DETERMINATION OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY IN METHANOLIC EXTRACT OF ROBUSTA AND ARABICA COFFEE LEAVES

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INTRODUCTION

Phenols contained in coffee beans may also found in the coffee leaves. Previous research showed that the arabica coffee leaves contain phenolic compounds [1]. Phenolic compounds are compounds that have one or more hydroxyl groups attached directly to an aromatic ring [2]. Phenolics compounds have many biological effects, including antioxidant activity [3].

Antioxidants are the first line for defense against free radical damage, and important for health. The need for antioxidants becomes even more critical with increased exposure to free radicals. Pollution, cigarette smoke, drugs, illness, stress, and even exercise can increase free radical exposure [4].

The aim of this study was to determine total phenolic content and antioxidant activity in methanolic extracts of robusta and arabica coffee leaves (old and young leaves), as potential sources of natural antioxidants. The relationship between phenolic content and antioxidant activity was also investigated.

MATERIAL AND METHODS

MATERIALS

2,2' - diphenyl - l - picrylhydrazyl (DPPH), chlorogenic acid standart, metanol p.a were purchased from Sigma-Aldrich, Folin–Ciocalteau reagent was purchased from Merck, vitamin C pharmaceutical grade (99.8%). All other reagents were analytical grades.

METHODS

Sample Preparation

Young and old leaves of arabica and robusta from a Indonesian Coffee and Cocoa Research Institute were washed, cut, dried and powdered.Young leaves are light green, smooth, young, and recently expanded. Old leaves are rough texture and intense green color.

Extraction

250 g of sample were extracted with methanol 70% (1:7 v/v) at room temperature for 3 day. The extracts obtained were filtered through filter paper and concentrated with a rotary evaporator at 50^{o}C. After that, it was heated in oven to get thick extract.

Determination of total phenolic content

Stock standard solution prepared by dissolving 25 mg of chlorogenic acid in 25 ml methanol. Working standard solutions were prepared by dilution of stock solution with methanol to get solutions in concentration of 100 µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml, 300 µg/ml, 400 µg/ml, 500 µg/ml, dan 600 µg/ml. For sample preparation, 25 mg extract was weighed. The extract was dissolved in 25 ml volumetric flask, and approximately 10 ml of methanol was added. The mixture was ultrasonic and diluted to 25 ml.

0.1 ml Folin–Ciocalteau reagent was mixed with 0.1 ml sample and 1.2 ml aquadest. That mixture held at room temperature for 5 min. Then 0.3 ml of 20 % sodium carbonate solution and 0.3 ml aquadest was added. After 30 min of incubation at room temperature, the absorbance of the solution was determined at 743 nm by spectrophotometer UV Vis. Quantitative measurements were performed, based on a standard calibration curve of eight concentrations. The total phenolic content was expressed as chlorogenic acid equivalents in milligram per gram of weight extract (mg CAE/g).

Determination of antioxidant activity

Stock vitamin C solution prepared by dissolving 25 mg of vitamin C in 25 ml methanol. Working vitamin C solutions were prepared by dilution of stock solution with methanol to get solutions in concentration of 4.99 µg/ml; 9.98 µg/ml; 14.97 µg/ml; 19.96 µg/ml; 24.95 µg/ml; and 29.94 µg/ml. For sample preparation, stock sample solution prepared by dissolving 25 mg of each extract in 25 ml methanol. Working solutions of old arabica and robusta extract were prepared by dilution of stock solution with methanol to get solutions in concentration of 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, dan 60 µg/ml. Then, working solutions of young arabica and robusta extract were prepared by dilution of stock solution with methanol to get solutions in concentration of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml, dan 120 µg/ml.

0.3 ml of sample solution with six different concentrations was added to 1.2 ml 0.1 mM DPPH. The mixture was shaken vigorously and kept for 30 min at room temperature in the dark. The absorbance at 515 nm was measured by a spectrophotometer after 30 min of incubation. The antioxidant activity of the samples was calculated according to the formula:

% Inhibition = $\frac{A0-A1}{A0}$ x 100 %

where A_0 = absorbance of the control

A_1= absorbance of the extract.

The percentage of antioxidant activity was plotted against the sample concentration (µg/ml) to obtain IC_{50}, defined as the concentration of the sample necessary to cause 50 % scavenging of DPPH radical calculated by linier regression curve.

