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The Characteristics of Some Commercial Arabica Coffee Beans in Indonesia

(Karakteristik Beberapa Biji Kopi Arabika Komersil di Indonesia)

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Abstract: Arabica coffee is one of the mainstay commodities in the Indonesian plantation sector. Geographical differences and the environment where coffee grow can affect the characteristic, phytochemical content and antioxidant activity of the beans. The aim of this study was to determine the phytochemical characteristics and antioxidant activity of 10 items of commercial Arabica coffee beans in Indonesia. The extraction of 10 coffee beans was carried out by kinetic maceration in 1 hour with 70% ethanol, then evaporated. Each extract was examined for morphology, phytochemical screening, assay of caffeine content using HPLC method, levels of flavan-3-ol, total flavonoid content (TFC), total polyphenolic content (TPC), antioxidant determination using DPPH and FRAP methods. All bean samples showed the presence of flavonoids, alkaloids, triterpenoids, coumarins, and tannins. The chemical content determination showed caffeine content of 1.5-2.9%; flavan-3-ol content 4.85-12.38cat/g extract; TFC 9.71–23.67QE/g extract; and TPC 21.8–76.26GAE/g extract. Tests of antioxidant capacity using the DPPH reveal an inhibiting effect with varying IC_{50} values from 19.49-81.41g/mL and using the FRAP method gave IC_{50} values from 18.01-61.2g/mL. This study indicates that coffee samples have the same phytochemical characteristics and also have an important antioxidant activity, which justifies its potential to be developed into antioxidant nutraceuticals.

Keywords: Antioxidant, caffein content, flavan-3-ol content, TFC, TPC

Abstrak: Kopi arabika merupakan salah satu komoditas andalan sektor perkebunan Indonesia. Perbedaan geografis dan lingkungan tempat kopi tumbuh dapat mempengaruhi karakteristik, kandungan fitokimia, dan aktivitas antioksidan biji kopi. Tujuan dari penelitian ini adalah untuk mengetahui kandungan senyawa fitokimia dan aktivitas antioksidan dari 10 jenis biji kopi arabika komersial di Indonesia. Telah dilakukan ekstraksi pada 10 biji kopi Arabika Indonesia secara maserasi kinetik 1 jam dengan pelarut etanol 70%, kemudian diuapkan. Masing-masing ekstrak diperiksa morfologi, penapisan fitokimia, uji kadar kafein menggunakan metode kromatografi cair kinerja tinggi, kadar flavan-3-ol menggunakan reagen vanillin dan HCl, kadar flavonoid total (TFC) dan kadar polifenol total (TPC) serta penentuan antioksidan menggunakan metode DPPH dan FRAP. Semua sampel biji kopi menunjukkan adanya alkaloid, flavonoid, triterpenoid, kumarin, dan tanin. Penentuan kandungan fitokimia menunjukkan kadar kafein 0,15-0,29%; kandungan flavan-3-ol 4,85-12,38cat/g ekstrak; TFC 9,71–23,67QE/g ekstrak; dan TPC 21,8–76,26GAE/g ekstrak. Pengujian kapasitas antioksidan menggunakan DPPH menunjukkan efek penghambatan dengan nilai IC_{50} yang bervariasi yaitu 19,49 - 81,41 g/mL dan menggunakan metode FRAP memberikan nilai IC_{50} 18,01-61,2 g/mL. Penelitian ini menunjukkan bahwa sepuluh sampel kopi Arabika Indonesia memiliki karakteristik fitokimia yang sama dan juga memiliki aktivitas antioksidan yang penting, sehingga sangat potensial untuk dikembangkan menjadi nutrasetikal antioksidan.

Kata kunci: Antioksidan, kandungan flavan-3-ol, kandungan kafein, TFC, TPC

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INTRODUCTION

COFFEE is one of the most common beverages in the world because it has a unique flavor. Moreover, it has a strong cultural, social, historical, and economic importance. Coffee is one of the most important tropical commodities tradings, including in Indonesia. Coffee plants (*Coffea* sp) are a member of the Rubiaceae family which includes almost 70 species, but those grown on a large scale and sold in Indonesia are Arabica coffee (*Coffea arabica* L) and Robusta coffee (*Coffea canephora* var. robusta). Arabica coffee is one traditional type of coffee with the best taste.

