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PHYTOCHEMICALS, ANTIOXIDANT, AND ANTIDIABETIC ACTIVITIES OF ETHANOL EXTRACT OF *Coleus atropurpureus* Benth. LEAVES

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ABSTRACT

Coleus atropurpureus Benth leaves are traditionally used in Central Sulawesi, Indonesia to treat diabetes mellitus. This study aims to determine the secondary metabolite levels of the ethanol extract of *C. atropurpureus* leaves, its antioxidant activity, and its effect on blood glucose levels in Streptozotocin-induced diabetic rats. Extract phytochemical screening was carried out qualitatively according to standard methods. Quantitative analysis of flavonoids, saponins, tannins, and alkaloids by UV-Vis Spectrophotometry. The DPPH method was used to determine antioxidant activity. Diabetic rats were treated with ethanol extract of *C. atropurpureus* leaves at doses of 150, 200, and 250 mg/kg BW for 28 days, then their blood sugar levels were calculated. The ethanol extract of *C. atropurpureus* leaves showed antioxidant activity (IC50) 56.96 µg/mL. The 250 mg/kg BW dose group significantly reduced blood glucose levels. It can be concluded that the ethanol extract of *C. atropurpureus* leaves has the potential as a source of antidiabetic.

Keywords: *Coleus atropurpureus* Benth, Secondary Metabolites, Antioxidant, Antidiabetic

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INTRODUCTION

The International Diabetes Federation (IDF) in 2021 recorded 537 million adults (age 20-79 years) or 1 in 10 people living with diabetes worldwide. This number is expected to increase to 643 million in 2030 and 783 million in 2045. Diabetes is responsible for 6.7 million deaths in 2021, or 1 every 5 seconds.[1] Even with the progress made in the treatment of diabetes with synthetic drugs, traditional treatments are still widely used around the world. For centuries, traditional medicine has used a variety of plants to treat diabetes. Medicinal plants are frequently more affordable than manufactured medications, have fewer side effects, and are even more successful at treating diabetes mellitus.[2] Free radicals are reactive chemicals produced by the body's metabolic functions, cellular respiration, and inflammatory responses. When present in excess, these free radicals can oxidize cellular constituents like proteins, lipids, nucleic acids, and DNA, which can lead to the onset of several illnesses, including diabetes.[3,4] Research is increasingly focusing on natural substances with antioxidant activities due to the perceived safety of natural antioxidants in comparison to synthetic counterparts.[5,6] Traditionally the people of Central Sulawesi, especially in Poso Regency, use *mayana* (*Coleus atropurpureus* Benth.) leaves to treat diseases, including diabetes mellitus. The Medical Health Guide reports that the genus Coleus is used to treat wounds, swellings, bruises, sprains, and cysts.

In traditional Indian medicine (Ayurveda) the genus Coleus is used as a tonic for heart problems, chest pain, lung ailments, digestive disorders, and sleeplessness (insomnia).[7] The Lamiaceae family is a potential source of therapeutic agents for the management of metabolic disorders, including diabetes.[8] Therefore, this study aims to determine the levels of secondary metabolites of the ethanol extract of *C. atropurpureus* leaves, as well as to clarify the antioxidant activity and effect of the extract on blood glucose.

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EXPERIMENTAL

Plant Materials and Extraction Procedure

The cultivated leaves of *C. atropurpureus* were obtained from Alitupu Village, Lore Utara District, Poso Regency, Central Sulawesi Province, Indonesia. According to the reference number 74/UN.28.UPT-SDHS/LK/2021, the plant underwent authentication by the Tadulako University Herbarium Celebense (CEB). To extract the filtrate, the leaves powder underwent maceration using 96% ethanol at a 1:10 (w/v) ratio for 3 cycles, each lasting 24 hours. Afterward, the filtrate underwent concentration using a vacuum rotary evaporator at a temperature of 60 ºC in a water bath, producing a crude extract with a concentration of 6.7% (w/w).

Phytochemical Screening of Secondary Metabolites

Phytochemical constituents of *C. atropurpureus* leaves were determined by different qualitative tests such as flavonoids (Shinoda test), saponins (foam test), tannins (Ferric chloride test), and alkaloids (Dragendorff test).[9,10]

Determination of Total Flavonoid

100 mg extract was combined with 2 mL of 4N HCl, followed by autoclaving at 110 °C for 2 hours and then cooling. Cultivated using ether and thereafter transferred into a 10 mL test tube. The ether was evaporated and then dried using N2 gas. 0.3 mL of a 5% NaNO2 solution was introduced. After 5 minutes, 0.6 mL of a 10% AlCl3 solution was added. After another 5 minutes, 2 mL of a 1 M NaOH solution was added to 10 mL of aquadest. After being diluted 250 times, measure the absorbance at 510 nm. The acquired data are graphed about the standard curve of Quercetin. The quantification of total flavonoid is denoted in milligrams of Quercetin equivalent per gram of extract.[11]

