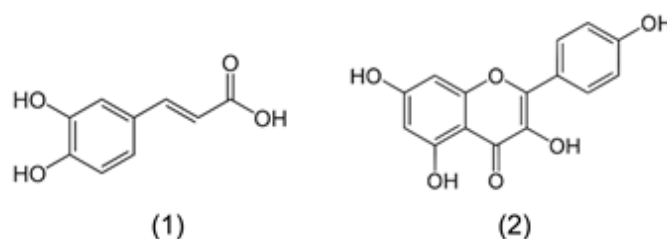

Figure 1. Extraction and Fractionation Scheme of ELG for the Isolation of the α-glucosidase Inhibiting Principles

Figure 6. Chemical Structures of (1) Caffeic Acid and (2) Kaempferol Identified in the Active Fraction of ELG

Conclusion

The AGI principles in lemongrass were better extracted in the ethanolic solvent. ELG showed a non-dose-dependent manner of inhibition of PPG elevation following all sugar challenges tested in this study, namely, sucrose, maltose, and glucose, with the lowest dose of 0.22 g/kg BW treatment showing the most optimum result. Further fractionation and identification of the bioactive compounds resulted in the identification of caffeic acid and kaempferol as the α-glucosidase inhibiting principles of ELG. This finding suggests the utilization of lemongrass in traditional and complementary medicine in the management of diabetes.

Acknowledgements

References

[1] Wild S.H., Roglic G., Green A., Sicree R., King H. 2004. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care. 27(5): 1047–1053, https://doi.org/10.2337/ diacare.27.5.1047

[2] Braun, C., Brayer, G.D., Withers, S.G. 1995. Mechanism-based inhibition of yeast α-glucosidase and human pancreatic α-amylase by a new class of inhibitors 2-deoxy-2, 2-difluoro-α-glycosides. J. Biol. Chem. 270(45): 26778–26781, https://doi.org/ 10.10 74/jbc.270.45.26778

[3] Campbell, L.K., White, J.R., Campbell, R.K. 1996. Acarbose: its role in the treatment of diabetes mellitus. Ann. Pharmacother. 30(11): 1255–1262, https://doi.org/10.1177/106002809603001110

[4] Ceriello A. 2005. Postprandial hyperglycemia and diabetes complications: Is it time to treat? Diabetes. 54(1): 1–7, https://doi.org/10.2337/diabetes.54.1.1.

[5] Dwek, R.A., Butters, T.D., Platt, F.M., Zitzmann, N. 2002. Targeting glycosylation as a therapeutic approach. Nat. Rev. Drug Discov. 1(1): 65–75, https://doi.org/10.1038/nrd708.

[6] van de Laar, F.A. 2008. Alpha-glucosidase inhibitors in the early treatment of type 2 diabetes. Vasc. Health Risk Manag. 4: 1189–1195, https://doi.org/ 10.2147/vhrm.s3119.

[7] Santoso F., Winarno J., Gunawan-Puteri M.D.P.T. 2018. Application of lemongrass (*Cymbopogon citratus*) as a functional food ingredient with alpha-glucosidase inhibitory activity. Adv. Eng. Res. 172: 205–209. https://doi.org/10.2991/fanres-18.2018.42.

[8] Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., Mann, A.S. 2011. Scientific basis for the therapeutic use of *Cymbopogon citratus*, stapf (Lemon grass). J. Adv. Pharm. Technol. Res. 2(1): 3–8, https://doi.org/10.4103/2231-4040.79796.

[9] Koop C.E. 2002. The future of medicine. Sci. 295(5553): 233–233, https://doi.org/10.1126/science.295.5553.233.

[10] Widiputri D.I., Gunawan-Puteri M.D.P.T., Kartawiria I.S. 2019. Benchmarking Study of *Cymbopogon citratus* and *C. nardus* for Its Development of Functional Food Ingridient for Anti-diabetic Treatment. Proceeding of International Conference on Innovation, Entrepreneurship And Technology (ICONIET) 2018. 2(2): 109–114, https://doi.org/10.33555/iconiet.v2i2.20.

[11] Adeneye A.A., Agbaje E.O. 2007. Hypoglycemic and hypolipidemic effects of fresh leaf aqueous extract of *Cymbopogon citratus Stapf.* in rats. J. Ethnopharmacol. 112(3): 440–444, https://doi.org/10.1 016/j.jep.2007.03.034.

