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Effect of pre-treatment processes and stability testing of lemongrass (*Cymbopogon citratus*) extract on α -glucosidase inhibitor (AGI) and α -amylase inhibitor (AAI) activities

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Abstract:

Lemongrass was proved in the previous studies to be one of Indonesian local plants with relatively high activity in inhibiting α -glucosidase and α -amylase enzymes and thus it can be useful to lower blood glucose level in diabetic patients. This health benefit of lemongrass has unfortunately not been widely explored in the herbal industries. Even though lemongrass has become one of the main raw materials in such industries, the use of lemongrass has been purposed mostly to obtain its aroma and taste. Commercialization of lemongrass as herbal medicine or functional food ingredients with α -glucosidase inhibitor (AGI) and α -amylase inhibitor (AAI) activities requires a closer study on how these activities can be affected by different pre-treatment processes of fresh lemongrass. In this work, the effect of different washing and drying scenarios during the pre-treatment process of lemongrass extraction on both AGI and AAI activities was studied. The result showed that a combination between 1 time washing and oven drying at 40°C offered the optimum AGI activity. The AAI level was found to be significantly decreased as lemongrass had gone through drying process. However, when compared among different drying methods and different sequences of washing process, the AAI level was found to be relatively unaffected. Stability testing of powdered lemongrass extract was additionally conducted in real time and accelerated conditions to make an estimation of the shelf-life. The shelf-life of powdered lemongrass extract was found to be ± 8 months (at 5°C), ± 3 months (at 25°C) and ± 1.5 months (at 30°C).

Keywords: lemongrass, AGI, AAI, diabetes

Introduction

Various Indonesian medicinal plants have been studied in previous researches to find the presence of α -glucosidase and α -amylase inhibitory activities [1], [2]. Among these plants, *Cymbopogon citratus*, commonly known as lemongrass, had been found to show high anti-diabetic potencies, while showing good stability when undergone heat challenges. Lemongrass extract obtained through an extraction from fresh plant using water at 70°C for 40 minutes resulted in a sucrase inhibitory activity that ranged from ± 70 -100% [2]. This ability of lemongrass extract to inhibit the breaking down of disaccharide into monosaccharide shows a promising potential to help diabetic patients in maintaining their blood glucose level. Thus, an effort to commercialize lemongrass extract as a functional food or herbal medicine ingredient is necessary to be made.

Unfortunately, the use of lemongrass in herbal industries has not been focused on taking benefit of its α -glucosidase inhibitor (AGI) and α -amylase inhibitor (AAI) activities yet. Indonesian herbal industries commonly use lemongrass as one of their main raw materials with a main purpose to obtain its taste and aroma, rather than its bioactive materials content. An industrial observation conducted in this work revealed the need to firstly study the impact of different pre-treatment processes of fresh lemongrass prior to extraction process, on the level of AGI and AAI activities of the resulting lemongrass extract. Moreover, since it is found to be more favorable to have the extract in powder form for further use as an ingredient, pulverization of the lemon grass extract and a stability test on this powdered extract become very essential to be conducted.

Materials and methods

Materials

Materials used in this research were *Cymbopogon citratus* or lemongrass plants and chemicals for analysis purposes. The fresh lemongrass used was obtained from a farm in Bogor, Indonesia. The chemicals listed were used for sucrase inhibition assay, porcine pancreatic amylase inhibition assay, and filler used in spray drying process. Rat intestinal acetone powders

needed for sucrase inhibition assay was provided by Sigma-Aldrich. Potassium phosphate buffer, ethylenediaminetetraacetic acid (EDTA), analytical grade sucrose, and other chemicals used for this purpose were purchased from Merck, Germany, whereas the glucose kit was purchased from Wako, Japan. For porcine pancreatic amylase inhibition assay the chemicals needed were citric acid, di sodium hydrogen phosphate dodecahydrate, and analytical grade starch obtained from Merck, Germany. Maltodextrin and Arabic gum used in the pulverization process were technical grade and purchased from PT Bratachem Indonesia.

