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ANTIBACTERIAL ACTIVITY OF GREEN COFFEE BEAN EXTRACT AGAINST STAPHYLOCOCCUS AUREUS AND SALMONELLA ENTERITIDIS

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ABSTRACT
Green coffee has a high content of chlorogenic acid. Chlorogenic acid has been known to be useful for lowering blood sugar levels and has been widely used to lose weight. But the benefit of green coffee as an antibacterial has not been widely studied. The purpose of this study was to determine the antibacterial effect of green coffee on gram-positive bacteria (*Staphylococcus aureus*) and gram-negative (*Salmonella enteritidis*). The method used to test antibacterial activity of green coffee beans extract against bacteria *S.aureus* and *S.enteritidis* by disc diffusion method. Green coffee beans extract was divided into 4 concentration of 20%, 15%, 10%, 5%, negative control DMSO and positive control is gentamicin disk 10µg/disk and Identification of active ingredients found in green coffee beans extract using Liquid Chromatography-Mass Spectrometry (LC-MS) method. The data obtained were analyzed using Two Way ANOVA test with confidence level p <0,05. Based on the data obtained and the results obtained the highest inhibitory zone in the treatment concentration of 20% is 26.20 mm, and the average of the lowest inhibition zone in the DMSO treatment of 6.00 mm. The average inhibition zone of green coffee extract activity against *Staphylococcus aureus* was 22.73 mm much higher than the activity of green coffee extract to *Salmonella enteritidis* of 16.67 mm.

KEY WORDS
Green coffee**,** antibacterial effect, S.aureus, S.enteritidis.

In the world, there are several types of coffee, namely arabica coffee, robusta coffee, coffee liberika, and ekselsa but the coffee group that has an economic value of arabica coffee and robusta coffee. Arabica type coffee is very well planted at the altitude of 1,000 - 2,100 above sea level. The higher the location of the coffee plantation, the taste produced by the coffee beans will be better.

The content of caffeine in 100 gr samples is polyphenols (gallic acid, catechin, caffeic acid, vanillin, chlorogenic acid, epicatechin and ferulic acid), covering total phenol of 1300-3700 mg, total flavonoid of 15-103 mg, gallic acid of 2.5 mg, catechin of 30 to 80 mg, caffeic acid 1200-2500 mg, vanillin of 100-150 mg, chlorogenic acid of 1,4- 2.8 g, and epicatechin of 11-30 mg [1] (Kreicbergs et al., 2011). Coffee contains active ingredients that are beneficial to health include antioxidants (hepatoprotective, hypoglycemia and antimicrobial and antispasmodic) [2].

In this study, we want to know the antibacterial activity capacity of the green coffee bean extract against Staphylococcus aureus and Salmonella enteritidis, especially Salmonella enteritidis bacteria because the bacteria are transmitted through food.

MATERIAL AND METHODS OF RESEARCH

Green Coffee Beans Samples. This research used green coffee beans (*Coffee canephora* var *robusta*) were purchased in Lampung, Indonesia. The robusta green coffee beans were extracted using ethanol 90% as the solvent.

Characterization of Green Coffee Extract. Identification of active ingredients found in green coffee beans extracts using Liquid Chromatography-Mass Spectrometry (LC-MS) method. Columns used with Hypersil Gold specification (50mm x 2.1mm x 1.9µm). UHPLC ACCELLA type 1250 made by Thermo Scientific. 10 µL sample was placed into a centrifuge tube, dissolved with methanol 10 mL, then added with Pb-acetate of 0,1 mL, having a sanitation for 15 minutes and centrifugation at 4000 rpm for 5 minutes, final step Filter of filtrate with 0.2-micron membrane filter, and inserted on vial bottle.

Bacteria Sample. The bacteria used are *Staphylococcus aureus* and *Salmonella enteritidis* was obtained from Laboratory of Microbiology, Faculty Medicine, University of Brawijaya, Indonesia. The bacteria were propagated in Tryptic Soy Agar (Oxoid^{TM}, CM0131), incubated at 37^0 C for 24 hours.

Antibacterial Activity. The method used to test antibacterial activity green coffee beans extract of against bacteria *S.aureus* and *S.enteritidis* by disc diffusion method. Inocula of each bacteria in TSA was transferred into phosphate buffered saline pH 7,4 until the level of bacteria turbidity equated to the 0,5 McFarland standard (1,5 x 10^8 CFU/mL), then 200 µL of the suspensions of bacteria were placed into Mueller Hilton Agar (Oxoid^{TM}, CM0337) and spread evenly by cotton swab sterile. Green coffee beans extract was divided into 4 concentration of 200mg/mL, 150mg/mL, 100mg/mL,50 mg/mL, negative control DMSO and positive control is gentamicin disk 10µg/disk (Oxoid^{TM}, CT0024B). Each of concentration green coffee beans extracts was taken 20µL then impregnated on a blank disk, incubated at 40^0C for 10 minutes until disc dries up, then was placed on the surface MHA already existing bacteria, incubated at 37^0 C for 24 hours.

