Study in Activity Combination of *Physalis angulata* and *Hibiscus sabdariffa* in 70% Ethanol Extract to Decrease Blood Sugar Levels and Histopathology of Pancreas Langerhans Island in Alloxan Induced Diabetic Rats

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Abstract: Ciplukan (*Physalis angulata* L) and roselle petals (*Hibiscus sabdariffa* L) have been shown to have blood sugar levels decreasing properties. This study aims to determine the effect of the Ciplukan and Roselle petals ethanol extract combination to the decrease in blood sugar levels in alloxan-induced diabetic rats (Sprague Dawley). The diabetic rats were divided into seven groups, each consisting of four rats. Group I (negative control), group II (positive control), group III (ciplukan 10mg/200g BW), group IV (roselle petals 60mg/200g BW), group V (ciplukan 5mg/200g BW and roselle petals 30mg/200g BW), group VI (ciplukan 5mg/200g BW and roselle petals 60mg/200g BW) and group VII (ciplukan 10mg/200g BW and rosella petals 30mg/200g BW). All groups were induced with alloxan. Parameters observed were decreased blood sugar levels on the 15th and 30th day. After that, surgery and histopathological observation of pancreatic organs were conducted at the 31st day. Blood sugar percentage was analysed using one-way ANOVA with 95% significance level and continued with the Tukey test. The results showed that group number VII combination of 70% ethanol extract ciplukan with 10mg/200g BW dose and roselle flower petal dose 30mg/200 g BW decreased blood sugar level equal to glibenclamide dose 0.052mg/200g BW ($p \geq 0.05$) that is equal to 66.74% and improvement activity of pancreatic islets of Langerhans, has been shown at the histology result.

1 INTRODUCTION

Diabetes mellitus (DM) is a disease or disorder of fat, carbohydrate, and protein metabolism caused by a disturbance of insulin secretion, insulin work (sensitivity), or both characterized by hyperglycemia. Symptoms in DM patients such as polyuria, polydipsia, polyphagia, weight loss, and lethargy by hyperglycemia are the most common early symptoms (Dipiro *et al*., 2014).

Synthetic antidiabetic drugs have side effects of gastrointestinal disorders, anorexia, lactic acidosis, vitamin B12 absorption disorders, and edema. Plants as traditional medicine are easy to obtain and generally has lower side effects when compared to synthetic drugs. Components in one ingredient (usually) have mutually supportive effects, and one plant has multiple pharmacological effects that makes it more suitable and safer for degenerative metabolic disease (MOH RI, 2008). The ciplukan (*Physalis angulata* L.) and roselle petals (*Hibiscus sabdariffa* L.) are alternatives that can be used in the treatment of DM (Sunaryo *et al*., 2012; Rosemary *et al*., 2014).

Sunaryo *et al*. (2012) reported that the fraction of chloroform ciplukan with the dose of 2mg/20g BW in mice has the antidiabetic effect and can improve the number of Langerhans pancreatic cells proportional to glibenclamide. Rosemary *et al*. (2014) reported that ethanol extract of roselle petals at 600mg/kg BW dose was able to lower blood sugar levels comparable with glibenclamide of 77.69% in streptozocin-induced mice.

Based on this background, this study wanted to prove the effect of ciplukan and roselle petals ethanol extract in combination to the lowering of sugar levels in alloxan-induced rats. The purpose of combining the two extracts is to obtain smaller doses and have activities that are comparable or even greater than the single herbal extract.

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2 MATERIALS AND METHODS

2.1 Plant and Preparation of Extracts

Physalis angulata L. was obtained from Merapi Farma Herbal Yogyakarta, and *Hibiscus sabdariffa* L. obtained from Balai Tanaman Obat dan Rempah (BALITTRO) Bogor. The reference drug used was glibenclamide. A total of 1500 g of dried herb ciplukan and 1500 g of dried roselle petals each made into a powder. It was then sieved with mesh no. 40, weighed, and recorded. As many as 900 g and 1000 g of powder were each macerated by using ethanol 70%. They were left soaking for the first 6 hours while stirring occasionally, and then let stand for 18 hours. The maceration was performed three repetitions using a new liquid. The macerate was then evaporated using a vacuum rotary evaporator until it turns into a viscous extract (MOH RI, 2008).

2.2 Phytochemical Screening

Phytochemical screening was performed using TLC method by preparing a 10 mg/ml test solution in ethanol for each extract. Prepared test solutions were bottled on a silica gel TLC plate GF254. The separation and detection systems of the compounds are shown in Table 1.

