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Test Of Secondary Metabolites And Total Phenols Of Robusta Coffee Grass (Coffea Canephora) Bener Meriah District

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Abstract: Coffee is one of the plantation commodities which has an important role in Indonesia's economic activities. The development of coffee processing, both on a small scale and on an industrial scale, will produce by-products called coffee waste. Coffee grounds waste is waste produced from the coffee processing process which reaches almost 45% of total production. The aim of this research was to determine the secondary metabolite and total phenol content of robusta coffee grounds in Bener Meriah Regency. The method used in this research is the experimental method. The resulting coffee grounds will be extracted using a solvent, and the resulting extract will be tested for secondary metabolites and total phenols. This test will be carried out in the chemistry laboratories of FKIP Unimal and FKIP USK. The results of testing the secondary metabolite content and total phenol content in Robusta coffee grounds were Alkaloids (Dragendrof, Mayer and Wagner), Saponins, Flavonoids, Polyphenols and Steroids, and the total phenol content was 87.38 (mg GAE/g extract).

Keywords: Secondary Metabolite, Total Phenol, Robusta Coffee

1. Introduction

Coffee is one of the plantation commodities which has an important role in Indonesia's economic activities. The development of coffee in terms of area, production and productivity in recent years has fluctuated. In 2019, the coffee area in Indonesia reached 1,245,358 ha with production of 752,511 tons [3].. Robusta coffee (Coffea canefora) is a type of coffee that is widely cultivated in Indonesia and is one of the leading commodities. Robusta coffee plants have been shown in several studies to be quite resistant to disease attacks, and have a characteristic taste that is more bitter, slightly sour and contains higher levels of caffeine than Arabica coffee.

According to research [4] robusta coffee beans from Aceh Regency are known to contain alkaloids, flavonoids and polyphenols, while in Gayo Regency robusta coffee contains alkaloids, flavonoids, terpenoids and polyphenols. The development of coffee processing, both on a small scale and on an industrial scale, will produce by-products called coffee waste. Skin, husk and coffee grounds are waste produced from the coffee processing process which accounts for almost 45% of the coffee fruit and can be valuable ingredients including the extraction of caffeine and polyphenols. Solid and liquid waste produced from the wet coffee processing stage is very high [9]. From 720 tons of coffee production, 324 tons of coffee grounds waste was obtained or around 45% of total production. Coffee waste contains several toxic chemicals such as

alkaloids, tannins and polypenolics which make it more difficult for the environment to degrade organic materials biologically [11]. However, the content of secondary metabolites and total phenols in coffee grounds can vary because it is influenced by geographical location and initial treatment of the coffee beans.

Based on the problems and background above, further research is needed regarding the content of secondary metabolites and total phenols in robusta coffee from Bener Meriah Regency. This content data can be used as a guide for making more useful use of coffee bean waste. This research aims to: 1) Knowing the secondary metabolite content found in Robusta coffee grounds (Coffea canephora) 2) Knowing the total phenol content found in Robusta coffee grounds (Coffea canephora)Robusta coffee is known as coffee that is resistant (robust) to various diseases and changing environments, has superior properties and grows very quickly, therefore robusta coffee is widely cultivated in Indonesia.

The following is the classification of Robusta coffee Kingdom: Plantae Sub-Kingdom: Angiosspermae Class: Dicotyldoneae Sub-Class: Sympetalae Order: Rubiales Family: Rubiaceae Genus: Coffea Sub-Genus: Eucoffea Species: Coffea canephoraRobusta coffee fruit is elliptical in shape with an average fruit length of 12 mm. Robusta coffee berries can be harvested after they are 10-11 months old. The size of robusta coffee beans is around 20-40% of the size of the fruit. Robusta coffee is often referred to as second class coffee beans, which have a slightly sour taste or even no sour taste at all [13].Coffee grounds are residue from processing soluble coffee. The daily volume of coffee grounds ranges from 0.91 to 1.86 kg for every kilogram of soluble coffee. Coffee grounds can be used as a planting medium for oyster mushroom cultivation by utilizing the remaining nutrients contained in coffee grounds left over from brewing. Basically, coffee grounds have adequate nutrition to be used as organic fertilizer. Coffee has an acidic pH level (6.9 to 6.2) so it helps speed up the composting process in the planting medium.The nutritional content of coffee grounds or stale coffee can be an alternative for fulfilling plant nutrition. Apart from the acidic pH level, the contents of coffee grounds are 2.28 percent nitrogen, 0.06 percent phosphorus and 0.6 percent potassium. Coffee grounds can increase the intake of nitrogen, phosphorus and potassium needed by plants so that they can fertilize the soil (Arya, 2017).

