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Identification of Antioxidants Activity and Phytochemical Compounds of Coffee Powder Robusta (*Coffea Canephora*), Robusta Lanang (*Peaberry Coffee*) And Arabica (*Coffea Arabica*), In Umkm Kopi Kare, Madiun Regency

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Abstract

Coffee is one of the plantation commodities in Indonesia which acts as a source of foreign exchange and is a source of income for one and a half coffee farmers. One of the regions in Indonesia possessing abundant coffee resources is Madiun Regency especially Kare Village. UMKM Kopi Kare produces three types of coffee products, specifically robusta, robusta lanang, and arabica. In order to determine the characteristics of the 3 coffee variants produced by UMKM Kopi Kare, an analysis of antioxidants and phytochemical compounds were carried out. Antioxindant activity was analyzed using DPPH method and phytochemical compounds were analyzed using qualitative method. Based on the antioxidant test results, robusta 83.41 ± 2.64%, robusta lanang 84.71 ± 3.45%, and arabica 84.32 ± 2.66%. The results of the phytochemical test showed that the three variants of UMKM Kopi Kare contained phytochemical compounds including flavonoids, saponins, tannins, and alkaloids.

Keywords: *Antioxidant; Coffee; Phytochemical; UMKM*

Introduction

Coffee is one of Indonesia's leading plantation commodities. The six largest coffee producing regions in Indonesia are in South Sumatra, North Sumatra, Aceh, East Java, South Sulawesi and Bali. The southern Sumatra region is the largest coffee-producing region in Indonesia. South Sumatra's total coffee production in 2020 reached 191,081 tons, while North Sumatra region reached 74,997 tons. The total coffee area in Aceh reached 126,289 hectares, with production in 2020 reaching 73,419 tons. The famous Acehnese coffee is Gayo coffee, which has a strong aroma and consistency. East Java produced 48,498 tons of coffee in 2020, making it the fourth-largest producer of coffee in Indonesia. South Sulawesi produced 33,728 tons and Bali produced 15,300 tons (BPS, 2021). East Java is one of the areas that have produced a great amount of coffee. Coffee-producing areas in East Java include Banyuwangi, Jember, Malang and Madiun regencies. One of the areas in Madiun Regency with abundant coffee resources is Kare village. Coffee production in Madiun Regency in 2020 amounted to 129.76 tons (BPS, 2021).

Coffee growth is strongly influenced by the surrounding environment, such as climate, altitude, rainfall, and wind conditions. Coffee has a bitter taste and it is easily soluble in water. Moreover, coffee has a high content of antioxidants and a variety of phytochemical compounds that can provide analgesic benefits (Arwangga et al., 2016, Farah et al., 2005). Naturally, coffee beans contain phytochemical compounds in the form of alkaloids that act as refreshing ingredients and caffeine, which can reduce pain. Antioxidants are compounds that have antibacterial and anticancer benefits. The area where coffee is grown affects the characteristics of the physico-chemical content of coffee. In this study, an analysis of antioxidant

activity and phytochemical compounds in coffee products was carried out in UMKM Kopi of Kare Village, Madiun Regency.

Method

The materials used in the sample extraction were 3 types of coffee (lanang coffee, arabica, robusta), 95% ethanol, DPPH solution, concentrated HCl, 2N HCl, zinc powder, and maye aquadest reagent.

The tools used were UV VIS 100 DA-X (B-one) spectrophotometer, analytical balance (RADWAG), waterbath (biostellar), electric stove, 100 ml volumetric flask (pyrex), whatman paper, aluminum foil, stirring rod, funnel, and 100 ml glass beaker (pyrex).

Identification of Antioxidant activity

The working procedure began with the making of 0.125 mM DPPH solution in 95% ethanol. Determination of the maximum wavelength was carried out by measuring the maximum absorption of 0.125 MM DPPH blank solution at a range of 510 - 520 nm with an ethanol blank. Furthermore, 2 ml of the sample solution with a concentration of 1 mg/ml was incubated with 3 mL of 0.125 mM DPPH for 30 minutes (dark conditions), in room temperature. Read at maximum absorbance. %antioxidants are determined based on the formula (Suena et al, 2020):

$$\%\text{antioxidan} = \frac{(abs.DPPH - abs.sample)}{abs.DPPH} x\ 100\%$$

Identification of phytochemical compound

Flavonoid

Weigh the sample with a concentration of 10 mg/ml, the sample was dissolved with distilled water until homogeneous in a test tube, 2 ml of the sample that was made was then taken and then transferred to another test tube, 0.15 g of zinc powder and 2 mL of 2 N hydrochloric acid were added and then left for 1 minute. Lastly, 10 drops of concentrated hydrochloric acid were added. If within 2 minutes to 5 minutes a greenish yellow color occurs, then it indicates the presence of flavonoids (glycoside-3-flavonol) (Malik et al, 2014).

