

The Effect Hypoglycemic of Ethanol Extract Combination Red Betel Leaf (*Piper crocatum*) and Dayak Onion (*Eleutherine palmifolia Merr*) in Streptozotocin-Induced

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ABSTRACT

Background: Red betel leaf and Dayak onion is used traditionally to treat diabetes mellitus, The use of this combination of extracts to assess the effectiveness of the combination therapy is getting better by working synergistically. **Objective:** This study aimed to determine the hypoglycemic effect and the effective dose of ethanol extract combination of red betel leaf and Dayak onion of male rats (*Rattus norvegicus*). **Methods:** The number of 35 male rats were divided into 7 groups and each group consisted of five rats. The first group (normal control) was given Na CMC; the second group (negative control) streptozotocin-induced ; the third group (positive control) glibenclamide , while the 4th, 5th,6th, and 7th were successively given a single dose of ethanol extract of red betel leaf 25 mg/KgBB, a single dose of Dayak onion 50 mg/KgBB, combination red betel leaf and forest onion bulbs, 25+50 and 50+100 mg/kg bb. Each rat group was intraperitoneally (IP) induced with STZ 40 mg/KgBB and then treated for 21 days. The hypoglycemic effect was tested by using the God- Pap method. **Result:** Phytochemical analysis revealed that flavonoids, tannins, saponins, phytosterols and phenols were present in ethanol extract of red betel leaf and forest onion bulbs. The study concluded that the combination of red betel leaf and Dayak onion at doses 25 mg/KgBB and 50 mg/KgBB were synergistic in reducing glucose. **Conclusion:** In conclusion, the ethanol extract combination of red betel leaf and dayak onion have hypoglycemic effects.

Key words: Hypoglycemic, Streptozotocin, Red betel leaf, Dayak onion.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorders characterized by deregulation in carbohydrate metabolism and high blood sugar levels over a prolonged period resulting from either destruction or impairment of insulin-secreting pancreatic β cells and target tissue.^{1,2} DM is a group of a metabolic disease of all age groups especially the middle age people.³ The long term diabetes leads to complications in various body systems such as diabetic kidney. The World Health Organization (WHO) published estimates for the years 2000 and 2030, using data from 40 countries but extrapolated to the 191 WHO member states. Other estimates have been produced by the International Diabetes Federation (IDF). The estimates produced here to update the previous IDF estimates, and include all 216 countries of the United Nations and in the year 2025 estimated that, there will be 300 million patients suffered from diabetes in world.^{4,5}

Indonesia is on 7th position among the world top ten countries with the highest diabetes mellitus incident after China, India, USA, Brazil, Rusia and Mexico with a diabetic population at age 20-76 years reached 7.6 million in 2012. Total Indonesian population affected by DM was 8.4 million in 2000 and is predicted to be 21.3 million in 2030.⁶

Indonesia is a country enriched with medicinal plants (herbal medicine). The slogan is back to

nature to minimize the side effects of chemical drugs and increase the use of medicinal plants. Regarding this aspect, one of the potential medicinal plants is red betel (*Piper crocatum*). Red betel is considered to have efficacy as a drug based on Evidence-Based Medicine. The widely reported medicinal properties of red betel the anti-hyperglycemic, anti-proliferative on cancer cells, antibacterial activity gram-positive and gram-negative against, antioxidant activity and others.⁷ The chemical contents of red betel leave 96 % ethanol extract are tannins, saponins, triterpenoids, and flavonoids. Flavonoids in red betel leave form anthocyanidins flavonon, Auron, isoflavones, catechins, and chalcones.

Bawang Dayak (local name) or onion dayak or *Eleutherine palmifolia* (L.) is a well-known plant among Dayak tribe living in south Kalimantan Island, Indonesia. The origin of *Eleutherine* plant is from South America. Others species from this genus for examples are *E. latifolia*, *E. Americana*, *E. bulbosa* and *E. plicata*. They are cultivated and naturalized in Indonesia, Malaysia, Philippines and Africa., Dayak tribe uses the plant to cure the various type of illness such as diabetes mellitus, , cholesterol ,cancer and ulcers.⁶ The onion dayak contain active chemical compounds are flavonoids, alkaloids, steroids, phenolics, and glycozide. Extracts dayak onion Act as α -Glucosidase or α -Amylase Inhibitor. These types of extracts can reduce the blood glucose level by inhibiting the gastric enzymes which are

