Ethanolic Extraction of Lemongrass in a Scaled-Up Laboratory Percolator

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Abstract. Lemongrass (*Cymbopogon citratus*) is widely recognized for its fragrant qualities and is commonly used as spices in Asian countries. Previous research has proven that lemongrass extract, which contains the polyphenols caffeic acid and kaempferol, can decrease the absorption of glucose in the human intestine by inhibiting the alpha-glucosidase enzyme. With this beneficial potential, the production of lemongrass extract on an industrial scale is becoming necessary. In previous research, an upscaling of lemongrass extraction process using water as a solvent has been carried out in a percolator to obtain optimum alpha-glucosidase inhibitory (AGI) activity. However, other research has also shown higher AGI level of ethanolic extract of lemongrass when compared to its aqueous extract. This study hence focused on the optimization of lemongrass extraction using ethanol in an upscaled laboratory percolator with four varied parameters, the ethanol concentration, maceration time prior to percolation, maceration temperature and the percolation time. The result showed that there was no significant difference between the use of 50% and 70%-v ethanol concentration in a mixture with water. Moreover, there was no significant difference found in the yield and AGI activity resulting from a maceration conducted at room temperature, at 30°C, 35°C, or 40°C, however, a maceration time of 5 hours was found to result in the best AGI activity. The optimum percolation time after maceration was found to be 40 minutes. In conclusion, the best conditions for the ethanolic extraction of lemongrass in this percolator are the use of 50%-v ethanol-water as a solvent and to conduct 5 hours period of maceration at room temperature prior to the 40 minutes percolation.

Keywords: lemongrass extraction, percolator, Alpha-glucosidase inhibitor, *Cymbopogon citratus*, Diabetes

1 Introduction

Lemongrass (Cymbopogon citratus) is a common plant that grows in tropical and subtropical climates throughout Asia. This aromatic plant belongs to the citrus terpene flavor family and is primarily utilized in Asian cuisine, mostly due to its taste and fragrance. In several Asian traditional herbal medicines, however, lemongrass is commonly used by diabetic patients to reduce and control their blood sugar levels, since it

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has been found to contain luteolin, flavonoids, and phenolic compounds, which can contribute to alpha-glucosidase inhibition (AGI) activity [1,2]. This AGI activity is a very beneficial anti-diabetic property. Alpha-glucosidase is an enzyme in the epithelium of the human small intestine, which breaks down complex sugars to monosaccharides such as glucose. The ability to inhibit α-glucosidase will result in the reduced production of glucose and will slow down the absorption of glucose into the bloodstream, thus preventing hyperglycaemia [3,4].

With this beneficial potential, the production of lemongrass extract on an industrial scale is becoming necessary. Based on scientific proofs from previous research, a study explored the optimum process conditions for a lemongrass extraction using water as a solvent in an upscaled size [5]. Since industrial scale extraction is more commonly performed in a percolator than in a maceration tank, the study attempted to adapt the same working principle of an industrial percolation in a pilot scale percolator with a capacity of 13 liters. This study obtained the optimum operating conditions of the pilot scale percolator for aqueous extraction of lemongrass, which recommended to perform 40-minutes maceration prior to a 70-minutes percolation at a flowrate of 94.64 cm3/s and a temperature of 70°C, using a dried sample-to-solvent (water) ratio of 1:20 (w/v) with the maximum amount of 300 grams. This extraction condition resulted in an aqueous lemongrass extract with an AGI of 49.89±3.476% and the yield of 39.45±1.59%.

Nevertheless, several studies showed that under similar maceration conditions, an extraction of lemongrass using ethanol resulted in significantly higher inhibitory activity when compared to its water extract [6,7]. Ethanol in a mixture with water was also found to be one of the common solvents used in the extraction of herbs and plants in the Indonesian herbal industries for its ability to extract most of the targeted bioactive compounds and its volatility, which can allow energy saving during solvent evaporation [8]. Therefore, it is of immense interest to the industries to study whether upscaling the ethanolic extraction of lemongrass in a percolator will give similar positive results as in the aqueous extraction performed previously.