Data Analysis. The data obtained in this study were analyzed qualitatively and quantitatively using two Way Annova with SPSS software (Statistical Package for Social Sciences) by having a confidence level of $p<0.05$.

RESULTS AND DISCUSSION

Characterization of Green Coffee Extract. This study exhaustively investigated about green coffee beans. Green coffee is not a kind of roasted coffee. In roasted-coffee processing, the main content of coffee, *i.e.* chlorogenic acid (CGA), will decompose causing the CGA level in roasted coffee will also decrease. Several studies have shown that chlorogenic acid acts as an antioxidant, anticancer and antibacterial agent [3].

To obtain the active ingredients from green coffee beans, extraction was done using 90% ethanol as the solvent. CGA is a polar material. Therefore, CGA can be obtained by using nonpolar ethanol. Besides, CGA is soluble in ethanol and acetone [4].

Here is the yield value formula of green coffee ethanol extract. Yield (*rendeman*) is the ratio of the number of extracts produced from the extraction of plants. The yield value of green coffee ethanol extract here was calculated by dividing the weight of coffee extract with the initial weight of coffee material (raw material), which was then multiplied by 100%. By applying this formula, it was obtained the yield value of 12%. According to [5], the lower yield value indicates the better extract quality.

$$Yield = \frac{weight\ of\ extract\ coffee}{weight\ of\ raw\ material}\ x\ 100\% = \frac{50\ gram}{414\ gram}\ x\ 100\% = 12\%$$

Moreover, in this study, the identification of green coffee-active ingredients with LC-MS obtained 5 peaks, covering: peak 1 with molar mass of 353 indicated as 3-O-caffeoylquinic acid or better known as chlorogenic acid; peak 2 with molar mass of 367 indicated as 5-*O*-feruloylquinic acid; peak 3 with molar mass of 515 indicated as dicaffeoylquinic acid; peak 4 with molar mass of 529 indicated as 4-*O*-caffeoyl-5-*O*-feruloylquinic acid; and peak 5 with molar mass of 529 and m/z 192.50 indicated as 3-*O*-feruloyl-4-*O*-caffeoylquinic acid [6]. The compounds of green coffee beans extract described are listed in Table 1 below

Table 1 – Characterization Compounds of Green Coffee Extract

Peak	MS	m/z	Compounds
1	1353	190.50-191.50	3-*O*-caffeoylquinic acid
2	367	190.50-191.50	5-*O*-feruloylquinic acid
3	515	352.50-353.50	dicaffeoylquinic acid
4	529	366.50-367.50	4-*O*-caffeoyl-5-*O*-feruloylquinic acid
5	529	192.50-193.50	3-*O*-feruloyl-4-*O*-caffeoyquinic acid

The active ingredients obtained in this study were slightly different from Mullen *et al.*'s [6] study that used coffee berry as the sample. From the study, it was identified 16 active ingredients while, in this study, it was identified 5 active ingredients only.

Furthermore, in this study, it was no indication of the main component of coffee that is caffeine. This is in line with the study of Kartasasmita *et al.* [7] stating that the caffeine content of decaffeinated coffee beans using ethanol solvent is 0.382% with the decrease in caffeine content of 79.713%.

Antibacterial Activity. The antibacterial activity of Green Coffee Beans extract was indicated by measuring the diameter of the inhibition zone (mm) as shown in Table 2.

Table 2 – Diameter of the Inhibition Zone Obtained with Green Coffee Beans Extract against *Staphylococcus aureus* and *Salmonella enteritidis*

Bacteria	Concentration	Inhibition zone (mm)					
		n1	*n2*	*n3*	*n4*	*n5*	X±SD
Staphylococcus aureus	DMSO	6	6	6	6	6	6±0
	Gentamicin 10µg	21	20	21	19	20	20,2±0,84
	200 mg/mL	32	34	33	31	31	32,2±1,30
	150 mg/mL	30	30	30	31	31	30,4±0,54
	100 mg/mL	28	27	25	25	27	26,4±1,34
	50 mg/mL	22	22	21	21	20	21,2±0,83
Salmonella enteritidis	DMSO	6	6	6	6	6	6±0
	Gentamicin 10µg	26	25	26	24	26	25,4±0,94
	200 mg/mL	21	21	19	20	20	20,2±0,83
	150 mg/mL	18	16	17	17	16	16,8±0,83
	100 mg/mL	16	18	16	17	17	16,8±0,83
	50 mg/mL	16	15	14	14	15	14,8±0,83

Note: diameter of disc 6mm.