Coffee (*Coffea arabica* L.) is known to have health benefits and one of them is due to its antioxidant properties. Regular coffee consumption has been associated with a reduced risk of mortality and chronic diseases, including cancer (liver, kidney, and to a lesser extent, premenopausal breast and colorectal cancers) and the incidence of melanoma[1,2]. Coffee is a rich source of caffeine (methylxanthines alkaloid) and chlorogenic acids (hydroxycinnamates)[3], Chlorogenic acids are a class of polyphenols and has effective in decreasing blood pressure in hypertensive rats and are safe for patients with mild hypertension[4]. Coffee oil contains triglycerides and fatty acids and has biological activity as anti-cancer, anti-inflammatory, anti-bacterial, anti-diabetic, and anti-atherosclerotic[5]. Coffee also showed the highest content of each class of phenolic group (tannins, flavan-3-ol, and procyanidins). Flavan-3-ol is the main subclass of the flavonoid group which has high antioxidant activity[6].

Geographical and environmental conditions affecting the physical and chemical qualities of coffee beans. In general, Arabica coffee is grown in tropical or subtropical countries. Arabica coffee grows at an altitude of 600-2000m above sea level. The higher the location of the coffee plantation, the better the taste produced by coffee beans because it is related to plant metabolic processes such as biochemical processes and the synthesis of secondary metabolites which also affect growth, morphological characters, and the content of active compounds[7-9].

This study was conducted to determine the phytochemical content and bioactivity of 10 samples of commercial Arabica coffee beans in Indonesia, which so far have only been known as drinks to be developed into nutraceuticals with potential antioxidant properties.

MATERIALS AND METHODS

MATERIALS. Nine Arabica coffee beans specimens were collected from local coffee suppliers. These samples were obtained from various well-known coffee-producing regions in Indonesia, namely Aceh (1), Bali (2), Flores (3), Gayo (4), Papua (5), Puntang (6), Semeru (7), Temanggung (8), and Toraja (10). Also, one specimen was obtained from *Kopina Coffee Farm*, Pangalengan, Bandung, West Java, Indonesia (9). Ethanol, concentrated HCl, ammonia, chloroform, Ferric (III), TPTZ, Na Acetate anhydrous, acetic acid, DPPH, NaOH, petroleum ether, sulfuric acid, methanol pro-HPLC. All chemical materials using analytical grade were purchased from Merck, Caffeine standard (Chem service Inc, Pennsylvania).

Tools. Microplate reader (Versamac), High-Performance Liquid Chromatography (Shimadzu) LC-20 AD.

METHODS. Arabica coffee beans aged 28-34 weeks after blooming (anthesis) have been roasted at a medium level. Physical/morphological examination of coffee beans was carried out. Furthermore, the coffee beans are ground using a coffee grinder to form a powder. All bean samples were tested qualitatively for the presence of phytochemical content such as alkaloids, tannins, steroids/triterpenes, flavonoids, and saponins as per the standard protocols[10]. The coffee bean powder was extracted by kinetic maceration 1 hour with 70% ethanol, remaceration 3 times, then evaporated with a rotary evaporator and dried.

Antioxidant Activity Determination. The microplate radical scavenger DPPH method was based on the 96-well microplate assay given by Bobo *et al* with some modifications[11]. The mixture as listed in Table 1. was pipetted into well Nunc 96, then incubated in the dark for 30 minutes, and absorbance was measured at a wavelength of 517 nm using a 96-well microplate reader (Versamac). The antioxidant activity was calculated according equation: %inhibition of radical = $[(OD_{control\ solution}-OD_{sample\ solution})/ OD_{control\ solution}]$ x 100. All tests were carried out in triples and then the IC_{50} value was calculated.

Table 1. The composition of the solution for the DPPH test.

	DPPH 150 µmol/L in methanol	Extract/standard (20 µg/mL)	Aquadest
Control solution	180 µL	-	20 µL
Sample solution	180 µL	20 µL	-
Blank	-	20 µL	180 µL

The FRAP antioxidant activity method was using the 96-well microplate method[12]. The mixture as listed in Table 2. was pipette into well Nunc-96, then incubated for 30 minutes at 37^{0}C, then read the absorbance with a 96-well microplate reader at a wavelength of 593 nm. The absorbance value obtained is used to calculate the % FRAP capacity (the equality of ferric ions with antioxidant activity (µmol/g)), with the equation %FRAP capacity = [($OD_{sample\ solution}$-$OD_{blank\ solution}$)- $OD_{control\ solution}$ / $OD_{sample\ solution}$] x 100. All tests were carried out in triplicates and then the IC_{50} value was calculated.

Table 2. The composition of the solution for FRAP testing.

