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Quantitative Analysis of Phytochemical Compounds and Antihyperglycemic Potential of Robusta Coffee from West Lampung

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West Lampung Regency in Lampung Province produces high quality robusta coffee with a distinct, strong bitter flavour. Bitter flavour indicates the amount of bioactive substances produced by plants called phytochemicals. The aim of this study is to analyse the phytochemical compounds and antihyperglycemic potential of robusta coffee beans from West Lampung, Indonesia. Quantitative phytochemical analysis was carried out using the thin layer chromatography method and spectrophotometry. Animal experimental design using robusta coffee on hyperglycemia conditions in mice which was induced by alloxan 170 mg/kgBW subcutaneously and given by 1 ml of brewed robusta coffee for 10 days. The robusta coffee from West Lampung contained total caffeine 4,014.87 µg/g, total flavonoid content (TFC) 93.6 mg quercetin equivalent per gram of sample, with total alkaloid content (TAC) 0.848 mg of quinine equivalent per gram of sample, total tannin content (TTC) 182.3 mg of tannic acid equivalent per gram of sample, and total saponin content (TSC) 24.2 mg of quillaja bark equivalent per gram of sample. The administration of 1 ml/mice/day of robusta coffee brewed for 10 days did not show a decrease in blood sugar level in hyperglycemic mice, due to the short duration of the study, so the role of coffee in decreasing hyperglycemia conditions has not been optimally observed.

Key words: alloxan, hyperglycemia, Lampung, quantitative phytochemical, robusta coffee

INTRODUCTION

Robusta, arabica, and liberica are the three coffee varieties grown in Indonesia. Lampung Province is the second largest producer of robusta coffee (*Coffea canephora*) in Indonesia with West Lampung Regency becoming the central area of robusta coffee plantations (BPS-Statistics Indonesia 2023). One of the most notable features of Lampung's robusta coffee beans is their intensely bitter taste. Bitter taste of beverages is typically linked to the amount of bioactive substances from the phenolic and alkaloid groups (Herawati *et al.* 2019). Bioactive substances primarily derived from plants are known as phytochemicals. Phytochemical analysis indicates the presence of bioactive compounds in different parts of robusta coffee plants. Bioactive component found in robusta coffee beans is composed of alkaloids, flavonoids, saponins, tannins, caffeine, and phenols (Wigati *et al.* 2019), while the leaves contain alkaloids, flavonoids, terpenoids, and phenols (Kurang and Kamengon 2021; Maxiselly *et al.* 2022).

Based on previous studies, the presence of bioactive phytochemicals in coffee beans has been linked to the positive effects of regular consumption of coffee (Mendoza and Silva 2018). Robusta coffee extract could inhibit the growth of several bacteria, including *Bacillus subtilis, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. It may also act as an antibiofilm due to its ability to inhibit *P. aeruginosa* biofilm (Muttaqin *et al.* 2022; Suryanti *et al.* 2023). On the other hand, robusta coffee from Jambi, located in the east coast of Sumatera, could decrease blood sugar levels in hyperglycemic mice (Riany 2019). Phytochemical analysis and antihyperglycemic potential of robusta coffee originating from Lampung has not been widely reported. Robusta coffee is a major commodity in Lampung Province, which makes this study extremely significant. Therefore, the aim of this study is to analyze the phytochemical compounds and antihyperglycemic potential of robusta coffee beans from West Lampung, Indonesia.

MATERIALS AND METHODS

Quantitative Phytochemical Analysis.

Robusta Coffee Preparation. Robusta coffee beans come from Batu Ketulis District, West Lampung Regency, Lampung Province (-5.029323582025426, 104.2525900542842). The coffee beans were roasted

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at 180°C for 5 minutes then ground into powder. The process of roasting coffee at this temperature is to maintain the bioactive content in robusta coffee (Supriana *et al.* 2020).