The results of the antibacterial activity of green coffee extract against *Staphylococcus aureus* and *Salmonella enteritidis* bacteria at various concentration treatments were analyzed statistically using ANOVA (Two Way Analysis of Variant) test with SPSS 22 software, indicating a significant difference of $p<0.05$. Meanwhile, the results of Tukey test showed a different notation. The data of the results of this study can be seen in Table 3 below.

Table 3 – Descriptive Data of Antibacterial Activity of Green Coffee Extract

		Average Inhibition Zone (mm)±SD
Concentration Treatment	Aquadest	6.00 ± 0.000^{a}
	Gentamicin 10µg	22.80 ± 2.860^{bc}
	200 mg/mL	26.20 ± 6.408^{c}
	150 mg/mL	23.60 ± 7.199^{c}
	100 mg/mL	21.60 ± 5.168^{bc}
	50 mg/mL	18.00 ± 3.464^{b}
Bacteria	*Staphylococcus aureus*	22.73^{b}
	Salmonella enteritidis	16.67^{a}

Based on Table 3, it is shown that the highest-average inhibition zone was 26.20 mm obtained from the concentration treatment of 200 mg/mL while the lowest-average inhibition zone was in the aquadest treatment of 6.00 mm. It can be concluded that the average inhibition zone of the antibacterial activity of green coffee extract against Staphylococcus

aureus was 22.73 mm, which is much higher than that against Salmonella enteritidis obtaining 16.67 mm.

Herbal ingredients can be said to have antibacterial activity if the inhibition zone > 8mm, but the specification of *sensitive, intermediate* and *resistant* depends on the bacteria being tested and the antibiotics used as positive controls. Here are the standards of the antibiotic-inhibition zone according to CLSI (2011).

Table 4 – Standards of Antibiotic-Inhibition Zone

Antibiotic Disk	Concentration	Bacteria	Inhibition Zone (mm)		
			Susceptible	Intermediate	Resisten
Gentamcin	10 µg	*S.aureus*	≥15 mm	13-14 mm	≤12 mm
		S.enteritidis	≥15 mm	13-14 mm	≤12 mm

Source: Clinical Laboratory Standards Institute (CLSI), 2011.

Based on the Certificate of Coffee Plant Determination, it is mentioned that the chemical contents of coffee beans include caffeine, trigonelline, polyphenol compound, caffeic acid, chlorogenic acid, diterpene ester, coffee oil, quinic acid, ethylphenol, dicaffeoylquinic acid, dimethyl disulfide, putrescine and amino acid. Aromatic and phenol compounds in coffee can inhibit the growth of bacteria by altering the structure and function of bacterial cytoplasm [9].

In this study, there were 6 treatments against *S.aureus* and *S.enteritidis* bacteria, covering negative control using aquadest, positive control using gentamicin of 10µg, and green coffee extract doses of 200mg/mL, 150mg/mL, 100mg/mL and 50mg/mL. To find out whether there was a difference of average inhibition zone between the treatments and bacteria, a test of Two Way ANOVA was carried out:

1. The aquadest treatment (notation a) was significantly different from other treatments.
2. The 10µg Gentacimin treatment was significantly different from the aquadest treatment. However, this treatment was not significantly different from the concentration treatments of 100 mg/mL, 50 mg/mL, 200 mg/mL, and 150 mg/mL.
3. The concentration treatment of 200 mg/mL was significantly different from the aquadest treatment and the concentration treatment of 50 mg/mL. However, the treatment was not significantly different from the 10µg Gentamicin treatment and the concentration treatments of 100 mg/mL and 150 mg/mL.
4. The concentration treatment of 150 mg/mL was significantly different from the aquadest treatment and the concentration treatment of 50 mg/mL. But, the treatment was not significantly different from the 10µg Gentamicin treatment and the concentration treatments of 100 mg/mL and 200 mg/mL.
5. The concentration treatment of 100 mg/mL was significantly different from the aquadest treatment. However, the treatment was not significantly different from the 10µg Gentacimin treatment and the concentration treatments of 50 mg/mL, 200 mg/mL and 150 mg/mL.
6. The concentration treatment of 50 mg/mL was significantly different from the aquadest treatment and the concentration treatments of 200 mg/mL and 150 mg/mL. However, the treatment was not significantly different from the 10µg Gentamicin treatment and the concentration treatment of 100 mg/mL.

CONCLUSION

Based on the results of this study, it can be concluded that the extraction of green coffee using ethanol solvent results in chlorogenic acid. Furthermore, green coffee extract has antibacterial properties by destroying the membrane cytoplasm of *S.aureus* and *S.enteritidis* bacteria.

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