In this study, the ethanolic extraction of lemongrass in pilot scale percolator was performed to observe the effect of several process parameters on the AGI and yield of the resulting extract. The parameters studied were the ethanol concentration, maceration time prior to percolation, maceration temperature and the percolation time.

2 Methods

This study was performed in 2 main stages as shown in Fig. 1, which include the optimization of the maceration process prior to percolation, followed with the optimization of the percolation condition for ethanolic extraction of lemongrass. A re-trial on aqueous extraction with the condition recommended by previous research [5] was also performed to have a direct comparison to the result of this study, since different sample of lemongrass was used. All of the results of each stage were analyzed based on their extraction yield (grams soluble solids/grams dried lemongrass sample) and AGI activity. Resulting data were analyzed by means of one -way and two-ways ANOVA, t-test, and slope analysis.

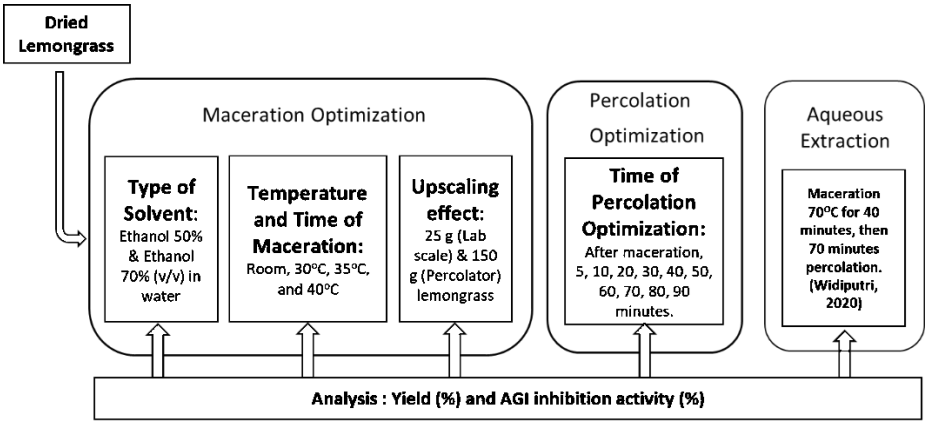


Fig. 1. Experimental design.

The materials used for the maceration and extraction process were dried lemongrass (obtained from PT. Deltomed Laboratories), ethanol 96%-v/v, and distilled water. To analyze the samples, chemicals such as dipotassium hydrogen phosphate powder from Merck (Germany), potassium dihydrogen phosphate powder from Sinopharm Chemical Reagent Co., Ltd. (Shanghai), sucrose powder from Kanto Chemical Co., INC (Japan), ethylenediamine tetraacetic acid disodium salt dihydrate powder from Sinopharm Chemical Reagent Co., Ltd (Shanghai), tris (hydroxymethyl) aminomethane powder from Merck (Germany), hydrogen chloride powder from Merck (Germany), Intestinal acetone powders from rat powder from Sigma (Germany), DMSO 100% liquid from Merck (Germany) and alpha-glucosidase inhibitor assay kit from Megazyme (Ireland) were utilized.

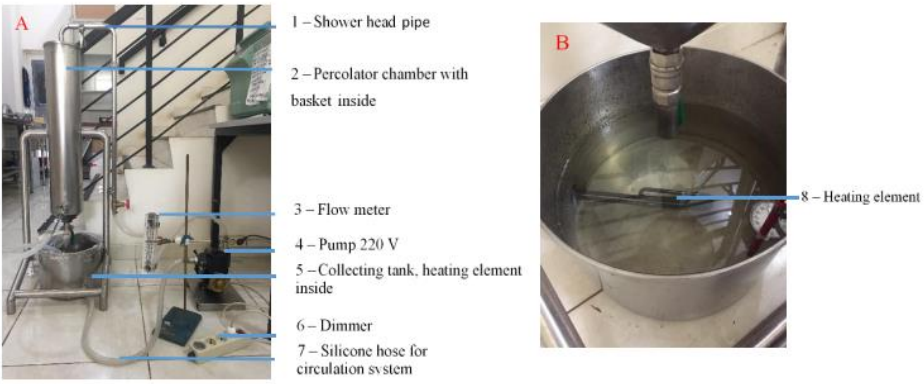


Fig. 2. Design of percolator used in this study [5]

