Phytochemical Screening And Antidiabetic Test Of Ethanol Extract Of Sapodilla Kecik Leaves (Manilkara Kauki (L.) Dubard) On Decreasing Blood Glucose Of Diabetic Rats

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Abstract.
The development of traditional medicine needs to be carried out so that Indonesia's natural wealth can be utilized as effectively as possible to improve people’s welfare. As with diabetes, some reputable traditional herbal medicines contain antidiabetic properties. Natural antidiabetic drugs derived from compounds isolated from plants can be used as an alternative to traditional medicine for diabetes because they have potential side effects. Diabetes can be treated alternatively by using various substances, especially those containing polyphenols, such as flavonoids. Sapodilla kecik (Manilkara kauki (L.) Dubard) from the Sapotaceae family is a type of plant used in traditional medicine; leaves, roots and bark can be used to treat diarrhea, fever, anthelmintic, and as an antileptaleptic. Secondary metabolites in sapodilla kecik leaves are flavonoids, alkaloids, tannins, triterpenoids and glycosides. This research was carried out by characterizing and screening phytochemicals to determine the content of compounds in Sapodilla kecik (Manilkara kauki (L.) Dubard) and carrying out antidiabetic tests on streptozotocin-nicotinamide-induced diabetic rats. The results showed that sapodilla kecik leaves fulfilled the simplicia characterization requirements. Sapodilla leaves contain secondary metabolites such as alkaloids, flavonoids, tannins, glycosides, saponins and steroids. The ethanol extract of sapodilla kecik leaves at a dose of 500 mg/Kg BW starting from the 4th day of treatment, a dose of 250 mg/Kg BW starting on the 8th day of treatment, and a dose of 125 mg/Kg BW starting on the 12th day was able to reduce the KGD of induced diabetic rats with nicotinamide and streptozotocin.

Keywords: Sapodilla kecik, (Manilkara kauki (L.) Dubard), antidiabetic.

I. INTRODUCTION
The public’s perception of traditional medicine continues to grow and expand. This situation is caused by various factors and current issues that demand a return to nature. According to observations/empirics from subsequent scientific evidence through preclinical trials and clinical trials, traditional medicines in various regions are hereditary. This traditional medicine needs to be developed so that Indonesia’s natural wealth can be utilized as effectively as possible to improve people’s welfare. As with diabetes, some reputable traditional herbal medicines contain anti-diabetic properties. More than 800 species of antidiabetic plants are known to exist, according to available literature [1]. Diabetes mellitus (DM) is a chronic or chronic disease in the form of a metabolic disorder characterized by an increase in blood glucose levels above normal. Diabetes mellitus is a complex chronic disease that requires ongoing medical care with multifactor risk reduction strategies beyond glycemic control [2]. Therefore, it is necessary to administer specific compounds with antidiabetic effects orally. Natural antidiabetic drugs derived from compounds isolated from plants can be used as an alternative to traditional medicine for diabetes because they have potential side effects. Diabetes can be treated alternatively by using various substances, especially those containing polyphenols, such as flavonoids. According to Patel et al. (2012), this substance has strong antioxidant properties and can protect the liver from reactions caused by benzoate oxidation [3].

Besides potent antioxidants, polyphenolic compounds can also break down proteins to inhibit carbohydrate-breaking enzymes such as glucosidase, which contribute to postprandial hyperglycemia. Studies show that the use of chemicals isolated from plants in treating diabetes, high blood pressure, and heart disease has long been known. Flavonoids can increase the amount of glucose produced in adipose tissue and other organs by increasing AMP: ATP levels, inhibiting gluconeogenesis, and activating AMPK [4]. In addition, flavonoids function as antioxidants through the formation of AGEs by trapping reactive dicarbonyl compounds such as methylglyoxal and glyoxal [5, 6]. Sapodilla kecik (Manilkara kauki (L.) Dubard) from the Sapotaceae family is a type of plant used in traditional medicine; leaves, roots and bark can

