The Effect of Differences in Extraction of Cassia Siamea. Lamk Leaves in Etoh 70% and Etoh 90% Against The Inhibition of The A-Glucosidase Enzyme

H Tanty1*, Aryusmar2, S D Permai3, Bustanussalam4

1,3 Mathematics Department, School of Computer Science, Bina Nusantara University, Jakarta, Indonesia 11480
2 Language Center, Computer Science Department, Faculty of Humanities, Bina Nusantara University, Jakarta Indonesia 11480
4 Bioteknologi, LIPI, Cibinong, Bogor, Indonesia

e-mail: syarifah.diana@binus.ac.id

Abstract. This research aims at examining the comparison of Different Solvent Extractions on antidiabetic activity tests of Cassia Siamea. Lamk leaf. It conducted in vitro using the α-glucosidase enzyme and maceration technique with ethanol concentration of 70% and 90%. In this case, each ethanol extract was fractionated using ethyl acetate and hexane. The results of the t-test statistic are greater than t table of 2.262 and the Sig value is smaller than α of 0.05. Therefore, It found that the difference in average antidiabetic activity for 70% and 90% concentrations in ethanol, ethyl acetate and hexane fractions produced positive values. However, the two ethanol concentrations differ in producing an average antidiabetic activity. Finally, the research concluded that the antidiabetic activity of C. siamea. Lamk leaf extract with an ethanol concentration of 70% is better than ethanol concentration of 90%.

Keyword: Extraction, cassia siamea lamk, inhibition, glucosidase enzyme

1. Background
Diabetes mellitus (DM) is a metabolic disease due to impaired insulin secretion, or the body cannot effectively use insulin produced by the pancreas, resulting in a buildup of glucose concentration in the blood (hyperglycemia). DM sufferers in the world continue to increase, according to the International Diabetes Federation (IDF) in 2015 it is estimated that diabetics sufferers in the world reach 415 million, and it is predicted to increase to 642 million in 2040. Indonesia ranks 7th country with the number of DM sufferers 10 million in 2015 and will increase to 16.2 million in 2040 [1]. There are 2 types of diabetes mellitus (DM), type 1 DM is caused by impaired insulin secretion, whereas type 2 DM occurs due to ineffective use of insulin. DM sufferers generally occur at the age of 15-64 years due to genetic factors, eating patterns that consume lots of carbohydrates, lack of exercise, smoking, hypertension and obesity [2].

Cassia siamea. Lamk has long been used by Indonesian people as traditional medicine by utilizing all parts of plants such as roots, bark, flowers and leaves. Boiled water from juar leaves is used for the treatment of skin (itching), malaria, and diabetes, while the flowers are used to treat intestinal worms [3]. Several types of chronic diseases that can be prevented by flavonoids contained in johar leaves include Cancer, TB, Tumors, Diabetes, and Stroke [4]. Heruna conducted an in vitro study of antidiabet activity of ethanol extracts of juar leaves, the results showed inhibition of the α-glucosidase enzyme for...
the n-hexane fraction by 52.319% at a concentration of 1000 ppm and IC50 value of 570,137 [5]. Whereas the in vivo test using Wistar strain white mice with the alloxan induction method showed that the ethyl acetate test fraction group dose 150 mg / kg BW gave the highest rate of decrease in blood glucose level (10.25%) and was not significantly different from the n-hexane fraction. (9.98%) at the same dose that is 150 mg / kg body weight, which is equivalent to the positive control given glibenclamide dose 0.65 mg / kg body weight [6].

The use of solvent concentration in the process of maceration (extraction) of herbal ingredients will affect the type of compound from the isolation. In general, for maceration process herbal ingredients are used methanol or ethanol solvent with a concentration of 96%, 90% or 70% which is then fractionated using n-hexane, ethyl acetate or chloroform. Arifianti et al's research results reported that the type of extracting solvent that can find sinensetin in the most amount on the leaves of Orthosiphon stamineus Benth. is a 96% ethanol solvent [7]. In addition, Rosnani et al extracted the leaves of the Kulu plant (Artocarpus Camansi) using the n-hexane solvent to produce a pure compound β-sitosterol propionate that can reduce blood sugar in Webster mice by 89 mg / L after 60 minutes of the compound [8]. J.A. Mashi et al used ethylacetate solvents to extract Persea Americana leaves, producing several compounds that could reduce glucose levels induced by alloxan rats [9]. Anton Smith et al extracted the leaves of DregeaVolubils [Benth.] With ethyl acetate solvents and with chromatography columns producing pure compounds that showed antidiabet activity [10]