Tanin

The sample was dissolved with distilled water with a concentration of 5 mg/ml. 2 ml of the sample was taken and heated for 5 minutes. Then, 2 drops of FeCl¬3 1% were added. If the solution forms a greenish-brown or blue-black color, it is positive that tannins are present (Marlinda et al. 2012).

Alkaloid

The sample was weighed 0.05 g and put into a dry test tube that was labeled. Then, 0.5 ml of 2N HCl was added to each test tube that already contained the sample. Following that step, 4.5 ml of distilled water was added in each test tube. The test tubes containing the sample were placed into a beaker containing distilled water and then heated on an electric stove for 20 minutes. Next, the samples were filtered using whatman paper to produce the filtrate for each sample. 2 drops of Mayer's reagent was then added to the filtrate and changes of each sample were observed. A white or yellowish precipitate that formed indicated the presence of alkaloids (Hanani, 2015).

Saponin

The method used was based on Hanani (2015) with slight modification. Coffee samples weighing 0.15 grams each were transferred into a test tube using a spatula and 15 ml of distilled water was added. The next step was shaking the test tube for 10 seconds and adding 1 drop of 2N HCl. Foam was then present and the changes that occurred in 3 samples were observed (Hanani, 2015).

Result and Discussion

Antioxidant acivity analysis

The antioxidant test method used was the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method, which is a quantitative test method to determine antioxidant activity (Molyneux P, 2004 in Suena, 2020). The principle of quantitative measurement of antioxidant activity using the DPPH method is that there is a change in the intensity of the purple color of the DPPH, which is proportional to the concentration of the DPPH solution. The free radical DPPH that has an unpaired electron will give a purple color. The color will turn yellow when the electrons are paired. This change in the intensity of the purple color occurs due to the reduction of free radicals produced by the reaction of the DPPH molecule with the hydrogen atoms released by the sample compound molecules to form diphenyl picryl hydrazine compounds and cause the DPPH color to decay from purple to yellow (Rizkayanti et al., 2017). Based on the data obtained, each type of coffee powder has antioxidant activity. Robusta coffee powder extract, robusta lanang and arabica as much as 1 mg/ml had antioxidant activity values of 83.41 ± 2.64%, 84.71 ± 3.45%, and 84.32±2.66%. According to Meidina (2015), the average antioxidant activity of robusta coffee extract at a concentration of 12.5% is 56.958%.

The antioxidant activity of coffee is influenced by the time of harvest. Harvest time is closely related to the formation of active compounds in plant parts. The right harvest time for coffee is when it contains large amounts of chlorogenic active compounds (Suena, 2020). Coffee beans contain chlorogenic acid, one of the antioxidents from phenolic compound that can inhibit oxidative damage and hence have an antioxidant effect. Chlorogenic acid belongs to a family of esters formed from the combination of quinic acid and several trans-cinnamic acids, most notably caffeine, pcoumaric, and ferulic acids. Antioxidant activity can deactivate the development of oxidation reactions by forming free radicals. The antioxidant activity of robusta

coffee beans grown in one area with other areas has different characteristics according to the age of the plant used, harvest time, and the environment where it grows or the ecology of the highlands (Amrullah, 2022).

In addition, the antioxidant activity is caused by the polyphenolic compounds contained in coffee. The main polyphenol groups found in coffee include chlorogenic acid (CGA), caffeic acid, and ferullic acid. The amount of chlorogenic acid reaches 90% of the total polyphenols in coffee. Chlorogenic acid is a powerful antioxidant. These compounds work by inhibiting lipid peroxidation, fighting hydroxyl radicals, and fighting superoxide anions. Ferulic acid in coffee has anti-inflammatory, anti-allergic, antibacterial, antiplatelet and antiviral effects. Pharmacologically as an antioxidant, this compound is able to inhibit lipid peroxidation on biological membranes and inhibit superoxide anions. Caffeic acid also works in inhibiting superoxide anion (Suena, 2020).

Phytochemical compound identification

Phytochemical tests include the content of flavonoids, saponins, tannins, and alkaloids which can be seen in **Table 1**.

Variant of Coffee	Flavonoids	Saponins	Tannins	Alkaloids
Robusta	+	+	+	+
Robusta Lanang	+	+	+	+
Arabic	+	+	+	+

Table 1: The results of phytochemical compounds identification of kopi kare variant.