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obligatory for the break the polysaccharides into the simple sugar.¹ The bioactive compounds contained in red betel leaves and Dayak onions have the potential to be used as antidiabetic drugs. The red betel leaf extract at a dose of 50 mg/kg body weight and a dose of 100 mg/kg body weight could reduce blood glucose levels in alloxan-induced rats and administered onion extract of water and ethanol with a dose of 100 mg/kg for 28 days. blood glucose levels of alloxan-induced diabetic rats.^{8,9}

Based on the description above, this research will use a combination of red betel leaf extract (*Piper crocatum*) and dayak onion extract (*Eleutherine palmifolia* Merr). The use of this combination of extracts to assess the effectiveness of the combination therapy is getting better by working synergistically where both extracts strengthen each other's properties or the effect decreases due to interactions between the two plant extracts. The purpose of this study was to determine the hypoglycemic effect of a combination of ethanol extract of red betel leaves and dayak onion on reducing glucose levels in streptozotocin-induced male rats and knowing effective dose combinations.

MATERIAL AND METHODS

Plant material

The plants used were red betel leaves and onion dayak cultivated in central Sulawesi Province. The material was determined by UPT of Biology Resources Tadulako University, Central Sulawesi. The plants were dried at 50 °C for 48 hours in an oven and then powdered.

Preparation of plant extract

Red betel leave extract (RBLE) and onion dayak extract (ODE) prepared by macerating in a 96 % ethanol for 3 x 24 hours. Maserat powder was excluded by using filter papers then the filter evaporated using rotary evaporator at 60°C, then concentrated on a water bath at 60°C until thick extract was obtained. The extract 96% ethanol RBLE and ODE were kept 4°C until use.

Phytochemical screening

Alkaloids

A total of 0.5 g of extract add 5 ml of hydrochloric acid 2 N and heat it over a water bath for 2 minutes. After that 2 drops, dragendorff LP was added. If the results give yellow, orange to red brick precipitate, the sample positive alkaloids.

Flavonoid

A total of 0.5 g of extract and then 100 ml of hot water were added, boiled for 5 minutes and filtered in hot conditions. The filtrate obtained is then taken 5 ml then added 0.1 g of magnesium powder and 1 ml of concentrated hydrochloric acid and 2 ml of amyl alcohol, shaken and allowed to separate. Flavonoids are positive if there is red, yellow, orange in the amyl alcohol layer.

Saponin

A total of 0.5 g of extract was added to the test tube with 10 ml of hot water added, cooled and then shaken for 10 seconds, if it was in the form of a solid foam as high as 1-10 cm which was stable not less than 10 minutes and not lost in the addition of 1 drop of 2 N hydrochloric acid reagent solution showed the presence of saponins.

Tannin

A total of 0.5 g of extract was extracted with 10 ml of distilled water and then filtered, the filtrate was diluted with distilled water until it was colourless. Taken 2 ml of the solution and then add 1-2 drops of 1% iron (III) chloride reagent. If the blue or blackish green colour shows the presence of tannins.

Steroids

A total of 0.5 g extract added 25 ml ethanol 96% and then heated and filtered. The filtrate is evaporated then ether is added. The ether layer was piped and tested on the spot plate by adding Lieberman Buchard reagent (3 drops of anhydride acetic acid and 1 drop of concentrated H₂SO₄). Red or purple indicates a triterpenoid and green indicates steroids.

Animal

The using animal in this study were male albino Wistar rats weighing 200- 250 g purchasing from the laboratory city of Bantul, Yogyakarta. They were acclimatized in room temperature 25 ° C, 12 hours light/12 hours dark cycle. All rats were kept in separate cages. Standard pellet and water ad libitum were given daily till 42 days. The experimental protocol was approved by Tadulako University ethics committee, Central Sulawesi, Indonesia.

Induction of diabetes in rats

A total of 35 rats were divided into 7 groups. The rats have fasted for 16 hours, The initial blood glucose level from the tail vein was then measured using GOD-PAP method then all groups were induced with streptozotocin at a dose of 40 mg/kg bb intraperitoneally. Streptozotocin 240 mg (Biorworld) dissolved using citrate-buffered saline (Merck) pH 4. One week later blood taken from the tail vein to determine blood glucose as a level of glucose increases. Furthermore, given treatment group I as a normal control, treatment II as a negative control using 0.5% Na CMC, III as a positive control was given glibenclamide, IV and V suspensions as a treatment with a single extract of red betel leaf dose of 50mg / kg BW and a forest onion dose of 100mg / kg BB, treatment to VI and VII as a treatment with a combination of extracts dose of 25mg / kg BB + 50mg / kgBB; 50mg / kg BB + 100mg / kgBB and 7, 14, 21 and 28 days later the glucose levels were measured again by using GOD- PAP method.