RESULTS AND DISCUSSION

Total phenolics content

Total phenolic content was determined by the Folin–Ciocalteu colorimetric method using chlorogenic acid as a standart [5]. A linear calibration curve of chlorogenic acid, in the range 100–600 µg/ml with r^2 value of 0.997, was constructed (Fig. 1).

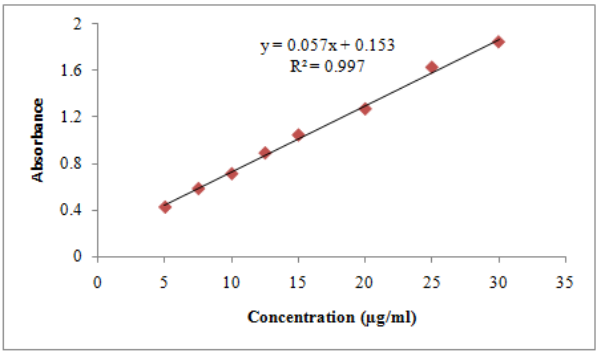

Fig. 1: Standart curve of chlorogenic acid

Phenolic contents of methanolic extracts of old robusta, old arabica, young robusta, and young arabica coffee leaves were shown in Table 1. Methanolic extracts of old robusta coffee leaves showed the highest total phenolic content among all methanolic extracts of coffee leaves, followed by methanolic extracts of old arabica, young robusta and young arabica were 399.403 ± 0.559;354.307 ± 1.204; 244.232 ± 1.761 and 190.916 ± 1.715 mg CAE/g extracts.

Based on the species, methanolic extract of robusta coffee (*Coffea canephora*) leaves had total phenolic content higher than methanol extract of arabica coffee (*Coffea arabica*) leaves in old leaves and young leaves. Previous research suggested that robusta coffee (*Coffea canephora*) beans containing a total phenol content higher than arabica coffee (*Coffea arabica*) beans [5]. High levels of total phenolic content in the seeds was also occurs in the leaves. The leaves and seeds were located in the same species possibility had the same compound.

Based on age, methanolic extract of old leaves had total phenolic content higher than methanol extract of young leaves in both species of coffee leaves. These results were consistent with previous research which determine the total phenolic content in young and old leaves from 8 different plant species [6]. These results suggested that average of total phenolic content in old leaves higher than young leaves.

Total phenolic content was significant difference between methanolic extracts of old robusta, old arabica, young robusta, and young arabica coffee leaves (ANOVA, $P<0.05$).

Determination of Antioxidant Activity

Antioxidant activity was determined by radical scavenging activity using DPPH radical [7]. The result of plotting percentage of antioxidant activity against the sample concentration (µg/ml) were shown in Fig 2.

Measured by DPPH method (Table 2), methanolic extracts of old robusta coffee leaves showed antioxidant activity among all methanolic extracts of coffee leaves, followed by methanolic extracts of old arabica, young robusta and young arabica were 7.519 ± 0.029; 8.317 ± 0.050; 13.678 ± 0.053 and 15.535 ± 0.089 µg/ml, respectively. Vitamin C showed higher antioxidant activity than all methanolic extracts of coffee leaves. IC_{50} value of vitamin C was 3,658 ± 0,032 µg/ml. The antioxidant activity of ascorbic acid was higher than sample. Based on that IC_{50} value, vitamin C and methanolic extracst of coffee leaves were potent antioxidant. A compound said to be potent antioxidants when the value of IC_{50} <50 µg/m [8].

Table 1: Total phenolic content in methanolic extracts of coffee leaves

Sample	Total phenolic content (mg CAE/g extract weight, n=3)
Methanolic extract of old robusta leaves	399,403±0,559[a]
Methanolic extract of old arabica leaves	354,307±1,204[b]
Methanolic extract of young robusta leaves	244,232±1,761[c]
Methanolic extract of young arabica leaves	190,916±1,715[d]

The values are presented as mean ± SD, the different superscript in each row are significantly different ($P > 0.05$).

