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Research Article

Antidiabetic Potential of Keji Beling (Strobilanthes crispus (L.) Bl.)

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Abstract: Background: Diabetes Mellitus (DM) is a disease that cannot be completely cured or cannot even be completely cured. The vile shard plant is empirically used by the community to treat diabetes (DM). This study aims to conduct phytochemical screening and test the activity of 96% ethanol extract of kejibeling leaves (Strobilanthes crispus (L.) Bl.) as a herbal antidiabetic in male white mice (Mus musculus) with alloxan induction. Method: This research is an experimental laboratory research with a true experimental posttest control design using a completely randomized design (CRD) with 5 treatments and 5 replications. Treatment P1 (without treatment) as normal control (N), P2 as positive control (+), P3 as negative control (-), P4 kejibeling leaf extract 250 mg/kg BW, P5 kejibeling leaf extract 500 mg/kg BW. Result:The results of phytochemical screening showed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids and steroids. SPSS results show that the data is normally distributed ($p>0.05$) and homogeneous ($p>0.05$). The results of the ANOVA on the treatment of giving keji beling leaf extract 250 mg/Kg BW showed a sig. 0.393 ($p>0.05$) and treatment of 500 mg/Kg BW obtained a sig value. 0.517 ($p>0.05$). Conclusion:The conclusion from the research results shows that administering doses of 250 mg/kg BW and 500 mg/kg BW of keji beling leaf extract can reduce blood sugar levels in mice. It is hoped that the results of this research will be useful for the community as an antidiabetic therapy using kejibeling leaves (Strobilanthes crispus (L.) Bl.).

Keywords: Antidiabetic; Diabetes Mellitus; Herbal Antidiabetic; Keji Beling Leaves; Phytochemical Screening.

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1. Introduction

Treatment for diabetes mellitus includes insulin and oral antidiabetic medications. Long-term use of these medications can cause side effects such as diarrhea, dizziness, headaches, nausea, and vomiting (Theresia et al., 2017).

Diabetes mellitus treatment is a chronic, lifelong treatment using external insulin and oral antidiabetic medications. Oral antidiabetic medications may be useful for people who are allergic to insulin, who do not use insulin injections, or in combination with insulin. Long-term use of insulin and oral antidiabetic medications can cause unwanted side effects. Therefore, it is necessary to find effective and safe medications with relatively few side effects (Guidelines et al., 2010), and to develop equally effective treatment methods with fewer side effects (Irawati et al., 2020). One alternative for diabetes treatment is traditional medicine utilizing natural ingredients such as plants (Diki-Dongga et al., 2016). Indonesia has a vast potential for plants to be used as traditional medicines. Plants can be used as medicines because they contain natural chemicals in the form of primary and secondary metabolites (Anastasia et al., 2016).

The keji beling plant (Strobilanthes crispus) has long been used by the public as a traditional herbal medicine due to its content of various secondary metabolites. Keji beling

leaves (Strobilanthes crispus) are a type of medicinal plant known to have many benefits, including treating kidney stones, gallstones, diabetes mellitus, hemorrhoids, constipation, and urinary incontinence (Roring et al., 2017).

The morphology of the keji beling plant (Strobilanthes crispus (L.) Bl.) at the Batu Materia Medica Herbal Laboratory UPT, Jalan Lahor 87, Batu City, Malang, on May 4, 2023, showed a shrubby habit, 1-2 m tall. Stem: Jointed, round, coarsely hairy, monopodial branching, green. Leaves: Single, opposite, lanceolate or oval, serrated edge, pointed tip, pointed base, 9-18 cm long, 3-8 cm wide, short-stemmed, pinnate veins, green. Flowers: Compound, spike-shaped; funnel-shaped crown, hairy, purple; short-haired petals, purple; four stamens, white; yellow. Fruit: Round, brown. Seeds: Round, small, flat, brown. Roots: Taproot, light brown. According to (Fahlevi & Dewi, 2019)); Tatt Lim et al, (2012) Kejibeling (Strobilanthes crispus) contains several chemical compounds such as saponins, flavonoids, glycosides, sterols, terpenes, fats and minerals (high levels of potassium, silicic acid, sodium, calcium). Keji beling also contains vitamins (ascorbic acid, riboflavin, and thiamine), phenolic acids (p-hydroxybenzoic acid, p-coumaric acid, caffeic acid, vanillic acid, gentinic acid and ferulic acid), caffeine, tannins, alkaloids, and catechins.

