ABSTRACT
Iler (Plectranthus scutellarioides (L.) R.Br.) leaves of extracts ethanol were previously shown to antidiabetic activity and the ethyl acetate fraction showed the highest activity of antidiabetic. The present study was undertaken to investigate the subfractions of ethyl acetate fraction of iler leaves which has the best antidiabetic activity. Diabetes was induced in mice by single intraperitoneal administration of alloxan monohydrate (255 mg/kg bw). Control normal group, as well as diabetic mice were divided into groups (n=4) receiving different treatments. Doses (100 mg/kg bw) of PSS 1, PSS 2, PSS 3 and PSS 4 were studied in alloxan-induced diabetic mice for a period of 8 days. Glibenclamide (0.7 mg/kg bw) was used as a positive drug. The results of the study indicates that PSS significantly (P<0.05) reduced the blood glucose level in alloxan induced diabetic mice. PSS 1 has good antidiabetic activity with the percentage decrease of blood glucose level relative 48.42%.

KEYWORDS: Iler (Plectranthus scutellarioides), Antidiabetic, Mice, Alloxan.

INTRODUCTION
Based on the World Health Organization, in 2016 diabetes mellitus (DM) becomes one of the health problems. DM is not a infectious diseases, DM is a metabolic disorder that is at number three highest in the world with the number of deaths as much as 100.4 million people.
per year. DM is a chronic metabolic disorder disease characterized by increased blood glucose levels (hyperglycemia). In addition, it occurs due to the interruption of carbohydrate, lipid, and protein metabolism as a result of the lack of insulin or the body is unable to use insulin (insulin function insufficiency) or both (ADA, 2016). DM disease will cause serious damage to the heart, blood vessels, eyes, kidneys and nerves (WHO, 2016).

One of the medicinal plants that can be used as an antidiabetic is iler (*Plectranthus scutellarioides* (L.) R.Br.) has been known as traditional medicine and cultivated in Indonesia (Moektiwardoyo *et al.*, 2011). Traditionally iler leaf is used for diabetes mellitus, constipation, fever, pelunt menstruation and inflammation of the ear. (Dalimartha, 2008). Leaves iler contains alkaloids, tannins and polyphenols, flavonoids, quinones, monoterpenes and sesquiterpenoids as well as triterpenoids (Rahmiyani *et al.*, 2015) caffeine acid, rosmarinic acid ester, β-sitosterol, stigmasterol (Padua *et al.*, 1999), ethyl salicylate, methyl eugenol, thymol, carvakrol (Dalimartha, 2008) and quercetin (Moektiwardoyo *et al.*, 2011).

Previous research has shown that extract ethanol of iler has good antidiabetic activity at dose 200 mg/kg bw with percentage of relative glucose decrease of 21.52% in white rat (*Rattus norvegicus*) strain Wistar induced with alloxan and glibenclamide as positive control drugs (Susilawati, *et al.*, 2016). The study showed the potential of ethanol extract of iler leaves as antidiabetic, it is necessary to know the subfractions that antidiabetic activity. This research will be conducted to separate of the ethyl acetate fraction and test the antidiabetic activity in vivo in mice.

**MATERIALS AND METHODS**

**Material**

The material used is leaves of iler (*Plectranthus scutellarioides* (L.) R.Br.) which has been dried obtained from Indonesian Spices and Medicinal Crops Research Institute, Bogor, West Java in October 2016. The chemicals used are Dragendorff reagent, Mayer reagent, Liebermann-Burchard reagent, H$_2$SO$_4$, silica gel, alloxan, glibenclamide, PGA 2%. The experimental animals used were white male mice (*Mus musculus*) Swiss Webster strains aged 2-3 months weighing 20-30 gram.

**Apparatus**

Macerators, Evaporators, Water bath, Separator funnel, Vacuum liquid chromatography, Glukometer, Glucose test strips.
Research Method
Iler leaves were determined in *Herbarium Bandungense*, School of Life Science and Technology, Bandung Institute of Technology, with the document number 4393/I1.CO2.2/PL/2016. The research was agreed by the Health Research Ethics Committee of Faculty of Medicine of Padjadjaran University, with the document number 547/UN6.C.10/PN/2017.