Total Caffeine. 100 mg of robusta coffee powder was put into a microtube, then 1 ml of absolute ethanol was added, vortexed and sonicated for 1 hour. Next, the solution was macerated for 24 hours, vortexed and centrifuged. Spotting 1 µL of sample on a silica gel 60 F254 plate, including the caffeine standard. The plate was inserted into a chamber containing a saturated mobile phase of ethyl acetate-methanol-water (100:113.5:10), then eluted to the limit, removed, dried and read at a wavelength of 272 nm Rf. 0.70 (Palacios *et al.* 2017).

Total Flavonoid Equivalent Quercetin. The standard used in determining flavonoids was quercetin. 50 mg of robusta coffee powder was added with 0.3 ml of 5% sodium nitrite, and waited 5 minutes. Then added 0.6 ml of 10% aluminum nitrate, and waited 5 minutes. Next, 2 ml of 1 M sodium hydroxide was added, adjusted to a volume of 10 ml, and diluted 50 times. The absorbance of the sample was read at a wavelength 510 nm (Malak *et al.* 2021).

Total Alkaloid Equivalent Quinine. The standard used in determining alkaloids was quinine. 50 mg of robusta coffee powder was added to 5 ml of 2N HCl, shaken, and then filtered. The solution was washed three times with 10 ml of chloroform in a separating funnel, and the chloroform phase was discarded. The solution was neutralized by adding 0.1 N NaOH, then 5 ml of BCG solution and 5 ml of Phosphate Buffer were added. After that, the solution was extracted with 5 ml of chloroform and stirred with a magnetic stirrer at 500 rpm for 15 minutes. The sample extraction with chloroform was repeated twice. The chloroform phase was collected and evaporated using nitrogen gas, then chloroform was added to bring the volume to 5 ml. The absorbance of the sample was read at a wavelength of 470 nm (Nurlaelasari *et al.* 2023).

Total Tannin Equivalent Tannic Acid. The standard used in determining tannin was tannic acid. 50 mg of robusta coffee powder was extracted with 10 ml of diethyl ether for 20 hours, then filtered and the remaining diethyl ether was evaporated. Following this, distilled water was added to the sample to reach a volume of 10 ml. A total of 1 ml of sample solution was taken and added with 0.1 ml of Folin Ciocalteu reagent, then vortexed and waited 5 minutes. The sample was added with 2 mL of 20% sodium carbonate, vortexed and waited for 5 minutes, then distilled water was added until it reached a volume of 10 ml. Samples were diluted 25 times, incubated for 30 minutes at room temperature, and absorbance of the sample was read at a wavelength 760 nm (Nurlaelasari *et al.* 2023).

Total Saponin from Quillaja Bark. The standard used in determining saponin is saponin from Quillaja bark. 50 mg of robusta coffee powder was combined with 2 ml of 25% H_2SO_4. Subsequently, it was placed into an autoclave for 120 minutes at 110°C. The filtrate was extracted with ether, dried, was added with 1 ml of water, and vortexed for 5 minutes. To the solution, 50 µL of anisaldehyde was added, shaken, and left to stand for 10 minutes. Following this, the solution was given with 2 ml of 50% sulfuric acid and heated in a water bath 60°C for 10 minutes. Water was added to reach a volume of 10 ml using a volumetric flask and then diluted five times. The absorbance of the sample was read at a wavelength 435 nm (Tandi *et al.* 2022).

The total flavonoid equivalent quercetin, total alkaloid equivalent quinine, total tannin equivalent tannic acid, and total saponin from quillaja bark of the samples were calculated using the formula (Wijayanti *et al.* 2023):

$$C = \frac{c \,.\, V \,.\, DF}{m}$$

Where:

C = total phytochemical content mg/g sample
c = sample concentration from calibration curve in mg/L
V = volume of sample in L
DF = dilution factor
m = mass of sample in gram