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be used to treat diarrhoea in children, seeds can be used as a febrifuge, anthelmintic, and as an antileprotic [7]. Previous research by Arsyad and Ayu (2016) stated that sapodilla leaves are used to treat diarrhoea [8], diabetes blood sugar levels can be reduced by drinking sapodilla juice [9]. Secondary metabolites in sapodilla leaves are flavonoids, alkaloids, tannins, triterpenoids and glycosides [10]. The flavonoid group is a compound commonly found in plant tissues [11]. In this study, characterization and phytochemical screening were carried out to determine the compounds contained in sapodilla kecik (*Manilkara kauki* (L.) Dubard) and to perform antidiabetic tests on streptozotocin-nicotinamide-induced diabetic rats.

II. METHODS

**Materials and Tools**

The plant material used in this study was sapodilla kecik leaves (*Manilkara kauki* (L) Dubard) identified at the Medanese Herbarium Laboratory, University of Sumatera Utara. The chemicals used in this study were iron (III) chloride, lead (II) acetate, sodium hydroxide, hydrochloric acid, concentrated sulfuric acid, chloralhydrate, Mollish reagent, Mayer reagent, Liberman-Bouchard reagent, Dragendorf, magnesium powder, concentrated hydrochloric acid, amyl alcohol, 96% ethanol, isopropanol, chloroform, methanol, toluene, n-hexane, nicotinamide (Brataco-Chem), streptozotocin (Nacalai et al., Japan), metformin tablets (Hexpharm Jaya), ketamine-Hamelin (PT. Combiphar), 0.9% NaCl, aquabides, sodium citrate, citric acid, Na-CMC. The tools used in this study were maceration equipment, rotary evaporator, glassware, blender, electric balance, simplicia drying cupboard, porcelain cup, desiccator, a set of water content determination tools, animal scales, syringes, oral sondes, animal restrainers, rat cages, glucometers, glucose test strips, furnaces, cover glasses, and other glassware.

**Making Simplicia**

Fresh sapodilla kecik leaves (*Manilkara kauki* (L.) Dubard) are first sorted and then cleaned of impurities by washing under running water until clean, drained, and weighed as wet weight. Sapodilla kecik leaves that have been washed are dried in a drying cupboard at a temperature of ± 40°C until dry (indicated when they are crushed brittle), then weighed as dry weight. The dried sapodilla kecik leaf simplicia is blended into a powder and then stored in a tightly closed container at room temperature to prevent moisture and other impurities.

**Examination of Simplicia Characteristics**

Examination of simplicia characterization includes macroscopic and microscopic examination, determination of water content, determination of water-soluble extract content, determination of ethanol-soluble extract content, determination of total ash content and determination of acid-insoluble ash content.

**Simplicia Phytochemical Screening and Extracts**

Phytochemical screening carried out includes an examination of Alkaloids, Flavonoids, Saponins, Tannins, and Terpenoids.

**Preparation of Sapodilla Kecik Leaf Ethanol Extract (EEDSK)**

As much as 500 grams of sapodilla kecik leaves were put into a glass vessel, then ethanol solvent was added until the simplicia powder was submerged with ten parts ethanol solvent, covered and soaked for the first 6 hours, and then left to stand for 18 hours. Macerate is separated by filtering using filter paper. Repeat the extraction process three times with half the solvent volume in the first extraction. Then the filtrate obtained was separated by a rotary evaporator to obtain a thick extract. The viscous extract in the rotary evaporator is placed in a glass beaker, covered with aluminium foil, and then stored in the refrigerator to prevent damage to the extract.

**Preparation of Sapodilla Kecik Leaf Ethanol Extract Suspension (EEDSK)**

Weigh each EEDSK dose of 125, 250, 500 mg/kg bw with a watch glass, then put into a mortar and add 0.5% Na-CMC suspension little by little while grinding until homogeneous, then put into a 10 mL volumetric flask. The volume comprised 0.5% Na-CMC suspension up to the marked line.