Determination of Total Saponin

100 mg extract was combined with 2 mL of 25% H2SO4 and autoclaved for 120 minutes at 100 °C. Subjected to ether extraction and subsequently dried. Add 1 mL of aquadest and agitate for 5 minutes. Add 50 µl of Anisaldehyde, agitate, and allow to stand for 10 minutes. A 2 mL solution of 50% H2SO4 was introduced and thereafter subjected to a 10-minute heating period at 60 °C. A volume of 10 mL of Aquadest was added. After being diluted by a factor of 10, measure the absorbance at a wavelength of 435 nm. The acquired findings are graphed about the established Quillaja bark curve. The quantification of total saponin is denoted in milligrams of Quillaja bark equivalent per gram of extract.[12]

Determination of Total Tannin

100 mg of the sample was subjected to extraction using 10 mL of methanol for 20 hours at ambient temperature, followed by filtration. The residual substance is subjected to boiling with 10 mL of distilled water, followed by cooling and filtration. The resulting extract was supplemented with 10 mL of aquadest. The extract solution was combined with 0.1 mL of Folin-Ciocalteu reagent and subjected to vortexing for 5 minutes. Subsequently, 2 mL of 20% Na2CO3 was added and subjected to vortexing for an additional 5 minutes. A volume of 10 mL of Aquadest was added, and the dilution was performed five times. The measurement of absorbance was taken at a wavelength of 760 nm following a 30-minute incubation period at room temperature. The acquired results are graphed depicting the relationship with the Tannic Acid standard curve. The quantity of tannin is quantified in milligrams of Tannic Acid equivalent per gram of extract.[13]

Determination of Total Alkaloid

100 mg extract was combined with 5 mL of 2N HCl and agitated. The solution was subjected to three washes using 10 mL of CHCl3 in a separating funnel, after which the CHCl3 phase was disposed of. They were nullified using a 0.1N NaOH solution. A volume of 5 mL of BCG and 5 mL of buffer phosphate were introduced. The solution was subjected to extraction using 5 mL of CHCl3, which was agitated with a 1500 rpm magnetic stirrer for 15 minutes. This process was repeated twice. The resulting CHCl3 phase was then collected and evaporated using N2 gas. Finally, 10 mL of CHCl3 was added to the solution. After diluting by a factor of 10, measure the absorbance at 480 nm. The collected values are graphed about the Quinine standard curve. The total alkaloid is quantified in milligrams of Quinine equivalent per gram of extract.[14]

Antioxidant Activity

The antioxidant activity of the extract (DPPH) was assessed by measuring the free radical reduction activity of 2,2-Diphenyl-1-picrylhydrazyl. The extract was dissolved in methanol at five distinct concentrations. A volume of 1 mL of extract was combined with 1 mL of a 0.1 mM DPPH solution. The mixture was then incubated for 30 minutes at room temperature and in a dark environment. Subsequently, the absorbance of the solution was quantified by employing a UV-Vis spectrophotometer at a specific wavelength of 517 nm. Pure methanol was used as the blank solution, whereas DPPH was employed as the control solution.[15] The percentage of DPPH radical inhibition for each concentration of the sample solution was determined using a formula:

$$\text{Inhibition (\%)} = [(\text{Control Abs} - \text{Sample Abs}) / \text{Abs. control})] \times 100$$

The IC50, which represents the 50% inhibitory concentration, was determined by graphing the percentage inhibition against the concentration of the sample. Quercetin served as the reference standard.

Animals

The male Wistar rats are 3-4 months old and weigh between 150-200 grams. The rats were maintained under controlled environmental conditions, including a temperature range of 24-26 °C, a light cycle of 12 hours a dark cycle of 12 hours, and a humidity level ranging from 70% to 75%. Rats were given a standard diet and ad libitum water during the experiment. The Ethics Committee on Medical and Health Research, Faculty of Medicine, Tadulako University approved the utilization of animals and experimental methods in this work (No: 1183/UN28.1.30/KL/2022).