Secondary metabolites are organic compounds produced by plants that do not have a direct function in photosynthesis, growth or respiration, solute transport, translocation, protein synthesis, nutrient assimilation, differentiation, formation of carbohydrates, proteins and lipids. Secondary metabolites which are often only found in one species or a group of species are different from primary metabolites (amino acids, nucleotides, sugars, lipids) which are found in almost all plant kingdoms [7]. Secondary metabolites are classified into three groups, namely terpenoids, phenolics, and alkaloids. Secondary metabolite compounds do not play a direct role in plant life but play a role in cell interactions with their environment, such as protecting plants against biotic and abiotic stress. Secondary metabolites are usually used as ingredients in medicines, flavorings, fragrances and insecticides. Secondary metabolites are formed by plants outside the carbohydrate and protein biosynthesis pathway (Dian, 2016)Phenolic compounds are the largest group of compounds synthesized by fruits, vegetables and other plants. The structure of the phenol compound can be seen in Figure 3.3. Phenolic compounds are divided into four main groups, namely phenolics with aromatic rings, quinones, and polymers. Phenolic compounds have antioxidant activity, the higher the phenol content of a plant, the stronger its antioxidant activity [13]. Phenolic compounds are secondary metabolite compounds found in plants with the characteristic of having an aromatic ring containing one or two hydroxy groups (OH). In plants, this group of compounds has several functions, namely: • Cell wall builders (lignin) • Flower pigments (anthocyanins) • Growth control (flavonols) • Defense (flavonoids) • Inhibit and stimulate germination (simple phenols) • Odor (vanillin , methyl salicylate).

2. Materials and Methods

Metabolite Tests and Total Phenols of Robusta Coffee Grounds (Coffea canephora) in Bener Meriah Regency was carried out for 8 (eight) months at the Chemistry Laboratory of FKIP Malikussaleh University and the Chemistry Laboratory of FKIP Syiah Kuala University. The sample for this research is robusta coffee grounds from Bener Meriah Regency taken from coffee shops that use robusta coffee from Bener Meriah and from coffee farmers in Bener Meriah Regency.Chemicals used 95% ethanol, aluminum foil, secondary metabolite testing materials include (mercury (II) chloride, potassium iodide, picric acid, bismuth (II) nitrate, hydrochloric acid, iodine, propanol, sulfuric acid, acetic acid, iron (III) chloride hexahydrate, gelatin, sodium hydroxide, ammonia, chloroform and lead (II) nitrate) filter paper, ethyl acetate, n-hexane, dichloromethane, distilled water, 50% glucose and Gallic AcidThe research tools used are digital scales, vacuum rotary evaporator, separating funnel, UV Vis and glassware.