**Preparation of Experimental Animals**

The experimental animals used were white male rats with a body weight of 180 - 250 grams divided into six groups, each consisting of 4 rats. Before the experiment, the experimental animals were acclimatized...
for two weeks to their environment. Food and drink during maintenance and experiments were given the same ad libitum. Animals are kept in cages with good ventilation, and cleanliness is always maintained; the animal's weight is weighed, and its behaviour is observed. From this study, researchers will use a sample of 24 rats and have received approval from the ethics commission with number 0620/KEPH-FMIPA/2022.

**Measurement of Blood Glucose Levels (KGD)**

The rat's blood was taken from the tip of the tail; the tail was cleaned with 70% alcohol and then cut 1 mm using scissors. The blood that came out was attached to a glucometer paper strip installed on the device then the number printed on the tool's screen was recorded.

**Test Animal Induction**

Twenty-four male rats weighing 180-250 grams fasted for 18 hours (drinking water is still given). Before being induced, the rats' weight and blood glucose levels were measured first to determine their initial body weight and blood glucose levels. Injected with nicotinamide solution of 230 mg/kg BW intraperitoneally, then 15 minutes later injected streptozotocin intraperitoneally at a dose of 65 mg/kg BW. Rats measured their blood glucose levels on day 3. Mice are considered diabetic if fasting blood glucose levels ≥ 200 mg/dL are considered diabetic and can be used for testing.

**Antidiabetic Activity Test of Sapodilla Kecik Leaf Ethanol Extract**

Test the antidiabetic activity using the EEDSK, given orally once daily. The test animals used in this experiment were white Wistar rats induced by nicotinamide and streptozotocin and divided into six groups. Each group consisted of 4 rats, namely:

- a. Group I is the negative control group
- b. Group II is the EEDSK 125 mg/Kg BW test group
- c. Group III is the EEDSK 250 mg/Kg BW test group
- d. Group IV is the EEDSK 500 mg/Kg BW test group
- e. Group V is the positive control group (Glibenclamide 45 mg/kg BW)
- f. Group VI is the normal group

Each group was given the test preparation orally, and then blood glucose levels were measured on days 4, 8, 12, 16, 20, 24, and 28 [12].

### III. RESULT AND DISCUSSION

**Macroscopic and Microscopic Examination**

The results of a macroscopic examination of fresh sapodilla kecik leaves showed that leaves have single leaves clustered at the ends of the twigs, have an inverted oval shape, and are widened to indented width, measuring 5-15 cm x 3-8 cm. The upper surface of the leaf is smooth, shiny dark green; the lower surface is finely hairy, resembling brownish-grey velvet; the base is sharp, and the tip is rounded to slightly notched. The central leaf veins protrude downwards, and the secondary leaf veins are 9-30 pairs, with a petiole length of 1.3-3.7 cm. Examination of the characteristics of the simplicia powder microscopically showed that there were bundles of spiral xylem vessels and calcium oxalate crystals in the form of rapid and stomata with a parasitic type. The results of the inspection can be seen in Figure 1.

**Fig 1.** Macroscopic and microscopic examination of sapodilla kecik leaves. a) macroscopic, (b) microscopic,

1. Parenchyma cells contain essential oil, 2.Cover hairs (Trichoma), 3.Spiral-shaped woody vessels, 4.Stomata are parasitic, and cells contain oil droplets

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Examination of Characterization of Sapodilla Kecik Leaf Simplicia

The results of determining the water content of sapodilla kecik leaf simplicia are 6.60%; this follows the general requirements for simplicia, namely, at most, 10% [13]. Determination of the simplicity's water content is essential to provide a maximum limit for the water content in the simplicia because a high amount of water can become a medium for the growth of bacteria and fungi, which can damage the compounds contained in the simplicia [13]. The water-soluble essence of the simplicia of sapodilla kecik leaves reaches 16.55%. Determination of water-soluble essence content to determine the levels of polar chemical compounds [13]. This study's ethanol content of the soluble sapodilla kecik leaf simplicia was 59.27%. Determination of ethanol soluble essence content to determine the concentration of soluble extracts in polar solvents, both polar and non-polar compounds. Extract extraction with water or ethanol solvents is used to determine the percentage of extractability with these solvents.