The research on the antidiabet inhibition of Wungu leaves macerated using ethanol with a concentration of 96% ethanol and 70% ethanol was carried out and the results showed an average value of α-glucosidase inhibition of 61.09% and 66.11%, respectively [11]. While Sayatus Sa'adah reported that the yield of onion tiwai extract with a water solvent was 8.75%; ethanol solvent 5.3%; and water - ethanol 8.31% [12].

This study aims to determine whether there are differences in the inhibitory antidiabet of extracted juar leaves extracted by maceration technique using 90% ethanol concentration and 70% ethanol. The statistical method that will be used to compare is paired t-test.

2. Methodology

2.1. Sample Preparation
Leaves samples were taken in February 2019 in Putat village, Sindang Laut Cirebon district. A total of 8 kg of fresh Juar leaves are dried in the room without being exposed to sunlight for 3 weeks, producing 4.27 kg of dried leaves, then smoothing with a blender, then sifted into powder.

2.2. Maceration and Fractionation
In 10 jar bottles, 300 grams of Juar leaf powder (10 x @ 300 gr) were each then extracted by maceration (without heat), each 5 bottles using 90% and 70% technical ethanol solvent for 24 hours. 3 times (3 x 24 hours). Every 24 hours 90% and 70% ethanol filtrate are separated. The filtrate from the separation was then concentrated with a vacuum evaporator and dried in an oven at 40oC, 90% and 70% ethanol dry extract.

In the fractionation process (partition) each of 10 grams of 90% ethanol extract and 70% was dissolved with 300 mL aquadest (water fraction), then put into a separating funnel and partitioned with 300 mL of n-hexane solvent (n-hexane fraction) and repeated 3 times (300 mL x 3) is then separated between the water fraction and the n-hexane fraction.

The water fraction was further repartitioned with 300 mL ethylacetate solvent (ethylsetate fraction) and repeated 3 times (300 mL x 3) and then separated between the water fraction and the ethylsetate fraction. The n-hexane fraction and the ethylacetate fraction were then concentrated by vacuum evaporator at 40°C.
2.3. Invitro Test with the α-Glucosidase Enzyme

The α-glucosidase enzyme solution used was 0.04 U / mL in concentration, samples were made in concentrations of 50, 100, 250, 500 and 1000 ppm. A 1% concentration control solution in DMSO. A sample mixture of 250 μL p-NPG 0.5mM as a substrate, 475 μL 0.1 M phosphate buffer pH 7 and 25 μL sample solution in 1% DMSO. After the mixture is incubated at 37°C for 5 minutes, then add 250 μL of the α-Glucosidase 0.04 U / mL and incubate again at 37°C for 25 minutes, then the reaction is stopped by adding 1000 μL Na₂CO₃ 0.2M p-nitrophenol formed is seen under ultraviolet light with λ 400 nm.

### Table 1. Mixing test of In Vitro ethanol extract, n-hexane fraction and ethyl acetate fraction each with a total volume of 2 mL.

| Samples \(^a\)      | Control (μL) | Blank (μL) | S1 (μL) | S0 (μL) |
|---------------------|--------------|------------|---------|---------|
| DMSO 1%             | 25           | -          | 25      | 25      |
| Phosphate buffer 0,1 M | 475          | 475        | 475     | 475     |
| PNPG 0.5 mM Substrate | 250          | 250        | 250     | 250     |
| A-glucosidase enzyme 0,04 unit/mL \(^b\) | 250          | -          | 250     | -       |
| Phosphate buffer 0,01 M \(^b\) | -            | 250        | -       | 250     |
| Na₂CO₃ 0,2 M \(^c\) | 1000         | 1000       | 1000    | 1000    |

