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Research Article

ANTIBACTERIAL ACTIVITY OF ROBUSTA COFFEE (*COFFEA CANEPHORA* L.) LEAVES TO *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI*

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ABSTRACT

Objective: This research aims to analyze the ability of robusta coffee leaves fraction extract to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* and also determine the minimum inhibitory concentration (MIC).

Methods: Antibacterial activity evaluated by the disc diffusion method observed in four types of fraction of extract robusta coffee leaves (n-hexane, ethyl acetate, ethanol, and water). Each extract divided into three various concentrations, 5%, 10%, and 15%. Determination of antimicrobial activity *in vitro* by the disk diffusion method.

Results: Ethyl acetate fraction of coffee leaves extract produced the largest diameter zone of inhibition of bacterial growth compared to other extraction fractions of 17.28 mm in *E. coli* and 18.58 mm in *S. aureus*. The MIC of coffee leaves extract fraction water, ethyl acetate, and n-hexane on *E. coli* and *S. aureus* is 5%, while the fraction ethanol MIC is 10%.

Conclusion: The antibacterial effect of ethyl acetate fraction of coffee leaves extract showed an antibacterial effect that was better than the fraction of n-hexane, ethanol, and water.

Keywords: Antibacterial, Coffee leaves, *Staphylococcus aureus, Escherichia coli.*

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INTRODUCTION

In Indonesia, two types of coffee widely cultivated, namely, Arabica (*Coffea arabica* L.) and Robusta (*Coffea canephora* L.). Coffee has strong historical, cultural, and economic values. For coffee connoisseurs, it may be rare to think that besides the seeds, coffee leaves can be used as a beverage. Indonesia is the 4th largest coffee producer in the world with the coffee plantation area in 2016 which is 26.780 hectares [1]. The use of coffee leaves to make tea has long been a tradition in West Sumatera, Ethiopia, Jamaica, India, Java, and South Sudan, but literature about the Sumatran use is limited. The Sumatran drink is called "Kahwa Daun" or "Kawa" [2].

Secondary metabolites such as mangiferin, caffeoylquinic acid, caffeine, hydroxycinnamic acid, allantoic acid, allantoin, theobromine, and theophylline have found in the leaves of some species of coffee [3-6]. The coffee is also an essential source of polyphenols including caffeic acids, chlorogenic acid, coumaric acid, ferulic acid, and sinapinic acid [7]. Researchers have shown interest in the phenolic compounds of plant and their potency in the prevention of degenerative diseases [8]. Recent study found a new potent anthelmintic agent that can be used as a potent drug for the treatment of helminth infection [9] and extracts of green coffee bean extract could decrease fasting blood glucose, profile lipid, blood pressure, and improved adiponectin level and homeostasis assessment model of insulin resistance index [10].

The previous research on the antibacterial activity of coffee including the strains of *Legionella pneumophila*, the bacteria involved in respiratory infections and identified the active substances as caffeic, chlorogenic, and protocatechuic acids [11,12]. Other studies showed that the growth of *Streptococcus mutans* could inhibit by coffee extract containing chemical compounds including trigonelline, chlorogenic acid, caffeic acid, and protocatechuic acid [13,14]. Research related to coffee plants and the antibacterial effects of most coffee beans only observed so it is interesting to examine the antibacterial effects of the coffee leaves extract. Screening the bacterial activity of the coffee leaves extract was tested against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*).

METHODS

Sample

The sample used in this study was Robusta coffee (*C. canephora* L.) which grew in the area of Curup Rejang Lebong Regency, Bengkulu Province, Indonesia. To get the maximum maceration results, the dried coffee leaves are pulverized to form a powder.

Microorganisms

The following microorganisms used in the experiments: *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923). The samples were unfrozen, inoculated on brain heart infusion broth, and incubated for 24 h. After that, they were seeded on nutrient agar (NA) and incubated for 24 h at 37°C. From the grown colonies, the suspension in NaCl at 0.9% corresponding to the 0.5 at the McFarland scale (1.5×10^8 CFU/ml) produced.

Extraction and fractionation

Coffee leaves powder weighed as much as 200 g. Then, the coffee is macerated using ethanol. The filtrate obtained from maceration was then evaporated using a rotary evaporator (Heidolph®) so that the concentrated ethanol extract obtained. After that the fractionation process was carried out using n-hexane and ethyl acetate.

Determination of *in vitro* antimicrobial activity with disc diffusion method

The inoculated test bacteria were taken with sterile Ose wire and then suspended into a tube containing 2 ml of 0.9% NaCl solution until the turbidity obtained was the same as the standard turbidity of solution

Table 1: Diameters of inhibition zones obtained with various fractions and concentration of robusta coffee leaves on SA and EC

Inhibition zone (mm)								
Control (+)*		Fraction	5%		10%		15%	
SA (n=3)	EC (n=3)		SA (n=3)	EC (n=3)	SA (n=3)	EC (n=3)	SA (n=3)	EC (n=3)
22.02±4.60	15.19±1.64	Water	11.45±0.00	10.06±0.03	13.00±0.00	11.71±0.00	12.51±1.00	11.15±1.00
		Ethanol	6.70±0.00	-	8.14±0.60	14.8±0.00	9.29±0.06	15.27±0.06
		Ethyl acetate	14.58±2.40	13.12±1.44	15.86±4.27	12.08±0.00	18.58±1.15	17.28±1.15
		n-Hexane	12.47±1.07	10.7±0.00	11.51±0.83	12.07±0.53	13.52±0.00	13.31±0.00

*Used tetracycline; SA: *Staphylococcus aureus* (ATCC 25923), EC: *Escherichia coli* (ATCC 25922)

Table 2: MIC of various fractions and concentration of robusta coffee leaves on EC and SA

Microorganisms	Minimum inhibitory concentration of various fractions			
	Water (%)	Ethanol (%)	Ethyl acetate (%)	n-hexane (%)
SA (ATCC 25923)	5	10	5	5
EC (ATCC 25922)	5	10	5	5

MIC: Minimum inhibitory concentration, SA: *Staphylococcus aureus*, EC: *Escherichia coli*

McFarland. The same treatment was carried out by other types of test bacteria [15]. NA media were poured into a Petri dish of 65 ml and left to solidify. After solidifying, 1 ml of the suspension mixture poured into each Petri dish as the second layer. Furthermore, the Petri dish is rotated ±600 as much as ×3 so that it forms a flat layer and is allowed to solidify on the surface of the top layer to put four paper discs and arranged in such a way that there is enough area to observe the inhibition zone that occurs.