Determination of Blood Glucose Levels

The male white rats are partitioned into six groups, with each group consisting of five rats. The groupings were categorized in the following manner: The experimental groups included the untreated group (normal control), the diabetic group receiving a 0.5% Na CMC suspension (negative control), the diabetic group receiving a 0.45 mg/kg bw/day Glibenclamide suspension (positive control), the diabetic group receiving a 150 mg/kg bw/day suspension of ethanol extract of *C. atropurpureus* leaves (dose 1), the diabetic group receiving a 200 mg/kg bw/day suspension of ethanol extract of *C. atropurpureus* leaves (dose 2), and the diabetes group receiving a 250 mg/kg bw/day suspension of ethanol extract of *C. atropurpureus* leaves (dose 3). The participants in the study were administered a single intraperitoneal injection of STZ at a dosage of 40 mg/kg body weight, which was formulated in 0.1 moles of citrate buffer at a pH of 4.5. The rats underwent an 8-hour fast on the third day following the induction, after which their blood glucose levels were once again assessed. Oral therapy is administered for 28 days when rat blood glucose levels have reached a state of hyperglycemia (> 200 mg/dl). The glucometer (Accu-Chek®) is utilized to measure blood glucose levels. The researchers collected and evaluated data on blood glucose levels before and after therapy at specific time intervals (7, 14, 21, and 28 days).

Statistical Analysis

Blood glucose level results were analyzed using analysis of variance (ANOVA) and followed by continued by the Mann-Whitney Test to determine significant differences ($p<0.05$).

RESULTS AND DISCUSSION

The ethanol extract of *C. atropurpureus* leaves was subjected to a phytochemical screening test, which revealed the presence of flavonoids, saponins, alkaloids, and tannins (Table-1). These substances have been identified as possessing pharmacological activity, particularly in the context of antidiabetic activity. Multiple investigations have demonstrated that the antidiabetic action of certain medicinal plants or herbs can be attributed to these specific chemicals.[17] The quantitative analysis revealed that tannins exhibited the highest concentrations in comparison to other secondary metabolites, as seen in Table-2 and Fig.-1.

Table-1: Phytochemical Screening of 96% Ethanol Extract of *C. atropurpureus* Leaves

Phyto-constituents	Test performed	Results
Flavonoid	Shinoda	+ ve
Saponin	Froth formation	+ ve
Tannin	FeCl3	+ ve
Alkaloid	Dragendorff's	+ ve
Yield (% w/w)		6,7

Table-2: Total Flavonoid, Saponin, Tannin, and Alkaloid of 96% Ethanol Extract of *C. atropurpureus* Leaves

Contents	Results (mg/g extract)
Total Flavonoid Equivalent Quercetin	21.613 ± 6.680
Total Saponin Equivalents Quillaja bark	52.967 ± 0.660
Total Tannin Equivalents Tannic Acid	91.520 ± 2.539
Total Alkaloid Equivalents Quinine	12.660 ± 3.547

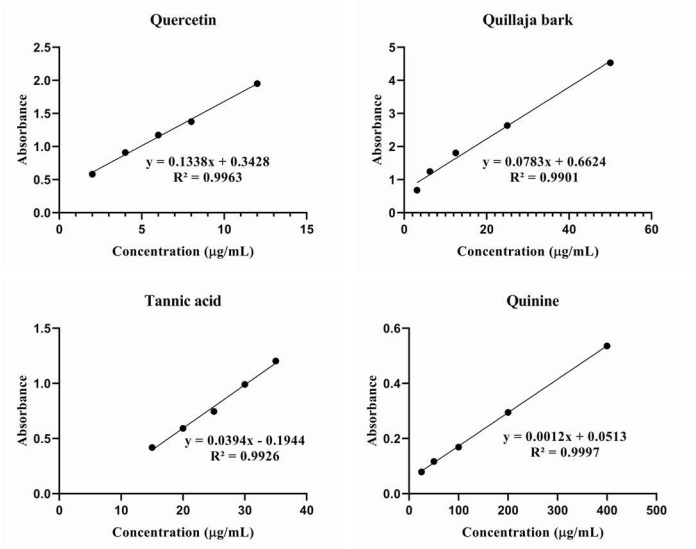

Fig.-1: Calibration Curve of Quercetin, Quillaja bark, Tannic acid, and Quinine

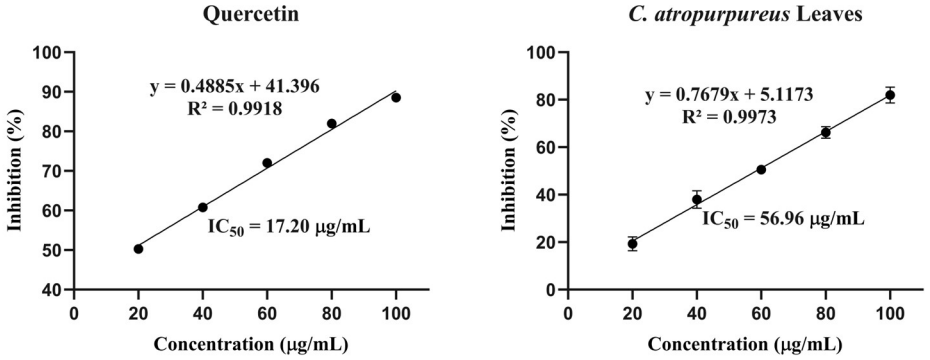

Fig.-2: Antioxidant Activity (IC50) of 96% Ethanol Extract of *C. atropurpureus* Leaves