Extraction with Maceration
Iler leaves were extracted with ethanol 70% by macerator apparatus, The extraction was done for 3x24 hours and accommodated every 24 hours, then the solvent was replaced with a new one. The extract of ethanol obtained in extract than concetrated by rotary evaporator. Then the viscous extract is evaporated over the water bath until the weight is constant.

Phytochemical screening of Iler
Iler extract was qualitatively tested for the presence of secondary metabolite in extract by using phytochemical screening (Farnsworth, 1966).

Fractionations by Liquid-Liquid Extraction
Ethanol extract (30 g) was suspended in distilled water (300 mL). Then the suspension obtained was placed into a 1L separatory funnel. Firstly, the solution was extracted with n-hexane (3x300 mL). Next, the aqueous layer was extracted with ethyl acetate (3x300 mL) to obtain the respective fractions. All of the fractions obtained were concentrated using the rotary evaporator. Concentrated fractions were kept in freeze drier for 24 hours to remove the remaining solvents.

Subfractionation of the ethyl acetate fraction
The ethyl acetate fraction sample was performed separating the compound by Vacuum Liquid Chromatography method (VLC) using eluent n-hexane: ethyl acetate (10: 0, 9: 1, 8: 2, 7: 3, 6: 4, 5: 5, 4: 6, 3: 7 , 2: 8, 1: 9, 0:10) and 100% ethanol and the results can be in TLC using eluent n-hexane: ethyl acetate (8: 2). The concentrated subfractions further will be referred as *Plectranthus scutellarioides* (L.) R.Br. subfraction (PSS).
Induction of diabetes
All Swiss Webster male white mice were acclimatization for 2 weeks before the experiment. Then induction of diabetes with alloxan dose 255 mg/kg bw intraperitoneally, except mice in the normal control group. After 48 hr, blood glucose was measured by glucometer.

Experimental procedure
The diabetic mice (glucose level > 200 mg/dl) were separated and divided into 6 different groups for experimental study, with each group containing 4 animals. Furthermore the group of normal mice and 6 groups of diabetic mice were daily oral treatment was administered for 8 days.

Group I-Normal control group, PGA 2% (not alloxan induction).
Group II-Negative control group, PGA 2%.
Group III-Positive control group, glibenclamide at dose of 0.7 mg/kg bw in PGA 2%.
Group IV-PSS 1 group, PSS 1 at dose 100 mg/kg bw in PGA 2%.
Group V-PSS 2 group, PSS 2 at dose of 100 mg/kg bw in PGA 2%.
Group VI-PSS 3 group, PSS 3 at dose of 100 mg/kg bw in PGA 2%.
Group VII-PSS 4 group, PSS 4 at dose of 100 mg/kg bw in PGA 2%.

Blood glucose measurements were performed daily from the first day of administration, using an amperometric method with glucometer. From the data of blood glucose levels obtained, calculated the percentage decrease of blood glucose level relative (P) from each test group by the formula.

\[
\text{Relative blood glucose} \times 100 = \frac{\text{Blood glucose at } t}{\text{Blood glucose first}}
\]

\[
P(\%) = \frac{\text{Relative blood glucose negative group} - \text{Relative blood glucose test group}}{\text{Relative blood glucose negative group} - \text{Relative blood glucose normal group}} \times 100
\]

Statistical analysis
The results of the study were subjected to one-way analysis of variance (ANOVA) followed by Duncan's test for multiple comparisons. Values with \( P<0.05 \) were considered significant.

RESULTS
Dried leaves extraction (1856.8 g) resulted in 345.78 g of ethanol extract (18.62%).
Table 1: Phytochemical screening of iler.