	FRAP solution (buffer acetate: TPTZ: $FeCl_3.6H2O$= 10: 1: 1)	Extract/Standard (2500 µg/mL)	Aquadest
Control solution	270 µL	-	30 µL
Sample solution	270 µL	30 µL	-
Blank	-	30 µL	270 µL

Assay of Caffeine . Caffeine content was determined using HPLC (Shimadzu) LC-20 AD according to the procedure[12]. Stationary phase (column): Rcyl C-18 (dimensions 150 x 4.6 mm and pore size 5µm) (Phenomenex), Mobile phase: methanol: water (70:30), Detector: UV-Vis spectrophotometer, Flow rate: 1 mL / min, Length wave: 273 nm. The system suitability test was carried out using a standard 6 ppm Caffeine solution, linearity test, determination of caffeine content in the sample, and accuracy test.

Assay of Flavan-3-ol. Briefly, a volume of 50 µL of extract (1000 and 2000 µg/mL) was added to 50 µLof vanillin reagent (4% in methanol solution freshly prepared) and after 5 min, add 50 µL of concentrated HCl. Blanks were prepared by replacing 4% vanillin solution with methanol. After 15 min incubation in a cold water bath, the absorbance of the sample was measured at 500 nm against a blank sample. The absorbance of the blank sample is subtracted from the absorbance of the sample containing vanillin (Δ E). The content of flavan-3-ol is calculated by the formula: (+)-catechin = 290.8 x ΔE and results are expressed as mg (+)-catechin/g[13].

TPC and TFC. The microplate total phenolic content (TPC) method was based on the 96-well plate using the Folin-Ciocalteu reagent (FCR) described by Bobo *et al* with some modifications[11]. Briefly, a 20 µL sample was incubated with 100 µL FCR (1:4) for 4 min at room temperature and then incubated with 80 µL Na_2CO_3 (7.5%) for 120 min at room temperature and light protected. The absorbance was then read at 750 nm against the reagent blank. Gallic acid (10-200mg/L) was used to generate a calibration curve that was used to calculate phenolic content and identify the mg GAE (gallic acid equivalent)/g extract.

The microplate TFC methodology was based on the 96-well microplate using the $AlCl_3$ reagent described by Horszwald et al[14] with some modifications. Aliquots of 20 µL of coffee extract were mixed with methanol (2000 µg/mL). Subsequently, 20 µl of $AlCl_3$ (10%w/w), 180 µL aquadest, and 20 µL sodium acetate (1M) were added. The sample was incubated at room temperature for 30 minutes. The optical density (OD) was then read at 415 nm against the reagent blank. Quercetin (25-175 µg/mL) in methanol was used to generate a calibration curve that was used to calculate TFC and expressed in mg QE (quercetin equivalent) per g extract.

RESULTS AND DISCUSSION

Macroscopic tests were carried out to determine the morphology of the Arabica coffee beans based on the physical form observed by shape, diameter, width, color, smell, and surface. Macroscopic examination showed all the beans were oval with the size of the bean diameter varying between 0.6-1.8 cm and width between 0.5-0.9 cm, dark brown color, characteristic aroma of coffee beans, and smooth surface. The center was cut in the form of a straight line on the beans of 6 types of coffee while in the other 4 samples there were lines that were not straight (Figure 1).

The phytochemical screening of all bean samples showed the presence of alkaloids, flavonoids, triterpenoids, coumarins, and tannins (Table 3).

The HPLC conditions used to determine the caffeine in samples are chromatographic conditions that have been optimized and validated in previous research reports[15]. This study results that the validated HPLC method is in the range of 50 to 400 g/mL with good linearity (r^2=0.99). The validation data prove that the method is accurate with percent recovery were 97.02 - 102.6 %. The result of the caffeine assay was obtained between 1.5-2.9% with an RSD value of 0.03-0.06% (Figure 2). This is in accordance with

Figure 1. Morphology of medium roasted Indonesian arabica coffee.

Table 3. Phytochemical screening of 10 coffee arabica in Indonesia.

Coffee sample	Yield (%)	Alkaloid	Flavonoid	Steroid/ Triterpenoid	Tannin	Coumarin	Saponin
1	1.84	+	+	+	+	+	-
2	1.98	+	+	+	+	+	-
3	1.62	+	+	+	+	+	-
4	1.52	+	+	+	+	+	-
5	1.66	+	+	+	+	+	-
6	1.56	+	+	+	+	+	-
7	1.26	+	+	+	+	+	-
8	1.21	+	+	+	+	+	-
9	1.79	+	+	+	+	+	-
10	1.32	+	+	+	+	+	-

