SPRAY DRIED AQUEOUS EXTRACT OF LEMONGRASS (Cymbopogon citratus) EXHIBITS IN VITRO AND IN VIVO ANTI HYPERGLYCEMIC ACTIVITIES

AKTIVITAS IN VITRO DAN IN VIVO ANTI HIPERGLISEMIA DARI EKSTRAK AIR SERAI (Cymbopogon citratus) YANG DIKERINGKAN DENGAN METODE SPRAY-DRYING

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ABSTRACT

Lemongrass was found to be a promising herb for anti-hyperglycemia treatment due to its activities to inhibit alpha glucosidase and alpha amylase in vitro activities and ability to improve blood glucose profile. Lemongrass potency through its anti-hyperglycemic ingredients requires evaluation of the functional stability during processing. In this study, the in vitro anti-hyperglycemic activities of spray-dried aqueous extract of lemongrass were determined by its inhibitory activity against rat intestinal glucosidase enzymatic hydrolysis of sucrose. In vivo activity was observed based on its ability to prevent blood glucose elevation in oral glucose, sucrose and maltose tolerance tests (OGTT, OSTT and OMTT). The in vitro evaluation showed that aqueous extraction, which involved stirring at 70 °C for 40 min, successfully increased the glucosidase inhibitory activity of lemongrass extract, while spray drying with inlet 130 °C had no significant impact to the activity tested in vitro. Spray-dried lemongrass powder was found to be effective for lowering blood glucose level in OGTT, OSTT and OMTT. This study provides support for further development of lemongrass extracts as functional ingredients for hyperglycemia treatment.

Keywords: anti hyperglycemia, aqueous extract, lemongrass, spray-dried powder

ABSTRAK


Kata kunci: anti hiperglisemia, ekstrak air, serai, bubuk spray-dry

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INTRODUCTION
Lemongrass potencies for diabetic treatment have been reviewed in previous studies (Shah et al., 2011; Geetha and Geetha, 2014), showing 125-500 mg/kg daily oral dosing of aqueous extract from fresh leaves could lower fasting plasma glucose in male Wistar rats after 42 days of treatment (Adeneye and Agbaje, 2007). Though evaluation of its immediate impact to blood glucose has not yet been done. Lemongrass also recently had been extracted in several solvents and evaluated for its in vitro alpha glucosidase inhibitory activities (Santoso et al., 2016; Gunawan-Puteri et al., 2017), resulting in the finding of lemongrass extracted in methanol and ethyl acetate having more than 50% inhibition activity against sucrase at 0.02 mg/ml (Santoso et al., 2016). Aqueous extract of lemongrass was optimized and the selected extract was then pulverized using spray drying methods for the production of lemongrass powder for functional food ingredients (Gunawan-Puteri et al., 2017). Spray drying is widely used in the Food industry due to the affordability and effectiveness of the operation and process which are relatively flexible, produce a good quality product and can prolong the shelf life of liquid products as it turns to powder (Munin and Edwards-levy, 2011). Despite the practicality of powder ingredients, impacts of the extraction optimization and further pulverization to produce spray-dried aqueous extract of Lemongrass on the sucrase inhibition activity in vitro and in vivo have not been observed.

METHODS
Materials
Sun-dried lemongrass was collected from Yogyakarta, Indonesia through the herbal supplier CV Sekar Utami. Rat intestinal acetone powder as a source of the glucosidase enzyme was acquired from Sigma-Aldrich, Singapore, while glucose kit C-II for glucose measurement was acquired from Wako, Japan. Potassium phosphate buffer pH 7.0 and ethylenediaminetetraacetic acid were acquired from Sinopharm Chemical Reagent Co., Ltd., China, while analytical grade methanol and maltodextrin were acquired from PT Bratchem, Indonesia. Aluminium oxide 60, sucrose (saccharose), maltose and D- (+)-Glucose anhydrous for biochemistry analysis, and other chemical reagents were acquired from Merck Millipore, Germany, unless stated otherwise.

Plant Samples Preparation
Sun-dried lemongrass was ground using a miller for 90 s to reduce the size. Maceration extraction methods were used with the ratio 3:5 (v/v) between dried lemongrass and water. The basic extraction was done for 24 h in room temperature while the optimized extraction was done for 40 min at 70 °C using stirring. Crude extracts were filtered using vacuum filtration and were subsequently concentrated using a rotary evaporator at reduced pressure at 50 °C and then stored in amber bottle glass at 4 °C prior to analysis or spray drying until the total soluble solid reached more than 10%. Selected samples underwent a spray drying process using 130 °C for the inlet temperature. The nozzle number of the spray dry machine was TD7-97 with the spray angle of 65°.