Determination of water-soluble extracts is used to determine the ability of extracts to be extracted in water solvents while determining ethanol-soluble extracts is more often used to determine whether extracts can dissolve in organic solvents. Water is intended to dissolve polar compounds, while ethanol dissolves less polar compounds in the extract. Both solvents are used because they are solvents that meet pharmaceutical requirements [14]. Examining the ash content uses the principle of heating the material to a temperature where organic compounds and their derivatives are destructed and evaporated, leaving only mineral and inorganic elements [15]. The total ash content of sapodilla kecik leaf simplicia is 5.24%. The total ash content is related to organic and inorganic minerals obtained internally and externally. The insoluble ash content of the simplicia of sapodilla kecik leaves is 0.48%. The acid-insoluble ash content aims to determine the amount of ash obtained from external factors sourced from impurities originating from sand or sick soil [13]. The standardization of a simplicia is fulfilling the requirements as a medicinal ingredient and determining the value of various product parameters so that quality can be guaranteed in storage [13]. The results of simplicia characteristics can be seen in Table 1.

Table 1. Results of examination sapodilla kecik leaves.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
<th>Requirements [16]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water content</td>
<td>6.60%</td>
<td>10%</td>
</tr>
<tr>
<td>Water soluble essence content</td>
<td>16.55%</td>
<td>&lt;30%</td>
</tr>
<tr>
<td>Ethanol soluble essence content</td>
<td>59.27%</td>
<td>&gt;28%</td>
</tr>
<tr>
<td>Total ash content</td>
<td>5.24%</td>
<td>&lt;18%</td>
</tr>
<tr>
<td>Acid insoluble ash content</td>
<td>0.84%</td>
<td>&lt;4.2%</td>
</tr>
</tbody>
</table>

Results of Phytochemical Screening of Sapodilla Kecik Leaves

The results of the phytochemical screening of sapodilla kecik leaf simplicia powder were obtained to obtain information on the class of secondary metabolites contained therein. Phytochemical screening was done on alkaloids, flavonoids, tannins, glycosides, saponins and steroids. The results of the phytochemical screening of sapodilla kecik leaf simplicia powder can be seen in Table 2.

Table 2. Results of Phytochemical Screening of sapodilla kecik leaf simplicia

(Manilkara kauki L.(Dubard))

<table>
<thead>
<tr>
<th>Secondary Metabolites</th>
<th>Reactants</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorff, Bouchardat, Meyer</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Mg Powder + Amyl Alcohol + HCl</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Molish+H$_2$SO$_4$</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Hot/shaken water</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>FeCl$_3$</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenes/Steroids</td>
<td>Lieberman-Bourchat</td>
<td>+</td>
</tr>
</tbody>
</table>

Examining the flavonoid compounds with the addition of magnesium powder and concentrated hydrochloric acid produces a red solution [16]. The addition of FeCl$_3$ gives a blackish-green colour, indicating a group of tannin compounds [17]. The sample, with hot distilled water and shaken vigorously, produced a stable foam; then, HCl 2 N was added, indicating the presence of a group of saponin compounds [16]. Examine the glycoside compound group by adding Molisch reagent and concentrated sulfuric acid to form a purple ring [16]. Examination of the triterpenoid/steroid compound groups by adding a few drops of

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Liebermann-Burchard reagent produces a pink or purple colour which indicates the triterpenoid compound group [18].

**Evaluation of Blood Glucose Levels of STZ and NA-Induced Diabetic Rats**

Before treatment, the test animals fasted for 18 hours so that the rats were in the same condition, which reduced the effect of the food consumed on the KGD measurement. Moreover, an STZ dose of 65 mg/kg BW intraperitoneally increased glucose levels (hyperglycemic) ≥ 200 mg/dl. The results of KGD measurements before and after induction can be seen in Table 3.