Extraction by Maceration. A total of 20 grams of the dried sample was weighed and put into a maceration vessel (jar), 250 ml of 96% ethanol was added to the sample while stirring repeatedly and left overnight at room temperature. The next day, the sample is filtered and poured into a clean beaker. The dregs were rinsed again with 20 ml of 96% ethanol to extract the remaining colored substances. The extract obtained is then closed and stored in a place protected from sunlight for a day, the precipitate formed is separated and then the filtrate is concentrated in a water bath (Okoduwa, 2015). The extract resulting from the maceration of the sample is then separated from the solvent using a rotary evaporator.Secondary Metabolite Testing (Phytochemicals)

Flavonoid Test. The flavonoid test is carried out as follows. Jamblang fruit peel extract was added with a few drops of 0.1 M lead acetate solution. The formation of a yellow precipitate indicated the presence of flavonoids. Alkaloid Test. The alkaloid test is carried out as follows. Samples of jamun fruit skin were added with 1 mL of 10% ammonia solution. Add sufficient chloroform and stir. Separated chloroform and placed in a test tube. Add 1 mL of 2 N HCl or 2 N H_2SO_4 solution, shake not too vigorously and let stand. Samples were separated into 6 parts. In part 1, 2 drops of Meyer's reagent solution were added. In part 2, 2 drops of Hager's reagent solution were added. In part 3, 2 drops of Dragendorff's reagent solution were added. In part 4, 2 drops of Wagner's reagent solution were added. In part 5, 2 drops of Burchard's reagent solution were added. Part 6 is used as a blank. Triterpenoids and Steroids. Triterpenoid and steroid tests are carried out as follows. Jamun fruit peel extract is dissolved in chloroform and filtered. The filtrate was dripped with a few drops of anhydrous acetic acid, then boiled and cooled. Add concentrated sulfuric acid to the cold solution. The formation of a brown ring indicates the presence of triterpenoids, while the green color indicates the presence of steroids. Polyphenol Test. The polyphenol test is carried out as follows: A total of 1 mL of jamun fruit peel extract was added to 1 mL of hot water (heated for 5 minutes). Add 1 mL of 1 M $FeCl_3$ solution. The color of the blue green or blue black solution indicates the presence of polyphenolic compounds. Tannin Test. The tannin test was carried out as follows: A total of 1 mL of jamun fruit peel extract was added to 1 mL of hot water. Add 1 mL of 10% gelatin solution to the sample. The formation of a cloudy white solution indicates the presence of tannin compounds. Quinones Test. The quinone test is carried out as follows:A total of 1 mL of jamun fruit peel extract was added to 1 mL of hot water (heated for 5 minutes). Add 1 mL of 1% NaOH solution to the sample. The formation of a red solution indicates the presence of quinone compounds. Saponin Test. The saponin test is carried out as follows:A total of 1 mL of jamun fruit peel extract was added to 1 mL of hot water (heated for 5 minutes). The sample was shaken for 10 seconds. The formation of foam that remains approximately 1 cm high and 1 drop of 0.1 N HCl is added indicates the presence of saponin compounds [8]

Testing Total Phenol Content A standard curve for gallic acid was made with varying concentrations of 0.4; 0.8; 1.2; 1.6; 2.0; and 2.4 mg/L and the absorbance was measured at 765 nm. The sample measurement procedure was carried out by inserting 0.4 mL of sample and 0.4 mL

of Folin-Ciocalteu reagent into a 10 mL measuring flask. The mixture was then shaken for five minutes. After that, add 4 mL of 7% Na2CO3, adjusting with distilled water until the volume is 10 mL. The solution was incubated for 40 minutes at 23 0C and the absorbance was read at λ=765 nm using a UV-Vis spectrophotometer.

3. Results and Discussion

3.1 Sample Preparation

Preparation of robusta coffee grounds is done by washing the coffee grounds using water until clean, then letting it air dry. The dried robusta coffee grounds are then blended until they become powder, this aims to increase the surface area so as to speed up the reaction in the maceration process. The samples used in the maceration process were filtered using a 36 mesh sieve.The extraction method in this research was carried out by maceration, namely by soaking Robusta coffee grounds using 96% ethanol solvent for 1x24 hours with several stirrings.

