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The Effectiveness of Ethanol Extract of Robusta Coffee Seeds on Blood Glucose, Urea, and Creatinine Levels of Male White Rats Induced by Streptozotocin

(*Efektivitas Ekstrak Etanol Biji Kopi Robusta terhadap Kadar Glukosa Darah, Urea, dan Kreatinin Tikus Putih Jantan yang Diinduksi Streptozotocin*)

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ABSTRACT

Background: Traditional medicine plays a role in alternative treatment systems for diabetes mellitus. **Objectives:** This study aimed to screen the secondary metabolite of the ethanol extract from robusta coffee (*Coffea canephora* Pierre) seeds. We aimed to clarify the effects of these extracts on blood glucose, urea, and creatinine levels in Streptozotocin-Induced Diabetic Rats. **Material and Methods:** Phytochemical screening of extracts was carried out qualitatively according to standard methods. The rats were administered ethanol extracts of C. canephora Pierre seeds at doses of 100, 200, and 300 mg/kg body weight for 28 days. Blood glucose, urea, and creatinine levels were then compared between the control and diabetes groups. **Results:** The ethanol extract of *C. canephora* Pierre seeds contain alkaloids, flavonoids, saponins, and tannins. Group doses of 100-300 mg/kg body weight can significantly reduce blood glucose, urea, and creatinine levels. **Conclusions:** The ethanol extract of *C. canephora* Pierre seeds showed the potential as an antidiabetic agent.

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INTRODUCTION

Health plays a vital role in human life as optimal health enables individuals to carry out their activities effectively. Nowadays, many diseases are not caused by microorganisms but rather by unhealthy lifestyles. Over time, lifestyles have changed, including unhealthy dietary habits and nutritional imbalances (Suiraoka, 2012). These changes have become one of the leading causes of degenerative diseases such as heart disease, hypertension, high cholesterol, and diabetes mellitus (Tandi et al., 2020). The number of diabetes patients continues to rise in Indonesia, ranking the country seventh out of ten with the highest number of diabetes patients in the world. The global prevalence of diabetes mellitus (DM) continues to increase significantly until 2030. According to WHO estimates, the number of type 2 diabetes patients in Indonesia is projected to rise from 8.4 million in 2000 to approximately 21.3 million by 2030. IDF also predicts that the number of DM patients in Indonesia will increase from 10.3 million in 2013-2017 to 16.7 million by 2045 (Perkeni, 2019; Soewondo et al., 2013).

Diabetes is a metabolic disorder caused by high levels of glucose in the blood, which can be potentially dangerous due to the increased reactivity of reactive oxygen species (ROS). The combination of ROS can result in lipid, DNA, and protein component changes in body tissues. Increased ROS activity interacting with double lipid layers in cells leads to lipid peroxidation. This process occurs naturally and also serves as a marker of oxidative stress, especially in the form of malondialdehyde (MDA). Reactive oxygen species' response to DNA fragments can damage the DNA structure. One indicator of genetic damage that can be measured in this context is 8-Hydroxy-Deoxy-Guanosine. Consequently, ROS can play a role in causing hyperglycemic conditions (Matough et al., 2012; Tangvarasittichai, 2015).

Hyperglycemia can damage venous blood vessels in the kidneys, impairing the glomerulus filtration process in blood vessels. As a result, kidney structure changes, leading to kidney function impairment. One sign of kidney damage that can be identified is examining urea and creatinine levels. Substances like urea and creatinine, which are byproducts of digestion, are excreted through the kidneys (Tandi et al., 2018). Urea concentration reflects the level of protein breakdown in the liver, which is then channelled to the kidneys and excreted in urine. On the other hand, creatinine is the end product of an endogenous process originating from creatine phosphate breakdown, and creatinine levels tend to be more stable. Increased creatinine and urea levels can be caused by elevated blood glucose levels, often in diabetes patients. This leads to automatic glucose oxidation, protein glycation, and polyol pathway metabolic activity, ultimately increasing the production of oxygen-responsive compounds (Kaufman et al., 2018).

The use of nature, especially for maintaining health and treating disease, is by using plants in the natural surroundings (Widodo, 2011). One type of medicinal plant that is used is the coffee plant. Out of 124 varieties, only three types are popular and widely developed, namely Robusta coffee, Arabica coffee, and Liberica coffee, which result from the crossbreeding of various coffee species. Robusta coffee (*Coffea canephora* Pierre) contains multiple active compounds, primarily corrosive chlorogenic acid, caffeine, trigonelline, and other polyphenolic compounds. Coffee can potentially boost the immune system due to its content of polyphenols and phenolic compounds with antioxidant properties. Coffee also contains antioxidants that can help combat the oxidative effects of diabetes, which, in turn, can help control blood sugar levels (Fadri et al., 2022). Based on the above explanation, researchers are interested in conducting a study related to testing the effectiveness of ethanol extract from Robusta coffee seeds in reducing blood glucose levels and creatinine and urea levels in male white rats induced by Streptozotocin.

