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RESEARCH PAPER

Comparison of antioxidant and antibacterial activity of Arabica coffee bean and Kawa Daun crude extract obtained from West Sumatra

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Abstract

Arabica coffee and Kawa Daun are considered to be a major contributor to antioxidant consumption that protect against the damaging effects of free radicals on the body. The objectives of this research were to assess the total phenolic content in the ethanolic crude extract of Arabica coffee beans and to analyze the antioxidant activity of the methanolic crude extract of Kawa Daun through the inhibition of the DPPH radical scavenging assay. The antibacterial properties of ethanolic crude extract of Arabica coffee beans and methanolic crude extract of Kawa Daun against pathogenic bacteria was determined by disk diffusion assay. The ethanolic crude extract of Arabica coffee beans at various concentrations, exhibited a total phenolic content of 5.640 mg GAE/g and achieved a DPPH radical scavenging assay of 90.57% at 800 mg/mL. Meanwhile, methanolic crude extract of Kawa Daun produced 89.27% at 400 mg/mL. The antibacterial activity of ethanolic crude extract of Arabica coffee bean resulted in higher reading of inhibition zone compared to methanolic crude extract of Kawa Daun. This study portrayed the significant potential of both Arabica coffee bean and Kawa Daun extracts as natural sources of antioxidants and antibacterial agents. This research highlights the promising antioxidant and antibacterial potential of both Arabica coffee beans and Kawa Daun. Kawa Daun, with their high phenolic content, demonstrate strong antioxidant properties, which contribute to their role in protecting the body from oxidative stress. In terms of antibacterial activity, both Arabica coffee and Kawa Daun possess significant inhibitory effects against common pathogenic bacteria, demonstrating their potential use as natural alternatives to synthetic antibiotics. The findings suggest that these plants could be further explored for their medicinal applications, potentially serving as ingredients in the development of functional foods or natural antibacterial agents.

1. Introduction

Coffee contains phytochemicals, including natural antioxidants, which have been proposed to contribute to all of the medical advantages linked with its consumption. The potential of Arabica coffee to serve as an antioxidant and a source of new antimicrobial agents can be unlocked, which in turn may encourage innovation in the fields of food science and public health. *Coffea arabica* is unique by its expression of secondary metabolites, including phenolic compounds. These compounds have the potential to serve as valuable raw materials for the extraction of bioactive chemicals that are of interest in the field of food science (Silva *et al.*, 2020). Arabica coffee beans have antimicrobial properties against a variety of bacteria. A study examined the antibacterial properties of coffee beans and coffee-derived compounds in vitro against drug-resistant strains. The study discovered that coffee bean and coffee by-product extracts, including Arabica leaf extract, could be used as an alternative treatment for multidrug-resistant (Rawangkan *et al.*, 2022). Previous studies have indicated that the chemical composition of Arabica coffee, encompassing components such as caffeine, flavonoids, trigonelline, choline, sucrose, and chlorogenic acids is subject to the influence of multiple

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factors, including genotype, environment, and processing (Ribeiro *et al.*, 2016). Additionally, this research also would offer valuable perspectives on the potential health advantages that can be attributed to the coffee extract. These insights would be obtained from an analysis of its phenolic content and antioxidant properties.

Robusta coffee (*Coffea canephora*) and Arabica coffee (*Coffea arabica*) are two commonly found species for coffee plantation sector in West Sumatra. Robusta coffee recorded a higher production compared to Arabica coffee. Moreover, coffee leaves are also utilized to produce a local drink known as Kawa Daun, which act as an alternative of coffee bean. Kawa actually refers to coffee while daun itself means leaves in Minangkabau, the term of Kawa Daun then popular in the market which refers to the final dried leaves and its drink. Process of producing Kawa Daun started by smoking the leaves of coffee plant for hours until it transformed into dry brownish green to dark brown dried leaves. A reference highlighted production of beverage from the coffee leaves in Minangkabau at least recorded since the Dutch era, particularly in cultuurstelsel system (Novita *et al.*, 2018). The herbal flavour and aroma of Kawa Daun tea resembled a normal cup of coffee because the presence of coffee in its properties. Nowadays, Kawa Daun drink is commonly served with sugar and also mix with sweetened condensed milk. Kawa Daun have a lineup of secondary metabolites such as mangiferin, allantoic acid and theophylline (Muslim and Dephinto, 2019).