2. Materials and Method

Laboratory research in the field of pharmacy requires a systematic experimental design and standardized procedures to ensure the validity and reproducibility of the results. Setiawan and Nurhayati (2022) emphasize the importance of thorough planning in laboratory research, including determining an adequate research duration, controlling relevant variables, and using standardized tools and materials. In addition, Prasetyorini et al. (2023) state that the consistent application of standard operating procedures (SOPs) in every stage of the research is crucial for maintaining data quality and ensuring that experimental results can be accurately reproduced in the future. Moreover, Kusuma and Hartono (2024) highlight that good laboratory management and strict quality control are essential to the smooth operation of research and the accountability of its results. Proper procedures and effective laboratory management play a key role in the success of experimental research, particularly in the fields of biomedical sciences and pharmacy.

This was an experimental laboratory study with a true experimental post-test control design. The research design used was a completely randomized design (CRD) with five treatments and five replications.

Procedure

Phytochemical Screening

a. Alkaloid Identification
   To 0.5 g of the extract in a test tube, 2 mL of 70% ethanol was added, stirred, and 5 mL of 2 N HCl was added, and heated in a water bath. After cooling, the mixture was filtered, and a few drops of Mayer's reagent were added to the filtrate. The sample was then observed until it became cloudy or sediment appeared (Mojab et al., 2003).
b. Flavonoid Identification
   2 mL of 70% ethanol was added to a 0.5 g extract in a cup, then stirred. 0.5 g of magnesium powder and 3 drops of concentrated HCl were added. The formation of an orange to red color indicates the presence of flavones, red to deep red indicates the presence of flavanols, and deep red to purplish red indicates the presence of flavanones (Mojab et al., 2003).
c. Saponin Identification
   2 mL of 70% ethanol was added to a 0.5 g extract in a test tube, then stirred. 20 mL of distilled water was added and shaken, then allowed to stand for 15-20 minutes. No foam indicates a negative result for saponins; foam greater than 1 cm indicates a weak positive result; foam greater than 1.2 cm indicates a positive result for saponins; and foam greater than 2 cm indicates a strong positive result (Mojab et al., 2003).
d. Identification Triterpenoids
   To 0.5 g of extract in a test tube, add 2 mL of 70% ethanol, stir, then add 1 mL of chloroform and 1 mL of acetic anhydride, and cool. After cooling, add H2SO4. A reddish color indicates the presence of triterpenoids (Ghosal et al., 2012).
e. Steroid Identification
   To 0.5 g of extract in a test tube, add 2 mL of 70% ethanol, stir, and add 2 mL of concentrated H2SO4 by slowly dripping from the side of the test tube. The formation of a red ring indicates the presence of steroids (Ghosal et al., 2012).

f. Tannin Identification
To 0.5 g of extract in a cup, add 2 mL of 70% ethanol, stir, and add 3 drops of FeCl3. If a characteristic blue, blue-black, green, or blue-green precipitate is obtained, the test results will be analyzed (Mojab et al., 2003).

Antidiabetic Test

a. Acclimatization
Male Wistar mice (Musmusculus) were exposed to their environment for 14 days in animal cages located in the Pharmaceutical Biology Laboratory of the Integrated Laboratory of PGRI Madiun University. This study used 30 mice divided into 5 groups, each consisting of 5 test mice and 1 reserve. Group 1 served as the normal control group (N). Group 2 served as the positive control (+) and was administered glibenclamide at 5 mg/kg body weight. Group 3 served as the negative control (-) and was induced by 0.9% NaCl at 10 mg/kg body weight. Group 4 was test group 1, administered 250 mg/kg body weight of kejibeling leaf extract (Strobilanthes cripus (L.) Bl.). Group 5 was test group 2, administered 500 mg/kg body weight of the extract. Treatment was administered orally. The dose was administered once, and blood sugar levels were checked every 15 minutes for 2 hours. The test was administered orally using an oral tube.

b. Alloxan Induction
According to the data, after the mice had acclimatized for 7-14 days, they were fasted for 8 hours and then injected with 200 mg/kg of alloxan intraperitoneally. Mice induced with alloxan were fed adequately and 24 hours after alloxan administration to prevent hypoglycemia (Agnia, 2015). Blood samples were taken 24 hours after induction to determine whether the mice had diabetes mellitus (DM), with blood sugar levels exceeding 200 mg/dl, taken through the tip of the mice's tail (Lal, 2016).

c. Administration of Keji beling Extract
Blood samples from diabetic mice were collected and measured using an Easy Touch glucometer and a sugar stick. The insulin leaf extract doses administered were 250 mg/kg body weight and 500 mg/kg body weight orally. The extract was administered once and monitored every 2 hours.

d. Blood Sugar Measurement
The mice's blood sugar levels were measured using an Easy Touch glucometer by taking blood samples from the tip of the mice's tail. Checks were performed at 15-minute intervals for 2 hours. The results were recorded for normality testing, homogeneity testing, and one-way ANOVA.