[12] Nivetha G., Vishnupriya V., Gayathri R. 2016. Comparative evaluation of anti-diabetic activity of Lemon Grass oil and Tulasi oil. Int. J. Pharm. Sci. Rev. Res. 39(1): 221–225.

[13] Gunawan-Puteri M.D.P.T., Bhandari M.R., Kawabata J. 2009. Indonesian medicinal plants and their anti-diabetic potencies. In Martirosyan D. (ed). *Functional Foods for Chronic Diseases, Vol. 4.* Functional Foods Center. Dallas. pp. 110–120.

[14] Gunawan-Puteri M.D.P.T., Josopandojo B., Adiyoga G.H., Kartawiria I.S., Widiputri D.I. 2017. Aqueous extraction optimization of *C. citratus* for development of food ingredients with alpha glucosidase inhibitory. In: Sukmana I., Ulvan A. (eds). *Integrated Sci-Tech: Interdisciplinary Research Approach, Volume 2.* Research Institute and Community Services University of Lampung, Lampung. pp. 55–61.

[15] Santoso F., Soedarma C., Adiyoga G.H., Gunawan-Puteri M.D.P.T., Sutanto H. 2016. Alpha-glucosidase inhibitory effect of methanolic extracts from Indonesian plants. Proceeding of the 2nd International Conference on Sustainable Global Agriculture and Food (ICSAF) 2016. pp 240–248.

[16] Gunawan-Puteri M.D.P.T., Rustandi F, Hendra P. 2018. Spray dried aqueous extract of lemongrass

(*Cymbopogon citratus*) exhibits in vitro and in vivo anti hyperglycemic activities. J. Pharm. Sci. Community. 15(2): 55–61, https://doi.org/10.24071/jpsc.1521531.

[17] Gunawan-Puteri M.D.P.T., Kawabata J. 2010. Novel α-glucosidase inhibitors from *Macaranga tanarius* leaves. Food Chem. 123(2): 384–389, https://doi.org/10.1016/j.foodchem.2010.04.050.

[18] Yeo, J.Y., Ha, T.J., Nam, J.S., Jung, M.H. 2011. Antidiabetic effects of *Vigna nakashimae* extract in db/db mice. Biosci. Biotechnol. Biochem. 75(11): 2223–2228, https://doi.org/10.1271/bbb.110538.

[19] Wongnawa M., Tohkayomatee R., Bumrungwong N., Wongnawa S. 2014. Alpha-glucosidase inhibitory effect and inorganic constituents of *Phyllantus amarus* Schum. & Thonn. ash. Songklanakarin J. Sci. Technol. 36: 541–546.

[20] Yusoff, N.A., Ahmad, M., Al Hindi, B., Widyawati, T., Yam, M.F., Mahmud, R., Razak, K.N.A., Asmawi, M.Z. 2015. Aqueous extract of *Nypa fruticans* Wurmb. vinegar alleviates postprandial hyperglycemia in normoglycemic rats. Nutrients. 7(8): 7012–7026, https://doi.org/10.3390/nu7085320.

[21] Eseyin O., Ebong P., Eyong E., Awofisayo O., Agboke A. 2010. Effects of *Telfairia occidentalis* on oral glucose tolerance in rats. Afr. J. Pharm. Pharmacol. 46: 368–72.

[22] Jo, S.H., Ha, K.S., Moon, K.S., Lee, O.H., Jang, H.D., Kwon, Y.I. 2011. In vitro and in vivo anti-hyperglycemic effects of Omija (*Schizandra chinensis*) fruit. Int. J. Mol. Sci. 12(2): 1359–1370, https://doi.org/10.3390/ijms12021359.

[23] Aronoff S.L., Berkowitz K., Shreiner B., Want L. 2004. Glucose metabolism and regulation: beyond insulin and glucagon. Diabetes Spectr. 17(3): 183–190, https://doi.org/10.2337/diaspect.17.3.183.

[24] Rosak C., Mertes G. 2012. Critical evaluation of the role of acarbose in the treatment of diabetes: patient considerations. Diabetes Metab. Syndr. Obes. 5: 357–367, https://doi.org/10.2147/dmso.s28340.