Leaves of robusta coffee are preferred in producing Kawa Daun over others due to its bigger size that will lead to a bigger yield of dried leaves. Flavonoid and chlorogenic acid in Kawa Daun carry antibacterial character because its compound was reported to damage the bacterial nucleotides (Anjani *et al.*, 2020). Previous research reported that extract of Robusta coffee (*Coffea canephora*) leaves contained 18.76-29.01 phenolic compound (mg Gallic acid equivalent/g DW extract), 14.46-20.09 flavonoid (mg Catechin equivalent/g DW extract), 15.36-27.43 (mg Tannic acid equivalent/g DW extract), and antioxidant activity 22.14-38.73 (mg Fe (II) equivalent/g DW extract) (Maxiselly *et al.*, 2022). This implied the chemical potency of coffee leaves to produce local drink as practiced by Minangkabaunese. Combination of chemical compounds of the leaves and smoke during processing might resulting in specific properties of the Kawa Daun. Kawa Daun show promise for treating specific illnesses based on array of secondary metabolites they contained. Research on properties in Kawa Daun can possibly lead in developing non-synthetic drug options. Regardless of the effectiveness of recent medication in treating illnesses, continual usage of synthetic drug can lead to chemical remnants buildup in body. Hence, development of microbial resistance towards the substances used (Prestinaci *et al.*, 2015). In recent years, opting for a more sustainable and safe treatment has grown. Kawa Daun is a sustainable option compared to synthetic drugs.

2. Materials and methods

2.1 Collection of plant materials

Arabica coffee beans and dried Kawa Daun were obtained from Desa Tuo Pariangan, West Sumatra. Both samples were processed into powder form to facilitate extraction. The coffee beans were precisely pulverized using an analytical grinder for two minutes, pausing at 15-second intervals to prevent overheating. The resulting powder was stored in an airtight container at an optimal temperature. The dried Kawa Daun were cut into smaller pieces for easier handling and then ground to a powder consistency, and was stored in a Schott bottle until the extraction process (Prestinaci *et al.*, 2015).

2.2 Extraction of Arabica coffee bean and Kawa Daun

The extraction of bioactive compounds from Arabica coffee beans and Kawa Daun was conducted to obtain crude extracts for antioxidant, total phenolic content, and antibacterial activity assays. For Arabica coffee beans, 20 g of dried powder was immersed in 200 mL of ethanol within a Schott bottle for 48 hours at room temperature. Following maceration, the mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator under vacuum at 175 bp for 45 minutes. The resulting crude extract was stored in amber glass containers at 6°C. Similarly, 20 g of Kawa Daun powder was immersed in 200 mL of 97% methanol for 48 hours, then filtered with Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator configured for methanol (Jafari *et al.*, 2019). The methanolic extract was stored at 4°C to maintain stability. These carefully tailored processes aimed to optimize the yield of phenolic compounds and bioactive constituents, with solvent selection, extraction duration, and storage conditions calibrated to preserve bioactivity for subsequent analyses.

2.3 Preparation of bacteria

For the antibacterial activity assays of both Arabica coffee bean and Kawa Daun extracts, three bacterial species were used, *Bacillus subtilis* and *Staphylococcus aureus* which are Gram-positive, along with *Escherichia coli*, a Gram-negative bacterium. These bacterial cultures were obtained from Unit Bank Kultur, UiTM Cawangan Negeri Sembilan. To prepare the bacterial cultures for both the Arabica coffee bean and Kawa Daun assays, bacterial strains were grown in nutrient broth and incubated overnight in an incubator shaker at 37°C.

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Following incubation, a 0.5 McFarland standard bacterial suspension was prepared for each species. For the Arabica coffee bean assay, the bacterial suspensions were spread onto Muller Hinton Agar (MHA) plates and for the Kawa Daun assay, the bacterial suspensions were spread onto nutrient agar plates using a hockey stick with both assays incubated at 37°C for 24 hours. This consistent preparation process allowed for effective testing of each extract's antibacterial properties against the selected bacterial species.

