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# Antioxidant Activity Of Ethanol Extract Of Papaya Leaves (*Carica Papaya* L.) On Reducing Blood Glucose Levels Of Streptozotocin-Induced Male Rats

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**ABSTRACT**

The purpose of this study was to determine the content and total amount of secondary metabolites and determine the antioxidant activity and the effect of ethanol extract of papaya leaves on glucose reduction induced by streptozotocin in male albino rats. Measurement of the total content of secondary metabolites and antioxidants was carried out using a UV-visible spectrophotometer using DPPH reagent, and 30 male albino rats were divided into 6 groups, with 3 groups as normal controls. The negative control group received the appropriate dose: 100 mg/kgBB, 200 mg/kgBB, and 300 mg/kgBB. Positive qualitative test results include secondary metabolites such as alkaloids, flavonoids, saponins, and tannins. Quantitative test results show a total alkaloid content of 0.4739% (equivalent to quinine), saponins 7.518% (equivalent to sapogenin), tannins 0.147% (equivalent to tannic acid), and flavonoids 1.653% (equivalent to quercetin). The IC50 result of the antioxidant activity of papaya leaf extract of 83.07 ppm is included in the strong category. Blood glucose measurement data showed that ethanol extract from papaya leaves effectively reduced blood sugar levels with an average value of 106 mg/dl at a dose of 100 mg/kg body weight.



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## INTRODUCTION

Indonesia is a country with a great diversity of flora and fauna. Most of these plants have been used as herbal medicines for generations. Due to the increasing cost of herbal medicine and its resistance to chemicals, the development and popularization of herbal medicine is gaining popularity. Herbal plants are another way to prevent the development of this resistance. One of the plants that contain large amounts of secondary metabolites is papaya leaves.

Papaya leaves are one of the plants used in traditional medicine and are also used by the community to treat skin diseases such as diarrhea and acne, increase appetite, and improve digestion (Maria, 2016). Papaya leaf extract contains secondary metabolites, namely saponins, flavonoids, tannins, and alkaloids. Active compounds such as saponins themselves have the ability as antioxidants that act as non-free electron delays, these compound

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components are part of secondary metabolite compounds (Maria, 2016). Secondary metabolites are specific small molecules that have varied structures, each compound has a different function and role as a reproductive protector in plants. secondary metabolites are organic molecules that are not directly involved in the normal growth and development of an organism. the function of secondary metabolite compounds is generally used in traditional medicine. Secondary metabolites are biomolecules that can be used as starting compounds in the discovery and development of new drugs. In plants, there are secondary metabolite compounds which are generally alkaloids, flavonoids, steroids, saponins, terpenoids, and tannins (Dewi, 2020).

Antioxidants are compounds that can neutralize or reduce free radicals, and inhibit oxidation in body cells, to prevent and reduce cell damage caused by free radicals. The mechanism of action of antioxidant compounds is by donating hydrogen atoms to radical compounds, this makes radical compounds stable. Based on the formation and origin, antioxidants in the body of living things are classified into two groups, namely endogenous antioxidants and exogenous antioxidants (Harni, et al., 2020). Oxidation reactions can produce free radicals and trigger chain reactions, causing cell damage.

Based on this background, it is necessary to research qualitative and quantitative analysis of secondary metabolites (alkaloids, flavonoids, saponins, tannins) in papaya leaves and antioxidants using spectrophotometric analysis and the DPPH test. I am interested. This study aims to identify secondary metabolites and antioxidant activity found in papaya leaves.

## METHODS

### Materials

The tools used in this study are Glassware, 40 mesh sieve, maceration vessel, blender, rat water bottle, glucometer, glucose strip test, test animal cage, rotary vacuum evaporator, injection syringe, test tube, oral syringe, rat feeder, analytical balance, gram scale, and water bath. The materials used were Distilled water, hydrochloric acid, iron (III) chloride, citrate-buffered saline (citric acid and sodium citrate), papaya leaves, drag-drop LP, 96% absolute ethanol, glibenclamide, handskun (sensi), cotton, label paper, filter paper, duct tape, Liebermann-Burchard, mask, magnesium powder, Na CMC, sodium hydroxide, chloroform, sodium chloride, streptozotocin and standard feed.

### Preparation of Papaya Leaf Ethanol Extract

Simplisia powder was extracted by maceration using 96% ethanol solvent. The simplicia powder was weighed as much as 1500 grams and then put into 3 maceration vessels of 500 grams each using 96% ethanol solvent as much as 1.5 L, then allowed to stand for 3 x 24 hours and protected from sunlight while occasionally stirring. The extract was filtered using filter paper and then the filtrate was obtained and evaporated using a rotavapor followed by thickening carried out using a waterbath (Tandi, 2016).