The function of stirring is so that the powder and solvent are mixed evenly. The advantage of this maceration method is that it is easy and does not require heating so there is little chance of the natural ingredients being damaged or decomposed [12] Then the filtrate is placed in a rotary evaporator and thickened using a hair dryer.The maceration process with 500 grams of robusta coffee grounds ethanol extract produces 30 grams of robusta coffee grounds ethanol extract. This extract has a characteristic blackish brown color and has a distinctive odor.

Figure 3.1. Sample Maceration

3.2 Secondary Metabolite Testing of Robusta Coffee Grounds (Coffea canephora)

Secondary metabolite testing on Kopu Robusta dregs was carried out through phytochemical testing. Phytochemical testing is carried out to identify the active compounds contained in plants. In this research, the test was carried out by taking a small sample of the macerated extract, then adding reagents according to the compound to be identified. The results of phytochemical tests on the ethanol extract of Robusta coffee grounds show that there are bioactive compounds, namely alkaloids, flavonoids, steroid saponins and polyphenols. Secondary metabolite test results can be seen in Table 3.1

UJI	POSITIF	NEGATIF	INFORMATION
1. Alkaloid			
a. Mayer	√		Formed brownish red
b. Dragendrof	√		An orange-brown precipitate is formed
c. Wagner	√		A reddish color forms
2. Saponin	√		Bubbles form

3. Tanin		√	No cloudy white solution is formed
4. Polifenol	√		A blackish blue solution is formed
5. Flavonoid	√		A yellow solution is formed
6. Kuinon		√	No Red Solution Formed
7. Steroid	√		Green Color Formed
8. Triterpenoid		√	No red solution formed

a. Alkaloids

Alkaloids are nitrogen base compounds that are generally found in plants. This compound has strong biological activity and is often used as a medicine or poison. Some tests for alkaloids are as follows: Mayer's test is used to detect alkaloids in samples. The positive reaction which produces a brownish red color is caused by the formation of a complex between the alkaloid and potassium mercury iodide in solution. Dragendroff's test involves the reaction between alkaloids and bismuth subnitrate in acetic acid solution. This reaction produces an orange-brown precipitate if alkaloids are present. The Wagner test detects alkaloids using a solution of iodine in potassium iodide. Positive reactions occur when iodine interacts with alkaloids, forming red or brownish red complexes, [4]

b. Saponin

Saponin is a compound that has surfactant properties, namely being able to reduce the surface tension of water. Saponins are usually found in plants and have activity as a foam producer. The saponin test is carried out by adding the sample to water and shaking it. Saponin will produce stable foam or bubbles because of its ability to reduce the surface tension of water. This reaction indicates the presence of saponin in the sample.

c. Polyphenols

Polyphenols are chemical compounds that have many hydroxyl groups (-OH) attached to aromatic rings. Polyphenols generally have antioxidant properties. The polyphenol test is carried out by adding $FeCl_3$ (ferrous chloride) solution to the sample. If polyphenols are present, a reaction will usually occur with iron which produces a greenish blue solution, indicating the presence of polyphenolic compounds [6].

d. Flavonoid

Test Flavonoids are a class of polyphenolic compounds widely found in plants, which have antioxidant and anti-inflammatory activity. Flavonoid tests usually use hydrochloric acid (HCl) and magnesium reagents. When flavonoids are detected, this chemical reaction will produce a yellow color. This color is produced due to the interaction between magnesium and the flavonoid structure which contains carbonyl groups [7].

e. Steroid

Test Steroids are lipophilic compounds that have the basic structure of cyclopentanoperhydrophenanthrene. Steroids are often found in various plants and animals. The steroid test uses Liebermann-Burchard reagent, which will produce a green color if steroid compounds are present. This reaction occurs due to the interaction between steroids and acetic anhydride and concentrated sulfuric acid, which produces a green color in certain compounds.