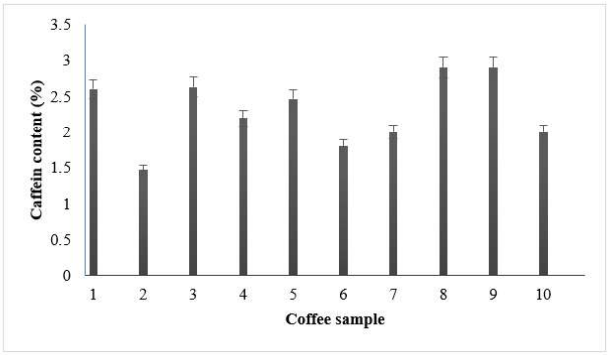

Figure 2. Caffeine content of Indonesian arabica coffee using HPLC method. Data were given in mean±SD, n=3

other studies that show caffeine levels in roasted coffee using HPLC method were 0.66-2.55%. The effect of roasting on coffee is that it significantly reduces the caffeine content of coffee beans, as well as their polyphenol content. Therefore, light or medium roasting is the recommended method for coffee processing, in order to preserve these compounds[13].

Flavan-3-ol is the main subclass of the flavonoid group which has high antioxidant activity. Flavan-3-ols compounds and chlorogenic acid contained in the coffee we consume, will be absorbed in the small intestine and arise in the circulatory system mainly as methylated, sulfate, and glucuronide metabolites[6,13]. The content of flavan-3-ol compounds can

be determined using the addition of vanillin and HCl reagents. The addition of vanillin reagent is intended to attract the metabolite compounds contained, while HCl serves to accelerate the breaking of glycoside bonds between glycone and aglycone compounds[13].

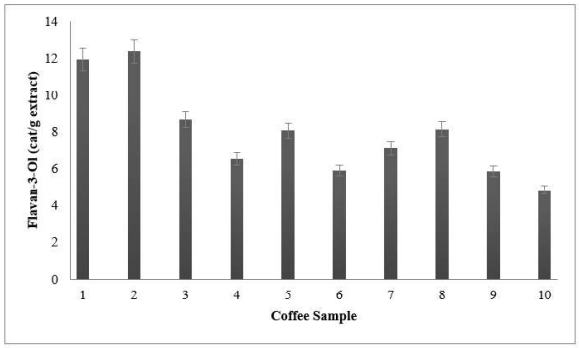

Figure 3. Flavan-3-ol content of Indonesian arabica coffee.
Data were given in mean±SD, n=3

Figure 3, showed that the flavan-3-ol content of Arabica coffee in Indonesia varies between 4.85±1.11 – 12.38±2.75 cat/g extract.

The phytochemical contents of Arabica coffee are polyphenol and their derivatives (such as chlorogenic acid/CGA), alkaloids (especially caffeine), and diterpenoid alcohols (such as kahweol and cafestol), lipids, carbohydrates, and volatile compounds. CGA is an ester of trans-cinnamic acid whose amount in coffee is significantly reduced during the roasting process[16]. The content of phenolic compounds has a significant influence in determining the quality of coffee and also plays an important role in the formation of coffee taste. A cup of coffee contains 200-550 mg of phenolic compounds, especially chlorogenic acid. Some researchs showed that the secondary metabolites are generally involved in plant adaptation to environmental conditions such as shade conditions (temperature and light intensity) which will also significantly affect the physical quality, sugar content, and phenol composition as well as the antioxidant activity of coffee beans[8-9].

Figure 4 shows the results of measuring the total flavonoid and phenolic content of some Arabica coffees in Indonesia, which vary from 9.71±0.82 – 23.67±1.39 QE/g extract and 21.8±7.1 – 76.26±4.2 GAE/g extract, respectively.

Coffee has been considered as a daily drink and has functional benefits as well as an important source of antioxidants in human intake, one of which is because it contains important secondary metabolites such as flavonoids, polyphenols, and caffeines. In recent decades, the compounds have been proposed as one of the most effective functional ingredients in foods and drink, with antiaging properties and able to neutralize the effects of oxidative damage on the skin, antibacterial, antiviral, anti-inflammatory, metalloproteinase expression suppression activity, and oxidative reduction. macromolecular damage[1-2,5,9,16-19]. The results of testing the antioxidant activity of Arabica coffee using the DPPH and FRAP methods gave IC_{50} 19.49± 2.04