<table>
<thead>
<tr>
<th>Secondary metabolite</th>
<th>Dried leaves</th>
<th>Concentrated Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinons</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Monoterpenoids/Sesquiterpenoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids/Triterpenoids</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: + : Detected  - : Undetected

Dried leaves extraction (1433.76 g) resulted in 267 g of ethanol extract, whereas the ethyl acetate fraction, the n-hexane fraction and the water fraction were 57.25 g (3.993%), 49.24 g (3.343%) and 132.23 g (9.223%), respectively.

Table 2: The PSS is combined based on the polar stripes.

<table>
<thead>
<tr>
<th>Dried leaves</th>
<th>Ethyl Acetate Fraction</th>
<th>PSS</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1001.75 g</td>
<td>40 g</td>
<td>0 (PSS 1-2)</td>
<td>0.2028</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 (PSS 3)</td>
<td>0.8326</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 (PSS 4-5)</td>
<td>1.3374</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 (PSS 6-7)</td>
<td>3.0437</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 (PSS 8-12)</td>
<td>31.0222</td>
</tr>
</tbody>
</table>

Stationary phase : Silika gel 60 F_{254}
Mobile phase    : n-hexane : ethyl acetate (8:2)
Detection       : A= Visible, B=UV 254 nm, C=UV 366 nm
D= Visible+H_{2}SO_{4}, E= UV 254 nm+H_{2}SO_{4}, F= UV 366 nm+H_{2}SO_{4}

Figure 1. TLC of ethyl acetate subfractions (PSS 12)
Figure 2: TLC of ethyl acetate subfractions combined (PSS 0-4).

Stationary phase: Silika gel 60 F$_{254}$
Mobile phase: $n$-hexane : ethyl acetate (8:2)
Detection: A= Visible, B=UV 254 nm, C=UV 366 nm
D= Visible+H$_2$SO$_4$, E= UV 254 nm+H$_2$SO$_4$, F= UV 366 nm+H$_2$SO$_4$
Note: 0= PSS 0, 1= PSS 1, 2= PSS 2, 3= PSS 3, 4= PSS 4
Tabel 3: Effect PSS on blood glucose level of alloxan-induced diabetic mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Blood glucose first</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75.50± 2.629</td>
</tr>
<tr>
<td>After induction</td>
<td></td>
</tr>
<tr>
<td>Basal Values</td>
<td>87.25± 1.931</td>
</tr>
<tr>
<td>After treatment</td>
<td></td>
</tr>
<tr>
<td>1st day</td>
<td>80.75± 5.056</td>
</tr>
<tr>
<td>2nd day</td>
<td>79.50± 3.968</td>
</tr>
<tr>
<td>3rd day</td>
<td>74.50± 2.754</td>
</tr>
<tr>
<td>4th day</td>
<td>86.00± 4.847</td>
</tr>
<tr>
<td>5th day</td>
<td>82.00± 5.049</td>
</tr>
<tr>
<td>6th day</td>
<td>86.25± 2.689</td>
</tr>
<tr>
<td>7th day</td>
<td>86.75± 4.732</td>
</tr>
<tr>
<td>8th day</td>
<td>80.00± 6.868</td>
</tr>
</tbody>
</table>

PSS was administered a dose 100 mg/kg bw. Blood glucose value is mean±SEM of 4 observation, *p<0.05 compared with their basal values of respective group.

Tabel 4: The percentage decrease of blood glucose level relative (P) compared with diabetic mices.

<table>
<thead>
<tr>
<th>Group</th>
<th>P (%)</th>
<th>PSS 1</th>
<th>PSS 2</th>
<th>PSS 3</th>
<th>PSS 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SEM</td>
<td>81.12±5.482</td>
<td>48.42±5.856</td>
<td>45.23±6.815</td>
<td>39.78±5.033</td>
<td>30.63±4.242</td>
</tr>
</tbody>
</table>

Relative blood glucose values are as expressed as mg/dl and each value is mean±SEM of 4 observation.
DISCUSSION