### Qualitative and quantitative analysis

Qualitative analysis of the chemical composition of papaya leaves includes testing the content of alkaloids, flavonoids, saponins, and tannins. The alkaloid test was carried out with

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the Drageendroff reagent, the flavonoid test was carried out with HCl preaction and magnesium, the saponin test was carried out with 2N HCl shaking, and the tannin test was carried out with FeCl3 preaction (Harni, et al., 2020).

**Antioxidant Activity Test**

Antioxidant activity was tested by mixing DPPH standard solution and samples in a series of concentrations. The mixture was homogenized and allowed to stand for approximately 30 minutes, then measured with a wavelength of 517 nm using a UV-Vis spectrophotometer. The antioxidant activity of the sample was calculated from the absorption resistance value of the DPPH solution as a percentage of inhibition:

$$\% \text{ Antioxidant} = \frac{A0-A1}{A0} \times 100\%$$

Description:

Ao = Control Absorbance

A1 = sample absorbance

According to the relationship between concentration and percentage inhibition, a regression equation was made to determine the $IC_{50}$ value. Linear regression equation y= ax + b where:

y = % inhibition

x = Concentration

**Preparation of 0.5% Na CMC suspension**

Sodium carboxymethyl cellulose (Na CMC) weighed as much as 0.5 grams and was sprinkled in a mortar containing 10 ml of heated distilled water, allowed to stand for 15 minutes until a transparent mass was obtained, then mixed until homogeneous. The Na CMC solution was transferred into a 100 ml volumetric flask. The volume was filled with distilled water up to 100 ml.

**Preparation of Glibenclamide Suspension 0.45 mg/kg BW**

Glibenclamide dose in adult humans is 5 mg per day, if converted to rats weighing 200 grams is 0.018 then the dose of glibenclamide for rats is 0.45 mg/kg BW. Weighed glibenclamide tablet powder equivalent to 3.6 mg then suspended in 0.5% Na CMC up to 100 ml then shaken until homogeneous.

**Preparation of Streptozotocin (STZ) Solution**

Streptozotocin was weighed as much as 0.32 grams and then dissolved using citrate-buffered saline with pH 4.5 to 100 ml, then induced in rats via intraperitoneal (IP). The dose of streptozotocin is 40 mg/kg BW.

**Preparation of Sample**

Ethanol extract of papaya leaves (*Carica papaya* L.) weighed 0.8 gram (dose of 100 mg/kg BW) was included in the 0.5% Na CMC suspension little by little, mixed until homogeneous, and added 0.5% Na CMC suspension up to 25 mL. At a dose of 200 mg/kg BW, weighed ethanol extract of papaya leaves (Carica papaya L) as much as 1.6gram was included in the 0.5% Na CMC suspension little by little, mixed until homogeneous and added 0.5% Na CMC suspension up to 25 mL. At a dose of 300 mg/kg BW, weighed ethanol extract of pa-

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paya leaves (Carica papaya L) as much as 3.2 grams was included in the 0.5% Na CMC suspension little by little, mixed until homogeneous and added 0.5% Na CMC suspension up to 25 mL.

### Blood Sampling

Blood sampling was conducted in the Instrumental and Biopharmaceutics laboratory of STIFA Pelita Mas Palu. Blood was taken on days 0, 7, 14, 21, and 28 through the tail of the rat using a tube that had been given Ethylene diaminetetraacetic Acid (EDTA) as much as 2 ml to be centrifuged into serum.

### Data Analysis

The data obtained in the form of blood glucose levels were analyzed statistically using One Way Anova analysis at the 95% confidence level, followed by a Post hoc Least Significant Difference (LSD) test to determine significant differences between treatments. Data processing was carried out using the SPSS software program.

## RESULT AND DISCUSSION

**Table 1**. Qualitative Test Results of Papaya Leaf Ethanol Extract

| No | Chemical Compounds | Reagent | Result |
|---|---|---|---|
| 1 | Alkaloids | Dragendrof | + |
| 2 | Flavonoids | Concentrated HCL and Mg Metal | + |
| 3 | Saponins | Shaken + HCL 2N | + |
| 4 | Tannins | Fecl3 | + |

**Table 2**. Quantitative Test Results of Ethanol Extract of Papaya Leaf

| No | Chemical Compounds | Result %b/b) |
|---|---|---|
| 1 | Total Alkaloid Equivalent of Quinine | 0,4739 |
| 2 | Total Flavonoids Equivalent of Quercetin | 1,653 |
| 3 | Saponin From Sapogenin | 7,518 |
| 4 | Total Tannin Equivalent of Tannic Acid | 0,147 |