Analysis Statical

Statistical analysis using one-way Anova followed by Least Singing Definition (LSD). Data were analyzed using the SPSS 23.

RESULT

Plant extraction

The maceration results RBLE and ODE green and dark extract with a percent yield of 5.09%.

Phytochemical screening

Phytochemical screening tests on an ethanol extract of red betel leaves and onion dayak were the presence of bioactive compounds contained in the extract. The results of the phytochemical screening are seen in Table 1:

Hypoglycemic activity

Blood glucose levels were measured before and after treatment for all groups of test animals, so that diabetic rats were given 40 mg / KgBB of streptozotocin induction intraperitoneally. The results of the measurement of blood glucose levels of extracts from the 1st, 7th, 14th and 21st days can be seen in Table 1 and Figure 1.

DISCUSSION

The samples used in this study were red betel leaf (*Piper crocatum*) and dayak onion (*Eleutherine palmifolia* Merr). Samples were obtained from the Palu city and Palolo village, Central Sulawesi. Before the research, the plants were determined at the UPT of Biology Resources at Tadulako University, Central Sulawesi.

Table 1: Phytochemical screening of piper crocatum and Eleutherine palmifolia.

| No | Component Bioactive | Reagent | Result | |
|----|---------------------|---|--|--|
| | | | Extract Ethanol leaf Piper crocatum | Extract ethanol bulbs Eleutherine palmifolia |
| 1 | Alkaloid | Dragendorff | Red precipitate | Red precipitate |
| 2 | Flavonoid | Magnesium dan HCl | Yellow precipitate | Yellow precipitate |
| 3 | Saponin | Foam test | Persistence of foam produced ±10cm for ten minutes | Persistence of foam produced ±10cm for ten minutes |
| 4 | Tanin | FeCl ₃ 1% | Blackish blue | Black |
| 5 | Polyphenol | FeCl ₃ 5% | Black green | Black green |
| 6 | Triterpenoid | Acid acetate anhydride and H ₂ SO ₄ | Red | Red |

+ = Positive

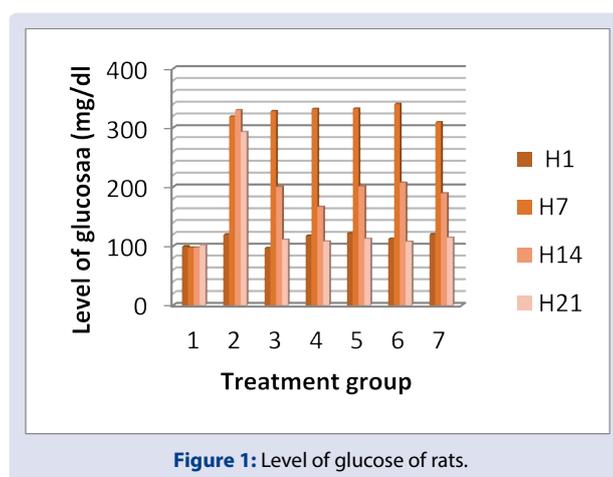


Figure 1: Level of glucose of rats.

The extraction method used is maceration because maceration has a simple working principle with simple equipment. The solvent used in this maceration process is 96% ethanol. The ethanol solvent was chosen because the compounds to be extracted were phenolic compounds¹⁰ The thick extract obtained from the results of the powder of red betel leaf and dayak onion is 50.99 grams obtained with the percent yield 5.09%.

The use of male rats as test animals because it can provide results that are more stable because and are influenced by the estrus cycle and pregnancy as in female white rats. Male white mice also have faster drug metabolic rates and a more stable body condition than female rats.¹¹ The results of phytochemical screening on an ethanol extract of red betel leaf and dayak onion showed that both positive plants contained alkaloid compounds, flavonoids, saponins, tannins, polyphenols, and triterpenoids. Saponins have antidiabetic activity against insulin secretion caused by modulation of calcium channels and regeneration of pancreatic β cells. Saponin has the same mechanism of action as sulfonylurea compounds, which interact with ATP-sensitive K channel on the membrane of β cells so that the flow of potassium out of cells becomes disturbed which will cause membrane depolarization and open calcium channels.¹² With the opening of calcium channels, calcium ions will enter the cytoplasmic cells so that they can activate calmodium in cells that can stimulate insulin-containing granules from vesicles expressed out of cells.² Flavonoids are antioxidant compounds that can be used to bind free radicals and form complexes with metals, this is because flavonoid compounds have at least two hydroxyl groups in the ortho position and para.¹³ The mechanism of flavonoids can inhibit lipid peroxidation, suppress tissue damage by free radicals and inhibit the activity of the glucokinase enzyme that can produce glucose compounds. Increasing antioxidants is very important in conditions of diabetes complications and insulin resistance because it can help reduce

