PROGRAM BOOK & ABSTRACT

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Antidiabetic Activity Test Of Ethyl acetate Extract Of Poguntanoh Leaves (Curanga fel-terrae Merr)

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Abstract

Background: Poguntanoh (Curanga fel-terrae Merr) is a species of Scrophulariaceae, it has been used to treat diabetes mellitus traditionally and give satisfactory results.

Objective: The Research was intended to know in vivo antidiabetic activity of ethyl acetate extract of plant leaves in white male mice.

Methods: The research include characterization, phytochemical screening of simplex, ethyl acetate extract of poguntanoh (Curanga fel-terrae Merr) leaves, testing in vivo antidiabetic activity performed in white male mice with alloxan induction method. Test of the antidiabetic effect of ethyl acetate extract of Curanga fel-terrae Merr. leaves was conducted by using alloxan induced diabetic mice by single dose of ethyl acetate extract. Diabetic Mice were divided into 3 groups and each group consisting of 6 mice. Group I were given suspension of 1.0 % CMC-Na, dose 1 % bw, Group II were given suspension of Metformin® with dose 50 mg/kg bw and Group III were given suspension of ethyl acetate extract of Curanga fel-terrae Merr. Leaves with dose 200 mg/kg bw. Suspension of test material (extract) was administered for 5 consecutive days orally and the BGL of mice were measured on the third, fifth, seventh, and tenth days after administration of the test material.¹⁰,¹⁵,¹⁷

Results: The results of the characterization of simplex and ethyl acetate extract of poguntanoh leaves (Curanga fel-terrae Merr) obtained water content 5.97%, 4.91%, respectively. Content of water soluble 25.66%, 0.50%, respectively. Content of ethanol soluble 19.32%, 0.08% respectively. Total ash content 4.01%, 13.94%, respectively. Content of acid insoluble ash 0.78%, 61.39%, respectively. Phytochemical screening of simplex showed the presence of flavonoid, glycosides, saponins, tannins and steroids/triterpenoids and ethyl acetate extract showed the presence of flavonoid, glycosides, saponins, tannins. Blood glucose reduction of test animals after treated with ethyl acetate extract up to tenth days was 53.96%

All of the data were analyzed statistically by analysis of variance (ANOVA) method, using SPSS (Statistical Product and Service Solutions) 17.0 software

Conclusion: Ethyl acetate extract of Curanga fel-terrae Merr, Leaves is effective as antidiabetic agent, ethyl acetate extract have ability to reduce BGL 53.96% up to tenth days.

Keyword(s): poguntanoh leaves, antidiabetic, Alloxan
INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous syndrome which all of the symptoms are characterized by increased of blood sugar level that caused relative or absolute insulin activity [1]. Hyperglycemic condition leads to microvascular and macrovascular complications and early death [2].

Poguntano (Curanga fel-terrae Merr.) have been used as diuretic, antipyretic, hepatoprotective, cardioprotective, antidiabetic, antioxidant, anti-inflammatory, anthelmintic, analgesic activities, inhibits hepatitis B (HB) e-antigen excreted by HepG2 2215 cell lines, suggesting to have anti-HB virus activity, anticancer activity in vitro and in vivo [6-17]. The purpose of this study was to determine antidiabetic activities of ethyl acetate extract of Curanga fel-terrae Merr. leaves.

EXPERIMENTAL

Plant and chemicals material

The materials used in this study were Curanga fel-terrae Merr. leaves which taken from Dairi, North Sumatera, Indonesia. The chemicals used are pro-analysis grade: alloxan (Sigma), sodium CMC (Merck), technical grade of n-hexane, ethyl acetate and distilled water.

Preparation of extract

The air-dried and powdered leaves of Curanga fel-terrae Merr. leaves. (1 kg) were repeatedly extracted by cold maceration with n-hexane (3x3 d, 10 L). The powder was dried in the air and extracted with ethylacetate (3x3 d, 10 L) at room temperature on a shake. The filtrate was collected, and then evaporated under reduced pressure to give a viscous extract and then freeze dried to give a dried extract [2,9].

Antidiabetic Assay

Animal Preparation

The animals used in this study are male mice weighing 25-35 grams. Before the experiment, mice were maintained for 2 weeks in a good cage to match the environment, i.e., the reception of light, 12 hours dark and 12 hours light.

Preparation of Extract Suspension and Alloxan Solution

Suspension of extract was prepared by using 0.5% CMC-Na with certain concentration. Solution of alloxan was prepared by dissolving alloxan in cold sodium chloride 0.9%.
Characterization of Simplex and Ethyl Acetate Extract

Characterization of simplex include determination of water content, determination of water-soluble extract, determination of the ethanol-soluble extract, total ash content determination and the determination of ash-not dissolve in acid content [8,9].

Phytochemical Screening

Determining the class of chemical compounds carried out on simplex, and ethyl acetate extract [17].

Preparation of Alloxan Induced Diabetic Mice

The mice were induced with alloxan solution 200 mg/Kg intra-peritoneal (ip). The blood glucose level (BGL) of mice was measured on the third day. On the third day, mice that have BGL higher than 200mg/dl were separated and used as test animals. Animals with BGL lower than 200 mg/dL, were induced back with STZ. If on the third day the BGL of the mice has been higher than 200 mg/dL, the animal is ready to be tested.