Research methodology

This study was divided into 4 stages, started with an industrial observation in 3 Indonesian reputable herbal industries. The result of this benchmarking study was then taken into consideration in the second stage, which was purposed to study the effect of different pre-treatment processes on the AGI and AAI activities of the lemongrass extract.

This stage was broken down further into 2 steps, which included an observation of washing and drying process. There were two options of washing sequences conducted; the first one is to perform the washing process one time, prior to drying. The second washing scenario was to perform it two times, prior to and subsequent to drying process (shown in Figure 1). After being had undergone the washing process observation, both lemongrass samples from each washing option were then dried by using an oven at 40°C until the moisture content was less than 10%. The resulted dried lemongrass were then milled and extracted with water, then the extract was analyzed to compare their AGI and AAI activities. The washing scenario that offered best result was then chosen to be used in the next observation, which was the drying process optimization (Figure 2). The effect of drying methods on AGI and AAI activities was observed using three different drying methods; these were sun drying, oven drying at 40°C, and oven drying at 60°C. Similarly with the previous step, the extract obtained from each drying variation was then analyzed to determine the most optimum drying method.

After having determined the effect of pre-treatment processes on AGI and AAI activities of lemongrass extract, pulverization of the liquid extract through spray drying was conducted. In a previous study by [2] an optimization of fresh lemongrass extraction process to yield the highest percentage of total soluble solids (TSS) content had been done. It was recommended that fresh lemongrass is extracted with water at 70°C for 40 minutes, with plants to water ratio of 3:10 under continuous stirring, where a TSS content up to 2.00% can be yielded.

In this work, the selection of the best filling agent (filler) to be used in spray drying process was conducted. Addition of filler might help protect the stability of the sample and even further improve their shelf life. The use of two types of filler were compared; the first type was 100% maltodextrin, and the other was mixing between Arabic-gum and maltodextrin with a ratio of 40:60. The result between these fillers were then compared through AGI and AAI assay.

In the last stage, stability testing of the pulverized lemongrass extract was performed. In food and drug industries, principle of kinetic degradation can be used to analyze the stability of certain compound or the shelf life of the product in certain condition. This can later be used to determine the best condition to store the product [3]. The lemongrass powder extract produced in the previous stage was tested in 2 different conditions; at real time for climate zone II ($\pm 25^{\circ}\text{C} \pm 2 / 60 \pm 5 \text{ RH}$) [4] and in an accelerated condition at $\pm 40^{\circ}\text{C} \pm 2 / 75 \pm 5 \text{ RH}$. A prediction of the shelf life of this powder extract in other climate zones that represents possible storing conditions in Indonesia was then made based on the stability testing.

Analysis techniques

The sucrase and α -amylase inhibitory activity was analyzed and determined using the method previously described in [5] with slight adjustments. For sucrase inhibition assay, a total of 100 μL extract in 50% dimethyl sulfoxide (DMSO) was placed in a tube containing a pre-incubated sucrose. The solution was then reacted by 200 μL enzyme and incubated at 37°C for 20 minutes. The reaction was stopped by adding Tris-HCl 2 M at pH 9, and the solution was then passed through aluminum oxide column. Afterwards, 50 μL of this solution was mixed with 200 μL of glucose kit. The mixture was incubated at 37°C for 10 minutes, and was then measured using micro-plate reader at the wavelength of 490 nm.

The α -amylase inhibitory activity was analyzed through porcine pancreatic amylase (PPA) assay. For this assay, 200 μL lemongrass extract in 50% DMSO was mixed with a pre-incubated starch azure solution (2 mg/700 μL) at 100°C , then reduced to 37°C , for 5 minutes at each temperature. Afterwards, it was reacted with PPA enzyme and incubated at 37°C for 10 minutes. The reaction was stopped using 50% acetic acid. The solution was then centrifuged at 4°C , 3000 rpm for 5 minutes and was read using micro-plate reader at the wavelength of 630 nm. The control was analyzed using the same method in each assay, only replacing the extract in 50% DMSO with 50% DMSO only.