MATERIAL AND METHODS

Plant Materials and Extraction Procedure

C. canephora Pierre seeds were obtained from Banyusari Village, North Lore District, Poso Regency, Central Sulawesi Province, Indonesia. The plant material was identified by the Celebense Herbarium (CEB) of Tadulako University with number 93/UN.28.UPT-SDHS/LK/2021. *C. canephora* Pierre seed powder was macerated using 96% ethanol with a ratio of 1:10 (w/v) for 3x24 hours until the filtrate was obtained. The filtrate was then concentrated using a vacuum rotary evaporator at 60°C and a water bath to produce a crude extract (10% w/w).

Phytochemical Screening of Secondary Metabolites

Qualitative tests were conducted to determine the phytochemical constituents in *C. canephora* Pierre seeds, including alkaloids (Dragendorff test), flavonoids (Shinoda test), saponins (foam test), and tannins (Ferric chloride test).

Animals

Male Wistar rats aged 3-4 months with body weights ranging from 150-200 grams were used in the study. The rats were housed under standard conditions with controlled temperature, light-dark cycles, and humidity. They were provided a standard diet and ad libitum access to water throughout the experiment. The study protocol was approved by the Ethics Committee on Medical and Health Research at Tadulako University (Approval No.: 6938/UN28.1.30/KL/2022).

Determination of Blood Glucose, Urea, and Creatinine Levels

The white male rats were divided into six groups: untreated, diabetic control, positive control (Glibenclamide), and three different doses of ethanol extract of C. atropurpureus leaves. Diabetes was induced in the rats by a single intraperitoneal injection of streptozotocin (STZ). Blood glucose levels were measured after fasting, and oral treatments were administered for 28 days. Blood glucose levels were monitored at specific intervals (Tandi et al., 2021).

The measurement of urea begins by separating the blood sample into serum through centrifugation for 15 minutes. A total of 10 µL of the sample is required for this process. Subsequently, 1000 µL of reagent 1 is added to the serum. Next, incubation is carried out for 5 minutes at a temperature of 25°C. After that, 1000 µL of reagent 2 is added to the mixture. The mixture is allowed to sit for 10 minutes at a temperature of 25°C, then measured using UV-VIS spectrophotometry (Elovution 201) at a wavelength of 578 nm, and the results of urea absorbance measurements are recorded (Tandi et al., 2023).

The creatinine measurement begins by separating a blood sample into serum through centrifugation for 15 minutes. 100 µL of serum is required, which is then mixed with picric acid reagent (reagent 1) in an amount of 100 µL and sodium hydroxide reagent (reagent 2) in an amount of 100 µL at a 1:1 ratio. This mixture is stirred until evenly distributed and left to stand for 30 seconds. Subsequently, the measurement is carried out using UV-VIS spectrophotometry (Evolution 201) at a wavelength of 492 nm. This measurement is performed twice, with the first measurement lasting for 30 seconds and the second for 2 minutes. The results of the creatinine absorbance measurement are recorded (Tandi et al., 2023).

Statistical Analysis

The results of blood glucose levels were analyzed using ANOVA followed by Duncan's test. Categorical data from pancreatic histology were analyzed using the Kruskal-Wallis and Mann-Whitney tests. Statistical significance was considered at $P < 0.05$.

RESULTS AND DISCUSSION

Phytochemical examinations were conducted on ethanol extract from *C. canephora* Pierre seeds, encompassing tests for alkaloids, flavonoids, saponins, and tannins. Qualitative outcomes of the phytochemical analysis indicated the presence of alkaloids, flavonoids, saponins, and tannins in the extracts, as shown in Table-1.

Table 1. Phytochemical Screening of Ethanol Extract of *C. canephora* Pierre seeds.

Phyto-constituents	Test performed	Results
Alkaloid	Dragendorff's	+
Flavonoid	Shinoda	+
Saponin	Froth formation	+
Tannin	FeCl3	+