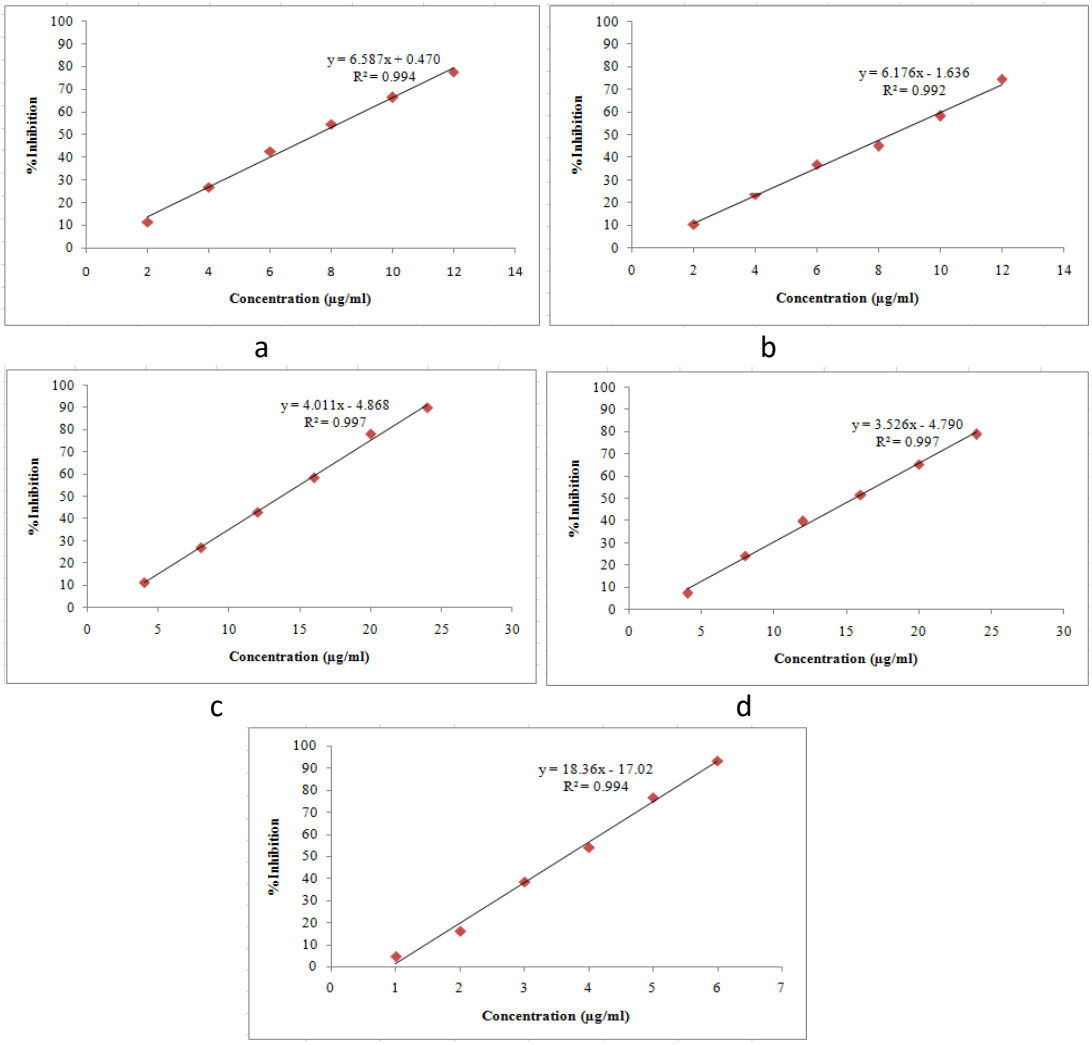

Fig. 2. Plotting percentage of antioxidant activity against samples concentration (n=3)
a) coffee leaves of old robusta, b) coffee leaves of old arabica, c) coffee leaves of young robusta, d) coffee leaves of young arabica, e) vitamin C

Table 2: IC_{50} value ascorbic acid and methanolic extracts of coffee leaves

Sample	IC_{50} value (µg/ml, n=3)
Vitamin C	3,650±0,032[a]
Methanolic extract of old robusta leaves	7,519±0,029[b]
Methanolic extract of old arabica leaves	8,317±0,050[c]
Methanolic extract of young robusta leaves	13,678±0,053[d]
Methanolic extract of young arabica leaves	15,535 ±0,089[e]

The values are presented as mean ± SD, the different superscript in each row are significantly different ($P < 0.05$)

Based on the species, methanolic extract of robusta coffee leaves (*Coffea canephora*) had higher antioxidant activity than methanolic extract of arabica coffee (*Coffea arabica*) leaves in old and young leaves. Previous research showed that robusta coffee beans contain higher antioxidant activity than arabica coffee beans in green and roasted coffee beans [9]. Based on the age, antioxidant activity of old coffee leaves higher than young coffee leaves because old leaves had a total phenol content higher than young leaves.

Antioxidant activity was significant difference in methanolic extracts of old robusta, old arabica,

young robusta, young arabica coffee leaves and vitamin C (ANOVA, P<0.05).