2.3 Preparation of Animal

The animals used for study were white male rats of Sprague Dawley strain, aged 2-3 months with a body weight around 150-300 g, obtained from the Bogor Agricultural University (IPB) of the Production Division of Working Meat and Animal Livestock. The experiment was completed with a randomized design, using 28 male rats divided into seven groups consisting of four rats. Before the experimental treatment, the animals were first acclimatized in the cage with standard feed and drink for approximately seven days. The protocol no. 17-05-0486 has been approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia no. 453/UN2.F1/ETIK/2017.

2.4 Measurement of Blood Sugar Levels

The rats fasted for 12 hours before treatment. Blood sample was collected through the eye area (sinus orbital). Blood glucose measurements were performed on day 15 and after administration of the test substance on day 30 using a spectrophotometer clinical 300.

2.5 Observation of Pancreatic Histopathology

The pancreatic histopathological observation process began with surgery in rats that have been anaesthetized with ketamine. The preparation stage of preparation included the taking of pancreatic organs, fixation, dehydration, clearing, embedding, cutting, staining with Haematoxylin-Eosin, closing the dosage, and observation by microscope (Olympus x21) with 4x10 magnification.

2.6 Data analysis

The percentage data of decreased blood sugar levels was statistically tested using ANOVA one way with

Table 1. Separation & detection system with TLC method

Compounds	Mobile Phase	Detection Reagent
Alkaloid	Ethyl acetate:methanol:ammonia (4:4:1)	Dragendroff
Saponin	Chloroform:methanol (1: 4)	Vanillin-Sulfuric acid
Tanin	Ethyl acetate: methanol : Formic acid 5% (4: 1: 1)	$FeCl_3$
Flavonoid	Acetic Acid: methanol : formic acid 5% (4: 1: 1)	Cytro-borate 5%
Terpenoid	*n*-hexane: Ethyl acetate (4: 1)	Liebermann-Bouchard

95% significance level. Then proceeded with the Tukey test to determine the significant differences between groups.

3 RESULTS AND DISCUSSION

3.1 *Physalis angulata* L. and *Hibiscus sabdariffa* L. extracts

Table 2 shows the results of the extract of ciplukan herb and roselle petals. Preparation of the dried powder was carried to enlarge its surface area so that the solvent used can be readily absorbed into dried powder and the active compound which is attracted more optimally (Vitasari, 2012), while pollination will help break the walls and cell membranes to maximize the extraction process (Koirewoa *et al*., 2016).

The dried powder was extracted by maceration using 70% ethanol. Ethanol is considered to be a more selective (mouldy and bacteria growing in ethanol 20% and above), non-toxic, neutral, and well-absorbed (Pratiwi, 2010). Using ethanol as a universal solvent is due to its easy-to-dissolve of polar, semi-polar, and non-polar active substances and its ability to precipitate proteins and inhibit the action of enzymes to avoid hydrolysis and oxidation processes (Dharma *et al*., 2010). According to Indraswari (2008), ethanol with 70% concentration is effective in attracting active compounds. The ethanol solvent has two sides consisting of a polar OH-group and a non-polar CH_2CH_3 group. The extraction by maceration was done because this method can be used for large quantities of samples, the implementation is simple, does not require special treatment and the possibility of decomposition of the active substance by the influence of temperature can be avoided because there is no heating process (Dharma *et al*., 2010). Percentage of extract weight to dried powder shows the effectiveness of the extraction process. The effectiveness of the extraction process is influenced by the type of solvent, particle size of dried leaves powder, method and duration of extraction (Istiqomah, 2013). The longer the extraction time, the higher the saturation point of the solution. The contact between the sample and the solvent can be increased while it was shaking, to keep the contact between the sample and the solvent frequent, resulting in a more perfect extraction process (Koirewoa *et al*., 2016). High percentage of extract weight to dried powder results indicate the possibility of the chemical compounds contained in the extract is also high (Isnawati *et al*., 2006).