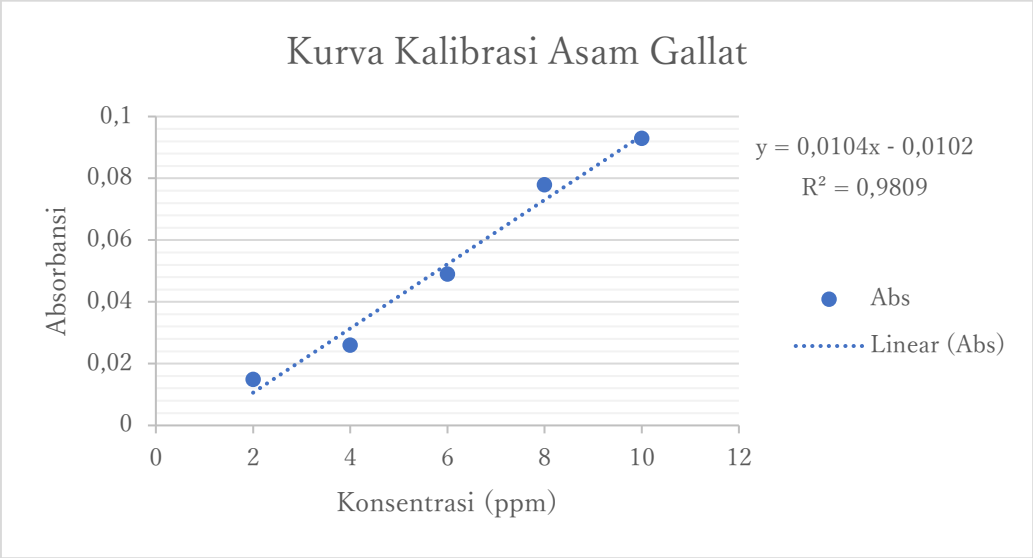
3.3 Testing Total Phenols of Robusta Coffee Grounds (Coffea canephora)

Total phenol content was measured on Robusta coffee grounds extract. The principle of measuring total phenol content with the Folin - Ciocalteu reagent is based on the reducing power of the hydroxy phenol group which is characterized by the formation of a blue complex compound. Gallic acid is used as a measurement standard because it is a derivative of hydroxybenzoic acid which is classified as a simple phenolic acid and is stable (Lee et al, 2003). The results of measuring the standard absorbance of gallic acid are presented in Table 3.2

Table 3.2 Results of Gallic Acid Standard Absorbance Measurements

Concentration (ppm)	Absorbance
2	0.015
4	0.026
6	0.049
8	0.078
10	0.132

From the data above, a standard curve for gallic acid can be created as shown in Figure 2 below.



The standard curve for gallic acid has a linear regression equation y = 0.0104x – 0.0102 with a regression coefficient of 0.9809. From this equation, the total phenol content can be obtained which is calculated based on this equation or the sample absorbance is plotted in a standard curve as shown in Table 3.3.

Table. 3.3 Total Phenol Content of Robusta Coffee Grounds Extract

Sample	Absorbance	Phenolic Content (mg GAE/g extract)	SD
Robusta Coffee Grounds	0.916	87.38	0.19

Based on the table above, it is known that the total phenols in Robusta coffee extract are 87.38 mg in 1 gram of extract. Phenolic Content in Coffee Coffee, especially Robusta coffee, is known to contain high levels of phenolic compounds, which act as antioxidants. Research shows that coffee grounds, even though they are considered waste, still contain quite high levels of phenolics and have the potential to be a source of useful active ingredients [2].

4. Conclusions

Based on the research results, it can be concluded: The secondary metabolite content found in Robusta coffee grounds extract is alkaloids, flavonoids, saponins, polyphenols and steroids. The total phenol content in Robusta coffee grounds extract is 87.38 mg in 1 gram of extract. This paper and the research behind it would not have been possible without the exceptional support of our teams, Chemistry Education, and Mechanical Engineering, Malikussaleh University. This research was supported by Lembaga Penelitian dan Pengabdian Kepada Masyarakat (LPPM) AKSI-ADB Malikussaleh University. The generosity and expertise of one and all have improved this study in innumerable ways and saved me from many errors; thank you so much.

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