Phytochemical screening of iler leaves showed the contain alkaloids, flavonoids, saponins and polyphenols (Table 1). Moektiwardoyo et al. (2011) who reported that alkaloids, saponins, phenols, flavonoids and tannins were present in the leaves of iler but in this study tannins are not detected due to the tannin content in the extract and the dried leaves is too small so it is not detected. Some researchers reported that flavonoids, tannins, saponins, alkaloids and polyphenols as bioactive antidiabetic principles. Mechanisms of Alkaloid has α-glucosidase enzyme inhibition and decrease glucose transport through the intestinal epithelium (Mishra et al., 2010; Patel et al., 2012). Mechanisms of flavonoids in lowering blood glucose levels is to reduce the absorption of glucose and increase secretion of insulin, decrease oxidative stress, inhibit the intestinal mucosa GLUT 2 and inhibit phosphodiesterase (Ajie, 2015).

Fractionation of iler have n-hexane fraction, ethyl acetate fraction and water fraction, then subfractionation of the ethyl acetate fraction by separation of VLC method could have 12 PSS (Fig. 1) and then TLC combined into 5 PSS (Table 2, Fig. 2) that some compound obtained in the PSS. After obtaining PSS 1-4, further testing has done activity antidiabetes with PSS 1-4. While PSS 0 was not tested because of the number of samples the least.

Alloxan is one of the common substances used for the induction of diabetes mellitus in addition to streptozotocin. Alloxan has ability to destructive beta pancreas cell of resulting in a decrease in endogenous insulin secretion and paves ways for the decreased utilization of glucose by body tissues (Yamamoto et al., 1981).

Glibenclamide is a type of medicine called a sulphonylurea. It is used to help control blood sugar levels in people with type 2 diabetes. Glibenclamide works mainly by stimulating the cells in the pancreas that produce insulin, causes the beta cells to produce more insulin (Syarif et al., 2007). Glibenclamide orally to positive group was used to compare an antidiabetic effect.

In this experiment, blood glucose level were measured in all groups showed that there is a treatment effect in each group. Data showed that negative control mices significant elevation ($P<0.05$) in blood glucose of the experiment as compared to their basal values, which was maintained over a period of 8 day although down but still in diabetic condition. Daily oral treatment with glibenclamide 0.7 mg/kg bw and PSS dose of 100 mg/kg bw showed
significant reduction ($P<0.05$) in blood glucose on successive days of the experiment as compared to their basal values (Table 3). At the end of experiment (8th day) blood glucose level PSS 1 group was 115.25±2.016 mg/dl in the diabetic mice treated with 100 mg/kg bw. showed better results than PSS 2, PSS 3 and PSS 4.

The most pronounced antidiabetes effect was obtained PSS 1 with dose of 100 mg/kg bw as shown in Table 4 with the percentage decrease of blood glucose level relative (P) compared with diabetic mice showed that PSS 1 (48.42 %), PSS 2 (45.23%), PSS 3 (39.78%) and PSS 4 (30.64%). Results indicated that PSS 1 showed the most antidiabetic activity which was probably the presence of several secondary metabolites as flavonoids, saponins, alkaloids and polyphenols as bioactive antidiabetic principles (Mukherji et al., 2006). In Fig. 1 and 2 PSS 1 has a yellow and orange color spot presumably indicating there is a phenolic compound such as flavonoid. Flavonoids have been reported to suppress glucose level significantly and the typical flavonoid has been found to be a strong inhibitor of $\alpha$-glucosidase (Kim et al., 2000).

CONCLUSION

The results of this study concluded that the subfractions from ethyl acetate fraction iler (*Plectranthus scutellarioides* (L.) R.Br.) leaves has good active antidiabetic activity and subfractions has the most antidiabetic activity is subfraction 1 (PSS 1) at dose of 100 mg/kg bw with the percentage decrease of blood glucose level relative 48.42% in white male mice (*Mus musculus*) Swiss Webster strains induced by alloxan and glibenclamide as positive control drugs.

ACKNOWLEDGMENTS

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REFERENCES