Table 2. Results of Ciplukan Herb Extraction and Rosella Flower Petals

No	Name	*Physalis angulata* L. herb extract	*Hibiscus sabdariffa* L. flower petals extract
1	Extract weight	98.80 g	234 g
2	Percentage of extract weight to dried powder	10.98 %	23.4 %

3.2 Phytochemical Screening

Phytochemical screening in this study used the thin layer chromatography (TLC) method. The purpose of using TLC is for more accurate and better identification of compounds contained in the extract. TLC is a way of physical separation with elements to be separated distributed between two phases (stationary phase and mobile phase). Separation is based on migration differences and distribution of compounds or ions (Astuti, 2016). The silica gel plate GF254 was used as stationary phase, while the eluent was used as the mobile phase varies for each of the secondary metabolites to be tested. A good eluent can separate the compound in large quantities that are marked by the appearance of stains. The stain that formed was not tailed, and the distance between the stains with each other was clear (Harborne, 1987). TLC plates were activated by the oven at temperature 115°C for 15 minutes to remove water contained in KLT plate (Sastrohamidjojo, 2007).

The results of screening tests can be found at Table 3. Stains obtained from the TLC were still not

Table 3. Phytochemical Screening Results

No.	Compounds	*Physalis angulata* L.	*Hibiscus sabdariffa* L.
1	Alkaloids	+	-
2	Flavonoids	+	+
3	Saponins	-	-
4	Tannin	+	-
5	Terpenoids	+	+

Note: (+) = detected
(-) = not detected

completely separated because the metabolite compounds were not yet perfectly attracted, possibly due to unsuitable eluents, inappropriate selection of TLC plates, and extract concentrations that were too high.

3.3 Results Measurement of Blood Sugar Levels

The result of blood glucose levels of each group before and after treatment can be seen in Figure 1. The percentage data of decreased blood sugar levels (Table 4) were inserted into statistics to test and determine their normality and homogeneity with Kolmogorov and Smirnov. The data of group I is not included in statistics because the results were different.

Table 4. Average percentage of reduced blood glucose levels

Treatment groups	Reduced blood glucose levels (%) ± SD
Negative	2.86 ± 0.81
Positive	63.02 ± 2.83
Ciplukan extract	60.86 ± 3.65
Roselle petals extract	60.12 ± 0.02
Combination I	56.77 ± 5.99
Combination II	60.50 ± 3.80
Combination III	66.74 ± 4.02

Note: The test was performed with four repetitions.

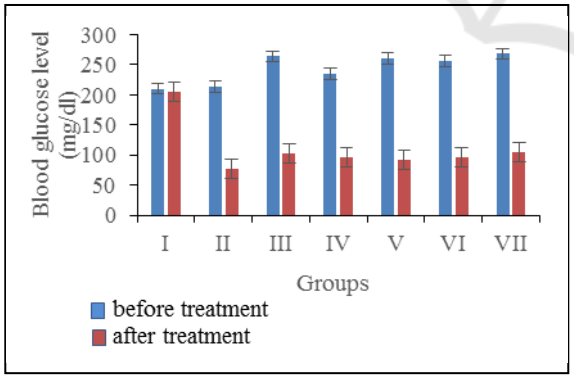

Figure 1. Graph of average blood glucose levels (mg/dl) of each group before and after treatment. (I) Negative control group. (II) Glibenclamid dose 0.052mg/200g BW group. (III) Extract Ciplukan dose 10mg/200g BW group.(IV) Extract Roselle petals dose 60mg/200g BW group. (V) Combination I (½ dose of Ciplukan: ½ dose of Roselle petals) group. (VI) Combination II (½ dose of Ciplukan: 1 dose of Roselle petals) group. (VII) Combination III (1 dose of Ciplukan: ½ dose of Roselle petals) group.

The data analysis showed normal data distribution {(p=0.819) ≥0.05} and homogeneity {(p=0.418) ≥0.05}. The analysis was continued by ANOVA one-way test to find out whether there was any significant difference or not in each treatment group with p≤0.05. Based on the analysis results, the value obtained was {(p=0.044) ≤0.05}. These results indicate that giving a combination of herbal extract ciplukan and roselle petals have a significant effect on the decrease in blood sugar levels of diabetic rats.