Rat intestinal glucosidase inhibitory activity assay
Glucosidase inhibitory activity was determined using methods described previously (Gunawan-Puteri and Kawabata, 2010; Ieyama et al., 2011; Arsiningtyas et al., 2014) with slight revision. Rat intestinal acetone powder was cold-ground and dissolved in 0.1 M potassium phosphate buffer (pH 7.0) containing 5 mM ethylenediaminetetraacetic acid and centrifuged at 11,000 rpm, 4 °C, for 60 min. The inhibitory activity against sucrose hydrolysis was measured by the following procedures. Two test tubes, as sample and control, containing 0.20 ml sucrose solution (56 mM) in potassium phosphate buffer (0.1 M, pH 7.0) and two test tubes, containing 0.40 ml potassium phosphate buffer (0.1 M, pH 7.0) as each blank were pre-incubated at 37 °C for 5 min. The control and control blank were defined as 100% and 0% enzyme activity, respectively. The working samples diluted in water (0.10 ml) were added to the sample and sample blank test tubes while 0.10 ml water was added to the control and control blank test tubes. Next, crude rat intestinal sucrase (0.20 ml) was added only to the test tubes containing sucrose solution (sample and control). The reaction was done at 37 °C for 20 min and stopped by adding Tris–HCl buffer (2 M, pH 6.3, 0.75 ml).

The reaction mixtures were then passed through a short column of Aluminium oxide 60 (1.5 g, 500 x 5 mm) for removing phenolics, which may interfere with the following glucose quantification. Each mixture (50 μl) was placed into a 96-well microplate and added with 200 μl
Spray dried aqueous extract of lemongrass was incubated at 37 °C for 15 min. The absorbance (Abs) was measured using a UV-Vis spectrophotometer at 492 nm wavelength.

Inhibitory activity was calculated by the following equation:

\[
\text{Inhibitory activity} (\%) = \frac{(\text{Abs}_c - \text{Abs}_{cb}) - (\text{Abs}_s - \text{Abs}_{sb})}{(\text{Abs}_c - \text{Abs}_{cb})} \times 100 \%
\]

with Abs = Absorbance, \([c]\) = control, \([cb]\) = control blank, \([s]\) = sample, \([sb]\) = sample blank

The experiments were done in triplicate for each concentration and the resulting sucrase inhibitory activity was plotted in the curve against concentration to derive the linear regression mathematical formula. Data were presented as IC50, which was defined as lemongrass concentration that inhibits 50% of sucrose hydrolysis into glucose and fructose in the presence of crude extract of rat intestinal acetone powder.

Test animals and housing
All experiments were performed on adult male Swiss mice (20 - 30 g) obtained from the Imono Laboratory, Sanata Dharma University, Indonesia. The animals were maintained under standard laboratory condition. They were housed in standard cages at temperature 22 ± 2 °C and 12:12 h light dark cycle. Standard pelleted diet and water were given ad libitum. All procedures described were reviewed and approved with approval number KE/KF/0618/EC/2017 by the Health and Medical Research Ethics Committee Faculty of Medicine Universitas Gadjah Mada - Dr. Sardjito General Hospital, Yogyakarta Indonesia.

Sugar tolerance test
Anti-hyperglycemic activity of spray-dried aqueous extract of lemongrass was tested using carbohydrate loads in mice. Overnight fasted mice were used to perform oral glucose tolerance test (OGTT), oral sucrose tolerance test (OSTT) and oral maltose tolerance test (OMTT). Male Swiss mice were divided into fifteen groups (n=5) randomly (negative control, sugar [glucose/sucrose] control, positive control and dose groups).

For glucose control Group I received 2 g/kg BW glucose solution orally and Group II were given acarbose 0.08 g/kg BW for the positive control. Group III-V (treated-glucose groups) received 4.33; 6.67; 10 g/kg BW of spray-dried aqueous extract of Lemongrass respectively. After 30 min of treatment, glucose solution was administered to all mice of Group II-V at 2 g/kg BW (James et al., 2009; Pattanayak et al., 2009; Rathod et al., 2011; Ali et al., 2013).

Group VI (sucrose control) received 4 g/kg BW sucrose solution orally. Group VII (positive control) was treated with acarbose 0.08 g/kg BW. Group VIII-X (treated-sucrose groups) received 4.33; 6.67; 10 g/kg BW of spray-dried aqueous extract of Lemongrass respectively. After 30 min of treatment, the animals were administered sucrose solution 4 g/kg BW (Ali et al., 2013; Yusoff et al., 2015).