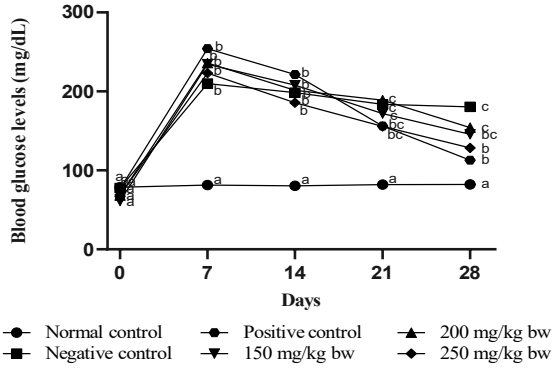

Fig.-3: Blood Glucose Levels in Experimental Rats after 1, 7, 14, 21, and 28 Days of Treatment. Different Letters Indicate Significant Differences, $p < 0.05$ (Man Whitney Test)

The ethanol extract derived from the leaves of *C. atropurpureus* exhibited the maximum antioxidant activity, as indicated by an IC50 value of 56.96 µg/mL. This antioxidant activity was approximately three

times lower compared to quercetin, as depicted in Fig.-2. The amount of antioxidant activity is significantly influenced by the presence of secondary metabolites, particularly flavonoids and tannins. Antioxidants that counteract oxidative stress can hinder and restore harm caused by free radicals, thus aiding in the prevention of issues associated with diabetes. The effect of the ethanol extract of *C. atropurpureus* leaves on the blood glucose levels of experimental rats is shown in Fig.-3. The blood glucose concentration on day 0 remains constant. Significant differences were observed between the negative control, positive control, and doses of the ethanol extract suspension of *C. atropurpureus* leaves compared to the normal control throughout the 7th to 14th day following STZ induction. The positive control and the administration of the ethanol extract solution *C. atropurpureus* leaves at a dosage of 250 mg/kg demonstrated a significant reduction in hyperglycemia compared to the other groups after the 21st and 28th days. However, it is important to note that this reduction did not reach the same level as observed in the normal control group. The experimental findings related to the ethanol extract derived from the leaves of *C. atropurpureus* showed the presence of flavonoids, saponins, tannins, and alkaloids, as presented in Table-1 and Table-2. The antidiabetic effect of each of these secondary metabolites is associated with their unique processes, as outlined in the following description. Flavonoids exhibit antidiabetic properties using GLUT4 synthesis and translocation, hexokinase activity enhancement in the liver, reduction of beta cell apoptosis, stimulation of peroxisome proliferator-activated gamma receptor (PPAR-γ) overexpression to enhance glucose uptake, activation of the AMPK pathway, inhibition of tyrosine kinase activity, and activation of nuclear factor-κB (NF-κB).[18] The hypoglycemic effect of saponins is attributed to their ability to restore the insulin response, enhance insulin signaling, elevate plasma insulin levels, and stimulate insulin secretion from the pancreas.[19] Tannins can impede the absorption of glucose in the intestines and postpone the development of insulin-dependent diabetes mellitus. This is achieved by exerting an insulin-like influence on tissues that are sensitive to insulin. Consequently, the regulation of the antioxidant environment of pancreatic β cells leads to a reduction in glucose levels.[20] Alkaloids have been found to effectively combat hyperglycemia by enhancing glucose utilization and glycogen production. They achieve this by modulating metabolism through the inhibition or induction of various molecules, such as AMP-activated protein kinase, glucose transporter 4, glycogen synthase kinase-3, sterol regulatory element binding proteins 1, glucokinase, glucose-6-phosphatase, acetyl-CoA carboxylase, peroxisome proliferator-activated receptor, and protein of tyrosine phosphatase 1B. These activities contribute to the stimulation of insulin.[21]

CONCLUSION

Secondary metabolites in the ethanol extract of *C. atropurpureus* leaves are thought to have a synergistic effect in reducing blood sugar levels in STZ-induced rats. Isolation of the chemical compound responsible for this effect and its mechanism requires further investigation.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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