The extraction process, including the preceding maceration and the percolation afterwards, was conducted in a percolator as shown in Fig. 2 [5]. The capacity of this percolator is 13 liters, with a 1:5 diameter-to-height ratio (15.03 cm in diameter and 75.15 cm in height). The percolator was designed based on the same working principle of industrial percolators and some considerations taken from scientific references [9-11]. In this percolator, the lemongrass sample is held in a sieve basket inside the chamber, and the heated solvent is circulated from the collecting tank into the showerhead and running it through the sample, thus extracting solutes as it percolates down. This circulation continues until an equilibrium concentration is achieved.

2.1 Maceration and Percolation Method

It has been pointed out in previous research that initiating the whole extraction process with a maceration and then followed with the percolation stage is preferable. Therefore, in this study, separate observation was performed on each stage, the maceration stage, and the percolation stage to obtain the most optimum process conditions.

To establish the substantial rise in yield and AGI activity during the initial maceration, two ethanol concentrations were compared. Ethanol 50%-v and ethanol 70%-v in a mixture with water were chosen for their applicability in industrial scale extraction. For this experiment, 50 g of dried lemongrass was used for each solvent type, and the solvent was prepared by diluting 96% ethanol with distilled water. The lemongrass to solvent ratio used was 1:20 (w/v in g/ml) with 50 g sample of dried lemongrass. Samples were then collected every hour until maceration period reached 6 hours and were analyzed for its yield and AGI activity. In the optimization of maceration, the solvent temperatures were varied to be at room temperature, 30°C, 35°C, and 40°C. The next stage of the experiment was conducted in the percolator shown in Fig. 2 by using 150 g of dried lemongrass to study the effect of upsizing the maceration process at the same condition. The maceration periods in the percolator were compared between 5 and 24 hours.

After determining the ideal maceration period, the time of ethanolic percolation was to be optimized. The optimum percolation condition for aqueous extraction, including period of percolation and the solvent flow rate was used as an initial trial. In this stage only the percolation period was varied, and both yield and AGI activity were analyzed to determine the best condition.

The extraction yield is defined as the percentage of the total soluble solids (TSS) in the extract in its ratio to the mass of dried lemongrass used. For the determination of yield, the extracts were taken as sample and weighed, then put in a dried crucible and oven dried for 24 h at 100°C. The crucible and the dried sample were then weighed again to know the final dried sample mass. The formula to calculate the yield percentage is given in Eq. 1.

$$Extraction\ Yield\ (\%) = \frac{(W_2 - W_1) \times Total\ Volume\ Solvent\ (ml)}{Sample\ Volume\ (ml) x\ Mass\ Dried\ Lemongrass\ (g)} x\ 100\% \quad (1)$$

W1 = Dry Crucible (g)
W2= Crucible and Dried Sample (g)

2.2 The AGI Assay Procedure

To quantitatively calculate the inhibition of AGI activity of the lemongrass, AGI assay procedure according to [12] was adapted. Based on this procedure, four types of solutions were needed: the sample solution, control solution, sample blank and control blank. The sample solution was made by adding 200 µL of sucrose solution, 100 µL of the extract sample, and 400 µL of rat intestinal enzyme inside a small test tube. The control solution was prepared by combining 200 µL of sucrose solution, 100 µL DMSO 50%, and rat intestinal enzyme. The sample blank solution consisted of 600 µL of buffer solution and 100 µL extract sample. Lastly, the control blank solution was prepared by adding 600 µL of buffer solution to 100 µL of DMSO 50% solution.