2.4 Determination of antioxidant activity

The Folin-Ciocalteu method was used to determine the total phenolic compound content in Arabica coffee bean. Gallic acid was used as the standard to measure the total phenolic content. A calibration curve was established by using gallic acid at different concentrations (100 – 800 µg/mL). A solution containing 0.5 mg of gallic acid was mixed with 10 mL of a 95% concentration. A solution with a concentration of 7.5% was prepared by disolving 7.5 grams of sodium carbonate in 100 mL of distilled water. A concentrated Folin-Ciocalteu reagent solution of 10 mL was diluted with 90 mL of distilled water to produce a diluted Folin-Ciocalteu reagent. For preparation reaction mixture, 0.20 mL of Folin-Ciocalteu reagent and 2.5 mL 7.5% Na_2CO_3 solution was poured into the test tube or cuvette. The solution mixed well with vortex or stirring to ensure proper progress of the reaction. The solution was then left to incubate in the dark for two hours. The absorbance of the blue colour for each concentration of gallic acid was determined using UV-Vis spectrophotometer at the wavelength of 765 nm. The mean of the absorbance was used to plot the calibration curve to determine the level of phenolic compound in the samples. Total phenolic content of the samples was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). Different concentrations were made 100, 200, 400, and 800 mg/L.

$$T = (C \times V) / m$$

where T is total phenolic contents (mg/GAE/g), C is concentration of gallic acid obtained from calibration curve (mg/ml), V is volume of extract (ml), and m is mass of extract (g).

Antioxidant activity of Arabica coffee bean and Kawa Daun methanolic crude extract was evaluated by performing DPPH radical scavenging assay. A stock solution was prepared by combining methanol with 2,2-diphenyl-1- picrylhydrazyl (DPPH). Next, 5 mL of freshly made stock solution was combined with 10 µL of extract from Kawa Daun at various concentrations which were 50 mg/mL, 100 mg/mL, 200 mg/mL, and 400 mg/mL. The absorbance of all concentrations was recorded after being kept for 30 minutes in total darkness. Additionally, 97% methanol was used as blank and a combination of ascorbic acid and methanol was used as control. The scavenging of free radical DPPH was calculated based on the following equation:

$$\text{Inhibition of free radicals (\%)} = \frac{Ao - As}{Ao} \times 100$$

Ao is the absorbance of the control reaction. As is the absorbance of solutions with the sample extract or standard.

2.5 Determination of antibacterial activity

The antibacterial activity of both Arabica coffee bean and Kawa Daun extracts was evaluated using a disk diffusion assay with various concentrations of each extract. For Arabica coffee beans, concentrations of 100 mg/mL, 200 mg/mL, 400 mg/mL, and 800 mg/mL of the ethanolic crude extract were prepared by diluting the stock solution with ethanol. For Kawa Daun, the methanolic crude extract was prepared at concentrations of 50 mg/mL, 100 mg/mL, 200 mg/mL, and 400 mg/mL. A 0.5 McFarland bacterial suspension of *B. subtilis*, *E. coli*, and *S. aureus* was spread onto the agar plates for both assays, with Muller Hinton agar (MHA) used for Arabica coffee bean and nutrient agar for Kawa Daun. Blank disks were then soaked with 20 µL of each concentration of the respective crude extracts and placed on the agar surface. Positive controls included gentamicin for Arabica coffee bean, and for Kawa Daun, gentamicin for *E. coli*, erythromycin for *B. subtilis*, and vancomycin for *S. aureus*, with distilled water and methanol serving as negative controls. Following incubation at 37°C for 24 hours, the inhibition zones were measured in millimeters, with each measurement averaged over three replicates for accurate recording.

2.6 Statistical analysis

The procedure of antibacterial activity was repeated in triplicates and the results were presented as mean ± standard error. After that, the results of antioxidant and antibacterial activity were analyzed by using one way analysis of variance (ANOVA) and suitable post-hoc test.