oxidative stress in type 2 diabetes patients.² Flavonoids also have been reported to stimulate Ca²⁺ uptake from isolated islet cells, hence it also effective even in non-insulin dependent diabetes.¹³

Tannin is known to modulate enzymatic carbohydrate solutions by inhibiting amylase and α -glucosidase enzymes. So that the glucose absorption of the gut becomes reduced which causes blood glucose levels to be controlled. Triterpenoid compounds have a stimulating effect on insulin-dependent and play a role in oxidative processes and also have an anti-insulin resistance effect.¹⁴ Polyphenol compounds are known to increase insulin secretion and insulin sensitivity, weaken the postprandial glycemic response and fasting hyperglycemia. The mechanism of action of phenol compounds includes inhibition of carbohydrate digestion and intestinal glucose absorption, stimulation of insulin secretion from β -pancreatic cells, modulation of glucose in the liver and insulin receptor activity.¹⁵

Profile measurement of blood glucose levels in male rats in group 1 of normal control, group 2 of negative control, group 3 of positive control (Glibenclamide), group 4 treatment of single extract of red betel leaves dose of 50 mg / kg BB, Group 5 single extract of forest onion 100 mg / kg BB, Group 6 combination 1 red betel leaf + debt onion bulb 25 + 50mg / kg BB and group 7 red betel extract + forest onion bulb dose 50 + 100mg / kg BB, showing hypoglycemic effect both in single use of each extract and use of combinations. The hypoglycemic effect of streptozotocin-induced diabetic rats on an eclectic combination is probably due to the synergistic effect of the secondary metabolite content contained in red betel leaf extract and dayak onion in regenerating pancreatic β cells to increase insulin secretion and peripheral tissue sensitivity to insulin.

Figure 1 and Table 1 showed blood glucose levels for the normal control group, negative controls, positive controls, single red betel doses 25 mg / kg bb, single-dose dayak onion of 50 mg / KgBB, combination

of red betel plants 25 mg / kg and black bulbs 50 mg / KgBB and the combination of red betel plants 50 mg / KgBB and dayak onion 100 mg / kg bb for 7 consecutive days obtained 97.8 ± 10.25 , 319 ± 68.17 , 328.4 ± 28.88 and 332.2 ± 56.86 , day 14 respectively 97.8 ± 7.62 , 330.2 ± 78.82 , 200.4 ± 31.98 , 166.6 ± 68.89 and 201.2 ± 37 , 61 21 days in a row amounting to 101.4 ± 8.41 ; 293.2 ± 123.12 ; 111.4 ± 4.50 ; 108 ± 9.51 ; 112.6 ± 10.08 ; 107.6 ± 8.20 and 114.4 ± 8.38 . In all groups treatment of seent animals increased blood glucose levels that varied, this is by the literature there is an increase in blood glucose levels after being induced with Streptozotocin, an increase in blood glucose levels caused by resistance to insulin action. Streptozotocin as a diabetic agent can trigger an increase in the production of excess free radicals and cause oxidative stress, varying glucose levels due to the condition of rats that are physiologically different so that the increase in blood glucose levels is different.¹⁶

Statistical analysis of blood glucose levels in test day 0 animals was carried out by analysis of one-way variants (One Way Anova). Based on statistical analysis, ANOVA showed significant results between normal controls and negative controls, a single dose of red betel leaf, the dose of dayak onion and combination 2. This is indicated by $P = 0.004$ ($p < 0.05$) which shows a significant difference in all treatments on day 0 so that it is followed by further testing of the Post Hoc LSD Test. Post Hoc LSD Test results showed normal control with negative controls, the single dose of red betel leaf, the single dose of dayak onion and the combination of extract 2 did not differ significantly from positive controls and extract combinations 1. This is because the blood glucose levels of test animals are still susceptible to normal. which is indicated by the average blood glucose level of rats in the range of 50-135 mg / dL.