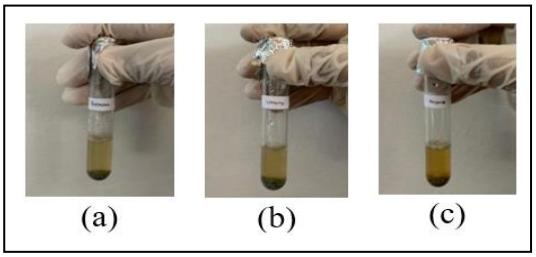

Figure 1: Flavonoid identification of (a) robusta, (b) robusta lanang and (c) arabica coffee.

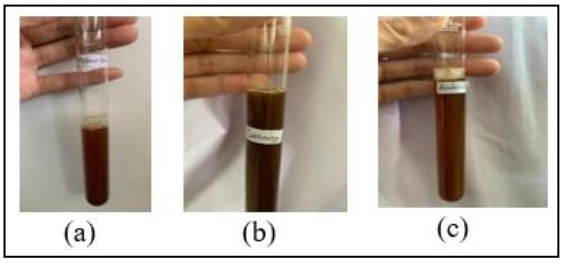

Figure 2: Saponin identification of (a) robusta, (b) robusta lanang and (c) arabica coffee.

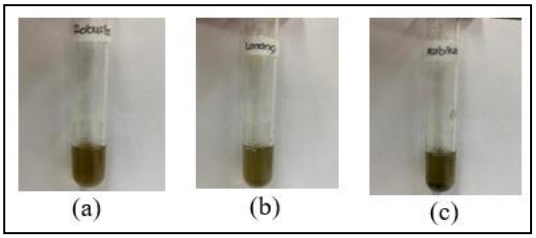

Figure 3: Tannin identification of (a) robusta, (b) robusta lanang and (c) arabica coffee

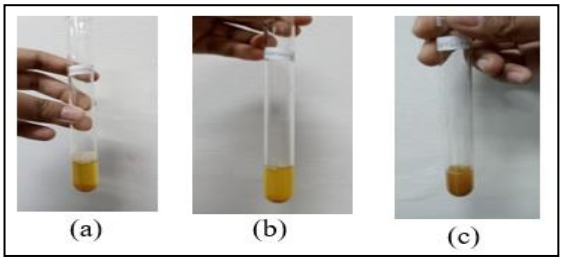

Figure 4: Alkaloid identification of (a) robusta, (b) robusta lanang and (c) arabica coffee

Based on the results of the flavonoid test that has been carried out, it states that arabica, robusta and lanang coffee show positive results, which means that the sample contains flavonoids. The statement that Arabica coffee contains flavonoids was also stated by Handoko (2020) that Arabica coffee showed positive results during flavonoid testing. In Wigati's research (2018), Robusta coffee also produced positive results because there was a change in color after adding metal powder. The color change that occurs in the flavonoid test is caused by the reduction process by hydrochloric acid and metals. This is indicated by a change in the color of the solution to greenish yellow in the sample to which zinc powder and concentrated HCl have been added. The addition of Zn (oxidizing agent) and HCl (helping the hydrolysis process) reduces the benzopyron core that composes the basic structure of flavonoids and forms flavilium salts (Rosidah et al, 2021). This is also supported by Azizah (2019), which stated that metal powder and hydrochloric acid added to the flavonoid test resulted in the reduction of flavonoid compounds contained in coffee, causing a color change in the solution to become greenish yellow which proves the presence of flavonoids.

According to the results of the saponin test, the three coffee samples—robusta coffee, arabica coffee, and lanang coffee—were all positive for saponins. Based on the results of this test, it was appropriate that the positive reaction of saponins formed a steady foam for not less than 10 minutes, as high as 1 cm to 10 cm (Rachman, et al. 2018). Saponins are a type of glycoside found in plants. Saponins are a complex group of natural compounds and have large molecular masses consisting of aglycones, either steroids or triterpenoids with one or more sugar chains/glycosides and based on their chemical properties. Steroidal saponins are mainly found in monocotyledonous plants (such as in the families Agavaceae, Dioscore aceae and Liliaceae), while triterpenoid saponins are mainly found in dicotyledonous plants (such as in the Fabaceae, Araliaceae and Caryophyllaceae families). Saponins have a positive effect that is useful for the body. The positive effects of saponins for health can function as antioxidants, inhibit dental caries activity and platelet aggregation. Saponins are compounds that have anti-inflammatory, analgesic, anti-function, and cytotoxic effects. Many studies have revealed the positive side of saponins, but in fact the use of saponins must be within predetermined limits because inappropriate use can cause adverse effects, so the most effective method for reducing saponin compounds must be determined. The properties of saponins include being soluble in water but not soluble in meters and easily damaged by heat; therefore, boiling and steaming are the preferred methods that can be applied. However, the most efficient temperature and time must be determined (Gunawan, D. H., 2018).