[25] Ali, R.B., Atangwho, I.J., Kuar, N., Ahmad, M., Mahmud, R., Asmawi, M.Z. 2013. In vitro and in vivo effects of standardized extract and fractions of *Phaleria macrocarpa* fruits pericarp on lead carbohydrate digesting enzymes. BMC Complement. Altern. Med. 13(1): 1–11, https://doi.org/10.1186/1472-6882-13-39.

[26] Sprague J.E., Arbeláez A.M. 2011. Glucose counterregulatory responses to hypoglycemia. Pediatr. Endocrinol. Rev. 9(1): 463.

[27] Temelkova-Kurktschiev, T.S., Koehler, C., Henkel, E., Leonhardt, W., Fuecker, K.A.T.J.A., Hanefeld, M. 2000. Postchallenge plasma glucose and glycemic spikes are more strongly associated with atherosclerosis than fasting glucose or HbA1c level. Diabetes Care. 23(12): 1830–1834, https://doi.org/10.2337/diacare.23.12.1830.

[28] Willis, H.J., Thomas, W., Eldridge, A.L., Harkness, L., Green, H., Slavin, J.L. 2011. Glucose and insulin do not decrease in a dose-dependent manner after increasing doses of mixed fibers that are consumed in muffins for breakfast. Nutr. Res. 31(1): 42–47, https://doi.org/10.1016/j.nutres.2010.12.006.

[29] Bhattacharya, S., Christensen, K.B., Olsen, L.C., Christensen, L.P., Grevsen, K., Færgeman, N.J., Kristiansen, K., Young, J.F., Oksbjerg, N. 2013. Bioactive components from flowers of *Sambucus nigra* L. increase glucose uptake in primary porcine myotube cultures and reduce fat accumulation in *Caenorhabditis elegans*. J. Agric. Food Chem. 61(46): 11033–11040, https://doi.org/10.1021/jf402838a.

[30] Girón, M.D., Sevillano, N., Salto, R., Haidour, A., Manzano, M., Jiménez, M.L., Rueda, R., López-Pedrosa, J.M. 2009. *Salacia oblonga* extract increases glucose transporter 4-mediated glucose uptake in L6 rat myotubes: role of mangiferin. Clin. Nutr. 28(5): 565–574, https://doi.org/10.1016/j.clnu.2009.04.018.

[31] Wright, E.M., Martín, M.G., Turk, E. 2003. Intestinal absorption in health and disease–sugars. Best Pract Res Cl Ga. 17(6):943–956, https://doi.org/10.1016/s1521-6918(03)00107-0.

[32] Fadini, G.P., Bonora, B.M., Avogaro, A. 2017. SGLT2 inhibitors and diabetic ketoacidosis: data from the FDA Adverse Event Reporting System. Diabetologia. 60(8): 1385–1389, https://doi.org/10.1007/s00125-017-4301-8.

[33] Kwon, O., Eck, P., Chen, S., Corpe, C.P., Lee, J.H., Kruhlak, M., Levine, M. 2007. Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. FASEB J. 21(2): 366–377, https://doi.org/10.1096/fj.06-6620com.

[34] Wood, I.S., Trayhurn, P. 2003. Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. Br. J. Nutr. 89(1): 3–9, https://doi.org/10.1079/bjn2002763.

[35] Zang Y., Sato H., Igarashi K. 2011. Anti-diabetic effects of a kaempferol glycoside-rich fraction from unripe soybean (edamame,*Glycine max L. Merrill. 'Jindai'*) leaves on KK-A^y mice. Biosci. Biotechnol. Biochem. 75(9): 1677–1684, https://doi.org/10.1271/bbb.110168.

[36] Al-Numair, K.S., Chandramohan, G., Veeramani, C., Alsaif, M.A. 2015. Ameliorative effect of kaempferol, a flavonoid, on oxidative stress in streptozotocin-induced diabetic rats. Redox Rep. 20(5): 198–209, https://doi.org/10.1179/1351000214Y.0000000117.

[37] Mohammed, F.Z., El-Shehabi, M. 2015. Antidiabetic activity of caffeic acid and 18β-glycyrrhetinic acid and its relationship with the antioxidant property. Asian J. Pharm. Clin. Res. 8(5): 255–260.