Research Article

Bioactive components and antibacterial activity in Robusta coffee leaves (*coffea canephora)*

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ABSTRACT

Purpose: To analyze bioactive components and antibacterial activity in brewed Robusta coffee leaves that processed by *Japanese Style Green Tea Process* (JGTP).

Methodology: This was an experimental research with the sample of Robusta coffee leaves and brewed Robusta coffee leaves. The coffee leaves were brewed in water at 70°C during 10 minutes. The analysis of nutrition contents by proximate method, total flavonoids by calorimeter method, chlorogenic acid and caffeine by HPLC method, antioxidant activity by DPPH method, total chlorophyll using the arnon formula and antibacterial activity by disk diffusion method in 5, 10, 15, and 20 g.

Results: There were significant differences in color, taste, and acceptability of brewed Robusta coffee leaves. The best acceptance of brewed Robusta coffee leaves was 5 g of dried coffee leaves. Robusta coffee leaves contain 3.1% water, 6.03% ash, 17.76% protein, 2.37% fat, and 70.83% carbohydrate. The content of flavonoids, chlorogenic acid and caffeine in Robusta coffee leaves were 0.81 mgQE/g, 16.92 mg/g, 0.46 mg while in the brewed were 0.10 mgQE/g, 3.49 mg/g, 0.19 mg/g, respectively. The inhibition of free radical in Robusta coffee leaves were 90.2 - 92.7%, while in the brewed were 83.3 - 86.1%. Total chlorophyll of dried leaves were 63.62 -69.39 mg/L and the brewed were 2.08 - 6.01 mg/L. Antibacterial activity *Escherichia coli* 7.65 – 9.89 mm and *Salmonella tiphy* 7.47-8.70 mm inhibition zone.

Applications/Originality/Value: The role of various bioactive components in Robusta coffee leaves on antioxidant and antibacterial activity.

Keywords: JGTP

INTRODUCTION

Coffee plant (*Coffea sp*.) is included in the family *Rubiaceae*. In general, there are three types of coffee, including Arabica coffee (*Coffea arabica*), Robusta coffee (*Coffea canephora*), and Liberica coffee (*Coffea liberica*). The most widely consumed coffee in Indonesia is Robusta coffee. Robusta coffee can be easily grown at lower altitude of less than 1000 meters above sea level and it resilient against disease and pests.(Kanisius, 1988)

The results of research on Arabica coffee leaves have nutritional content based on proximate components. Proximate components of Arabica coffee leaves from the Ethiopian region include 5.5-7.8% moisture, 8.8-12.4% ash content, 14.4-19.0% protein, 4.5-12.5% fat, and 51.0-63.9% carbohydrate.(Woldesenebet, 2005) Robusta coffee leaves contain phytochemicals that vary depending on leaf development.(X.-M. Chen, Ma, & Kitts, 2018) The younger the coffee leaves, the higher the chlorogenic acid content even 10 times higher than the old coffee leaves.(Campa et al., 2017) The content of chlorogenic acid in Robusta coffee is higher compared to Arabica coffee, respectively 11.3% and 4.1%.(Ky et al., 2001) The caffeine content in young Robusta coffee leaves (0.29-0.5%) is lower than the caffeine content in its fruit (1.6-2.4%).(Khotimah, 2014)

Robusta coffee leaves also contain phenolic acid and total flavonoids, respectively 27.04 µg/g and 10.90 µg/g higher than Arabica coffee leaves respectively 21.80 µg/g and 8.08 µg/g.(Nayeem, Denny, & Mehta, 2011) Flavonoids as natural antioxidants can prevent the occurrence of diseases, such as cancer, metabolic syndrome, diabetes mellitus, and atherosclerosis which associated to oxidative stress.(X. Chen, 2019; Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004)

