QUALITATIVE AND QUANTITATIVE DETERMINATION OF SECONDARY METABOLITES AND ANTIDIABETIC POTENTIAL OF Ocimum basilicum L. LEAVES EXTRACT

Joni Tandi1,2, Tien Wahyu Handayani1 and Agustinus Widodo2
1Department of Pharmacy, Sekolah Tinggi Ilmu Farmasi Pelita Mas, Palu- 94111, Indonesia
2Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Tadulako University, Palu-94148, Indonesia
Corresponding Author: jonitandi757@yahoo.com

ABSTRACT
Traditional herbal substances are used in alternative diabetes mellitus care programs. This research aimed to determine the secondary metabolite levels of ethanol extract Ocimum basilicum L. leaves, clarify the effect of extracts on blood glucose levels, and histopathological pancreatic tissue Streptozotocin-induced diabetic rats. Phytochemical screening of extracts was carried out qualitatively according to standard methods. UV-Vis Spectrophotometric was the quantitative estimation of alkaloids, flavonoids, saponins, and tannins. The rats were treated with ethanol extract of O. basilicum L. leaves with a dose of 200, 400, and 800 mg/kg bodyweight for 28 days, then calculated with blood levels and histopathology of pancreatic tissue in the group treated and compared to the group of diabetes. Group doses of 400 and 800 mg/kg body weight could significantly reduce blood glucose levels, and according to histological results, can regenerate mouse pancreatic cells. It could conclude that the ethanol extract of O. basilicum L. leaves is a potential source of antidiabetic.

Keywords: Ocimum basilicum L., Diabetes, Pancreas, Histopathology

INTRODUCTION
Diabetes is a chronic metabolic disease that happens when the pancreas is no longer capable of producing insulin or when the body can not use the insulin it renders correctly. Diabetes shows a rise in blood glucose levels, which causes severe damage to the nerves, blood vessels, eyes, heart, and kidneys. In 2019, some 463 million people suffer from diabetes, and this figure is forecast to increase by around 25% by 2030, and approximately 51% by 2045. The prevalence of diabetes, in general, is rising faster in middle and low-income countries. Although diabetes treatment using synthetic drugs has experienced much progress, traditional herbs are still widely used throughout the world. Some herbs have been used in traditional medicine for centuries to treat diabetes. Medicinal plants are more affordable and have fewer side effects than synthesized drugs in general, and some are more effective in treating diabetes mellitus. Traditional health practitioners confirm basil (Ocimum basilicum L.) leaves to be used for diabetes management. Previous research has shown that O. basilicum L. has antidiabetic activity. O. basilicum L. water extract contains antioxidants that can inhibit α-glucosidase and α-amylase. Extracts of O. basilicum L. air parts have an antidiabetic effect thought to be through suppressing the release of endogenous glucose, inhibiting glycogenolysis, and stimulating glycogenesis. This study aims to determine secondary metabolite levels ethanol extract of O. basilicum L. leaves and clarify the effects of extracts on blood glucose and determine the possible impact on pancreatic tissue.

EXPERIMENTAL
Plant Materials and Extraction Procedure
O. basilicum L. leaves were obtained from cultivated plants in Bobalo Village, Palasa District, Parigi Moutong Regency, Central Sulawesi Province, Indonesia. This plant was identified by Herbarium Celebense (CEB) of Tadulako University under number 82/UN.28.UPT-SDHS/LK/2017. Simplicia O.
**Phytochemical Screening of Secondary Metabolism**

Phytochemical screening of alkaloid compounds, flavonoids, saponins, and tannins using standard methods for qualitative testing.

**Determination of Total Alkaloid**

100 mg extract was added with 5 ml of HCl 2N, shaken out. Washed the solution with 10 mL CHCl₃ in a separating funnel 3 times, discarded the CHCl₃ phase. They neutralized with NaOH 0.1N. 5 mL BCG and 5 mL buffer phosphate were added. 5 mL of the solution was extracted with 5 mL CHCl₃, stirred with a 1500 rpm magnetic stirrer for 15 minutes, repeated 2 times, and the CHCl₃ phase was collected, evaporated with N₂ gas, then CHCl₃ added to 10 mL. Diluted 10 times, read absorption at λ 470 nm. The results obtained are plotted against the Quinine standard curve. The total alkaloid is expressed as mg of equivalent Quinine/g extract.

**Determination of Total Flavonoid**

100 mg extract was added 2ml HCl 4N, then autoclave for 2 hours 110 ° C, then cold. Extracted with ether, then put in a 10ml test tube. Evaporated ether, dried with N₂ gas. 0.3 ml of 5% NaNO₂ was added after 5 minutes added 0.6 ml of 10% AlCl₃, waited 5 minutes, added 2 ml of 1 M NaOH added aquadest to 10 ml. Diluted 250 times, read absorption at λ 510 nm. The results obtained are plotted against the Quercetin standard curve. The Total flavonoid is expressed as mg of Quercetin equivalent/g extract.