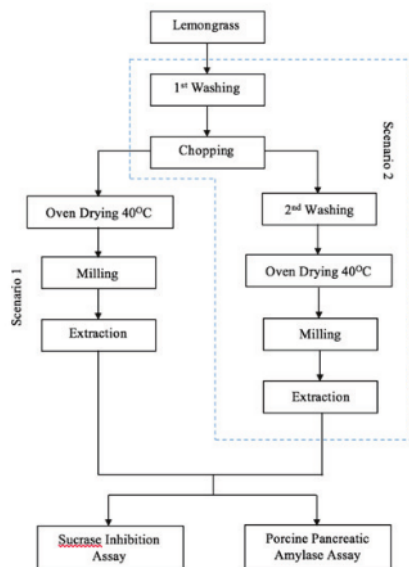


Figure 1 Optimization of washing process

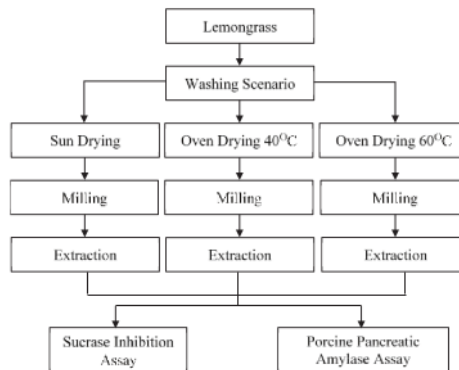


Figure 2 Optimization of drying process

Results and discussion

Effect of pre-treatment process on AGI and AAI activity

This study started with an industrial observation in 3 reputable Indonesian herbal industries, who produce different types of herbal products and medicines. The visit to these industries was purposed to learn, whether lemongrass was utilized as a raw material in their production processes and the reason for this utilization. The result of this observation showed that lemongrass was indeed one of the main raw materials used in producing herbal products, with the main purpose to obtain the typical aroma and taste of this plant. Several health benefits of lemongrass were also targeted through the use of this plant in herbal products, as it is believed that lemon grass can help in treating anemia, it can act as anti-inflammatory remedy as well as anti-microbial agent [6]. However, there has not been any attempt made to industrially produce lemongrass extract for the purpose of diabetes therapy. Complete information resulted from this industrial observation is summarized in Table 1.

Based on the results of this industrial observation, it was concluded that processing lemongrass in industrial scale requires the plants to be dried after harvesting, since in dried condition, this material can be transported in a more efficient, practical and safer way.

Moreover, it was learned that once the dried plants was delivered to an industry, it would be possible that a standardization of quality must take place. This pre-treatment of raw materials can involve washing, drying and grinding processes. Additionally, it was also found out, that the most common drying methods used in the herbal industry to pre-treat their raw materials are sun drying and oven at 40°C - 60°C. These information was taken into account in the next stages of this experiment, in order to study further how the AGI and AAI activity of lemongrass could be affected after being pre-treated before even going through the extraction process.

Table 1 Result of benchmarking study to Indonesian herbal industries

	INDUSTRY 1	INDUSTRY 2	INDUSTRY 3
<i>Plants as raw material</i>	Most of the raw materials were bought from distributors in dried condition. Lemongrass is utilized in fresh condition, to obtain scent and flavors.	All raw materials were provided by distributors in dried condition and were ready to be used in production.	All raw materials were bought from farmers in fresh condition.
<i>Standardization of raw materials</i>	Re-standardization: Sorting, Washing, Drying, Grinding, Sieving	Sampling to conduct several quality assurance tests in laboratory.	All plants were ensured to be fresh at delivery by giving farmers trainings on necessary SOPs (standard operating procedures) previously.
<i>Pre-treatment process of raw materials on-site</i>	Washing to remove soil and dirt Drying using hot air conveyor Drying using oven at 60°C Grinding	No pre-treatment process necessary, sun drying had been conducted by farmers before delivering to the industry.	Washing Drying using spinner Chopping Drying using air dryer Drying using hot air dryer at 40°C