Data Analysis

The data analysis technique for phytochemical screening was qualitatively analyzed using several tests (flavonoids, alkaloids, tannins, saponins, steroids, and triterpenoids). The activity of the ethanol extract of kejibeling leaves as an herbal antidiabetic agent tested on white mice was descriptive and quantitative, based on the reduction in blood sugar levels. The data that has been obtained and collected is then subjected to a computerized data analysis process using SPSS version 25. The method used is One Way Anova (Analysis of Variance) with a confidence level of 95% ($p<0.05$).

3. Results and Discussion

Organoleptic Test Results

Organoleptic testing and phytochemical screening are specific parameters for extract standardization. The results of the organoleptic test on the 96% concentrated ethanol extract of keji beling leaves are shown in Table 1.

Table 1. Organoleptic test results of the 96% ethanol extract of kejibeling leaves (Strobilanthes crispus (L.) Bl.).

Organoleptic Test	
Color	Deep Green
Distinctive	Odor of Kejibeling
Taste	Astringent
Form	Thick Extract

Phytochemical Screening

The results of the phytochemical screening of the 96% ethanol extract of Keji Beling leaves (Strobilanthes crispus (L.) Bl.) are shown in Table 2.

Table 2. Results of the phytochemical screening of the 96% ethanol extract of Keji Beling leaves (Strobilanthes crispus (L.) Bl.).

Compound Class	Results
Alkaloids	+
Flavonoids	+
Saponins	+
Triterpenoids	+
Steroids	+
Tannins	+

Antidiabetic Test

The results of the antidiabetic activity test for reducing blood glucose levels in mice are shown in Figure 1.

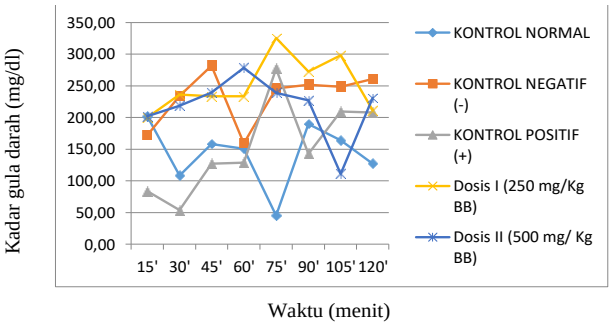

Figure 1. Decreased glucose levels.

Discussion

Organoleptic Testing

Organoleptic testing is a test using the five senses, including color, odor/aroma, taste, and shape. Organoleptic testing is a simple, objective initial assessment. Organoleptic testing is conducted by observing shape, color, odor, and taste (BPOM RI, 2000).

Based on the organoleptic test results in Table 1, the color of the extract is dark green. This is because the extracted material comes from simple folium/leaf extracts. The leaves of the keji beling plant are dark green, resulting in a dark green extract. The odor/aroma of the keji beling leaf extract is characteristic of keji beling leaves. The taste of the extract is astringent and the extract is thick.

Phytochemical Screening

According to Table 2, the active compounds/secondary metabolites of the keji beling leaf extract are alkaloids, flavonoids, saponins, triterpenoids, steroids, and tannins. This is in accordance with research (Roring et al., 2017) the results of phytochemical tests of keji beling leaf extracts were positive for containing active compounds flavonoids, alkaloids, saponins, triterpenoids, steroids and tannins. Alkaloids are able to stimulate insulin secretion because alkaloids are able to stimulate sympathetic nerves (sympathomimetic) which has the effect of increasing insulin secretion. Meanwhile, the work of secondary metabolite compounds in lowering blood glucose levels uses extra-pancreatic mechanisms, namely by increasing glucose transport in the blood, inhibiting glucose absorption that occurs in the intestine, as well as inhibiting glucose synthesis and increasing glucose oxidation. Flavonoids are phenolic compounds that can protect pancreatic β cells from free radicals which act as antioxidants ((Lukačínová et al., 2008)). In addition, flavonoids function to inhibit the alpha glycosidase enzyme which functions to break down carbohydrates. Inhibition of the alpha glycosidase enzyme causes a delay in glucose absorption which ultimately will also lower blood glucose levels ((Hasim et al., 2019)).

Saponins function to inhibit the activity of the alpha glucosidase enzyme in the small intestine, reducing glucose absorption in the intestine, resulting in decreased blood glucose levels (Hasim et al., 2019). Similarly, flavonoid saponins work to inhibit the function of the alpha glycosidase enzyme, where this enzyme is useful for breaking down carbohydrates. Thus inhibiting glucose absorption and reducing blood glucose levels (Hasim et al., 2019). Saponins have an inhibitory effect on the α-glucosidase enzyme. The α-glucosidase enzyme is an enzyme found in the small intestine that functions to convert disaccharides into glucose, with the α-glucosidase inhibitor effect of saponins, glucose absorption that occurs in the small

intestine can be inhibited (Rahma et al., 2017)). The inhibitory effect of the α-glucosidase enzyme is also possessed by flavonoids (Iryani et al., 2017).