**Determination of Total Saponin**

100 mg extract was added 2 mL 25% H₂SO₄, then autoclave 120 minutes 100 ° C. Extracted with ether and then dried. 1 mL aquadest is added, then vortex for 5 minutes. 50 µl Anisaldehyde added, shaken out, then let stand for 10 minutes. Added 2 ml of 50% H₂SO₄, then heated in a water bath for 10 minutes 60°C. Aquadest added up to 10 ml. Diluted 10 times, read absorption at λ 435 nm. The results obtained are plotted against the standard Quillaja bark curve. The total saponin is expressed as mg of Quillaja bark equivalent/g extract.

**Determination of Total Tannin**

100 mg of the sample was extracted with 10 mL of methanol for 20 hours at room temperature, then filtered. The residue is boiled with 10 mL of distilled water, then cooled and filtered. The extract obtained added aquadest up to 10 mL. 1 mL extract solution was added with 0.1 mL Folin-Ciocalteu reagent and vortex, let stand 5 minutes, added with 2 mL 20% Na₂CO₃ and vortex, let stand 5 minutes. Aquadest added up to 10 mL, carried out 5 times dilution. The absorbance was read at λ 760 nm after incubation for 30 minutes at room temperature. The results obtained are plotted against the Tannic Acid standard curve. The total tannin is expressed as mg Tannic Acid equivalent/g extract.

**Animals**

Male Wistar rats aged 3-4 months, with 150-200 grams of body weight. The rats were kept in standard conditions, with a temperature of 24-26°C, a light cycle of 12 hours and a dark cycle of 12 hours, and a humidity level of 70-75%. Rats were given a standard diet and ad libitum water during the experiment. The use of animals and experimental protocols in this study was approved by the Ethics Committee on Medical and Health Research, Faculty of Medicine, Tadulako University (No.: 3380.A/UN28.1.30/KL/2019).

**Determination of Blood Glucose Levels**

The white male rats are divided into 6 groups, each of 5 rats. The groups were classified as follows: the untreated group (normal control), the diabetic group who were given 0.5% Na CMC suspension
Ocimum basilicum L. LEAVES EXTRACT

Joni Tandi et al.

(negative control), the diabetic group who were given 0.45 mg/kg bw/day Glibenclamide suspension (positive control), the diabetic group given 200 mg/kg bw/day suspension of ethanol extract of O. basilicum L. leaves (dose 1), the diabetic group given 400 mg/kg bw/day suspension of ethanol extract of O. basilicum L. leaves (dose 2), the diabetes group given 800 mg/kg bw/day suspension of ethanol extract of O. basilicum L. leaves (dose 3). The group was made diabetic with a single intraperitoneal injection of STZ 40 mg/kg bw prepared in 0.1 moles of citrate buffer (pH 4.5). Rats fasted for 8 hours the third day after the induction, then blood glucose levels were again measured. When rat blood glucose levels have reached a state of hyperglycemia (> 200 mg/dl), oral treatment is given for 28 days. Blood glucose levels are measured using a glucometer (Accu-Chek®). Data on blood glucose levels before and after treatment (14, 21, and 28 days) were recorded and analyzed.

Histopathological Examination of The Pancreas

Pancreatic histology was performed after treatment on the 28th day. Test animals are sacrificed with neck dislocations that have been previously anesthetized. The rat pancreas was taken and put in a container containing 10% formalin. The pancreatic organs fixation prepares pancreatic preparations with 10% Buffered Neutral Formalin (BNF) for 48 hours. The specimen is then cut as thick as 0.5-1 cm. The cut specimen is put into the cassette embedding and then processed for 20 hours in a tissue processor, then transferred to the embedding center and then blocked using paraffin. The blocks were cut using a microtome with a thickness of 5-6 μm, stretched on a gelatin solution surface in a 40 °C floating bath. The results are cut using a glass object and placed on a heating plate. The coloring is done using Hematoxylin and Eosin (H&E) dyes. Observations were made under an Olympus Bx-51 microscope with a magnification of 400 times. Pancreatic histology was observed, and ratings were based on the following categories: score 0 if there was no cell necrosis, score 1 if <10% cell necrosis, score 2 if <40% cell necrosis, score 3 if >40% cell necrosis.14

Statistical Analysis

Blood glucose level results were analyzed using analysis of variance (ANOVA) and followed by Duncan’s test to determine significant differences (P <0.05). The pancreatic histology data results, which are categorical based on scores, were statistically analyzed using the Kruskal-Wallis test and continued by the Mann Whitney Test to determine significant differences (P <0.05).