**Table 3**. Antioxidant Activity of Papaya Leaf

| Compounds | Concentration (ppm) | % Inhibition | $IC_{50}$ ppm |
|---|---|---|---|
| Quercetin | 10 | 17.99 | 21.22 |
| | 15 | 32.32 | |
| | 20 | 41.41 | |
| | 25 | 56.52 | |
| | 30 | 76.27 | |
| | 35 | 96.01 | |
| Papaya Leaf Extract | 40 | 16.71 | 83.07 |
| | 60 | 34.23 | |
| | 80 | 48.24 | |
| | 100 | 62.73 | |
| | 120 | 80.25 | |
| | 140 | 96.01 | |

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**Table 4.** Mean Blood Glucose Level

| Day | Mean ± SD Blood Glucose Level (mg/dl) | | | | | |
|---|---|---|---|---|---|---|
| | Normal Control | Negative Control | Positive Control | Dose 100 mg/kg BB | Dose 200 mg/kg BB | Dose 300 mg/kg BB |
| 0 | 87 ± 3 | 87 ±1 | 88 ±2 | 87 ± 2 | 87 ± 2 | 87 ± 3 |
| 7 | 105 ±15 | 306 ±79 | 320±102 | 337 ± 36 | 331± 41 | 331 ± 47 |
| 14 | 92 ±6 | 309 ±97 | 254 ±47 | 195 ± 50 | 166 ± 53 | 167 ± 32 |
| 21 | 102 ±6 | 332 ±24 | 122± 19 | 138 ± 11 | 129 ± 16 | 120 ± 12 |
| 28 | 89 ±4 | 329 ±62 | 111 ±8 | 106 ±16 | 117 ±12 | 110 ±6 |

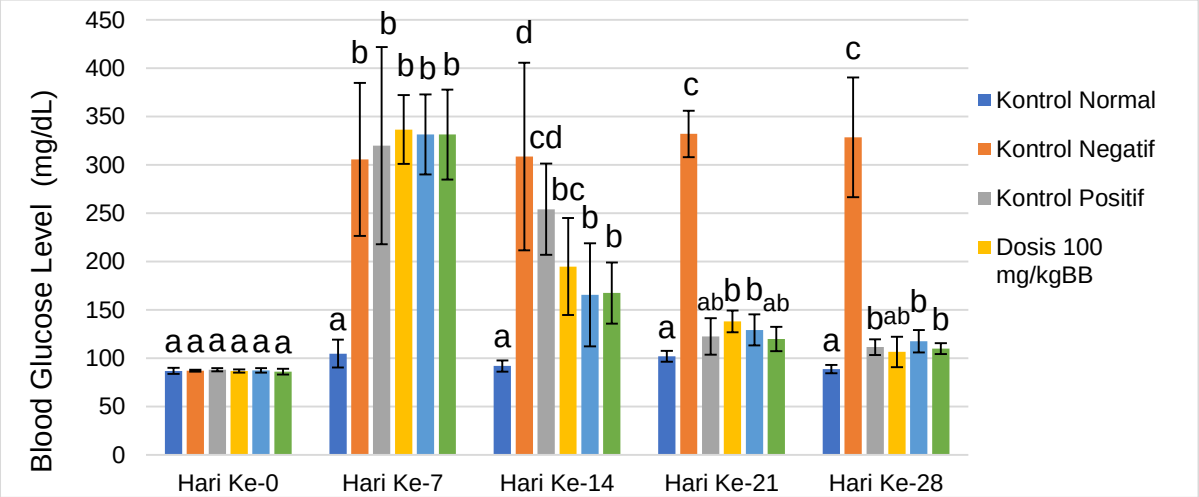


**Figure 1.** Graph of Blood Glucose Level Measurement Results

This study used papaya leaf test material obtained from the Maku area of Central Sulawesi. Then the concentrated ethanol extract of papaya leaves obtained was qualitatively identified by phytochemical screening test, and secondary metabolism in papaya leaf ethanol extract was determined by phytochemical screening test. determine whether the product exists. Furthermore, quantitative analysis was carried out using UV-visible spectrophotometry to determine the total amount of secondary metabolites contained in papaya ethanol extract (Mahatriny, et al., 2014).