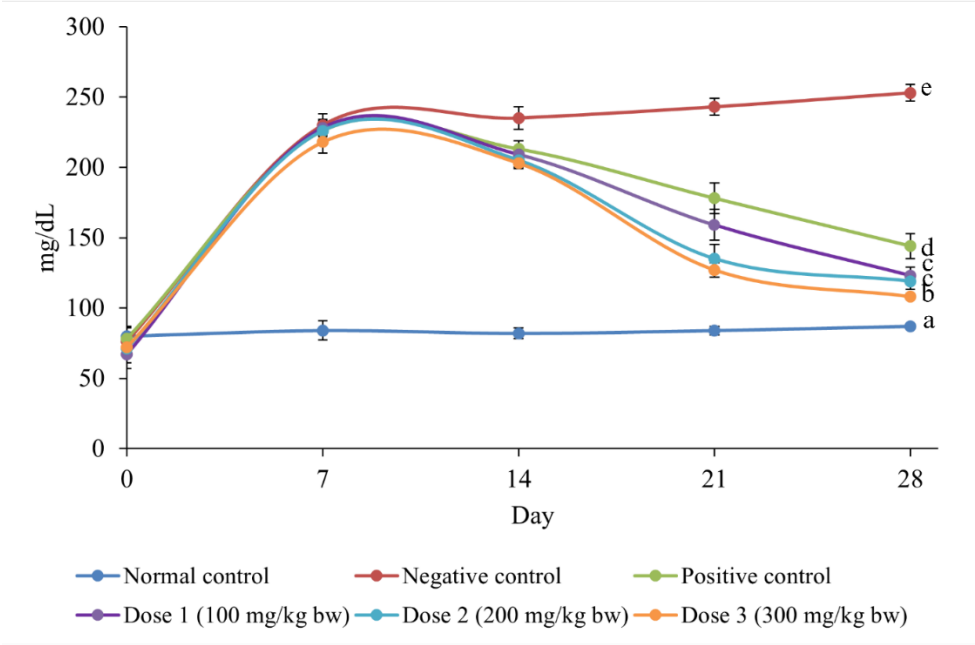

Figure 1. The effect of the ethanol extract from *C. canephora* Pierre seeds on the blood glucose concentration (mg/mL) throughout the experiment.

The effect of ethanol extract of *C. canephora* Pierre seeds on the blood glucose levels of the experimental rat is shown in Figure 1. Based on data analysis using ANOVA and followed by Duncan Test during the trial period, the normal control group rat did not show significant variations in blood glucose levels compared to other groups. The ethanol extract of *C. canephora* Pierre seeds at 100-300 mg/kg dose significantly reduced hyperglycemia compared with the negative control group ($P < 0.05$).

Secondary metabolites possess pharmacological properties, including antidiabetic effects. Alkaloid-rich plants are known for their antidiabetic potential, acting through various mechanisms. These mechanisms include inhibiting carbohydrate-hydrolyzing enzymes like α-Glucosidase and α-Amylase, blocking protein tyrosine phosphatase 1B, dipeptidyl peptidase-4 (DPP4), activating 5'-adenosine monophosphate-activated protein kinase (AMPK), promoting GLUT4 translocation, and facilitating

pancreatic regeneration and insulin release through stimulation and regeneration of pancreatic β-cells (Rasouli et al., 2020).

Flavonoids, recognized as antioxidants, are crucial in preventing or managing diabetes mellitus. In vitro studies have shown that certain flavonoids exhibit antioxidant and antidiabetic properties by inhibiting α-glucosidase and dipeptidyl peptidase-4 (DPP4). Flavonoids exhibit diverse antidiabetic actions, affecting multiple pathways. They can stimulate GLUT4 synthesis and translocation, increase hepatic hexokinase activity, reduce beta-cell apoptosis, activate the peroxisome proliferator-activated gamma receptor (PPAR-γ) to enhance glucose uptake, activate AMPK pathways, inhibit tyrosine kinase activity, and activate nuclear factor-κB (NF-κB) (Al-Ishaq et al., 2019).

Saponins derived from various marine organisms have been reported to possess hypoglycemic properties, regulating blood glucose levels and mitigating diabetes-related complications due to their antioxidant activity. Saponins achieve their hypoglycemic effects by promoting glycogen synthesis, inhibiting disaccharide activity, modulating insulin release from pancreatic beta cells, and inhibiting α-glucosidase activity (El Barky et al., 2017).

Tannins, valuable polyphenolic compounds in medicinal plants and various food sources offer numerous health benefits. Reports suggest that tannins from medicinal plants play a significant role in managing diabetes and its complications. Tannins exhibit antidiabetic potential by lowering glucose levels through delayed intestinal glucose absorption (inhibition of α-amylase and α-glucosidase activity), mimicking insulin's effects in insulin-sensitive tissues, and delaying the onset of insulin-dependent diabetes mellitus by regulating the antioxidant environment in pancreatic β-cells (Ajebli & Eddouks, 2019).

The effect of ethanol extract of *C. canephora* Pierre seeds on the urea and creatinine levels of the experimental rat is shown in Figures 2 and 3. Based on data analysis using ANOVA followed by Kruskal-Wallis and Mann-Whitney tests during the trial period, the normal control group rat did not show significant variations in urea and creatinine levels compared to other groups. The ethanol extract of *C. canephora* Pierre seeds at 100-300 mg/kg dose significantly reduced hyperglycemia compared with the negative control group.