Animal Experimental Design: Effect of Robusta Coffee on Hyperglycemia Conditions in Mice. There were 3 mice used in this test. All mice had their initial blood sugar levels checked (Day 1), then alloxan was induced subcutaneously at a dose of 170 mg/kgBW dissolved in 0.3 ml of aqua pro injection. Alloxan induction aims to cause hyperglycemia in mice. Alloxan can partially degrade pancreatic islet beta cells (β) and subsequently reduce the quality and quantity of insulin produced by these cells (Ighodaro *et al.* 2017). After 5 days post-alloxan induction (Day 6), the mice's blood sugar levels were measured to ensure that the mice experienced hyperglycemia (blood sugar levels above 200 mg/dl). Robusta coffee powder was brewed with hot water at 95°C, then the brewed robusta coffee was given to mice at a rate of 1 ml/mice/day for 10 days. Next, the decrease in mice's blood sugar was measured twice, on Day 11 and Day 16. All blood sugar measurements were fasting blood sugar, where mice were fasted for 6 hours before their blood sugar was checked (Dewi *et al.* 2018).

RESULTS

The result of quantitative phytochemical analysis which was conducted on robusta coffee from West Lampung, including Total Caffeine, Total Flavonoid, Total Alkaloid, Total Tannin, and Total Saponin, as shown in Table 1. The robusta coffee used in this study contained 4,014.87 µg/g of total caffeine. The total flavonoid content (TFC) on robusta coffee contained 93.6 mg of quercetin equivalent per gram of sample, with total alkaloid content (TAC) contained 0.848 mg of quinine equivalent per gram of sample, total tannin content (TTC) 182.3 mg of tannic acid equivalent per gram of sample, and total saponin content (TSC) 24.2 mg of quillaja bark equivalent per gram of sample.

In this study, robusta coffee was tested *in vivo* on mice to evaluate its antihyperglycemic effects. The results of blood glucose measurements in mice are attached in Figure 1.

Based on Figure 1, on Day 1 before induced by alloxan, the three mice showed normal initial blood sugar, 82 mg/dl, 109 mg/dl, and 111 mg/dl. After alloxan induction and waiting for 5 days, on the Day 6 all mice showed an increase in blood sugar above 200 mg/dl (hyperglycemia), namely 225 mg/dl, 386 mg/dl, and 587 mg/dl. Alloxan was induced in mice as a diabetogenic agent which can cause hyperglycemia in mice (blood sugar above 200 mg/dl). Furthermore, the administration of 1 ml/mice/day of brewed robusta coffee for 10 days did not show a decrease in blood sugar level in mice. Only the Mice-2 showed a decrease in blood sugar from 386 mg/dl (Day 6) to 366 mg/dl (Day 11) and further to 342 mg/dl (Day 16), while the other two mice (Mice-1 and Mice-3) showed fluctuations in blood sugar level on Day 11 and Day 16. However, the normal condition of hyperglycemic mice has not been achieved. This may be due to the short duration of the study, so the role of coffee in decreasing hyperglycemia conditions has not been optimally observed.

Table 1. Quantitative analysis of phytochemical compounds on robusta coffee

Parameter	Result	Equivalent
Total caffeine	4,014.87 µg/g	-
Total flavonoid	93.6 mg/g	Quercetin
Total alkaloid	0.848 mg/g	Quinine
Total tannin	182.3 mg/g	Tannic acid
Total saponin	24.2 mg/g	Quillaja bark

DISCUSSION

In general, robusta coffee originating from West Lampung contains various phytochemical compounds such as caffeine, flavonoid, alkaloid, tannin, and saponin in line with study conducted by (Wigati *et al.* 2019) where extract from robusta coffee beans cultivated in Bandung, Bogor, and Garut, West Java contained alkaloids, flavonoids, saponins, and tannins, the same as Robusta coffee grown in Central Java (Temanggung, Boyolali, Wonosobo) and East Java (Jombang, Malang, and Kediri) (Utami *et al.* 2018). Furthermore, the results of the phytochemical characteristic test showed that the robusta coffee seed extract obtained from Malang, East Java contains flavonoids, alkaloids, terpenoids, tannins, and saponins (Nada *et al.* 2021). The crude methanolic extracts of