Based on the results of the tannin test on samples of arabica, robusta and lanang coffee grounds, it was proven positive to contain tannins. This was indicated by a change in color to greenish brown after the addition of 2 drops of 1% FeCI3. With the addition of 1% FeCl3 concentration in the three coffee grounds, they will react with Fe3+ ions to form complex compounds (Harborne, 1987). Positive results for terpenoids and steroids are indicated by the presence of red, purple or blue, and green. The color change is formed due to the oxidation of terpenoid or steroid compounds through the formation of conjugated double bonds (Siadi, 2012). The results of the tannin test have been strengthened by other studies, namely Phytochemical Screening and Antioxidant Activity Test of Methanol Extracts of Full-Kerinci River Traditional Processed Coffee Powder and Kayu Aro Tea Using the DPPH Method (1,1-Diphenyl-2-Pikrylhydrazil). This study tested the tannins from the maceration of coffee grounds using methanol with the addition of FeCI3, causing the color to turn green, red, purple, blue-black or dark green. Furthermore, it can also turn blackish green, which means the test is positive for tannins (Oktaviani et al., 2019) **[19]**.

Tannins are polyphenolic compounds that are soluble in water, resistant to biodegradation, and have a wide prevalence in plants. The tannin test was carried out with FeCl3 reagent and the formation of a green, dark blue, or greenish black color indicating the presence of tannins. According to Gunalan et al (2012) **[20]**, chemical components in arabica coffee are tannins, alkaloids, flavonoids, coumarins, quinones, phenols and essential oils. Based on the test results, it can be seen that the fermented arabica coffee contains tannins, which is characterized by a change in color to greenish brown. Chlorogenic acid is a group of tannin compounds contained in arabica coffee with an amount of about 5.5-8,0% (Clarke and Macrae, 1987) **[21]**. Chlorogenic acid is one component that contributes to the acidity of coffee drinks. The results of the phytochemical test showed that the higher the concentration, the lower the tannin in the sample.

The tannin content in robusta and robusta lanang coffee is affected by the roasting temperature. The content of these tannins plays an important role as a resistant agent against microbial decomposition, mainly due to the ability of these molecules to inhibit microbial growth by binding strongly to proteins and polysaccharides, such as cellulose and pectin. Condensed tannins are more resistant to microbial decomposition, while hydrolyzed tannins are more easily degraded by some microorganisms. Tannins occur universally in higher plants and are present in significant quantities in many food crops. Tannins precipitate with heavy metals and alkaloids, thus being often used to treat gastrointestinal toxicity (Linh et al., 2014) **[22]**.

According to the alkaloid test result on samples of robusta, robusta lanang, and arabica, a yellow precipitate formed after the Mayer reagent was added, indicating the presence of alkaloid chemicals. These deposits can occur due to a deposition reaction due to ligand replacement. The nitrogen atom which has a lone pair of electrons in the alkaloids replaces the iodine ion in Mayer's reagent. This results in the formation of a yellowish white precipitate on the addition of Mayer reagent because nitrogen in the alkaloids will react with metal ions K+ from potassium tetraiodomercurate (II) to form a precipitated potassium-alkaloid complex (Marliana et al., 2005 in Sangi et al., 2008). The results of this study are supported by research on powders and ethanol extracts of arabica coffee leaves conducted by Wenas et al (2020), which revealed that the identification of alkaloids using Mayer's reagent with positive results will indicate the formation of a white/yellow precipitate. The results of this study showed that the simplicia powder showed negative results for the content of alkaloids, while the extracts showed positive results. Alkaloids are derivatives of amino acids, have a bitter taste, and are secondary metabolites from plants, animals, fungi, and can be extracted from the source using acids (usually sulfuric acid or hydrochloric acid) (Maharti, 2007 in Fitriani, 2014) **[25]**. Caffeine is an alkaloid compound in the form of white crystals found in coffee (Tanauma et al., 2016).

Conclusion

The antioxidant activity and phytochemical profiles from the extracted robusta (Coffea Canephora), robusta lanang (Peaberry Coffe Bernas), and arabica (Coffea Arabica) were studied. The antioxidant activity of robusta, robusta lanang and arabica is found to be 83.41 ± 2.64%, 84.71 ± 3.45% and 84.32±2.66% respectively. The presence of flavonoids, tannins, alkaloids, and

saponins is identified from these extracts. Further studies are required to analyze other physicochemical properties of these extract.

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