Understanding the possibility for the lemongrass to undergo 2 times of washing process; first washing is done by the farmer directly after harvesting and prior to sun drying, and the second one is on-site done by the herbal industry, it became necessary to study whether there could be a reduction of AGI and AAI activity due to these washing processes. An observation on the enzymes inhibition (%) is shown in Figure 3, which depicts a significant reduction of AGI activity when the lemongrass was washed two times (p-value = 0.002). The reduction of α -glucosidase inhibition in 2 times washing was expected to happen due to the leaching of some water soluble contributing bio-active compounds during the process, which are suspected to be mostly phenolic compounds [7]. This was also proven through an analysis of AGI activity in the rinsing water, which resulted in \pm 9% inhibition of α -glucosidase enzyme. However, the inhibition of α -amylase enzyme was not significantly affected by doing multiple washing (with a p-value of 0.08).

In the next step, an observation on the drying methods' effect on AGI and AAI activity level was conducted. Figure 4 showed a comparison of the inhibition level of both α -glucosidase and α -amylase enzymes between different drying methods. The results were also compared to the AGI and AAI level of lemongrass extract obtained from its fresh condition (undried). From this figure, it can be seen that the highest inhibition activity was actually obtained when the extract came from fresh (undried) lemongrass. However, when obtaining lemongrass in fresh condition in a large scale is not an option, it can be seen from Figure 4 that oven drying at 40°C will deliver the highest AGI activity. Meanwhile, the activity of AAI was found to be insignificantly affected by the observed drying methods. Despite having the lower activity of AGI and AAI when compared to fresh lemongrass extract, dried materials are still more preferable in industries because it can facilitate a long term storage of raw materials, and can enable more effective and efficient processing, such as the need of a smaller amount of solvent for extraction [8].

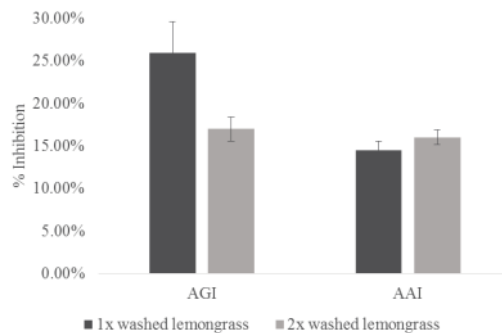


Figure 3 Comparison of AGI and AAI activity between 1 times and 2 times washed lemongrass

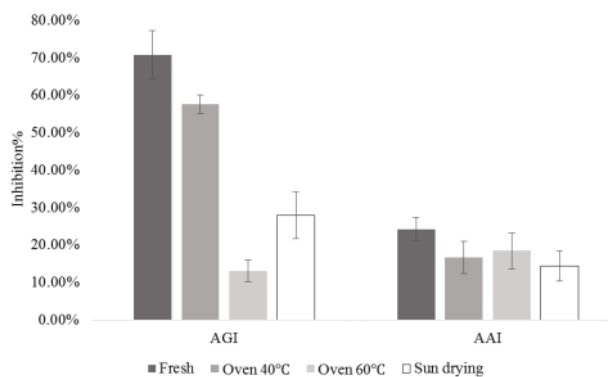


Figure 4 Effect of drying processes of lemongrass on AGI and AAI activity

Stability testing of lemongrass powder extract

In the subsequent stage to studying the effect of different pre-treatment processes on the AGI and AAI activity level, a stability testing of lemongrass powder extract was performed in this work. The method used to pulverize the liquid extract is as previously discussed in the research methodology part of this paper. However, since heat treatment can cause denaturation of organic compounds, the application of spray drying as one of the most widely used methods for pulverization in the food and pharmaceutical industries is concerned to lower the AGI and AAI activity of lemongrass extract. Thus, an addition of filling agent (filler) is required, which is expected to act as an encapsulation agent that can enhance product stability even when exposed to high temperature.

As can be seen in Table 2, the α -glucosidase (sucrase) and α -amylase inhibition of powder extracts using two types of filler were analyzed, and compared with the inhibition of liquid extract (prior to spray drying). The extract used in this part of experiment was obtained using fresh (undried) lemongrass plants. The results showed that there is no significant difference between the use of 100% maltodextrin and mixture of maltodextrin-Arabic gum (60:40). The reduction of AGI activity after spray dried was $\pm 38.5\%$ and of AAI was $\pm 40\%$. However, due to practicability and economical reason, 100% maltodextrin was selected to be used in producing the powder extract. The stability test was then performed on this powder extract.