The solutions were then mixed using a vortex and put inside the pre-heated water bath at 37°C for 55 minutes for incubation. After the incubation, 750 µL of Tris HCl was then added to each of the solutions and were then filtered using the aluminum oxide and cotton filter. Afterwards, 10 µL of each solution was placed onto a microplate and added with 300 µL of glucose kit. The microplate was then put inside the incubator for 20 min at 50°C and read using a microplate reader at 492 nm wavelength. The data resulted from the microplate reader will be the absorbance, which can be converted to determine the alpha-glucosidase inhibition activity (%) using Eq. 2.

$$Inhibition\ (\%) = 1 - \frac{(A_{Sample} - A_{Sample\ Blank})}{(A_{Control} - A_{Control\ Blank})} x100\% \quad (2)$$

A= Absorption

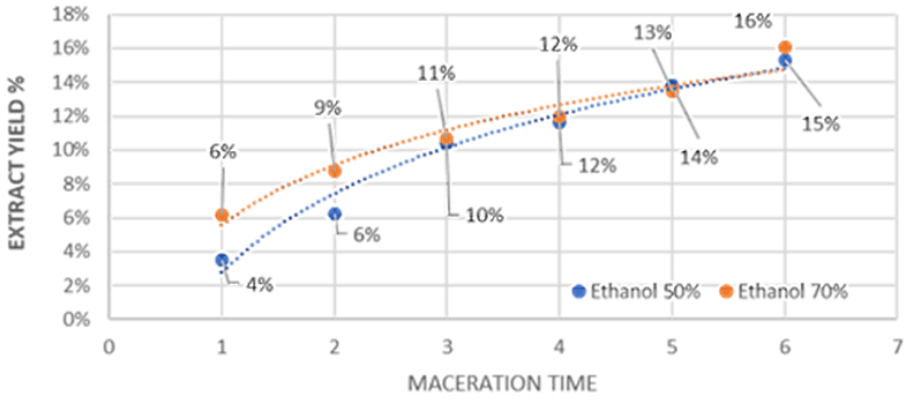
3 Results and Discussion

3.1 Optimization of Maceration Process

The optimization of maceration process prior to percolation was done by observing the effect of different solvent concentrations used, the maceration period and solvent temperature on the yield and AGI activity of the resulting extract. For this stage, the maceration was done in laboratory scale in an Erlenmeyer flask with 50 g dried lemongrass and 1,000 ml solvent. The results are explained in separate parts in the following subchapters.

Effect of Solvent Concentration. The solvent used in this research as mentioned in the methodology part was ethanol in a mixture with water. The concentration of ethanol solution was varied: 50% and 70% (v/v) ethanol-water, with the consideration that these are the common ethanol concentrations used as solvent for extraction in the Indonesian herbal industries. Figure 3 shows the yield percentage from the two different solvents

at every hour. The data followed a logarithmic profile, showing that the extraction achieved an equilibrium condition after a certain time. The p-value found from the T-test analysis on these results was 0.746 ($p > 0.05$), which indicates that there is no significant difference between the use of 50% and 70% ethanol from the perspective of its effect on the yield.



Solvent	Extraction Yield(%)	Logarithmic R^2
Ethanol 50%	9.13±3.48a	$R^2 = 0.9754$
Ethanol 70%	8.94±3.66a	$R^2 = 0.9482$

Extraction Yield % (Total Soluble Solids (g)/ Mass Lemongrass (g) .Numerical values on the table are written in the format Mean ± Standard Deviation. The alphabetical letter following the numerical value for each column represents their significance according to the Tukey HSD test with ($p<0.05$).

Fig. 3. Effect of ethanol concentrations on yield.

Different ethanol concentrations were also studied on its effect on the extract's AGI activity. The AGI activity was performed at different concentrations depending on the soluble solids of each sample, and the results are shown in Table 1. The result implies that there is no significant difference in terms of extracted yield and AGI activity between ethanol 50% and 70%, thus making ethanol 50% the more preferrable solvent to be used in the extraction process.