The content of caffeine, flavonoids, and chlorogenic acid of coffee leaves can also

function as the prevention of diarrhea. Caffeine can inhibit protein and DNA synthesis by inhibiting the merging of adenine and thymidine. Caffeine also increases genotoxicity after DNA damage.(Pruthviraj, Shital, Suchita, & Shilpa, 2011) Flavonoids as an antibacterial by inhibiting nucleic acid synthesis, inhibiting energy metabolism, and changes in cytoplasmic membrane function.(Farhadi, Khameneh, Iranshahi, & Iranshahy, 2018) In addition to flavonoids, there are chlorogenic acid contents that function as antibacterial. Chlorogenic acid is a phenolic bioactive compound causes physiological changes in bacterial cell membranes that encourage damage to bacterial nucleotides.(Kabir, Katayama, Tanji, & Nakamura, 2014; Lou, Wang, Zhu, Ma, & Wang, 2011)

The processing of coffee leaves to obtain optimal antibacterial is done by minimizing chemical and enzymatic reactions to prevent damage in phytochemical content. The process can be obtained using green tea processing.(X.-M. Chen et al., 2018) The processing of green tea is able to produce phenolic components 6 times higher than black tea. Green tea processing aims to maintain the content of natural polyphenols which have beneficial effects on health. (Chacko, Thambi, Kuttan, & Nishigaki, 2010). Therefore, the aim of this research was to examine bioactive components, including caffeine, chlorogenic acid, total flavonoids, antioxidant activity, total chlorophyll, and nutritional contents and antibacterial activity in Robusta coffee leaves by green tea processing.

MATERIALS AND METHODS

The samples used *Robusta canephora* Pierre ex Froehner from Gambung village. Coffee leaves were processed using the Japanese Style Green Tea Process (JGTP) method at the Tea Quality Processing and Testing Laboratory, Tea and Quinine Research Center (PPTK) Gambung, Bandung Regency, West Java, Indonesia. Preliminary research was conducted to obtain the JGTP procedure at the withering stage, including 2 minutes steaming method and 45 s, 60 s, and 75 s of blanching. The best withering method was chosen based on the results of the best organoleptic tests conducted by trained panelists. The best withering result that will be used in the processing of coffee leaves was the blanching method for 75 s.

This study was an experimental study with one factor completely randomized design (CRD) with 2 samples, dried Robusta coffee leaves and brewed Robusta coffee leaves. Dried Robusta coffee leaves were brewed with 200 ml of water in 70ºC for 10 minutes. Samples were analyzed in duplicate with three replications.

Processing Robusta Coffee Leaves with JGTP Method

JGTP method was carried out through 5 steps, picking; blanching; cooling, crushing, and drying. Coffee leaves were picked from 2nd, 3rd, and 4th leaf of each branch of coffee plants, sorted, then carried out the blanching process for 75 s. Leaves were soaked in water and put in seducer for ± 15 minutes while separating the leaves from its midrib. The next process was crushing the leaves three times using a crushing-tearing-curling machine (CTC) (CNC, Sri Lanka). The last process was the drying process. The drying process was 4-5 hours long by a rack drier machine (CNC, Sri Lanka) at 80°C.

Proximate Analysis

Proximate analyses using dried Robusta coffee leaves samples processed by JGTP to analyze the moisture, ash, fat, protein, and carbohydrate contents with AOAC 2005 method.(Analysis., 2005)

Total Flavonoid Analysis

5 grams of dried Robusta coffee leaves in 200 ml of methanol were homogenized with a magnetic stirrer for ± 30 minutes. The solution was taken 60 ml for centrifugation, then coffee leaves produced supernatant in methanol. Meanwhile, 5 grams of dried Robusta coffee leaves were brewed in 200 ml of hot water (70ºC) for 10 minutes and stirred. After being dissolved in water, 60 ml was taken to be centrifuged, coffee leaves supernatant will be produced in brewed water. Afterwards, two samples were analyzed.