The test solution for each fraction with various concentrations (5%, 10%, and 15%), 5 mg/5 ml tetracycline solution as a positive control, each solution was used for antibacterial testing with the paper disc diffusion method. Then, the Petri dishes were incubated in an incubator at 37°C for 1×24 h. Observations were made after 1×24 h, the incubation period with three repetitions for each bacterium. Observe the inhibition zone formed around the disc paper and then measured the diameter of the inhibition zone in units of millimeters (mm) horizontally and vertically using a scale bar.

RESULTS

Extraction of 200 g of coffee leaves by maceration using 96% ethanol obtained n-hexane fraction 16.46 g, ethyl acetate fraction 17.17 g, ethanol fraction 5 g, and water fraction 16.69 g. The coffee leaves extract fraction was then tested on *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 using concentrations of 5%, 10%, and 15% with positive controls using tetracycline antibiotics. The Ministry of Health states that microbes were stated to be sensitive to the antibacterial origin of plants if they have a diameter size of resistance (bright area) 12–24 mm [16].

DISCUSSION

The coffee leaves used to come from coffee plantations in Kepahiang district. The leaves of the coffee plantations are routinely cut so that the growth of the coffee fruit more and more. The leaves are only used as compost. In this study, coffee leaf samples were collected and then cleaned and dried to have ±20% moisture content. Dried coffee leaves are ground to powder, which aims to expand the specific surface of the coffee leaves so that when the maceration is obtained optimal macerate.

Coffee leaf powder weighed about 200 g and extracted using a maceration method and then fractioned using n-hexane, ethyl acetate, ethanol, and water. Testing the effectiveness of antibacterial of each coffee extract fraction was carried out with three replications (triple) against *S. aureus* and *E. coli* so that the average diameter of the inhibition zone can be taken. The fractions obtained were then tested for *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 using the disc diffusion method, respectively.

The antibacterial effect of coffee leaves extract fraction on *S. aureus*

The results of testing the diameter of the inhibition zone growth of *S. aureus* using n-hexane, ethyl acetate, ethanol, and water fraction of coffee leaves showed that the ethyl acetate fraction had more significant antibacterial effect than other fractions. The bacterial growth inhibition produced by the largest ethyl acetate fraction was at a concentration of 15% (18.58±1.15 mm) (Table 1). All concentrations tested showed a steady growth inhibition response to the *S. aureus* bacteria, which located between 10 mm and 20 mm. The minimum inhibitory concentration (MIC) of coffee leaves extract fraction water, ethyl acetate, and n-hexane on *S. aureus* is 5%, while the fraction ethanol MIC is 10% (Table 2).

Coffee leaves contain alkaloids, caffeine, saponins, flavonoids, and polyphenols [17]. In ethyl acetate fraction dissolved in semipolar organic substances, one of the substances that dissolve in ethyl acetate fraction is alkaloid [18]. The mechanism of action of the alkaloid as an antibacterial is predicted by inhibiting cell wall synthesis, thereby causing the cell to become lysis [19]. One type of alkaloid in coffee plants is coffin.

The antibacterial effect of coffee leaves extract fraction on *E. coli*

The results of testing the inhibition zone diameter of the growth of *E. coli* using hexane, ethyl acetate, ethanol, and water fraction of coffee leaves showed that ethyl acetate fraction had more significant antibacterial effect than the other fractions. The bacterial growth inhibition produced by an ethyl acetate fraction is at a concentration of 15% (17.28±1.15 mm) (Table 1). The diameter of the inhibition zone of ethyl acetate fraction at a concentration of 5%, 10%, and 15% showed a strong response to growth inhibition on *E. coli* which is located between 10 mm and 20 mm. The test results of the antibacterial effect of ethanol fraction on *E. coli* also showed a strong antibacterial effect that was at concentrations of 10% and 15%, whereas at a concentration of 5%, it did not show a diameter inhibition zone of bacterial growth. The MIC of coffee leaves extract fraction water, ethyl acetate, and n-hexane on *E. coli* is 5%, while fraction ethanol MIC is 10% (Table 2).

Substances of secondary metabolites that dissolve in polar solvents such as ethanol were polyphenols. Some compounds in coffee plants such as volatile, non-volatile organic acid, phenols, and aromatic compounds have antimicrobial activity [20]. The active substance that is thought to be found in coffee leaves and has antimicrobial effectiveness is polyphenols (tannins) [21]. Antibacterial effects of tannins include reactions with cell membranes, enzyme inactivation, and destruction or inactivation of genetic material functions [22].

CONCLUSION

Robusta coffee (*C. canephora* L.) leaves fractionation extract had an antibacterial effect, and the best inhibitory effect of E. coli and S. aureus

was at ethyl acetate fraction compared to n-hexane, ethanol, and water fractions.

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AUTHORS' CONTRIBUTIONS

The first author (ZM) initiated, conducted the research, and prepared the manuscript. The second author (YD) conducted the microbial test and interpretation data. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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