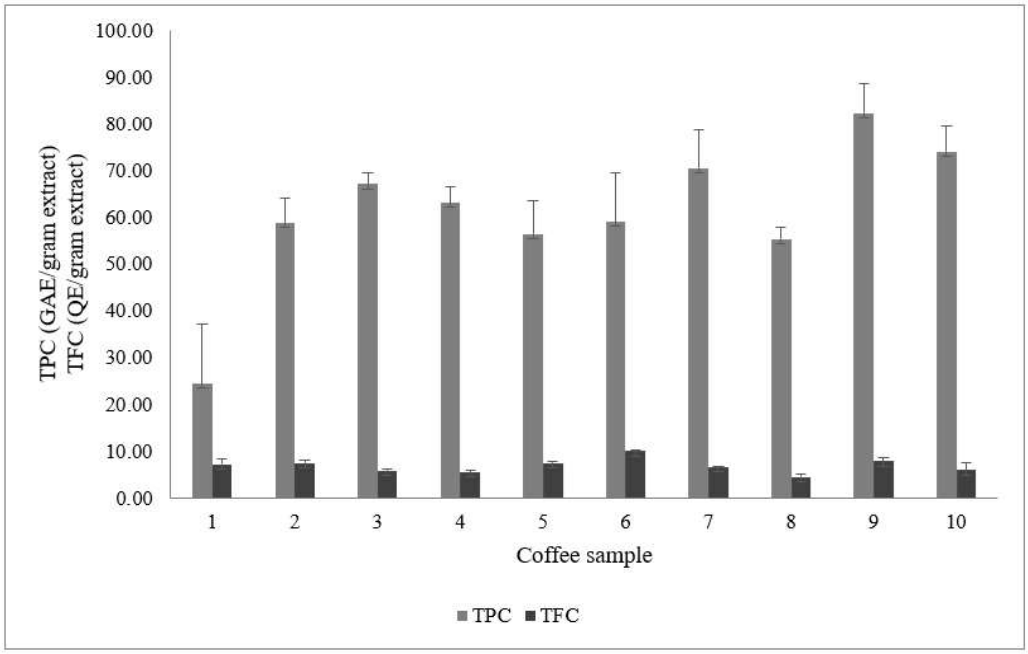

Figure 4. Total phenolic and total flavonoid content of Indonesian arabica coffee.
Data were given in mean±SD, n=3

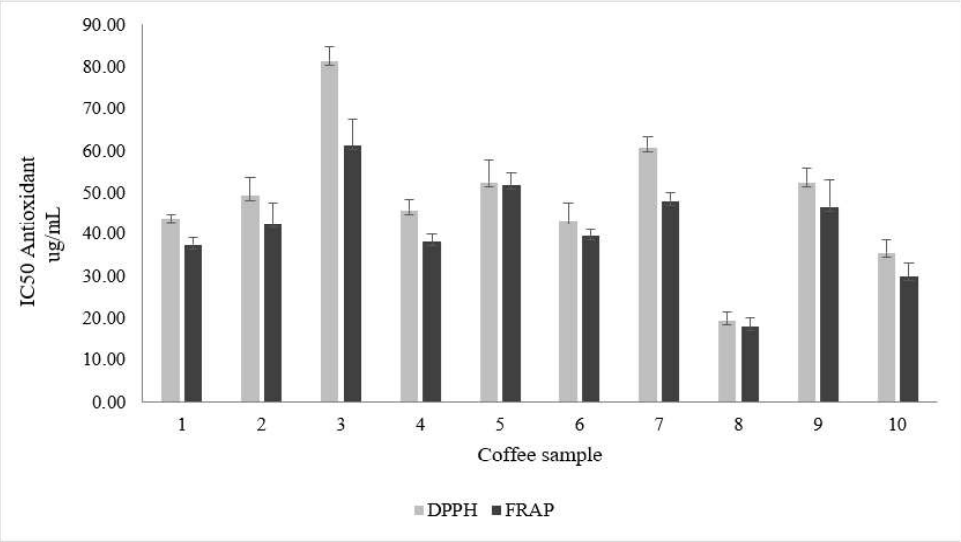

Figure 5. Antioxidant activity of Indonesian arabica coffee. Data were given in mean±SD, n=3

to 81.41±3.29 g/ml and 18.01±1.9 to 61.2±6.32 g/ml of 5-6, respectively (Figure 5). Antioxidant potency is usually associated with the content of phenolic compounds due to their extensive conjugated π-electron systems that facilitate the donation of electrons from the hydroxyl moieties to oxidizing radical species[20,21]. This data further strengthens that Arabica coffee in Indonesia has the potential as a strong antioxidant.

CONCLUSION

The present study shows that ten samples of Indonesian Arabica coffee have phytochemical characteristics in the form of caffeine, flavan-3-ol, flavonoid, and phenolic compounds that support antioxidant activity as well as other bioactivities so that it is very potential to be developed into antioxidant nutraceuticals or other types of preparations such as cosmeceuticals, quasi-drugs, etc.

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