Total flavonoid analysis using aluminum chloride ($AlCl_3$) calorimetry method with absorbance measurement using UV-VIS spectrophotometer. 1 ml of sample solution was mixed with 9 ml of methanol. Pipette 2.8 ml sample solution; 0.4 ml $AlCl_3$ 5%; 6.8 ml of 5% acetic acid. Incubation for 30 minutes, measure the absorbance (λ 428 nm). Calibration curves used the standard quercetin in methanol (0.005 mg / ml) with varying concentrations of 0 µg/ml, 5 µg/ml, 10 µg/ml, 20 µg/ml, 40 µg/ml, and 60 µg/ml. Standard was carried out using the same procedures as samples. (Rhayanne T. M. Ramos, Ferreira, & Soares, 2017; Silva, Pezzini, & Soares, 2015)

Analysis of Chlorogenic Acid and Caffeine

Analysis of chlorogenic acid using 20 g of dried coffee leaves extracted with 150 mL methanol:water (50:50 v/v) in soxhlet. After 4-5 cycles, soxhletation was stopped and cooled

down at room temperature. The extract was transferred into a 200 mL flask and the volume was adjusted to 200 mL with a mixture of methanol-water. Meanwhile, caffeine analysis using 10-20 g of coffee leaves were added to 5 g of MgO and put into 200 mL of water. Furthermore, the samples were heated in a water bath at 90°C and sterilized for 20 minutes. Then the extracts were filtered with 0.45uL, 25 mm cellulose filter cartridge. The filtrates were injected into the HPLC.(Naegele, 2016a, 2016b)

Total Chlorophyll Test

The total chlorophyll test was carried out by 5, 10, 15 and 20 grams of brewed Robusta coffee leaves compared to the samples dissolved with 80% acetone. Total chlorophyll test was conducted using 663 nm and 645 nm wave spectrophotometry. Chlorophyll levels were calculated using the Arnon formula:(Prawira-Atmaja et al., 2018)

Chlorophyll a = (12.7 x A663) - (2.69 x A645)
Chlorophyll b = (22.9 x A645) - (4.68 x A663)
Total chlorophyll = (20.2 x A645) + (8.02 x A663)

Analysis of Antioxidant Activity

Sample preparation was performed as a total flavonoid test. Antioxidant activity was analyzed using the DPPH method (1,1-*diphenyl*-2-*picrylhydrazyl*). A DPPH 0.4 mM solution was prepared by 0.00394 g of DPPH powder and dissolved in absolute methanol to 25 ml. The test was done by piping 0.1 ml of samples and sufficient volume with absolute methanol into a 5 ml measuring flask, then vortex. Measurement of antioxidant activity was done by adding 1 ml of DPPH 0.4 mM solution, then vortex for ± 1 minute. Incubate the solutions for 30 minutes in a dark place, measured the absorbance by UV-VIS spectrophotometry (λ517 nm). A blank solution was obtained by piping 5 ml absolute methanol and 1 ml DPPH solution, left for 30 minutes in a dark place and measuring the absorbance as with the samples.(Hasanah, Maharani, & Munarsih, 2017)

Antibacterial Activity Test

Antibacterial activity test was carried out by the disk diffusion Kirby-Bauer method.(Rohdiana, Arief, & Budiman, 2013) This method was used to see the bacterial growth inhibition response based on the diameter of the clear zone of *E. coli* and *S. typhi*. Antibacterial activity test began by dissolving nutrients with aquades. The media stock and the equipment used were put into a 121°C autoclave for 20 minutes. The next step was making a suspension by taking an ose of pure bacteria and implanting it in a solution of NaCl. Next, applied the suspension to the hard agar using a sterile cotton swab. Blank discs that have been soaked in 5, 10, 15, and 20 grams of brewed Robusta coffee leaves for 20 minutes were placed on hard agar and incubated at 37°C for 24-hour incubation periods. Classification of bacterial growth inhibition was observed by clear zone diameter consists of 4 groups: weak response (diameter ≤ 5 mm), moderate (diameter 5-10 mm), strong (diameter 10-20 mm), and very strong (diameter ≥ 20 mm).(Sadino, Sahidin, & Wahyuni, 2018)

Organoleptic Test

Organoleptic test was carried out by 30 semi-trained panelists of Nutrition Sciences students with variations in weight of 5, 10, 15, and 20 grams of dried coffee leaves brewed with water at 70°C. The assessment of quality attributes of brewed coffee leaves used 4 favorite scales, 1 (dislike very much), 2 (dislike slightly), 3 (like slightly), and 4 (like very much).