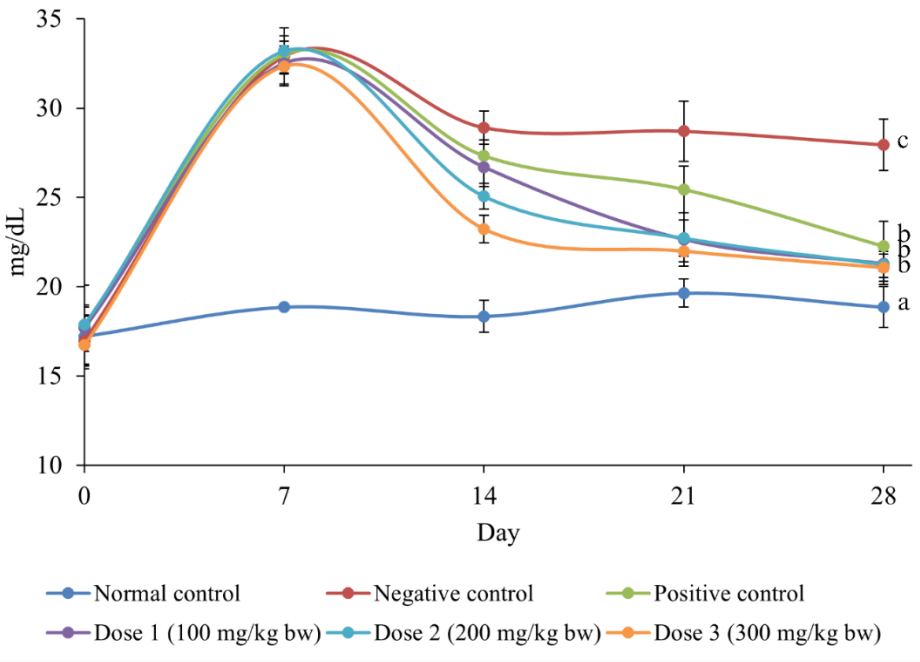

Figure 2. The effect of the ethanol extract from *C. canephora* Pierre seeds on the urea level (mg/mL) throughout the experiment.

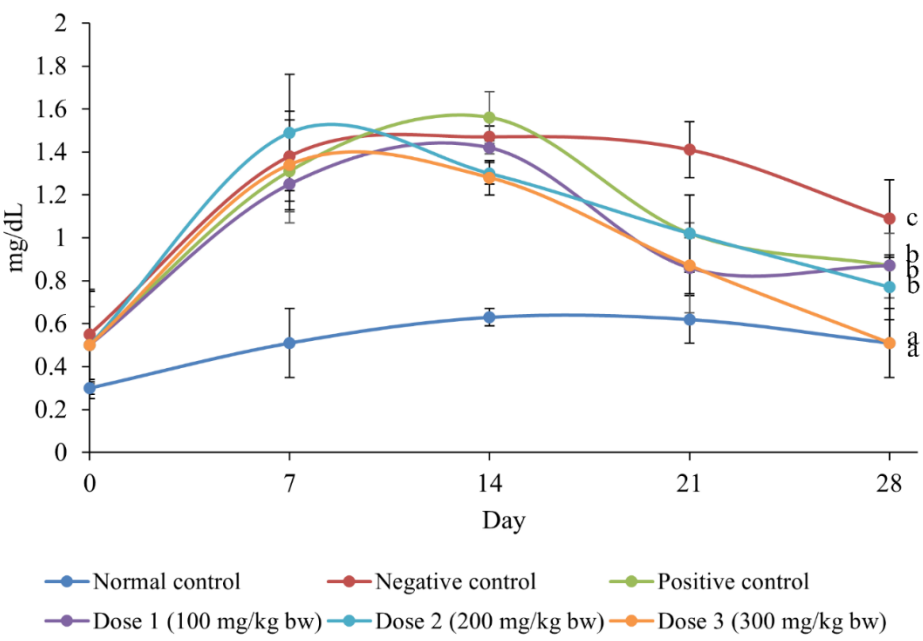

Figure 3. The effect of the ethanol extract from *C. canephora* Pierre seeds on the creatinine level (mg/mL) throughout the experiment.

The decrease in urea and creatinine is thought to occur due to the effects of the active substances flavonoids, alkaloids, saponins, and tannins. Some secondary metabolite compounds are antioxidants,

which can ward off and prevent damage caused by free radical compounds (Tandi et al., 2023; Widodo et al., 2022). These compounds function as substances that can improve the metabolic system in the body by repairing damage to pancreatic beta cells and kidney cells.

CONCLUSION

The ethanol extract of robusta coffee (*Coffea canephora* Pierre) seeds contains alkaloids, flavonoids, saponins, and tannins. Administered doses of 100-300 mg/kg BW of *C. canephora* Pierre seeds can reduce glucose, urea, and creatinine levels in rats induced by streptozotocin.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

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