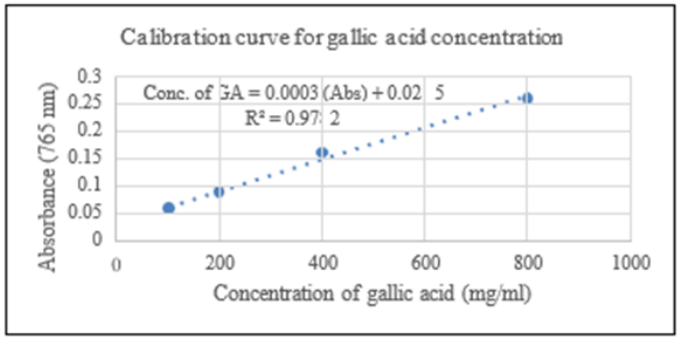

Figure 1. Calibration curve of standard gallic acid against absorbance.

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3. Results and discussion

3.1 Antioxidant activity of ethanolic Arabica coffee bean and methanolic Kawa Daun crude extract

The calibration curve of standard gallic acid against absorbance measured at 765 nm is shown in Figure 1. The regression equation was derived from the calibration curve, resulting in a $\gamma = 0.0003 + 0.0215$ equation. The R^2 value of 0.9782 indicates a strong correlation. Based on this, the total phenolic content of the extracts was determined and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g).

A higher concentration of ethanolic crude extract of Arabica coffee beans specified the potential antioxidant activity increases with its phenolic content. Phenolic compounds can stabilize free radicals by donating hydrogen atoms or electrons, which is the basis for this relationship, thereby averting cellular damage. This is because phenolic compounds have a well-earned standing for having antioxidant qualities that aid in scavenging free radicals and lowering oxidative stress (Yust *et al.*, 2023).

Table 1. Antioxidant activity of different concentration of Arabica coffee beans and Kawa Daun crude extracts.

Concentration (mg/ml)	% Antioxidant of Arabica coffee beans	% Antioxidant of Kawa Daun
800	90.57[a]	-
400	75.54[b]	89.27[a]
200	69.08[b]	81.45[b]
100	57.30[c]	70.0[c]
50	-	55.82[d]

Note: Superscript letters (a, b, c, d) differ to show significant differences between the concentrations within each extract

The antioxidant activity of both the ethanolic crude extract of Arabica coffee beans and the methanolic crude extract of Kawa Daun shows a concentration-dependent increase. Based on Table 1, the Arabica coffee bean extract reaches its highest antioxidant activity of 90.57% at 800 mg/ml, which was higher than the maximum observed in Kawa Daun (89.27%) at 400 mg/ml. At lower concentrations, the antioxidant activity of Arabica coffee beans was noticeably lower, with only 57.30% at 100 mg/ml, whereas Kawa Daun maintain a higher antioxidant capacity at 70.00% at the same concentration. This suggests that Kawa Daun may be more efficient in scavenging free radicals at lower concentrations compared to Arabica coffee beans, potentially due to a higher initial concentration of phenolic compounds or other antioxidant factors present in Kawa Daun. In a study by Anjani *et al.* (2020), it was found that Kawa Daun exhibited a DPPH radical inhibition of over 50%, leading to high antioxidant activity due to their ability to capture free radicals.

The reason for the difference in antioxidant activity can be attributed to the distinct phenolic profiles and bioactive compounds present in each extract. While both Arabica coffee beans and Kawa Daun contain phenolic compounds known to exhibit antioxidant properties, the concentration, and types of phenolics may vary between the two, affecting their ability to neutralize free radicals. Kawa Daun may have a more efficient or diverse set of antioxidants that act more effectively at lower concentrations, while Arabica coffee beans show a stronger overall antioxidant activity only at higher concentrations. This reflects the complex nature of antioxidant activity, which can depend not only on the total phenolic content but also on the specific antioxidant compounds and their interaction with free radicals. A higher concentration of crude extract leads to an increase in the total phenolic content. The scavenging activity increases as the concentration of extract increases. It is evident that the concentration of the crude extract directly affects the increase in DPPH scavenging activity. It is clear from the research conducted by Molole *et al.* (2022) that the antioxidant activity increases as the concentration of the crude extract rises when measuring the DPPH assay. In fact, phenolic compound in Kawa Daun, besides from the raw leaves itself, is also contribute from the firewood substances used during the smoking of the leaves. As cinnamon wood is preferable as source of firewood for coffee leaves smoking, the plant is supposed contributed to the phenolic antioxidant obtained in this present study (Novita *et al.*, 2018). This is in line with the report of another study who detected the significant of phenolic compounds in cinnamon wood smoke (Budaraga *et al.*, 2016).