STATISTIC ANALYSIS

Statistical analysis using univariate and bivariate analysis. If the data is normally distributed, use the paired t test, whereas if the data is normally distributed, use the Wilcoxon test. Analysis of antioxidant activity using the One Way Anova statistical test if normally distributed, and using the Kruskal Wallis test if the distribution is not normal. The analysis was performed with a 95% confidence level with p value 0.05 and $\alpha = 0.05$.

RESULTS

Processing Robusta Coffee Leaves with JGTP Method

Based on the appearance and color aspects, brewed coffee leaves with the blanching method had an average value from 7.5 to 8 (1-10) while the steaming method got a value of 4 (1-10) as seen in Figure 1. The taste of brewed using the blanching method had an average value from 34 to 35 (20-49) and obtaining a value of 27 for brewed by steaming method. The aroma of brewed and pulp of coffee leaves had a value of 5-5.50 (1-10) for the method of blanching water and steaming. From the aspect of the aroma of coffee grounds pulp obtained a value of 9 for each blanching method and 4 for the steaming method (1-10). Based on organoleptic test, the 75-second blanching method was a processing method that had the highest average score.

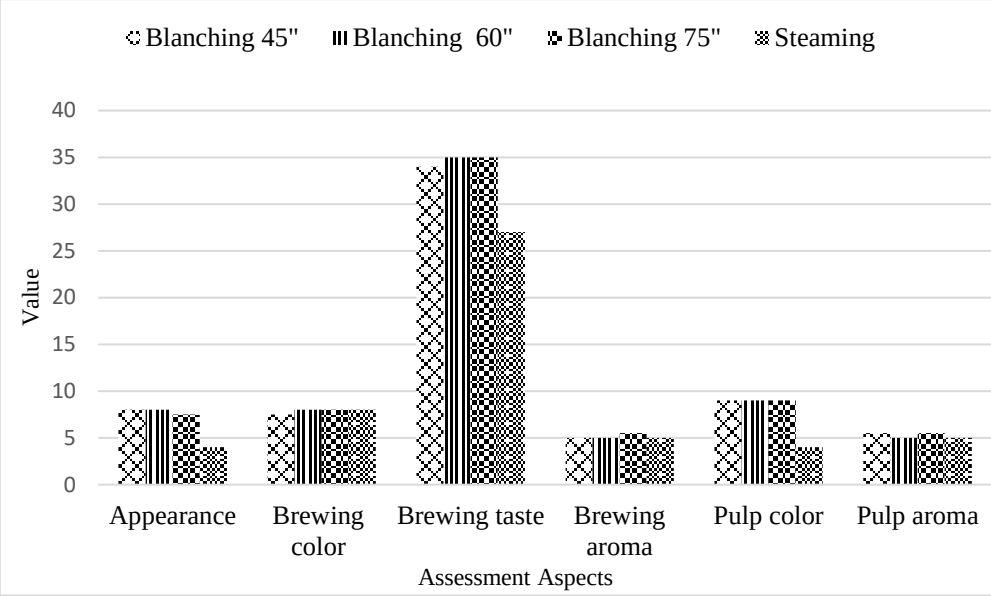

Fig.1: Selection of Withering Methods

Nutrient Content

The material used in this study was Robusta coffee leaves which have been dried through the Japanese Style Green Tea Process (JGTP) method as seen in Table 1 .