Group XI as maltose control received 3 g/kg BW orally. Group XII (positive control) was treated with acarbose 0.08 g/kg BW. Group XIII-XV (treated-maltose groups) received 4.33; 6.67; 10 g/kg BW of spray-dried aqueous extract of Lemongrass, respectively. After 30 min of treatment, the animals were administered maltose solution 3 g/kg BW (Wongnawa et al., 2014; Bae et al., 2015).

Blood collected from the tail vein of the mice and blood glucose levels were measured at 0, 15, 30, 60, 90 and 120 min using a glucometer (GlucoDr, All Medicus Co. Ltd) (Yeo et al., 2011; Wongnawa et al., 2014). Area under the blood glucose-time curve up to the last sampled time-point (AUC) was calculated using the trapezoid method formula (Eseyin et al., 2010; Jo et al., 2011; Yusoff et al., 2015).

Statistical analysis
Results are expressed as mean ± standard deviation (SD). Data were analyzed using Kruskal-Wallis analysis of variance followed by post-hoc Mann-Whitney tests using SPSS 22. A p-value <0.05 was considered statistically significant.

\[
\text{AUC} = \left( \frac{C_1-C_0}{T} \times t_1 - t_0 \right) + \left( \frac{C_2-C_1}{T} \times t_2 - t_1 \right) + \ldots + \left( \frac{C_n-C_{n-1}}{T} \times t_n - t_{n-1} \right)
\]

with \(T\) = time, \(C\) = concentration of glucose.
Table I. The Glucosidase Inhibitory Activity of Lemongrass Extract and Powder

<table>
<thead>
<tr>
<th>Samples</th>
<th>Treatments</th>
<th>Glucosidase IC$_{50}$ (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG Extract 1</td>
<td>Extraction: Maceration, room temperature, 24 h</td>
<td>132.89</td>
</tr>
<tr>
<td>LG Extract 2</td>
<td>Extraction: Maceration, stirring, 70 °C, 40 min</td>
<td>14.46</td>
</tr>
<tr>
<td>LG Powder</td>
<td>Extraction: Maceration, stirring, 70 °C, 40 min; Spray drying:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inlet 130 °C, spray angle 65°</td>
<td>18.22</td>
</tr>
</tbody>
</table>

Note: Values were presented as IC$_{50}$, defined as Lemongrass concentration to inhibit 50% of sucrose hydrolysis reaction in vitro.

RESULT AND DISCUSSION

Our results showed that the optimized extraction method using water solvent at 70°C increased the glucosidase inhibitory activity of the lemongrass extract despite the very short extraction time (40 min) compared to the basic extraction at room temperature for 24 h (Table I). While further study is required to show the active compound responsible for the sucrase inhibitory activity, previous study showed positive correlation of phenolic content and alpha glucosidase inhibitory activity of Indonesian medicinal plants tested, including lemongrass (Santoso et al., 2016). Aqueous extract of lemongrass is known to contain phenolics, such as caffeic acid, chlorogenic acid, catechol, elicicin, and hydroquinone (Shah et al., 2011), and some of the aforementioned compounds, such as caffeic and chlorogenic acids, were already known for its alpha glucosidase inhibitory activity in vitro (Oboh et al., 2015). Previous observations of the effect of extraction solvents, temperature and time on the total phenolic content in Salvia officinalis L. showed that higher temperature in combination with polar solvent increased phenolic solubility and diffusion coefficient. Best extraction conditions according to the higher total phenolic content were acquired using 30% ethanolic extraction at 60 °C for 30 min, followed by aqueous extraction at 60 °C for 90 min and water extraction at higher temperature was shown as the most desirable extraction method to acquire more caffeic acid in other plants (Dent et al., 2012).

Sudden heat exposure in the spray drying process was seen to reduce 26.09% of the sucrase inhibitory activity. Previous study shown that the addition of both maltodextrin and combination of maltodextrin and Arabic gum seemed to reduce the inhibitory activity even more (Gunawan-Puteri et al., 2017). Encapsulation is a common practice in pulverization using spray drying due to its ability to protect products from heat and decrease sticking possibility inside the spray dryer (Nogueira et al., 2014). Maltodextrin of DE 20-21 has been found as the best encapsulation agent for anthocyanin, while the combination of maltodextrin and Arabic gum (3:2) was shown to be the best encapsulating agent of polyanilines (Munin and Edwards-Levy, 2011). However, both encapsulating agents are a polysaccharide carbohydrate and though it may protect the active compound in the Lemongrass extract, it may also hydrolyze by heat in spray drying process or enzymatic treatment in sucrase inhibitory activity analysis contributing to the higher content of glucose in the end of analysis (Parikh et al., 2014).