RESULTS AND DISCUSSION

Phytochemical tests of O. basilicum L. leaves ethanol extract include alkaloid, flavonoid, saponin, and tannin tests. Phytochemical test results qualitatively showed positive extracts for analyzing alkaloids, flavonoids, saponins, and tannins (Table-1). Phytochemical test results quantitatively indicate that flavonoid compounds have the highest levels of other secondary metabolite compounds (Table-2).

<table>
<thead>
<tr>
<th>Phyto-constituents</th>
<th>Test performed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>Dragendorff’s</td>
<td>+ ve</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Shinoda</td>
<td>+ ve</td>
</tr>
<tr>
<td>Saponin</td>
<td>Froth formation</td>
<td>+ ve</td>
</tr>
<tr>
<td>Tannin</td>
<td>FeCl₃</td>
<td>+ ve</td>
</tr>
</tbody>
</table>

Secondary metabolites are known to have pharmacological activity, one of which is antidiabetic. Plants with alkaloids are known to have antidiabetic activity through several mechanisms, namely carbohydrates-hydrolyzing enzymes inhibitors: α-Glucosidase inhibitors and α-Amylase inhibitors, protein tyrosine phosphatase 1B inhibitors, dipeptidyl peptidase-4 inhibitors, 5’-adenosine monophosphate-activated protein kinase (AMPK) activators, GLUT4 (glucose transporter type 4) translocation activator, insulin secretion stimulators, and pancreatic β-cells regenerators.15,16
Table-2: Total Alkaloid, Flavonoid, Saponin, and Tannin Contents of Ethanol Extract of *O. basilicum* L. Leaves

<table>
<thead>
<tr>
<th>Contents</th>
<th>Results (mg/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Alkaloid Equivalents Quinine</td>
<td>187</td>
</tr>
<tr>
<td>Total Flavonoid Equivalent Quercetin</td>
<td>236</td>
</tr>
<tr>
<td>Total Saponin Equivalents Quillaja bark</td>
<td>93.7</td>
</tr>
<tr>
<td>Total Tannin Equivalents Tannic Acid</td>
<td>24.5</td>
</tr>
</tbody>
</table>

Flavonoids are antioxidants that play an essential role in the prevention and treatment of diabetes mellitus. In vitro, antioxidant and antidiabetic activities of some flavonoids have been reported to affect the inhibition of $\alpha$-glucosidase and dipeptidyl peptidase-4 (DPP4).\(^7\) Flavonoids have a variety of antidiabetic actions where one flavonoid can target several pathways. Flavonoid effects can be through activate the synthesis and translocation of GLUT4, increase hexokinase activity in the liver, reduce beta-cell apoptosis, activate the expression of peroxisome proliferator-activated gamma receptor (PPAR-$\gamma$) to improve glucose uptake, activate AMPK pathways, inhibit the activity of tyrosine kinase, and activate nuclear factor-$\kappa$B (NF-$\kappa$B).\(^18\)

Saponins from various marine plants and animals have been reported to have hypoglycemic activity. Saponin activity regulates blood glucose levels and prevents diabetes complications related to its antioxidant activity.\(^18\) The hypoglycemic action of saponins is through activation of glycogen synthesis, inhibition of the activity of disaccharide, modulate the release of insulin from the beta-cell islets, and inhibition of the activity of $\alpha$-glucosidase.\(^19-23\)

Tannin is one of the valuable secondary metabolites of plants that provides many benefits for human health. Tannins are polyphenol compounds found in many medicinal plants and food sources such as fruits, nuts, seeds, spices, and drinks. Various reports indicate that tannin compounds from medicinal plants play a major role in controlling the development of diabetes and its complications.\(^24-25\) Tannin has antidiabetic potential, as it can lower glucose levels by delaying the absorption of intestinal glucose (inhibits the activity of $\alpha$-amylase and $\alpha$-glucosidase), effects such as insulin in insulin-sensitive tissue, and delay the onset of insulin-dependent diabetes mellitus by regulating the pancreatic $\beta$-cell antioxidant environment.\(^26\)

![Fig.-1: Effects of Ethanol Extract *O. basilicum* L. leaves on Blood Glucose Level (mg/mL) During the Experiment](image)