3.2 Antibacterial activity of ethanolic Arabica coffee bean and methanolic Kawa Daun crude extract

Table 2 showed that the biggest inhibition zones for *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* were 17.00 mm, 15.33 mm, and 12.67 mm respectively at the concentration of 800 mg/mL, the highest concentration tested on Arabica coffee bean crude extract. Higher concentrations (400 and 800 mg/ml) showed more significant inhibition zones, indicating stronger antibacterial effects. For the positive control Gentamicin, the result on *B. subtilis* was 31.00 mm, for *E. coli* was 25.67 mm, and for *S. aureus* was 41.00 mm. The positive control, Gentamicin, consistently showed the largest inhibition zone, particularly against *S. aureus*, confirming its strong antibacterial effect. The positive control consistently shows the largest inhibition zones, serving as a benchmark for maximal antibacterial activity. The positive control showed impressive inhibitory effects, as demonstrated by the substantial zone of inhibition, which concurred with the observation in a previous study conducted by Yosboonruang *et al.*

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Table 2. Inhibition Zones (mm) of Arabica coffee bean and Kawa Daun crude extracts.

		Inhibition zone (mm±SE)	
Bacteria	Concentration (mg/ml)	Arabica coffee beans (mm ± SE)	Kawa Daun (mm ± SE)
Bacillus subtilis	800	17.00 ± 1.53	-
	400	12.33 ± 1.20	17.0 ± 2.08
	200	3.00 ± 3.00	9.3 ± 0.88
	100	2.00 ± 2.00	7.7 ± 0.88
	50	-	6.3 ± 0.88
	PC	31.00 ± 3.46 (Gentamicin)	21.7±1.20 (Erythromycin)
	NC	0	0
Escherichia coli	800	15.33 ± 2.19	-
	400	10.00 ± 0.58	11.0 ± 3.00
	200	8.00 ± 1.00	8.0 ± 0.00
	100	6.33 ± 0.33	7.5 ± 0.50
	50	-	7.0 ± 0.00
	PC	25.67 ± 2.33 (Gentamicin)	27.5 ± 2.50 (Gentamicin)
	NC	0	0
Staphylococcus aureus	800	12.67 ± 0.88	-
	400	10.33 ± 0.33	12.5 ± 1.20
	200	8.67 ± 0.88	9.0 ± 0.58
	100	7.33 ± 1.20	8.3 ± 0.67
	50	-	5.7 ± 2.96
	PC	41.60 ± 0.40 (Gentamicin)	14.3 ± 0.67 (Vancomycin)
	NC	0	0

Data are given as mean of inhibition (mm) of three reading; Keys: (-) Indicates no test been done

(2022). Conversely, it can be inferred that the negative control did not exhibit inhibitory effects since none of the tested strains were able to generate a zone of inhibition. The numerous types of genetic variations present in coffee plants may contribute to the antibacterial properties shown by crude coffee extracts.