Figure 1. Effects of spray-dried lemongrass powder in oral glucose (a), sucrose (b), and maltose (c) loading test on mice. Values are expressed as mean (n=5). Gluc: glucose, Sucr: Sucrose LG: Spray-dried Lemongrass powder.
Table II. The Area Under Curve in Oral Sugar Loading Test after Administration Acarbose and Spray-Dried Lemongrass Powder on Mice

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Area Under Curve (mg.min/dl)</th>
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<tbody>
<tr>
<td></td>
<td>OGGT</td>
</tr>
<tr>
<td>Sugar control</td>
<td>25530 ± 1924&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acarbose 0.08 g/kg BW</td>
<td>14624 ± 2001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LG 4.33 g/kg BW</td>
<td>23996 ± 745&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LG 6.67 g/kg BW</td>
<td>16034 ± 2045&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LG 10 g/kg BW</td>
<td>11888 ± 1974&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Treatments were co-administered orally and respectively with glucose (2 g/kg BW) in OGGT test, sucrose (4 g/kg BW) in OSTT test and maltose (3 g/kg BW) in OMTT. Values are expressed as mean ± SD of five animals in each group; a: p<0.05 vs acarbose; b: p<0.05 vs glucose; c: p<0.05 vs sucrose; d: p<0.05 vs maltose. LG: Spray-dried Lemongrass powder.

In the in vivo study, sugar tolerance tests in mice were employed to observe the anti-hyperglycemic activity of spray-dried lemongrass powder. The blood glucose levels increased 15 min after administration of glucose (2 g/kg BW), consistent with previous results (Mustaffa et al., 2014) (Figure 1A). Similar patterns were observed in sucrose loading (4 g/kg BW) and maltose loading (3 g/kg BW) (Figure 1B and 1C).

Administration of lemongrass extract for 30 days caused a steady decrease in blood glucose levels in normal rats (Ademuyiwa et al., 2015). However, no sugar tolerance test has been reported yet. Spray-dried lemongrass powder with oral dose of 6.67 g/kg BW was found to be effective in lowering blood glucose level in glucose and sucrose tolerance tests, while a significant decrease in AUC at dose 4.33 g/kg BW was observed in the maltose tolerance test (Table II). This finding suggested that spray-dried lemongrass powder probably exerted its antidiabetic effect via suppressing postprandial hyperglycemia. The present study confirmed the result of our previous study that shown lemongrass had an inhibition activity against sucrase (Nivetha et al., 2016; Santosoo et al., 2016; Gunawan-Puteri et al., 2017). Glucose derived from the diet and body synthesized needs transporters (SGLT, GLUT) to be transported into the bloodstream and cells (Wright et al., 2003), and before glucose is transported to the cells and stored for a source of energy, glucose exists in the bloodstream (Aronoff et al., 2004). There are several mechanisms to decrease the amount of glucose in the bloodstream, such as inhibition of glucose transporter and acceleration of the number of glucose transporters (Wood and Trayhurn, 2003). The effect of spray-dried lemongrass powder in prevention of blood glucose rise in OGGT test indicates the possibility that lemongrass might possess other anti-hyperglycemic activities than sucrase inhibitory activity.

The oral dose of 10.00 g/kg BW seemed to lower blood glucose to levels that are lower than acarbose treatment (Table II). Acarbose is a commercial anti-hyperglycemic medicine that has been used for more than 20 years to control hyperglycemia (Rosak and Mertes, 2012) and its ability to reduce hyperglycemia in sucrose tolerance tests has been proved (Ali et al., 2013). Nonetheless, the effect caused by the treatment of oral dose of 10.00 g/kg BW lead to the conclusion that the dose might not be suitable to be further observed in humans since it caused abrupt blood glucose lowering impact that may lead into hypoglycemia and subsequent drawbacks.

**CONCLUSION**

This study found that the aqueous extraction method, which involved stirring at 70 °C for 40 min successfully increased the sucrase inhibitory activity of lemongrass extract, while spray drying with inlet 130 °C did not have significant impact. The spray-dried lemongrass powder proved to be effective in lowering blood glucose level on OGGT, OSTT and OMTT.

**ACKNOWLEDGEMENT**

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