The antioxidant activity of papaya ethanol extract was tested to determine the antioxidant activity of papaya leaf extract. The method used in this study was 1-1-diphenyl-2-picrylhydrazyl (DPPH). This method is superior to the Ferry Reducing Antioxidant Power (FRAP) and Ferrous Ion Chelation (FIC) methods (Reni, 2018). It contains quercetin, which is a much more powerful antioxidant than vitamin C.

This antioxidant is measured from the fact that DPPH which lacks a pair of electrons turns purple when the sample releases a molecule of hydrogen atoms to form a diphenylpicrylhydrazine compound. When using UV-Vis spectrophotometry, the color change can change the absorption value at the maximum DPPH wavelength. The absorbance value can

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be derived from the radical capture activity value known as the inhibitory concentration (IC50) (Tristantini et al., 2016).

Measurement of secondary metabolite levels in papaya leaf extract was measured using UV-Vis spectrophotometry. In the form of the phytochemical screening test. This study aims to determine whether quantitative analysis can successfully determine the total amount of secondary metabolites using UV-Vis spectrophotometry. Total alkaloid content was determined using spectrophotometry. The total alkaloid content was determined by regression equation y=0.0474x+0.0593 and correlation coefficient R2=0.9966. Based on the regression equation, the equivalent total alkaloid content of quinine in the sample was quantitatively calculated, and the equivalent percentage of the total alkaloid content of caffeine from the ethanol extract of Carica papaya L was 0.4739% w/w. Alkaloids are widely used by humans for the manufacture of medicines. The role of alkaloids in plants is to protect plants from pests and diseases and regulate their growth. Alkaloids can be pesticides with low toxicity (Surya, 2017).

Total flavonoid content was determined spectrophotometrically. Total flavonoid content was determined y = 0.0006x+0.0271, correlation coefficient $R^2$ = 0.9985. The total flavonoid content of each sample was calculated and repeated three times. The average result of total flavonoid content was 1.653% w/b. Flavonoids are used as antiviral, antibacterial, antibacterial, anticholesterolemic, and insecticidal agents. The role of flavonoids in the world of health are used as antibiotics to treat cancer and kidney disease, as well as reduce blood clotting and overcome liver function disorders (Tuntun, 2016).

The total amount of saponins was determined spectrophotometrically. Total saponin content was determined using the regression equation y = 0.0004x + 0.0176 and correlation coefficient R2 = 0.9998. The average total saponin content was calculated based on the regression equation and amounted to 7.518% w/b. The relevance of these compounds in the health sector is that saponins prevent damage to biomolecules by free radicals by inhibiting the increase in vascular permeability, thus preventing excess oxides due to the formation of hydroperoxide intermediates. (Tandy et al., 2020).

Total tannin content was determined using the regression equation y = 0.0679x-0.0749, correlation coefficient R2 = 0.9979. Based on the regression equation, the total tannin content of each sample was calculated and repeated three times. The average total tannin content was 0.147% w/b. The health benefits of tannins are, as antioxidants, tannins capture free radicals, balance oxidants, and antioxidants, and allow cells to repair and maintain the stability of free radicals. Tannins also stimulate glucose and fat metabolism and prevent their accumulation in the blood. Tannins can act as an astringent, constrict the epithelial membrane of the small intestine, slow the absorption of food and sugar, and prevent the rate of increase in blood sugar levels from becoming too high (Tuntun, 2016). To determine the antioxidant activity of papaya fruit, an antioxidant activity test of ethanol extract of papaya leaves was conducted. In this study, the 1-1-diphenyl-2-picrylhydrazyl method and the iron ion chelate (FIC) method were used (Maesaroh et al., 2018). The comparator used is quercetin which is a very strong antioxidant compared to vitamin C. If vitamin C has 1 antioxidant, quercetin has 4.7. Saponin Sapogenin functions to donate protons into free radical compounds, and the

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unpaired electrons obtained there are resonantly delocalized so that quercetin radicals become reactive radicals due to their low energy (Nanggu, 2016).

The antioxidant activity test was carried out by preparing DPPH solution, followed by preparing quercetin stock solution with a concentration of 2000 ppm, measuring comparative antioxidant activity, and measuring the absorbance of papaya leaf samples. Measurements were made using UV-Vis spectrophotometry at a wavelength of 514 nm, and the maximum wavelength measured previously was between 400 and 600 nm. The maximum wavelength is generally 517 nm, and the results obtained were for a wavelength of 514 nm. The maximum wavelength may vary due to different experimental conditions, such as B. Differences in a solvent, measurement time, and the person performing the measurement (A'yun & Laily, 2015).