**Table 3. Results of KGD measurements before and KGD after NA - STZ induction**

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood Glucose Levels (mg/dL); Average (Mean ± SD, n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Induction</td>
</tr>
<tr>
<td>Normal Control</td>
<td>87 ± 9.6</td>
</tr>
<tr>
<td>CMC Na 0.5%</td>
<td>93.5 ± 13.2</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>78.5 ± 5</td>
</tr>
<tr>
<td>EEDSK 125 mg/Kg BW</td>
<td>92 ± 10</td>
</tr>
<tr>
<td>EEDSK 250 mg/Kg BW</td>
<td>94.7 ± 9</td>
</tr>
<tr>
<td>EEDSK 500 mg/Kg B2</td>
<td>98.5 ± 12.8</td>
</tr>
</tbody>
</table>

Information:

* significantly different from the CMC Na 0.5% control group

b significantly different from the glibenclamide comparison group
c significantly different from the normal group

Mice were induced intraperitoneally with NA 230 mg/kg and STZ 65 mg/kg BW; the KGD was measured from day 4 to day 28. The treatment was started after the rats tested positive for diabetes, namely on day 3, the rats were given the test preparation every day for four weeks, and the KGD was measured on the 4th, 8th, 12th, 16th, 20th, 24th, and 28th day. The average of the KGD between groups was calculated for each group of rats, then statistically analyzed using ANOVA and continued with the Post Hoc Tukey HSD test to see significant differences between treatments. The results of KGD measurements can be seen in Figure 2. Based on Figure 2, it can be seen that the EEDSK dose of 125 mg/kg BW, 250 mg/kg BW, and 500 mg/kg BW can lower blood glucose levels seen a decrease on day 8, day 12, day 16, day 20, day 24 and day 28. Research by Assagaf et al. (2020) stated that the total level of flavonoids in sapodilla leaves (M. zapota L.) was 14.52 per cent [19]. The mechanism of flavonoids related to the healing of diabetes is as an antioxidant through the binding of ROS. Flavonoids will release their hydrogen atoms and electrons to give to hydroxyl, peroxyl, and peroxynitrite radicals [20]. One of the flavonoid compounds is myricetin. Myricetin is a compound from the flavonol group that has the potential as an antioxidant [21]. Flavonoids work to repair beta cells in the pancreas through excessive free radical scavenging [22].

![Fig. 2. Graph of average blood glucose levels after treatment](http://ijstm.inarah.co.id)
Flavonoids can reduce blood glucose levels by inhibiting gluconeogenesis by increasing the ratio of AMP, thereby activating AMPK, increasing glucose uptake in skeletal muscle and adipose tissue, increasing insulin sensitivity, increasing insulin secretion, reducing apoptosis, increasing proliferation pancreatic β cells, inhibit the α-glucosidase enzyme, inhibit glucose absorption and increase glucose tolerance [4, 23, 24]. The mechanism of tannins lowers blood glucose levels, namely increasing glucose uptake through activation of insulin signalling pathway mediators, such as PI3K and p38 MAPK, GLUT-4 translocation, regenerating pancreatic β cells, increasing insulin activity, increasing glycogenesis, as an astringent substance which can cause wrinkling of the intestinal epithelial membrane thereby reducing food absorption [25, 26]. Saponins have a hypoglycemic effect by inhibiting gluconeogenesis by activating AMPK, restoring insulin response, increasing insulin signalling, increasing insulin secretion, activating glycosyn synthesis, inhibiting α-glucosidase activity, inhibiting expression of mRNA glycogen phosphorylase and glucose six phosphatases, and increasing GLUT-4 expression [27].

IV. CONCLUSION

Based on the discussion and results of the study, it was concluded that sapodilla kecik leaves meet the simplicia characterization requirements according to WHO and the Indonesian Pharmacopoeia requirements. Sapodilla kecik leaves also contain secondary metabolites such as alkaloids, flavonoids, tannins, glycosides, saponins and steroids. The ethanol extract of sapodilla kecik leaves at a dose of 500 mg/Kg BW starting from the 4th day of treatment, a dose of 250 mg/Kg BW starting on the 8th day of treatment, and a dose of 125 mg/Kg BW starting on the 12th day was able to reduce the KGD of induced diabetic rats with nicotinamide and streptozotocin.

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