In the present study, there was linier correlation between antioxidant activity and phenolic contents of methanolic extracts of coffee leaves (coefficient r = 0.9865) (Fig. 3). These results suggested that the phenolic compounds contributed significantly to the antioxidant capacity of the investigated plant species. These results were consistent with many research that reported the positive correlation between total phenolic content and antioxidant activity 10]. Antioxidant activity may also come from the presence of other antioxidant secondary metabolites such as alkaloid (caffein dan trigoneline), saponin, and α-tokopherol [11] [12].

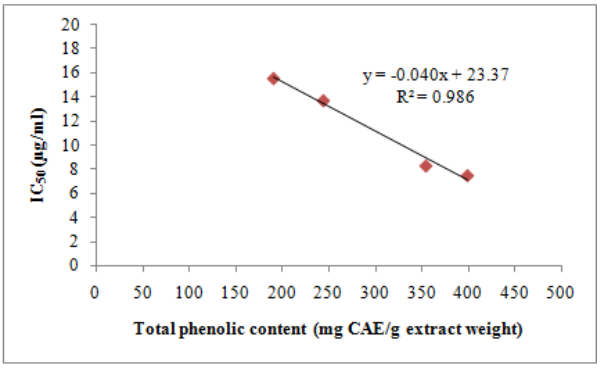

Fig. 3: Relationship between antioxidant activity (IC_{50}) and total phenol content in methanolic extracts of coffee leaves

CONCLUSIONS

Old leaves present a higher of total phenolic content and antioxidant activity than young leaves. Robusta leaves present higher total phenolic content and antioxidant activity than that of arabica leaves. Total phenolic content and antioxidant activity methanolic extract of old robusta coffee leaves, old arabica, young robusta, and young arabica were significantly different. The positively high correlation between total phenolic content and antioxidant activity was given by methanolic extracts of coffee leaves.

REFERENCES

1. Salgado PR, Favarin JL, Leandro RA, Filho OF. Total phenol concentrations in coffee tree leaves during fruit development. *Scientia and Agricola*. **65** (4), 354-359 (2008).
2. Vermerris W, Nicholson R. Phenolic Compound Biochemistry. Netherlands : Springer; (2006).
3. Apak R, Guclu K, Demirata B, Ozyurek M, Celik SE, Bektasoglu B et al. Comparative Evaluation of Various Total Antioxidant Capacity Assay Applied to Phenolic Compounds with The CUPRAC Assay. *Molecules*.**12**: 1496-1547 (2007).
4. Percival M. Antioxidants. *Clinical Nutrition Insights*.; **31** : 1-4 (1998).
5. Perez-Hernandez LM, Chavez-Quiroz K, Medina-Juarez LA, Meza NG. Phenolic Characterization, Melanoidins, and Antioxidant Activity of Some Commercial Coffees from Coffea arabica and Coffea canephora. *Journal of Mexico Chemistry and Society*.; **56** (4): 430-435 (2012).
6. Achakzai AKK, Achakzai P, Masood A. Kayani SA, Tareen RB. Response of Plant Parts and Age on The Distribution of Secondary Metabolites on Plants Found in Quetta. *Pakistan Journal of Botani*. **41**(5): 2129-2145 (2009).
7. Molyneux P. The Use of The Stable Free Radical Diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant Activity. *Journal of Science and Technology*. **26** (2): 211-219 (2004).
8. Zuhra CF, Tarigan JB, Sihotang H. Aktivitas Antioksidan Senyawa Flavonoid Dari Daun Katuk (*Sauropus Androgunus* (L) Merr.). *Jurnal Biologi Sumatera*. **3** (1): 7-10 (2008).
9. Yashin A, Yashin Y, Wang JY, Nemzer B. Antioxidant and Antiradical Activity of Coffee. Antioxidants **2**: 230-245 (2013).
10. Javanmardi J, Stushnoff C, Locke E, Vivanco JM. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food Chemistry. **83**, 547-550 (2003).
11. Erna C. Uji Aktivitas Antioksidan dan Karakteristik Fitokimia pada Kopi Luwak Arabika dan Pengaruhnya terhadap Tekanan Darah Tikus Normal dan Tikus Hipertensi. *Tesis. Depok: Program Studi Magister Ilmu Kefarmasian*; (2012).
12. Farah, A. Coffee constituents in Coffee: Emerging Health Effects and Disease revention. First Edition. United Kingdom: Blackwell Publishing Ltd; (2012).