Table 2 Filler selection

Sample	Filler	Inhibition	
		AGI (%)	AAI (%)
Pre evaporation	Without filler	100.00 \pm 0.00	81.77 \pm 0.25
Post evaporation	100% Maltodextrin	61.22 \pm 0.23	48.53 \pm 0.55
Post evaporation	(60:40) Matrodextrin-Arabic gum	61.69 \pm 0.62	49.49 \pm 1.52

The stability or shelf-life testing was conducted in 30 consecutive days under 2 different conditions. The first condition was labelled Real Life (RL) which represented a sample storing at 25°C and the second condition represented the accelerated condition at 40°C. The results of AGI and AAI activity level observation were plotted using Arrhenius kinetics principles, to

find out that the inhibition activity degradation of the lemongrass powder extract followed a first order reaction. Equation 1 below was used to find the activation energy of the degradation reaction. By using the same equation, different rate constants (k) at different temperatures can be determined.

$$\ln \frac{k_2}{k_1} = \frac{E}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right) \quad (1)$$

where k is constant rate at temperature (K), E is the activation energy (J/mol), and R is universal/ideal gas constant (8.314 J/mol.K).

The shelf-life of the lemongrass powder extract in different temperatures was determined using equation for calculating the half-life of a product (equation 2) with an adjustment that the final concentration should be 90% of the initial condition instead of 50%. Table 3 shows the α -glucosidase (sucrase) and α -amylase inhibition degradation down to its 90% and 50% of its initial inhibition at different storage temperature. Based on its climate condition, Indonesia was grouped into zone 4 with a condition of 30°C/ 70% RH [4]. Other possible storing condition of the lemon grass extract would be under refrigerated condition, which can be taken as an average of $\pm 5^\circ\text{C}$. At these two temperatures, based on the AGI activity only, the shelf-life of lemongrass powder extract is ± 1.5 months and ± 8 months respectively.

$$t_{1/2} = \frac{\ln \frac{C_A}{0.5C_A}}{k} \quad (2)$$

where $T_{1/2}$ or $t_{C=0.5}$ denotes the half-life or t-half (time) and C is the concentration.

Conclusions

Pre-treatment processes of fresh lemongrass affect the α -glucosidase and α -amylase inhibitory activity of its extract. Results of this study showed that conducting multiple washing sequence and drying process can decrease the AGI activity significantly, whereas the AAI is only slightly affected. The pulverization of lemongrass extract can be done through spray drying with the addition of maltodextrin as a filler, where a reduction in the inhibition activity of around 40% will be resulted. The shelf life based on AGI activity of the lemongrass powder extract at room temperature 25°C, in Indonesian climate zone (30°C) and under refrigerated condition (5°C) was found to be around 3, 1.5 and 8 months respectively.

Table 3 Shelf life testing at different temperatures

Inhibitor	Temperature (°C)	Rate constant (k) %inh. Day ⁻¹	t _{C=0.5} (Months)	Shelf-life (Months)
α-glucosidase (sucrase)	40	1.20.10 ⁻⁰²	1.92	0.29
	30	2.80. 10 ⁻⁰²	8.25	1.25
	27	1.80. 10 ⁻⁰³	12.83	1.95
	25	1.30. 10 ⁻⁰³	17.77	2.70
	5	4.63.10 ⁻⁰⁴	49.94	7.59
α-amylase	40	8.60. 10 ⁻⁰³	2.68	0.41
	30	1.70. 10 ⁻⁰³	13.59	2.06
	27	1.00. 10 ⁻⁰³	23.10	3.51
	25	7.00. 10 ⁻⁰⁴	33.00	5.02
	5	1.62.10 ⁻⁰⁴	142.53	21.67

t_{C=0.5}: the time required to achieve 50% inhibition activity of initial

Shelf life : the time required to achieve 90% inhibition activity of initial

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