Table 1. Effect of ethanol concentrations on AGI activity

Solvent	Analytical Concentration (mg/mL)	AGI activity Inhibition (%)
Ethanol 50%	45.64±17.39	35.13±25.54[a]
Ethanol 70%	44.72±18.31	32.67±12.51[a]

The analysis concentration is ten times the concentration of the initial sample. Numerical values on the table are written in the format mean ± standard deviation. The alphabetical letter following the numerical value for each column represents their significance according to the Tukey HSD test with ($p<0.05$).

Effect of Maceration Period and Temperature. The next step of the maceration optimization was the determination of the optimum solvent temperature and period. In this stage, the same sample-to-solvent ratio of 1:20 was also used. The yield obtained at different temperatures (room temperature, 30°C, 35°C and 40°C) and maceration times (from 1 h up to 6 h) can be seen in Fig. 4. The analysis for the yield was carried out using two-ways ANOVA with replications with alpha 0.05. The results showed that there is a significant difference between the factor time, temperature as well as an interaction between time and temperature in affecting the extraction yield. Even though the trend of these curves seems to increase with time, they fit at best to a logarithmic profile with $R2 \geq 0.975$ for all variations of temperature and are approaching equilibrium at maceration time of above 5 hours.

Furthermore, to evaluate if there was a significant increase in yield, a slope test analysis was performed using Excel with alpha of 0.05. This analysis aimed at finding out whether maceration temperature affected the yield significantly. All p-values from the slope test regression analysis were bigger than 0.05, which indicated that there was no significant difference on yield when different temperature was chosen. After further analysis using one-way ANOVA, the analysis showed that there is significant difference in the effect of time but not of temperature on the extraction yield.

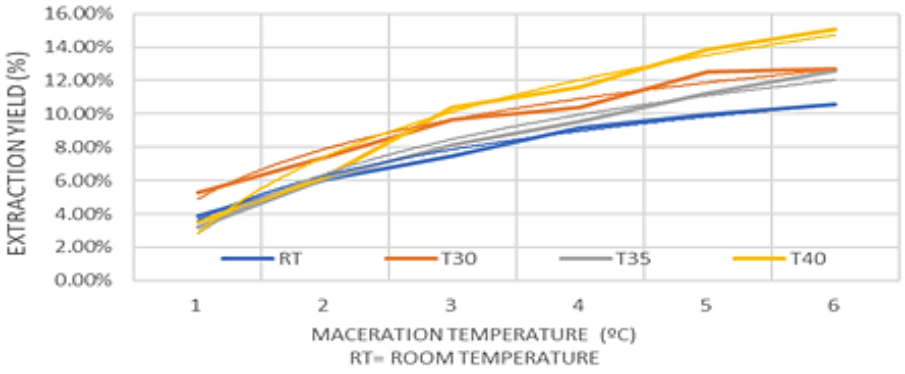


Fig. 4. Effect of maceration time and temperature on yield.

The analysis on AGI was performed to extract samples taken at the 5th hour for every variation of temperature. The sample at the fifth hour was selected, since the yield was considered to have already started to approach its equilibrium at this point of time. As could be seen in Table 2, no significant differences between the AGI activity for each different temperature was found. This implies that neither the time nor the temperature of the maceration process contributes to the inhibition activity of alpha-glucosidase.

Table 2. Effect of solvent temperature in 5 hours maceration on AGI activity

Temperature	Analysis Concentration (mg/mL)	AGI activity Inhibition (%)
RT	49.80±2.01	43.3±6.21a
30°C	62.65±5.48	53.9±11.73a
35°C	56.1±3.55	64.64±6.80a
40°C	69.15±4.24	33.68±15.09a

RT= Room Temperature
The analysis concentration is ten times the concentration of the initial sample. Numerical values on the table are written in the format Mean ± Standard Deviation. The alphabetical letter following the numerical value for each column represents their significance according to the Tukey HSD test with ($p<0.05$).