Statistical analysis of ANOVA glucose levels of test animals on the 7th, 14th and 21st days showed significant results characterized by $P = 0.000$ ($p < 0.05$) which showed a significant difference in all treatments. The LSD further test results showed that the normal control was significantly different from the negative control, positive control, single dose of forest onion tuber, the combination of extract 1 and combination of extract 2 and not significantly different from the single dose of red betel leaf. Negative controls differed significantly from all treatments. Positive controls differed significantly from normal controls and the remaining negative controls were not significantly different. The most effective decrease in blood glucose levels of male rats was a combination dose of 25 mg + 50 mg of red betel leaf and dayak onion which was 107.6 ± 8.20 almost comparable to the blood glucose level of rats given glibenclamide. where it is known this is because in red betel leaves there are phytochemical compounds namely alkaloids, saponins, tannins, and flavonoids. So that it causes a decrease in blood glucose levels comparable to glibenclamide.⁸

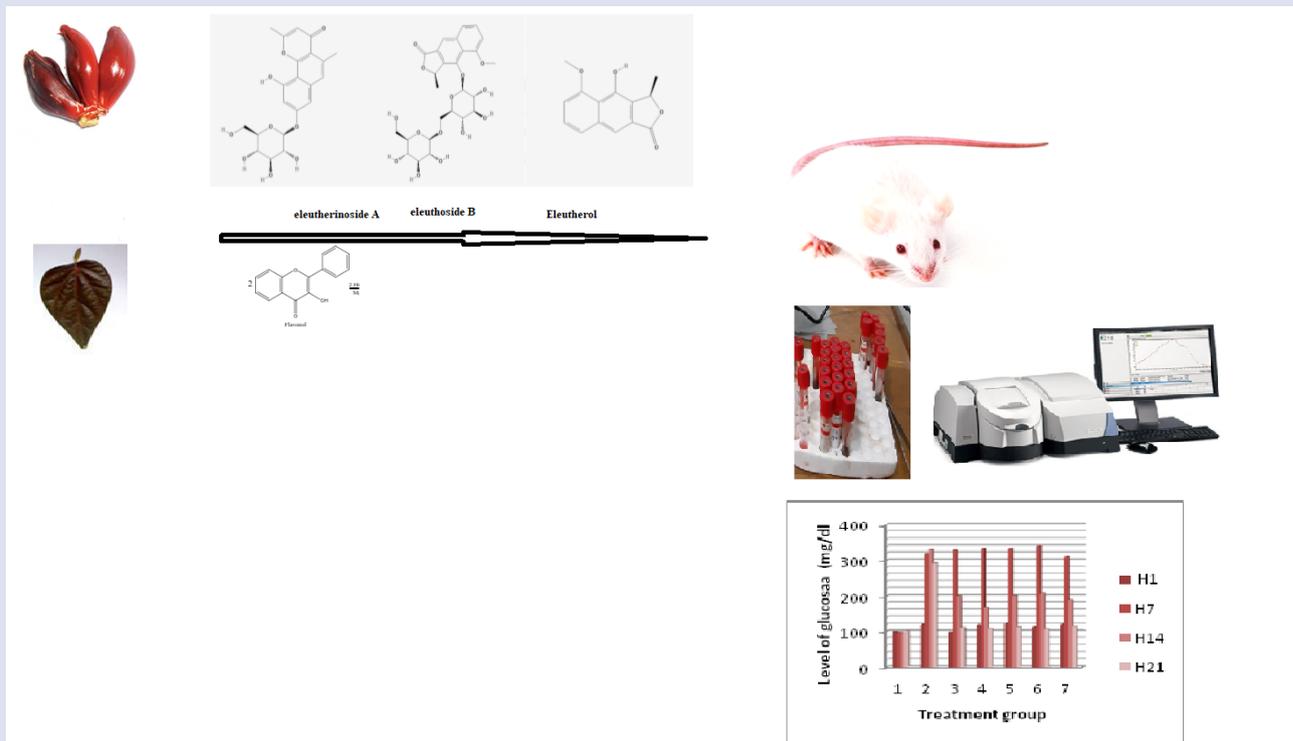
CONCLUSION

The dose of 25 + 50 mg/kg BB combination of red betel leaf (*Piper crocatum*) and dayak onion (*Eleutherine palmifolia* Merr) has the most effective effect in reducing blood glucose levels in male albino rats (*Rattus norvegicus*).

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GRAPHICAL ABSTRACT



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