\(^a\) Samples were made in concentrations: 50, 100, 250, 500 and 1000 ppm

\(^b\) Incubate 37°C for 5 minutes

\(^c\) Incubate 37°C for 25 minutes

\[\text{% inhibition} = \left(\frac{C - S}{C}\right) \times 100\]

Where:
- C = absorbance control - blank
- S = absorbance S1 - S0

The IC\(_{50}\) (Inhibition Concentration 50) value is an antidiabetic concentration (ppm) which can inhibit 50% of the α-glucosidase enzyme. IC\(_{50}\) value is obtained from the intersection of the line between 50% resistance to the concentration axis, then entered into the equation \(Y = a + bX\) where \(Y = 50\) and the \(X\) value indicates IC\(_{50}\).

### 3. Result and Discussion

In Vitro test results of the antidiabetic activity of 90% ethanol extract with ethyl acetate and hexane fractions can be seen in table 2 and the inhibitory graphs respectively can be seen in figures 1,2 and 3.

### Table 2. Antidiabetic inhibition of ethanol extract 90%, ethyl acetate fraction and hexane fraction

| Name of Samples | Concentration (ppm) | Inhibition (%) | IC\(_{50}\) (ppm) |
|-----------------|---------------------|---------------|------------------|
| Ethanol extract | 50                  | 2.968         |                  |
|                 | 100                 | 6.308         |                  |
|                 | 250                 | 6.308         | 3114.099         |
| Concentration (ppm) | 90% Ethanol Extract | Ethyl Acetate Extract | Hexane extract |
|---------------------|----------------------|-----------------------|----------------|
| 50                  | 7.607                | 9.091                 | 48.794         |
| 100                 | 19.109               | 8.534                 | 48.857         |
| 250                 | 1268.633             | 9.091                 | 48.794         |
| 500                 | 9.091                | 16.698                | 50.464         |
| 1000                | 42.857               | 42.857                | 50.464         |

**Figure 1.** Graph of antidiabetic inhibition of 90% Ethanol extract

**Figure 2.** Graph of antidiabetic inhibition of 90% Ethyl Acetate extract
Figure 3. Graph of antidiabetic inhibition of 90% Hexane extract

The results of In Vitro antidiabetic activity of 70% ethanol extract with ethyl acetate fraction and hexane fraction can be seen in table 3 and the inhibitory graphs respectively can be seen in figures 4, 5 and 6.

Table 3. Antidiabetic inhibition of ethanol extract 70%, ethyl acetate fraction and hexane fraction

| Name of Samples       | Concentration (ppm) | Inhibition (%) | IC<sub>50</sub> (ppm) |
|-----------------------|---------------------|----------------|------------------------|
| Ethanol extract       | 50                  | 7.047          |                        |
|                       | 100                 | 8.150          |                        |
|                       | 250                 | 8.272          |                        |
|                       | 500                 | 18.321         | 1539.310               |
|                       | 1000                | 19.608         |                        |
| Ethyl acetate extract | 50                  | 6.556          |                        |
|                       | 100                 | 9.314          |                        |
|                       | 250                 | 10.417         | 3694.284               |
|                       | 500                 | 12.806         |                        |
|                       | 1000                | 18.689         |                        |
| Hexane extract        | 50                  | 7.414          |                        |
|                       | 100                 | 17.708         |                        |
|                       | 250                 | 28.248         | 482.906                |
|                       | 500                 | 76.342         |                        |
|                       | 1000                | 79.718         |                        |
Figure 4. Graph of antidiabetic inhibition of 70% Ethanol extract

\[
y = 0.0277x + 7.3611 \\
R^2 = 0.8054
\]

Figure 5. Graph of antidiabetic inhibition of 70% Ethyl Acetate extract

\[
y = 0.0116x + 7.1463 \\
R^2 = 0.9696
\]

Figure 6. Graph of antidiabetic inhibition of 70% Hexane extract

\[
y = 0.0788x + 11.947 \\
R^2 = 0.8178
\]

To determine the antidiabetic inhibitory power of the three solvents used, namely ethanol, ethylacetate fraction and hexane fraction using 70% and 90% concentrations in the extract of juar leaves, analyzed using statistical paired sample t-test. The results can be seen in the following table.
3.1. Ethanol Solvents