This antioxidant is measured from the fact that DPPH which does not have a pair of electrons turns purple when the sample releases a molecule of hydrogen atoms to form a diphenylpicrylhydrazine compound. When using UV-Vis spectrophotometry, the color change can change the absorption value at the maximum DPPH wavelength. This uptake value can be considered as a radical capture activity value and calculated as an inhibitory concentration value (IC50). (A'yun & Laily, 2015).

Based on Table 3, the IC50 value of quercetin was 21.22 ppm making it a very strong antioxidant. IC50 value of 10-50 ppm is said to be a strong antioxidant. While the IC50 of the papaya leaf extract sample was 83.07 ppm. It can be concluded that the comparison of the IC50 value of quinine is stronger than the papaya leaf extract sample. A compound is said to be a very strong antioxidant if its IC50 is less than 10 g/ml, and a compound is said to be a very strong antioxidant if its IC50 is in the range of 10-50 g/ml. strong antioxidant if it has a value in it It is 10-50 g/ml. If the value is in the range of 50-100 g/ml, it is said to be a strong antioxidant, and the IC50 value is in the range of 100-100 g/ml. 250 g/ml, is weak, if the IC50 value is higher than 250 g/ml then it is not effective (Hdayani et al., 2020). The difference in antioxidant activity of ethanol extract of clove leaves (Artocarpus camansi Blanco.) compared to quercetin is due to the concentration value of flavonoid secondary metabolites in clove leaves of 0.085%, the value is classified as a moderate antioxidant (A'yun & Laily, 2015).

**Measurement of Blood Glucose Levels**

The results of blood glucose measurements on day 0 showed normal conditions in all treatment groups. Seven days after giving a high-cholesterol diet and streptozotocin induction, there was an increase in blood glucose levels in all treatment groups, meaning there was an induction effect. On day 21, ethanol extract from Kuruwi leaves proved effective in reducing blood sugar levels in male albino rats. On day 28, the ethanol extract of papaya leaves proved effective in reducing blood glucose levels in male albino rats at a dose of 300 mg/kg body weight, comparable to normal and positive controls (Figure 1).

The decrease in blood sugar levels is thought to be caused by the effects of alkaloids, flavonoids, saponins, and tannins contained in the ethanol extract of papaya leaves (Table 1). Alkaloids have been shown to have regenerative abilities. In this case, it is proven that alkaloid

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extracts do have the ability to regenerate damaged pancreatic beta cells. The work of alkaloids to reduce blood sugar levels occurs through extrapancreatic mechanisms: increased glucose transport in the blood, inhibition of glucose absorption in the intestine, stimulation of glycogen synthesis, and inhibition of glucose synthesis (Tandi, et al., 2017).

Flavonoid compounds have antidiabetic effects and can protect the body from DNA damage due to reactive oxygen compounds (ROS). The mechanism of flavonoids in protecting the body from free radicals is by breaking down oxygen radicals and protecting cells from lipid peroxidation thus breaking the chain of radical reactions. Flavonoids act as antioxidants that can regenerate damaged pancreatic beta cells. In addition, flavonoids can also slow down, delay, and prevent the process of lipid oxidation and increase the sensitivity of insulin receptors, thus helping to overcome insulin deficiency and repair pancreatic and kidney cells damaged by free radicals (Tandi, 2018). Saponins can reduce blood sugar levels by inhibiting gastric emptying. Slower gastric emptying means food takes longer to be absorbed and blood sugar levels increase. Tannin compounds act as astringent or chelating agents that contract the smooth membrane of plants to reduce the absorption of food juice, thus inhibiting the absorption of sugar, and the rate of increase in blood sugar levels is not too high (Suyudi, 2017). Tannins have a hypoglycemic effect. That is, by increasing the process of sugar production in muscle tissue, there is an improvement in the function of pancreatic beta cells and kidney cells. Improvement of kidney cells causes a decrease in blood sugar, creatinine, and urea levels (Tandi, 2018).

## CONCLUSION

Ethanol extract of papaya leaves (Carica papaya L) contains alkaloid compounds, flavonoid saponins, and tannins. The total alkaloid test parameter (caffeine equivalent) is the total content of alkaloid compounds 0.4739% b/b, the total flavonoid test parameter (quercetin equivalent) is 1.653% b/b flavonoids, the total saponin test parameter saponin quantification error (acid equivalent) is 7.518% b/b, and the total tannin test parameter (tannin equivalent) is 0.147% b/b. Ethanol extract from papaya leaves has antioxidant activity with an IC50 value of 83.07 ppm included in the category of very strong antioxidants and reduces blood sugar levels at an effective dose of 100 mg/ml. It has the effect of lowering blood glucose.

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