Based on the results in Table 2, *Bacillus subtilis* exhibits the most significant inhibition zone at a concentration of 400 mg/ml, measuring 17 mm. In comparison, the inhibition zones for *Staphylococcus aureus*, and *Escherichia coli* were recorded at 12.5 mm and 11.67 mm, respectively. Furthermore, *B. subtilis* demonstrates a statistically significant difference at the 400 mg/ml concentration when compared to other concentrations of the Kawa Daun methanolic crude extract. Notably, *B. subtilis* also shows the largest inhibition zone diameter at a lower concentration of 50 mg/ml, which was 6.33 mm, surpassing the other bacterial strains. It is evident that the zone of inhibition increases with the rising concentration of the Kawa Daun methanolic crude extract. This trend can be attributed to the higher concentration of bioactive compounds present in the sample, which enhances the efficacy of the extract as the quantity of these compounds increases. *Bacillus subtilis* exhibited the largest inhibition zone diameter among the pathogenic bacteria tested, measuring 17 mm at the highest concentration of 400 mg/ml. According to Table 2, this result can be attributed to the fact that *Bacillus subtilis*, a Gram-positive bacterium, demonstrates significantly lower resistance compared to its Gram-negative counterparts (Breijyeh *et al.*, 2020). Furthermore, the structural differences in the thickness and composition of their cell walls contribute to the varying effectiveness of these bacteria in response to different conditions (Ida Muryany and Nur Amira, 2023).

Based on the Table 2, it can be seen that ethanolic crude extract of Arabica coffee bean produced higher reading of inhibition zone compared to methanolic crude extract of Kawa Daun. Thus, ethanolic crude extract of Arabica coffee bean has better position as antibacterial because coffee beans are proved to contain more concentration of caffeine, which also act as one of antibacterial properties than coffee leaves (Dado *et al.*, 2019).

Based on Figure 2, the inhibition zones for both Arabica coffee beans and Kawa Daun show differences in antibacterial effectiveness across the various concentrations. At higher concentrations, both extracts exhibit similar activity against *Bacillus subtilis*, with Arabica coffee beans showing a slightly larger inhibition zone (17.00 mm) compared to Kawa Daun (17.0 mm). However, at concentrations below 400 mg/ml, the Arabica coffee bean extract demonstrates a more significant reduction in inhibition zone size, particularly with *Bacillus subtilis* and *Staphylococcus aureus*. This indicates that Kawa Daun may be more consistent in its antibacterial activity at lower concentrations. For *Escherichia coli*, Arabica coffee beans show a higher inhibition at 800 mg/ml, nevertheless at 400 mg/ml and below, both extracts exhibit similar inhibition zones. These results suggest that Kawa Daun might be more