The effect of ethanol extract of *O. basilicum* L. leaves on the experimental rat blood glucose levels is shown in Fig.-1. During the trial period, the normal control group rat did not show significant variations in blood glucose levels compared to other groups. The ethanol extract of *O. basilicum* L. leaves at a dose of 400 mg/kg and 800 mg/kg significantly reduced hyperglycemia compared with the negative control group. However, it has not been able to decrease to the same level as the normal control and positive
control groups. Based on data analysis using ANOVA and Duncan Test, therapy with 400 mg/kg and 800 mg/kg was not statistically significantly different from normal control and positive control (P < 0.05). The ethanol extract of *O. basilicum* L. leaves is effective in controlling hyperglycemia in STZ induced diabetic rats. Previous studies have shown that *O. basilicum* L. extract has suspected antidiabetic activity through the mechanism of suppressing endogenous glucose release, inhibiting glycogenolysis and stimulating glycogenesis, through antioxidant activity, inhibiting α-glucosidase and α-amylase, increasing the translocation of GLUT4 to the muscle plasma membrane order, and increases insulin-stimulated. The ethanol extract of *O. basilicum* L. leaves is effective in controlling hyperglycemia in STZ induced diabetic rats. Previous studies have shown that *O. basilicum* L. extract has suspected antidiabetic activity through the mechanism of suppressing endogenous glucose release, inhibiting glycogenolysis and stimulating glycogenesis, through antioxidant activity, inhibiting α-glucosidase and α-amylase, increasing the translocation of GLUT4 to the muscle plasma membrane order, and increases insulin-stimulated. 

The results of our tests on the ethanol extract of *O. basilicum* L. leaves showed alkaloids, flavonoids, saponins, and tannins (Table-2). The secondary metabolites are associated with antidiabetic activity through their respective mechanisms, as described above. Various studies have shown that the chemical content of plant extracts could be a diabetes mellitus therapy. The phenolic, alkaloid, and flavonoid content of the *Kigelia africana* stem and the *Sterculia foetida* stem are related to the antioxidant and antidiabetic activity. Phytochemical screening reports indicate that the ethanol extract of the *Bambusa arundinacea* root contains flavonoids, tannins, and phenolics, and is associated with antihyperglycemic activity. *Dendrophtoe pentandra* (L.) Miq leaves extracted using several solvents shows the potential for bioactivity as an antioxidant and antidiabetic, whose activities are related to the content of saponin compounds, terpenoids, steroids, and specifically flavonoids.

Table-3: Histopathological Scores of Rats Pancreas Induced by STZ and Treated by Ethanol Extract of *O. basilicum* L. Leaves

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0 ± 0⁵</td>
</tr>
<tr>
<td>Negative control</td>
<td>3 ± 0⁶</td>
</tr>
<tr>
<td>Positive control</td>
<td>0.8 ± 0.8⁴</td>
</tr>
<tr>
<td>Dose 1 (200 mg/kg bw)</td>
<td>1.4 ± 0.5⁴</td>
</tr>
<tr>
<td>Dose 2 (400 mg/kg bw)</td>
<td>0.8 ± 0.8⁴ac</td>
</tr>
<tr>
<td>Dose 3 (800 mg/kg bw)</td>
<td>0.2 ± 0.4⁴</td>
</tr>
</tbody>
</table>

⁴ Means with different letters were statistically significant.

Fig.-2: Photomicrograph of Pancreatic Islets of Experimental Rats After 28 Days of Treatment
The effect of ethanol extract of *O. basilicum* L. leaves on the experimental rats' pancreatic histology is shown in Table-3 and Fig.-2. The normal control group rat did not show cell necrosis, while the other groups showed different cell necrosis levels. Based on data analysis results using the Kruskal-Wallis Test and continued by the Mann Whitney Test, therapy with 400 mg/kg and 800 mg/kg was not statistically significantly different from normal control and positive control (P <0.05).

STZ at high doses can cause depleted beta-pancreatic cells. STZ 40 mg/kg can increase plasma glucose and pancreatic necrosis at the cellular level. Histopathological studies conducted show recovery of damaged pancreatic cells after treatment with ethanol extracts of *O. basilicum* L. leaves. The results of this study support the possibility of regeneration of pancreatic cells to produce new β cells. The content of secondary metabolites such as alkaloids, flavonoids, saponins, and tannins is each thought to be associated with antidiabetic activity and pancreatic cell regeneration, as described earlier.

**CONCLUSION**

Antidiabetic and histopathological studies of pancreatic diabetic rats induced by STZ showed recovery of glucose levels and regeneration of damaged pancreatic cells after treatment with ethanol extract of *O. basilicum* L. leaves. The secondary metabolite compound in the ethanol extract of *O. basilicum* L. leaves is thought to have a synergy effect of preventing and alleviating diabetes mellitus. Isolation of the actual chemical compounds responsible for this effect and its mechanism requires further investigation.

**ACKNOWLEDGEMENT**

The authors thank the Integrated Research and Testing Laboratory-Gadjah Mada University and the Pathology Laboratory of the Maros Veterinary Center for technical assistance and facilitating the laboratory for this research.

**REFERENCES**

2. [https://www.who.int/health-topics/diabetes#tab=tab_1](https://www.who.int/health-topics/diabetes#tab=tab_1)

[RJC-5990/2020]