Other compounds such as tannins, alkaloids, and saponins can also induce the growth of new pancreatic β cells (Sarel & Simanjuntak, 2020). Saponins can also increase the effectiveness of insulin in the small intestine, thereby promoting normal intestinal function (Sandi A et al., 2022). Steroids and terpenoids play a role in lowering blood glucose levels by inducing insulin release from the pancreas, thereby lowering blood glucose levels (Guidelines et al., 2010). The synergistic presence of active compounds such as alkaloids, flavonoids, terpenoids, saponins, tannins, and steroids can lower blood sugar levels. According to (Astiti et al., 2018), diabetes mellitus can be treated using foods or plants containing antioxidants and flavonoids.

Antidiabetic Activity Test

Based on the results of the reduction in blood glucose levels shown in Figure 1, it can be seen that the two test doses of keji beling leaf extract can lower blood glucose levels in mice. At each test dose, the decrease in blood glucose levels was shown in different times. Based on Figure 1, it can be seen that at a dose of 250 mg/kgBW, it can reduce blood glucose levels in mice at the 120th minute. Meanwhile, at a dose of 500 mg/kgBW, it can reduce blood glucose levels at the 75th minute, but at the 120th minute it increased again. This is likely because during the test period the mice were still given food and drink to prevent hypoglycemia. According to David (2016), actually, normal blood glucose levels in the blood can change over time, such as before eating and after eating. The normal value of blood sugar levels at any time when not eating for 8 hours (fasting) is <100 mg/dl, before eating 70-130 mg/dl, after eating (1-2 hours) <180 mg/dl and before bed 100-140 mg/dl. The decrease in blood glucose levels at a dose of 500 mg/kgBW is faster than a dose of 250 mg/KgBW. This is likely due to the fact that at a dose of 500 mg/Kg BB the secondary metabolite content is greater.

The test results were analyzed using statistical methods using SPSS version 25. The SPSS test involved three comparisons: the normal control group versus the 5 mg glibenclamide-positive group, the normal control group versus the 5 mg glibenclamide-negative group, and the 5 mg glibenclamide-positive group versus the 250 mg and 500 mg kejibeling extract test groups.

The data from the normality, homogeneity, and ANOVA tests using three comparisons are presented as follows:

a. The results of the normality test for the comparison between the normal control and the positive control using the Shapiro-Wilk test yielded a significance value of 0.088 (>0.05) for the normal control, indicating that the data for the untreated group were normally distributed. The results of the normality test for the positive control showed a value of 0.300 (>0.05), indicating that the data were normally distributed. The homogeneity test for the comparison group between the normal control and the positive control yielded a value of 0.763 (>0.05), indicating that the data were significant between the two groups and derived from the same or homogeneous variation. The ANOVA test for the comparison group between the normal control and the positive control yielded a value of 0.926 (>0.05), indicating that the value was insignificant. The conclusion from the three comparison group tests between the normal control and the positive control was that the normal control did not affect blood sugar levels, but the positive control did affect blood sugar levels, meaning H1 was accepted and H0 was rejected.
b. Further testing was conducted on the comparison group between the normal control and the negative control. The results of the normal control and negative control normal control normal control comparison group are presented in Table 4.6, with a Shapiro-Wilk value of 0.88 (>0.05) in the normal group and 0.987 (>0.05) in the negative group, indicating that the data were normally distributed. The homogeneity test for the normal control and the negative group yielded a significance value of 0.797 (>0.05), indicating that the data derived from the same or homogeneous variation. The ANOVA test results showed a significance value of 0.465 (>0.05), indicating insignificant results. Therefore, it was concluded that the normal and negative groups had no effect on reducing blood sugar levels.
c. The results of the normality test for the comparison groups between the positive control and the test controls at doses of 250 mg/kg BW and 500 mg/kg BW using the Shapiro-Wilk test yielded a significance value of 0.300 (>0.05) for the positive control, a sign value of 0.079 (>0.05) for the test control at a dose of 250 mg/kg BW,

and a sign value of 0.385 (>0.05) for the test control at a dose of 500 mg/kg BW. These results indicate that the data were normally distributed among the three groups tested. The homogeneity test results showed a significance value of 0.649 (>0.05), indicating that the data were derived from the same or homogeneous data set. The results of the ANOVA test showed a value of 0.649 (>0.05) which is defined as an insignificant value, so it was concluded that the extract of keji beling leaves can replace / be an alternative to the positive control, namely glibenclamide.

4. Conclusion

Keji beling leaf extract contains active compounds such as alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids. Keji beling can be used as an antidiabetic therapy as a substitute for glibenclamide.

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