Study of the antidiabetic effect of ethanol extract of Curanga fel-terrae Merr. leaves was conducted by using alloxan induced diabetic mice by single dose of ethyl acetate extract. Mice were divided into 3 groups and each group consisting of 6 mice, they were:

Group I : Diabetes mice were given suspension of 1.0 % CMC, dose 1 % of body weight (BW)

Group II : Diabetic mice were given suspension of Metformin® with dose 65 mg/Kg BW

Group III, IV and V : Diabetic mice were given suspension of ethyl acetate extract with dose 200 mg/Kg BW.

Suspension of test material (ethyl acetate extract) was administered everyday orally and the BGL of mice were measured on the 1st, 3rd, 5rd, 7rd, 9rd, 11rd, 13rd and 15rd days after administration of the test material [10].

Statistical analysis

All data were analyzed with descriptive using SPSS 17.0.

RESULTS AND DISCUSSION

The result of simplex and extract characterization were showed in Table 1. As can be seen in Table 1, it is shown that the result of characterization not listed in monograph.
Table 1: The result of simplex and ethyl acetate characterization

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter</th>
<th>Result (%)</th>
<th>Requirements (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Simplex</td>
<td>Extract</td>
</tr>
<tr>
<td>1</td>
<td>Water content</td>
<td>5.97</td>
<td>4.91</td>
</tr>
<tr>
<td>2</td>
<td>Water-soluble extract content</td>
<td>25.66</td>
<td>13.94</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol-soluble extract content</td>
<td>19.32</td>
<td>61.39</td>
</tr>
<tr>
<td>4</td>
<td>Total ash content</td>
<td>4.01</td>
<td>0.50</td>
</tr>
<tr>
<td>5</td>
<td>Ash-not soluble in acid content</td>
<td>0.78</td>
<td>0.08</td>
</tr>
</tbody>
</table>

The results of phytochemical screening is presented in Table 2. As can be seen in Table 2, it is shown that the result of phytochemical screening. The result determining the class of chemical compounds of Curanga fel-terrae Merr. simplex show presence of flavonoids, saponins, tannins, glycosides, and triterpenoids/steroids group compound. In ethyl acetate extract show presence of flavonoids, saponins, tannins and glycosides group compound.

Table 2: The result of phytochemical screening

<table>
<thead>
<tr>
<th>No</th>
<th>Screening</th>
<th>Simplex</th>
<th>EEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Antrakuinon glycoside</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Triterpenoids/ steroids</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Antidiabetic activity of ethyl acetate extract was observed by depletion of blood glucose level (BGL) after induction with alloxan. Fasting blood glucose level in mice before and after treatment with extract were shown in Table 3 and Table 4.

Table 3. Fasting Blood Glucose Level Before and After Alloxan Induction

<table>
<thead>
<tr>
<th>Group</th>
<th>BGL Before Alloxan Induction (mg/dL)</th>
<th>BGL After Alloxan Induction (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMC-Sodium</td>
<td>81.67 ± 1.02</td>
<td>413.17 ± 6.14</td>
</tr>
<tr>
<td>Metformin</td>
<td>82.67 ± 1.74</td>
<td>491.17 ± 3.81</td>
</tr>
<tr>
<td>Ethyl Acetate Extract</td>
<td>82.67 ± 1.80</td>
<td>512.67 ± 1.89</td>
</tr>
</tbody>
</table>

Table 4. Fasting Blood Glucose Level After Ethyl Acetate Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Day-3 (mg/dL)</th>
<th>Day-5 (mg/dL)</th>
<th>Day-7 (mg/dL)</th>
<th>Day-3 (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMC-Sodium</td>
<td>403.00 ± 6.68</td>
<td>403.10 ± 2.75</td>
<td>395.00 ± 3.13</td>
<td>394.10 ± 3.15</td>
</tr>
<tr>
<td>Metformin</td>
<td>447.17 ± 6.92</td>
<td>378.33 ± 7.72</td>
<td>226.83 ± 15.7</td>
<td>135.00 ± 11.4</td>
</tr>
<tr>
<td>Ethyl Acetate Extract</td>
<td>485.67 ± 13.3</td>
<td>427.00 ± 6.45</td>
<td>288.33 ± 14.9</td>
<td>236.00 ± 12.6</td>
</tr>
</tbody>
</table>
Alloxan has been shown to cause direct irreversible damage to β-cells of pancreatic islet of Langerhans, resulting in degranulation and loss of insulin secretion. Clarification of the regenerating potential in experimentally-induced diabetic animals would be of interest as an alternative therapy for diabetes [11]. A preliminary phytochemical analysis of the ethyl acetate were shown flavonoids, tannins, glycosides and saponins. Flavonoids, their glycosides and saponins have been found to be responsible for blood glucose decreasing activity through increased insulin secretion, as evidenced in our experiment by alloxan-induced diabetic mice, which is capable of stimulating pancreatic secretion [12]. Flavonoids was found in EEPH and their role are to decrease sRAGE level. Flavonoids in general were presented anti-glycation properties. Quercetin is a flavonols member which found to be an inhibitor of glycation. Extract of cloves, ground Jamaican allspice and cinnamon amongst to be the most effective inhibitors of glycation. Resveratrol as a natural phytoestrogen found in grapes to be potent inhibitors of AGEs formation.

CONCLUSION
The result of this study showed that ethyl acetate extract posses antidiabetic activity.

DECLARATIONS
Conflict of Interest
No conflict of interest associated in this work

Contribution of Authors
The authors declare that this work was finished by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them

REFERENCES