Table 1: Nutrient Content of Dried Robusta Coffee Leaves

Proximate Component	Mean±SD (%)
Moisture	3.1±0.00
Ash	6.03±0.90
Protein	17.76±2.06
Fat	2.37±0.25
Carbohydrate	70.83±2.83

Note : SD = standard deviation

Bioactive Components (total flavonoids, chlorogenic acid, caffeine)

The results of the analysis showed that the content of bioactive components, as total flavonoids, chlorogenic acid and caffeine in Robusta coffee leaves as seen in Table.

Table 2: The Content of Bioactive Components

Treatment	Total flavonoids (mg QE/g)	Chlorogenic acid (mg/g)	Caffeine (mg/g)
	Mean±SD	Mean±SD	Mean±SD
Dried coffee leaves	0.81 ± 0.02	16.58 ± 0.48	0.456 ± 0.08
5 g of brewed coffee leaves	0.10± 0.01	3.49 ± 1.62	0.194 ± 0.00

Note: 5 g of dried coffee leaves were brewed with 200 mL of water at 70°C for 10 minutes

Total Chlorophyll

The results of chlorophyll analysis showed a significant difference in the groups of brewed coffee leaves (p = 0.000). While the results of chlorophyll analysis showed significant differences in the coffee leaves group (p = 0.014) and brewed coffee leaves (p = 0.000). The results of total chlorophyll analysis showed significant differences in each coffee leaves group (p = 0.006) and brewed coffee leaves group (p = 0.000). There were significant differences in chlorophyll a, chlorophyll b and total chlorophyll between coffee leaves and brewed coffee leaves (p = 0,000) as seen in Antioxidant Activity

The results of the analysis showed that there were no significant differences in the Robusta coffee

leaves group (p = 0.206) and brewed (p = 0.131) as seen in Table 3.

Table 2.

The results of the analysis showed that there were no significant differences in the Robusta coffee leaves group (p = 0.206) and brewed (p = 0.131) as seen in Table 3.

Antioxidant Activity

Table 2: Total Chlorophyll

Samples	Chlorophyll a		Chlorophyll b		Total Chlorophyll	
	Dried coffee leaves Mean ± SD	Brewed coffee leaves Mean ± SD	Dried coffee leaves Mean ± SD	Brewed coffee leaves Mean ± SD	Dried coffee leaves Mean ± SD	Brewed coffee leaves Mean ± SD
5 g	23.66±0.35 [a]	0.74±0.04 [a]	39.98±2.37 [a]	1.35±0.11 [a]	63.62±2.01 [a]	2.08±0.14 [a]
10 g	23.75±0.17 [a]	1.16±0.09 [b]	42.65±0.63 [ab]	2.19±0.13 [b]	66.37±0.67 [ab]	3.35±0.22 [b]
15 g	23.97±0.39 [a]	1.73±0.31 [c]	43.17±0.61 [ab]	3.19±0.44 [c]	67.13±0.99 [ab]	4.91±0.75 [c]
20 g	24.66±0.73 [a]	2.12±0.02 [c]	44.76±0.80 [b]	3.91±0.52 [d]	69.40±1.45 [b]	6.01±0.09 [d]
P[x]	0.098	0.000*	0.014*	0.000*	0.006*	0.000*

Note: One-way-anova.test. Dried coffee leaves were brewed with 200 mL of water at 70°C for 10 minutes.. *Significant (p < 0.05). Numbers followed by superscript letters (a,b,c,d) differ to show significant differences.