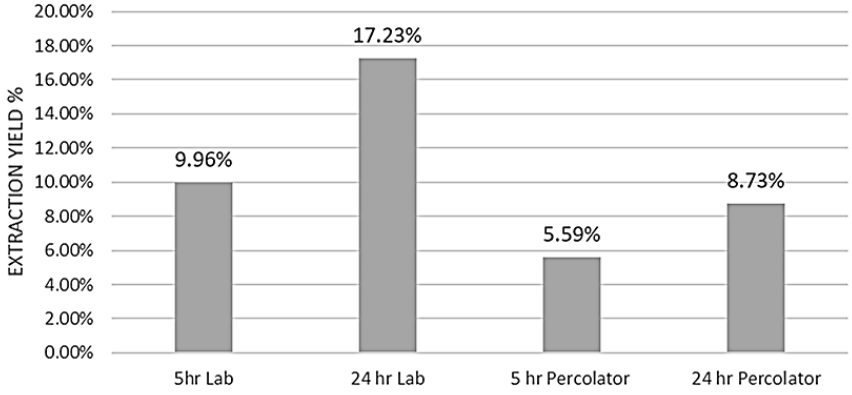
3.2 Optimization of Ethanolic Extraction in a Percolator

Upscaling Effect of Maceration Process in a Percolator. In this stage, the maceration process conducted in a laboratory scale (using 25 grams of dried lemongrass in an Erlenmeyer flask) was upscaled with a factor of 6 in its amount and performed in the percolator shown in Fig. 2 using 150 grams of dried lemongrass and 3,000 ml 50% ethanol as a solvent. The maceration time in both the Erlenmeyer flask and in the percolator was varied to be 5 and 24 hours. The 5 hours period of maceration was chosen from previous result of the optimization, while the 24 hours maceration time was selected as a comparison since it has been recommended from practical experiences at smaller scale herbal industries.

Figure 5 shows that the laboratory-scale maceration process for 5 and 24 hours resulted in a yield of 9.96% and 17% respectively. Meanwhile the yield resulted from maceration process performed in a percolator with a 6 times greater size for 5 and 24 hours are 5.59% and 8.73% respectively. The significant decrease of yield in this upscaling could be the result of some limitations of the equipment. The dried lemongrass was expected to expand in its volume during the maceration process, since the solvent will be absorbed and retained. As a result, the height of lemongrass placed in the sieve basket became higher than the solvent surrounding it, causing some part of the lemongrass to be not thoroughly soaked during the maceration. This might have been the reason of the decreased yield.

In terms of the AGI activity of the extracts, as shown in Table 3, although the analysis concentration of each solution varied based on the yield analysis, there was no

substantial change in AGI activity. However, understanding that with the lowest extract concentration, a comparable high inhibition activity was resulted, the extract from the maceration conducted in percolator for 5 hours showed the strongest ability to inhibit alpha-glucosidase enzyme. Therefore, the maceration for 5 hours was selected for further exploration on the optimization of percolation.



Time (hours)	Maceration Scale	Extraction Yield(%)
5	Lab	9.96±0.04b
24	Lab	17.23±0.19a
5	Percolator	5.59±0.19d
24	Percolator	8.725±0.21c

AM = After Maceration Aqueous Extraction ; FP = Final Percolation Aqueous Extraction
Extraction Yield % (Total Soluble Solids (g)/ Mass Lemongrass (g) .Numerical values on the table are written in the format Mean ± Standard Deviation. The alphabetical letter following the numerical value for each column represents their significance according to the Tukey HSD test with ($p<0.05$).