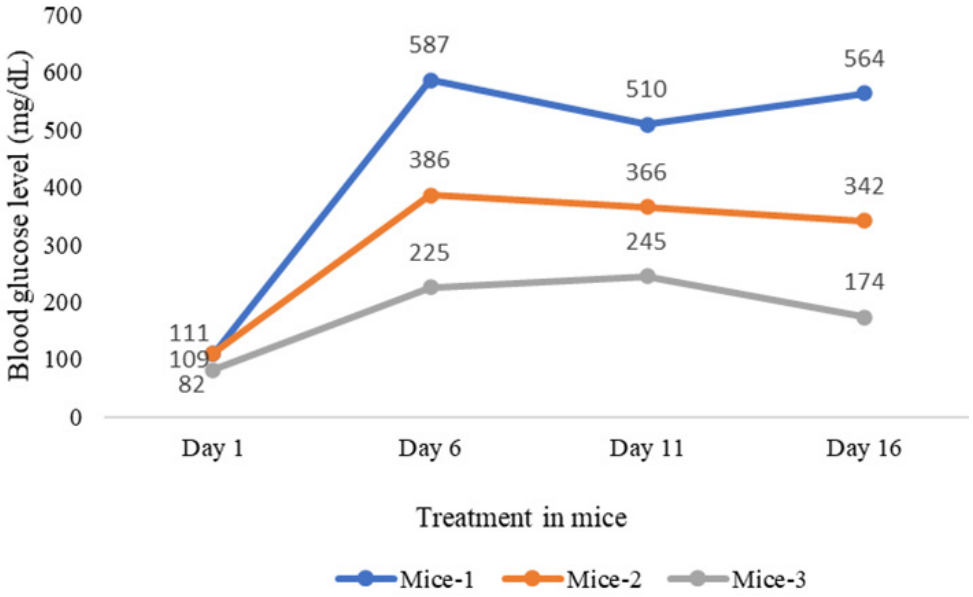

Figure 1. Blood glucose level in hyperglycemic mice. Day 1: initial (before induced by alloxan); Day 6: alloxan-induced hyperglycemia; Day 11: 5 days after treated by 1 mL brewed robusta coffee; Day 16: 10 days after treated by 1 ml brewed robusta coffee (all blood sugar measurements were fasting blood sugar, where mice were fasted for 6 hours before their blood sugar was checked)

robusta coffee from Karnataka, India showed results for phenolic, flavonoid, steroid, flavonoid, saponin, and terpenoid, also carbohydrates and proteins (Mahajan and Kapoor 2018).

The results of quantitative analysis of phytochemical compounds were shown in Table 1. Based on several studies, the total caffeine content of robusta green coffee from India of 182 mg/100 g coffee (1.82 mg/g), which was higher than arabica green coffee of 154 mg/100 g (Caracostea *et al.* 2021). The amount of flavonoids in robusta coffee varies, with a value of 7.98 mgQE/g (robusta coffee from Situbondo, East Java) (Ngibad *et al.* 2023), the crude methanolic extract of robusta coffee from Karnataka, India had an amount of flavonoids 76.04±0.23 mgQE/g (Mahajan and Kapoor 2018), the robusta coffee extract from West Lampung had total flavonoid with a value of 127.33±3.79 mg QE/g extract, and total alkaloid 356.90±41.51 mg caffeine equivalent/g extract (Suryanti *et al.* 2023). In other hand, Robusta coffee pulp from Jambi, Indonesia had total flavonoid content (TFC) 6.38±0.45 mg catechin equivalent/100gDW, total tannin content (TTC) 10.45±0.77 mg tannic acid equivalent/100 gDW, and total saponin content (TSC) 0.50±0.02 g vanillin equivalent/100 gDW (Maxiselly *et al.* 2023). Caffeine acts as a mild psychoactive stimulant drug. Many plant species, including coffee, cocoa beans, cola nuts, and tea leaves, contain varying amounts of caffeine in their seeds, leaves, or fruits (Muhammed *et al.* 2021). Flavonoid is categorized as an antioxidant with the ability to donate hydrogen atoms or to chelate metals (Kurang and Kamengon 2021). Naturally, saponin acts as a chemical barrier in the plant defense system to counter pathogens and herbivores (Augustin *et al.* 2011). Tannins are plant polyphenols with the properties of binding to proteins and antioxidant activities (Okuda and Ito 2011).