Table 3: Antioxidant Activity with DPPH Method

Treatment	Inhibition (%)		p
	Dried coffee leaves Mean ± SD(%)	Brewed coffee leaves Mean ± SD (%)	
5 gram	92.7 ± 0.61	84.9 ±0.23	0.109[p]
10 gram	91.8 ± 0.76	86.1 ± 0.85	0.019*[q]
15 gram	92.0 ± 2.00	85.6 ± 1.20	0.021*[q]
20 gram	90.2 ± 1.36	83.3 ± 1.47	0.000*[q]
p	0,206[z]	0,131[y]	

Note: Dried coffee leaves were brewed with 200 mL of water at 70°C for 10 minutes. *Significant (p <0,05). [p] Wilcoxon test, [q]Paired t test, [z]One way Anova, [y]Kruskal Wallis test

Antibacterial Activity Test

The results of the antibacterial activity analysis in *Escherichia coli* and *Salmonella typhi* showed significant differences in the groups of brewed coffee leaves (p = 000) as seen in Table 4.

Table 4: Antibacterial Activity

Samples	*Escherichia coli*		*Salmonella typhi*	
	Mean ± SD	Inhibition	Mean ± SD	Inhibition
5 g	7.65±0.10[a]	Medium	7.47±0.28[a]	Medium
10 g	8.47±0.15[b]	Medium	8.20±0.05[b]	Medium
15 g	9.17±0.15[c]	Medium	8.55±0.26[b]	Medium
20 g	9.89±0.26[d]	Medium	8.70±0.19[c]	Medium
p	0,000*		0,000*	

*One Way Anova test. *Significant (p < 0.05). Numbers followed by superscript letters (a,b,c,d) differ to show significant differences.

Acceptability of Brewed Coffee Leaves

There was a significant difference in the color and Taste of brewed coffee leaves (p = 0,000) and did not show a significant difference in the aroma of brewed coffee leaves (p = 0.392) as seen in Table 5.

Table 5: Acceptability of Brewed Coffee Leaves

Treatment	Color		Aroma		Taste	
	Median	Note	Median	Note	Median	Note
5 g	3(1-4)[a]	Like slightly	3(1-4)[a]	Like slightly	3(2-4)[a]	Like slightly
10 g	3(2-4)[ab]	Like slightly	3(2-4)[a]	Like slightly	2(1-3)[a]	Dislike slightly
15 g	2,5(1-4)[bc]	Dislike slightly	3(1-4)[a]	Like slightly	1(1-4)[b]	Dislike very much
20 g	2(1-4)[c]	Dislike slightly	3(1-4)[a]	Like slightly	1(1-2)[c]	Dislike very much
p	0,000*		0,392		0,000*	

Note: Kruskal Wallis test. *Significant ($p < 0.05$). Numbers followed by superscript letters (a,b,c,d) differ to show significant differences.

DISCUSSION

In this study, Robusta coffee leaves were processed by the Japanese Style Green Tea Process (JGTP). JGTP method is one of the tea processing without fermentation process. Previous study has explained that the JGTP method in Arabica coffee leaves has higher phytochemicals concentration compared to other tea-making methods.(X.-M. Chen et al., 2018). The blanching process was used at the withering stage. Blanching can deactivate the enzyme polyphenol oxidase (PPO) which can cause oxidation.(Pourcel, Routaboul, Cheynier, Lepiniec, & Debeaujon, 2007)

Oxidation is a degenerative process triggered by the presence of free radicals which causes rancidity and decreasing components in food. The oxidation process causes the loss of phenolic components of food.(X.-M. Chen et al., 2018) The blanching process increases the polyphenol content during extraction.(Nayak, Liu, & Tang, 2015) In addition, the processing of green tea maintain the composition of chlorophyll including chlorophyll a (Chl a) and chlorophyll b (Chl b) that acts as an antibacterial.(Li et al., 2018)

Based on acceptability of brewed coffee leaves, the best acceptance was 5 g of brewed coffee leaves. The more the amount of leaves that are brewed, it will produce a thick yellowish-green color. Coffee leaves products using the JGTP method do not undergo oxidation of polyphenol compounds so that the resulting color was greenish or yellowish-green. Green is the main color determined by the chlorophyll content. Chlorophyll a on the leaves will produce a dark green color and chlorophyll b will produce a yellowish-green color. In addition to the chlorophyll content, flavonol content also plays a role in producing a yellowish color on the coffee leaves by the JGTP method.(Chaturvedula & Prakash, 2011)