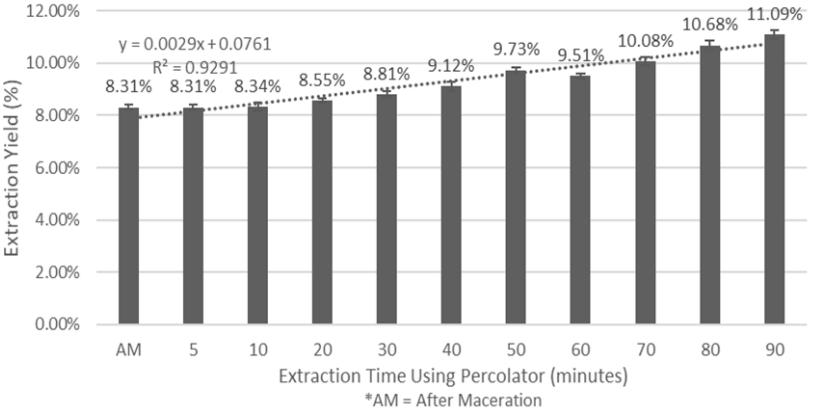
Fig. 5. Effect of upscaling the maceration process in a percolator on yield.

Table 3. Effect of upscaling the maceration process in a percolator on AGI activity

Time (hours)	Maceration Scale	Analysis Concentration (mg/mL)	AGI activity Inhibition (%)
5	Lab	49.80±2.01	43.31±6.21[a]
24	Lab	86.15±0.96	41.29±2.21[a]
5	Percolator	27.95±0.94	36.82±9.07[a]
24	Percolator	43.63±1.07	54.06±16.10[a]

The analysis concentration is ten times the concentration of the initial sample. Numerical values on the table are written in the format Mean ± Standard Deviation. The alphabetical letter following the numerical value for each column represents their significance according to the Tukey HSD test with ($p<0.05$).

Optimizing Percolation Time. After optimizing the maceration process of lemongrass ethanolic extraction, the next step in this research was to optimize the percolation time. The conditions selected based on previous results in this experiment are the use of 50% ethanol as the solvent of extraction, and to perform a maceration process at room temperature for 5 hours prior to the percolation process. Additionally, several recommended conditions based on previous research [5] were also applied, including the solvent flow rate of 1.5 gal/min (94.64 cm^3/s) and the sample-to-solvent ratio of 1:20 (g/ml). During this optimization of percolation time, 300 g of dried lemongrass in 6,000 ml of 50% ethanol was used. The samples of extract were taken after the first 5 minutes of percolation and every 10 minutes afterwards, until the no significant change in yield could be observed. Figure 6 shows that the end point of this observation was 90 minutes.



Percolation Time	Extraction Yield(%)
AM	10.12±0.09bc
5	8.31±0.17f
10	8.33±0.31f
20	8.55±0.16ef
30	8.81±0.20ef
40	9.12±0.36de
50	9.73±0.25cd
60	9.51±0.1cd
70	10.07±0.3bc
80	10.68±0.34ab
90	11.09±0.31a

AM = After Maceration Aqueous Extraction ; FP = Final Percolation Aqueous Extraction
Extraction Yield % (Total Soluble Solids (g)/ Mass Lemongrass (g) .Numerical values on the table are written in the format Mean ± Standard Deviation. The alphabetical letter following the numerical value for each column represents their significance according to the Tukey HSD test with ($p<0.05$).

Fig. 6. Effect of percolation time of extraction yield.

During the percolation, 50 ml of sample was taken at the first 5 minutes after the solvent circulation was started. After that, samples were taken every 10 minutes until a complete circulation time of 90 minutes has been performed. The data shown in Figure 5 show the extraction yield of the experiment. Here it is shown that the data followed an

increasing linear trend, thus making the result to be inconclusive. However, the observation had been stopped at 90 minutes, since an insignificant increase in yield has been detected between samples taken at minute 80 and 90.

Table 4 on the other hand shows that the AGI activity of the extracts increased after the solvent circulation had started but only up to minute 40, then it started to decrease again even though there was still an increase in the extraction yield. The reason that this phenomenon could happen is that after 40 minutes of percolation, not only the phenolic compounds contributing to AGI activity are extracted, but also other types of phytochemicals. Since the main goal of this research aimed more at the achieving of best AGI rather than the yield alone, a percolation time of 40 minutes is considered to be more preferable.