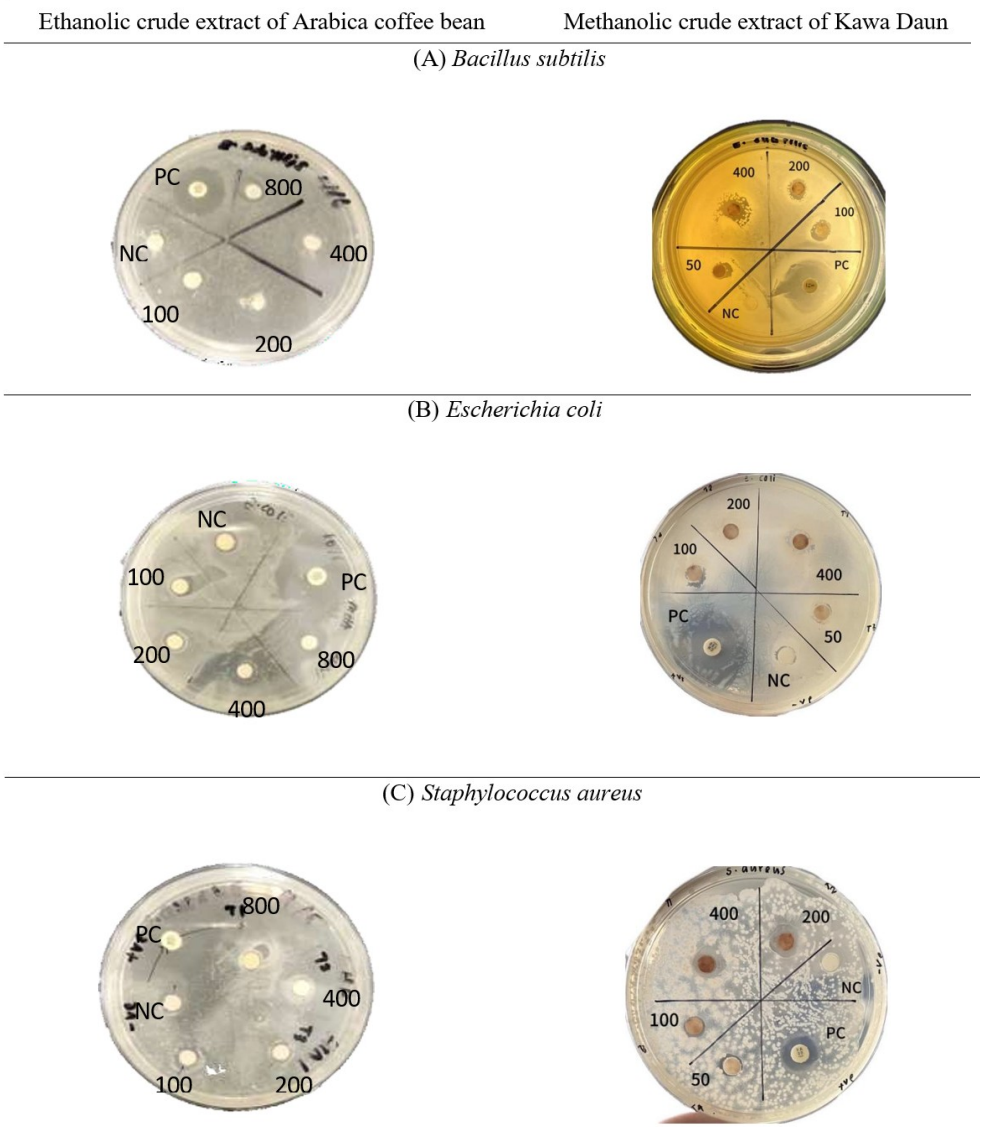

Figure 2. Inhibition zones of (A) *Bacillus subtilis*, (B) *Escherichia coli,* (C) *Staphylococcus aureus* after being treated with ethanolic crude extract of Arabica coffee bean at different concentrations (800, 400, 200, 100 mg/ml), and methanolic crude extract of Kawa Daun at different concentrations (400, 200, 100, 50 mg/ml), PC (positive control) and NC (negative control).

effective at maintaining its antibacterial activity over a range of concentrations. Moreover, previous study also obtained the effectiveness of smoke from cinnamon wood as antibacterial agent (Putri *et al.*, 2021) as a common firewood source of smoke for drying the Kawa Daun.

Several factors could explain these differences in antibacterial effectiveness. The variations in inhibition zones could be attributed to the chemical composition of the extracts, such as the types and concentrations of bioactive compounds like phenolics, flavonoids, or alkaloids. Arabica coffee beans are rich in phenolic compounds, which are known for their potent antioxidant properties and may also contribute to their antibacterial activity. In contrast, Kawa Daun may contain other bioactive compounds that are more effective against specific bacteria, potentially explaining the differences in activity, especially at lower concentrations. The method of extraction (ethanolic vs. methanolic) may also play a role in determining which compounds are more readily extracted, influencing the overall antibacterial potency of the extracts.

Conclusion

In conclusion, this study demonstrated the significant potential of both Arabica coffee bean and Kawa Daun extracts as natural sources of antioxidants and antibacterial agents. The antioxidant activity of the methanolic crude extract of Kawa Daun showed a significantly higher statistical reading, indicating its superior antioxidant potential over the ethanolic crude extract of Arabica coffee beans. Hence, the methanolic crude extract of Kawa Daun exhibits significantly higher antioxidant potential compared to the ethanolic crude extract of Arabica coffee beans.

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The methanolic crude extract of Kawa Daun is a superior antioxidant, demonstrating significantly higher antioxidant activity compared to the ethanolic crude extract of Arabica coffee beans. However, for antibacterial activity, the ethanolic crude extract of Arabica coffee beans showed stronger results than Kawa Daun. Therefore, Kawa Daun leaves are recommended as a potent antioxidant, while Arabica coffee beans are more effective as an antibacterial agent.

Conflict of interest

The authors declare no conflict of interest.

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