Based on the aroma described by panelists, the aroma of brewed coffee leaves such as the aroma of fresh leaves. The more the amount of leaves in brewed, the smell will be thicker and increasingly disliked by panelists. The aroma of the leaves is caused by the high compound of catechins.(Ho, Zheng, & Li, 2015) The taste of brewed coffee leaves was bitter. The bitter taste is influenced by the presence of chlorogenic acid and caffeine.(Belay, 2011; Gokul G & Lakshmanan, 2016) Caffeine is a natural compound in food and drinks that contributes to affect the bitter taste. Some studies show that the main taste of caffeine is bitter. Caffeine generally amounts to 2-5% of the dry weight of tea. Caffeine will cause a bitter taste on the tongue depending on the level of concentration.(Keast & Roper, 2007; Zou, Xiao, Wang, & Zhang, 2018) The study explained that the bitter taste began to emerge when the caffeine content was 75-155 mg/L. Chlorogenic acid also plays a role in acidity and bitterness.(Belay, 2011)

The moisture and ash content in dried Robusta coffee leaves processed by the JGTP method met the Indonesian National Standard (SNI) green tea requirements, where the moisture of green tea is set to a maximum of 8% and ash content of 4-8%.(SNI, 2016) The moisture in accordance with SNI can prevents the growth of bacteria and fungi, and prevent oxidation process. Protein content in dried Robusta coffee leaves with JGTP method included in the range of green tea, where protein content in green tea about 15-20%. Protein has the potential as an additional antioxidant in food because it can inhibit fat oxidation through several pathways including inactivation of Reactive Oxygen Species (ROS) through antioxidant enzymes (superoxide dismutase (SOD) and catalase), scavenge free radicals, chelating prooxidative transition metals, reducing hydroperoxide, and reducing hydroperoxides, and enzymatically removes specific oxidants.(Elias, Kellerby, & Decker, 2008) Protein also acts as an antimicrobial. Previous study explained that protein extracted from lemon, orange and grapefruit seeds could inhibit the growth of some pathogenic bacteria.(Karabiber, Yilmaz, & Zorba, 2018)

The fat content in Robusta coffee leaves processed by the JGTP method was lower than in Arabica coffee leaves with the same method. Based on previous research, Arabica coffee leaves have higher fat content about 4.5-12.5%. Meanwhile, the carbohydrate content in Robusta coffee leaves was higher than in Arabica coffee

leaves. In previous study, carbohydrate content of Arabica coffee leaves was about 51.99-63.93%.(Woldesenebet, 2005)

In this study, Robusta coffee leaves processed by JGTP method have bioactive components of flavonoids, chlorogenic acid, caffeine, and chlorophyll. Previous study has shown that coffee leaves contain flavonoids such as quercetin, routine, and kaemferol.(Martins, Araújo, Tohge, Fernie, & DaMatta, 2014) Flavonoids can act as antioxidants to scavenge free radicals, prevent the formation of reactive oxygen species (ROS), and increase endogenous antioxidants (superoxide dismutase, catalase, and glutathione peroxidase). The mechanism of flavonoids in preventing damage by free radicals is by giving hydrogen atoms to free radicals to form stable flavonoxy radicals (flavonoids (O•)). In addition, flavonoids can protect the phospholipid membrane by giving one of the hydrogen ions to the radical lipid peroxyl, so that radical reactions can be stopped.(Hamid, Usman, Aiyelaagbe, & Ameen, 2010; Irina & Mohamed, 2012) Flavonoids can also function as antibacterial by inhibiting nucleic acid synthesis, inhibiting energy metabolism, changes in cytoplasmic membrane function, reducing cell adhesion and biofilm formation and causing damage to the cytoplasmic membrane.(Farhadi et al., 2018)