Table 4. Effect of percolation time on AGI activity

Percolation Time	Analysis Concentration (mg/mL)	AGI activity Inhibition (%)
AM	50.62±0.46	26.19±4.86g
5	41.57±0.87	37.75±4.60ef
10	41.68±1.54	48.67±0.47cde
20	42.77±0.81	55.75±3.18bcd
30	44.05±1.01	45.75±1.77de
40	45.60±1.79	75.91±0.34a
50	48.65±1.24	42.33±2.99e
60	47.53±0.83	65.00±4.24b
70	50.38±1.69	45.97±0.67cde
80	53.42±1.71	56.74±0.18bc
90	55.45±1.53	27.19±4.94fg

AM= After Maceration

The analysis concentration is ten times the concentration of the initial sample. Numerical values on the table are written in the format Mean ± Standard Deviation. The alphabetical letter following the numerical value for each column represents their significance according to the Tukey HSD test with ($p<0.05$).

3.3 Comparison between Ethanolic and Aqueous Extract in Percolator

In the final stage of this study, a re-trial of previous experiment using water as a solvent in the same percolator used in this research was performed. The optimum process conditions for the aqueous extraction were applied, whose resulted extract will be compared to the extract resulting from the optimum process conditions for ethanolic extractions obtained in this research, in terms of their yield and AGI activity. The result can be seen in Table 5.

Table 5. Comparison between ethanolic and aqueous extract

Conditions/Parameters	Ethanolic Extraction	Aqueous Extraction [5]
Sample-to-solvent ratio (g/ml)	1:20	1:20
Amount of dried lemongrass (g)	300	300
Solvent used	50%-v ethanol	Water
Amount of solvent (ml)	6,000	6,000
Maceration Temperature (°C)	Room Temperature	70
Maceration time (min)	300 (5 h)	40
Percolation time (min)	40	70
Results		
Yield (%)	9.12±0.36	16.68±0.24
AGI activity (%)	75.91±0.34	81.32±12.07
Extract concentration (mg/mL)	45.60±1.79	83.4±1.18

From the yield perspective, the optimized aqueous extraction showed a higher yield (16.68±0.24%) than the optimized ethanolic extraction (9.12±0.36%). The t-test analysis was performed to compare these two sets of data, and the result showed that with an alpha of 0.05, the p-value in two tails was smaller than 0.05. This concluded that there is a significant difference between ethanolic extraction and aqueous extraction in terms of the resulting yield.

In terms of the AGI activity, it can be seen in Table 5 that in order to inhibit 81.32±12.07% of alpha-glucosidase, the extract concentration required is 83.41±1.18 mg/mL. Whereas in the ethanolic extract, to inhibit 75± 0.034% of alpha-glucosidase enzyme, the required concentration is 45.60 mg/mL. This comparison shows that the ethanolic extraction has higher ability to inhibit the AGI activity despite the lower concentration. This validated a study by [6], stating that the ethanolic extraction from lemongrass gives higher results in AGI activity inhibition. Moreover, the extraction with ethanol does not require heating of the solvent, which can be another advantage for the industry in saving energy.

From the operational perspectives, one possible reason why the extraction with ethanol resulted in lower yield than in the aqueous extraction is the number of circulations, which represents how many times the solvent had circulated through the raw material. As a consequence of the chosen solvent flowrate and the percolation time, the number of circulations in the aqueous and in ethanolic extraction was around 67 and 38, respectively. However, increasing the number of circulations in the ethanolic extraction, if done by lengthening the percolation time, will negatively affect the AGI activity, as discussed in Table 4.

4 Conclusion

In this research the extraction process of lemongrass using ethanol as a solvent in an upscaled laboratory percolator was studied. The process conditions recommended by this research are to use 50%-v ethanol in water as a solvent, to perform maceration at room temperature for 5 hours prior to 40 minutes percolation, and to use a sample-to-

solvent ratio of 1:20 (g dried lemongrass/mL solvent). This extraction condition will deliver an extraction yield of 9.12±0.36% (g TSS/g dried lemongrass) with and AGI activity of 75.91±0.34% at a concentration of 45.60±1.79 mg/mL.

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