Table 4. Comparison of antidiabetic results of C. siamea. Lamk leaves 70% Ethanol and 90% Ethanol

| Paired Differences | t  | Sig. |
|--------------------|----|------|
| Mean               | Std. Deviation |   |
| 0.11990            | 0.12343        | 3.072 | 0.013 |

H₀: μᵰ = 0 (There is no antidiabetic average difference of 70% Ethanol and 90% Ethanol)
H₁: μᵰ ≠ 0 (There is an antidiabetic average difference of 70% Ethanol and 90% Ethanol)

Based on the table 4, the t test statistic value of 3.072 is obtained where the value is greater than 2.262, then reject H₀. In addition, the Sig < α value of α is 0.05, so reject H₀. Because H₀ is rejected, it can be concluded that there are differences in antidiabetic activity between 70% Ethanol and 90% Ethanol. The antidiabetic mean difference between 70% Ethanol and 90% Ethanol is 0.1199. This shows that the antidiabetic activity on Ethanol is 70% higher than Ethanol 90%. So it can be concluded that the antidiabetic activity on Ethanol 70% is better than Ethanol 90%.

3.2. Ethyl Acetate Solvent

H₀: μᵰ = 0 (There is no antidiabetic average difference of 70% Ethyl acetate and 90% Ethyl acetate)
H₁: μᵰ ≠ 0 (There is an antidiabetic average difference of 70% Ethyl acetate and 90% Ethyl acetate)

Table 5. Comparison results of antidiabetic leaves of C. siamea. Lamk 70% Ethyl Acetate with Ethyl Acetate 90%

| Paired Differences | t  | Sig. |
|--------------------|----|------|
| Mean               | Std. Deviation |   |
| 0.13040            | 0.13659        | 3.019 | 0.015 |

Comparison of Ethyl acetate 70% solvent with 90% Ethyl acetate using statistical methods, namely the t test. T test results produce a Sig value of 0.015, where the value is smaller than α of 0.05. In addition, the t test statistic value is 3.019, where the value is greater than the t table value of 2.262. Therefore it can be decided to reject H₀. Since H₀ is rejected, it can be concluded that there are differences in the antidiabetic mean of Ethyl acetate 70% and Ethyl acetate 90%. The difference between the two concentrations in the Ethyl acetate solvent is 0.1304. This means that the average value of antidiabetic activity on Ethyl acetate is 70% greater than 90% concentration. This shows that the average antidiabetic activity on Ethyl acetate is 70% better than 90% concentration.

3.3. Hexane Solvents

Table 6. Comparison results of antidiabetic leaves of C. siamea. Lamk fraction of hexane ethanol 70% solvent with ethanol fraction hexane 90%

| Paired Differences | t  | Sig. |
|--------------------|----|------|
| Mean               | Std. Deviation |   |
| 0.12110            | 0.13927        | 2.750 | 0.022 |

H₀: μᵰ = 0 (There is no antidiabetic average difference of hexane 70% and hexane 90%)
H₁: μᵰ ≠ 0 (There is an antidiabetic average difference of hexane 70% and hexane 90%)
The results of the comparison of hexane 70% and 90% solvents show that there are differences in antidiabetic activity in the two solvent concentrations. This is known because $H_0$ was rejected. The decision was obtained because the statistical value of the test on the t test was 2.75 and Sig was 0.022. T value is greater than 2.262 and Sig value is smaller than $\alpha$ at 0.05. The average difference in hexane antidiabetic activity was 70% and 90%, 0.1211. Because the value is positive, it can be concluded that the average antidiabetic activity of hexane is 70% better than hexane 90%.

4. Conclusion
The results of the comparison of juar leaves extract using 70% ethanol and 90% ethanol ethylacetate fraction and hexane fraction showed that the two concentrations differed in producing an average antidiabetic activity. This is obtained because the t test statistic value is greater than t table of 2.262 and the Sig value is smaller than $\alpha$ of 0.05. Difference in average antidiabetic activity for concentrations of 70% and 90% in all three solvents yielded positive values. This shows that the antidiabetic activity of C.siamea.Lamk leaf extract with ethanol solvent concentration of 70% is better than ethanol concentration of 90% as well as the ethylacetate fraction and its hexane fraction.

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