Chlorogenic acid is an ester formed from hydroxynamic acid (caffeic acid, ferulic acid, p-kumaric acid) and quinic acid and into the phenolic acid group.(Liang & Kitts, 2016) Chlorogenic acid can chelate transition metals like Fe^{2+} to scavenge free radicals and break radical chains. Chlorogenic acid scavenge DPPH radicals, superoxide anions, hydroxyl radicals, and protect DNA from damage caused by oxidative stress.(Azam, Hadi, Khan, & Hadi, 2003) Chlorogenic acid also function as antibacterial. Chlorogenic acid is a phenolic bioactive compound that can cause physiological changes in bacterial cell membranes.(Kabir et al., 2014) Chlorogenic acid causes cell membrane rupture and release of the content inside the cellular. Chlorogenic acid changes in bacterial cell membrane permeability that will cause damage to bacterial nucleotides.(Lou et al., 2011)

Robusta coffee leaves with processed by JGTP method also contained caffeine. Caffeine prevents lipid oxidation by inhibiting the production of free radicals including hydroxyl radicals, peroxyl radicals, and singlet oxygen.(Azam et al., 2003) Caffeine also acts as antibacterial agent. It can inhibit protein and DNA synthesis by inhibiting the merging of adenine and thymidine. Besides, caffeine also increases genotoxicity after DNA damage.(Pruthviraj et al., 2011)

The amount of chlorophyll in brewed Robusta coffee leaves was very low compared to the amount of chlorophyll in tea leaves. Chlorophyll acts as antioxidants, where it can prevents the Fenton reaction, by chelating Fe(II). Chlorophyll directly scavenges the hydroxyl free radicals generated from H_2O_2.(Hsu, Chao, Hu, & Yang, 2013) It can also acts as antibacterial by increasing membrane permeability in bacteria.(Kustov et al., 2018) This will cause changes in membrane structure and accelerate intracellular leakage thereby destroying membrane integrity which will facilitate the entry of antibacterial.(Wu et al., 2016)

The results showed that Robusta coffee leaves with the JGTP method had a DPPH radical inhibition. Based on the results of this analysis, Robusta coffee leaves and brewed coffee leaves have high antioxidant activity, being able to capture free radicals by 50%. The results of this study are in line with research on green tea, where green tea has a DPPH radical inhibition more than 50%.

The results of antibacterial of brewed coffee leaves showed the inhibitory power of 5 g, 10 g, 15 g, and 20 g brewed Robusta coffee leaves against *Escherichia coli* and *Salmonella typhi* bacteria. *Escherichia coli* and *Salmonella typhi* belong to gram-negative bacteria. The greater the amount of leaves in the brewed process, the greater the diameter of the inhibition zone formed. The effectiveness of antibacterial substances will be influenced by the concentration of these substances. An increase in the concentration of the substance causes an increase in the content of active compounds such as flavonoids, caffeine and chlorogenic acid which functions as an antibacterial so that its ability to inhibit bacterial growth increases.(Mufti, Bahar, & Arisanti, 2017; Muslim & Dephinto, 2017) Previous study conducted on CTC brewed black tea with a comparison of tea weight and water volume (w/v) 1:50, 2:50, and 3:30 resulted in an area of 29.89 successive barriers; 33.64 and 45.02 mm^2.(Rohdiana et al., 2013)

CONCLUSION

Robusta coffee leaves in this research were processed by JGTP method with 75-second blanching. The best organoleptic based on color, aroma, and taste was 5 g of dried Robusta coffee leaves. Brewed Robusta coffee leaves processed by the Japanese Style Green tea Process (JGTP) have contain flavonoids 0.10 mg QE/g, chlorogenic acid 3.49 mg/g, caffeine 0.194 mg/g and high antibacterial activity in 20 g of brewed Robusta coffee leaves.

CONFLICT OF INTEREST

No conflict of interest.

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