The data then continued with the Tukey test to know the difference between each treatment groups. Based on the Tukey test, there was no significant difference (p≥0.05) between the positive control group and single ciplukan group, single roselle petal extract, combination I, combination II and combination III. This result means that in this case, all test groups have activities that are proportional to

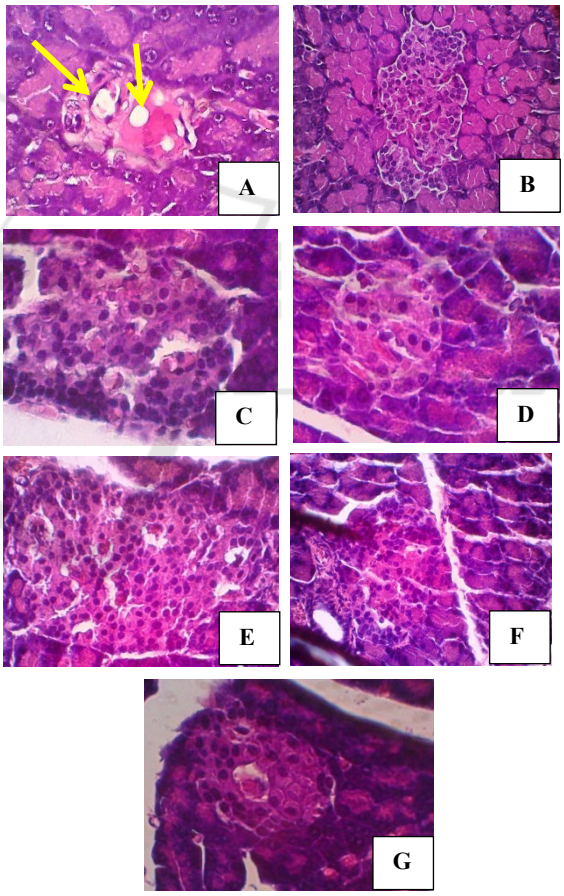

Figure 2. Histology of Pancreatic Islets Diabetic Rats with Magnification 4x10: (A) The arrows indicate the empty spaces (necrosis) of the Langerhans pancreas island cell in the negative control group; (B) Positive control group, no necrosis; (C) Ciplukan Extract group; (D) Flower Rosela extract group; (E) Combination I group; (F) Combination II group; (G) Combination III group

the positive control of glibenclamide in lowering blood sugar levels.

3.4 Observation of Pancreatic Histopathology

The results of pancreatic histologic preparations can be seen in Figure 2. Necrosis is cell or tissue death due to a reversible degeneration process. This condition can be caused by several things including toxins, drugs, low blood supply, no nerve preservation, temperature, light, radioactive and mechanical trauma (Berata *et al*., 2011). The alloxan mechanism in principle occurs through several processes that stimulant produce damage effects on pancreatic β cells. The reactive oxygen that is formed can cause damage to pancreatic beta cells. The destruction of pancreatic beta cells made a decreased insulin secretion (Szukudelski, 2001).

The results of this study have shown that the decrease in blood sugar levels and improvement of the pancreatic Langerhans islet cells induced the insulin secretion to compensate for high blood glucose levels and subsequently lowered it. The mechanism of decreased blood sugar levels and the improvement of the islet cells of Langerhans were derived from the compounds of flavonoids, anthocyanins, and terpenoids contained in ethanol extract 70% of ciplukan and roselle petals. Flavonoids are antioxidants that can clean up excessive free radicals, break the chain of free radical reactions, and binding metal ions (chelating). Flavonoids also have an inhibitory effect on the α-glucosidase enzyme (Taufiqurrohman, 2015). Anthocyanin has hypoglycaemic activity by inhibiting α-glucosidase in the intestinal lumen and improving insulin sensitivity (Kowalczyk *et al*., 2013). Terpenoids have antidiabetic activity that can stimulate the regeneration of Langerhans island cells so that the cell damage of Langerhans Island in particular β cells can be reduced gradually, the increased number of Langerhans island cells also allows the secretion of insulin so as to compensate for high blood sugar levels and then decrease it (Sunaryo *et al*., 2012).

4 CONCLUSIONS

The combination of 70% ethanol extract of ciplukan and roselle petals successfully decreased blood sugar levels in alloxan-induced rats. The combination of 70% ethanol extract of ciplukan and roselle flower petals in dose I showed lower blood sugar level, combination dose II equal to 60.50%, and combination dose III equal to 66.74%.

Based on the statistical study, there was no significant difference ($p \geq 0.05$) between the positive control group and single ciplukan group, single roselle petal, combination I, combination II and combination III. This result means that in this case all test groups have activities that are proportional to the positive control of glibenclamide in lowering blood sugar levels.

The combination of 70% ethanol extract of ciplukan and roselle petals was able to provide an improvement of the Langerhans island cell in alloxan-induced rats compared with the negative control group.

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