Normal blood glucose conditions of hyperglycemic mice had not been achieved in this study. This is similar with Adriansyah *et al.* (2020), reporting that the administration of functional robusta coffee powder fortified with cherry leaf powder for 8 days was not able to decrease the blood sugar levels of mice to normal. Basically, robusta coffee has the potential to be an antihyperglycemic agent, several studies have reported that infusion of Sidikalang coffee beans (*Coffea canephora* var. robusta) is effective in reducing fasting blood sugar levels in Wistar rats (*Rattus norvegicus* L.) (Rangkuti and Mourisa 2023). The administration of green coffee bean water extract can provide protection against pancreatic tissue damage, especially in the Langerhans islets, in diabetic mice. Robusta green coffee bean water extract has the highest chlorogenic acid content and it offers the greatest protective effect compared to arabica and liberica. This demonstrates that green coffee bean water extract can be considered as an alternative treatment to control blood sugar levels in diabetes conditions (Khairunnisa *et al.* 2022). In the current study, brewed robusta coffee treatment was carried out over a period of 10 days (Day 6 to Day 16), whereas the treatment given by Khairunnisa *et al.* (2022) lasted for 14 days. As a result, the protective effect of robusta coffee through blood glucose reduction has not yet been observed. On the other hand, ethanol extract of robusta coffee beans (*Coffea chanefora* L.) at a dose of 400 mg/kgBW has a very effective effect in reducing fasting blood glucose levels and temporary blood glucose levels in rats (*Rattus norvegicus*) with type II diabetes mellitus. Type II diabetes mellitus is the condition when response to insulin is diminished or insulin resistance (Rusman *et al.* 2022). Compared to previous study, the result of this recent study showed that the effect of brewed robusta coffee given for 10 days did not lower blood sugar levels in mice because alloxan selectively and cytotoxically affects pancreatic β-cells, causing β-cells destruction and type 1 diabetes mellitus (Yin *et al.* 2018). In addition, the administration of robusta coffee in this study was in the form of brewing (water extract). According to Evacuasiany *et al.* (2005), the antidiabetic ability of ethanol extract at the same dose is stronger than that of water extract in hyperglycemia mice induced by alloxan.

Additionally, robusta coffee bean powder also has an effect for speeding up the wound closure time in male mice with hyperglycemia induced by alloxan (Susanto *et al.* 2009). The positive effect of coffee can reduce blood sugar levels because it contains chlorogenic acid (Napitupulu and Kristineke 2019). Chlorogenic acid is the second most abundant component in coffee after caffeine. Chlorogenic acid contained in coffee has been proven to lower blood glucose levels, as evidenced by the significant difference in blood glucose levels before and after consumption (Maulidia and Jatmiko 2021). Various studies have proven the benefits of chlorogenic acid on blood glucose, such as delaying intestinal glucose absorption and inhibiting hepatic glucose output. Chlorogenic acid has a mechanism for reducing intracellular hyperglycemia and acts as a polyphenolic compound which works as a strong antioxidant in coffee (Yustisiani *et al.* 2013; Feyisa *et al.* 2019).

In conclusion, the robusta coffee from West Lampung contained total caffeine 4,014.87 µg/g, total flavonoid content 93.6 mg quercetin equivalent per gram of sample, with total alkaloid content 0.848 mg of quinine equivalent per gram of sample, total

tannin content 182.3 mg of tannic acid equivalent per gram of sample, and total saponin content 24.2 mg of quillaja bark equivalent per gram of sample. Furthermore, the administration of 1 ml/mice/day of robusta coffee brewed for 10 days did not show a decrease in blood sugar level in hyperglycemic mice, due to the short duration of the study, so the role of coffee in decreasing hyperglycemia conditions has not been optimally observed. Further research with various concentrations of robusta coffee extract over a longer period of time is required to observe the potential effect as antihyperglycemic.

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