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Screening of the antimicrobial activity of Indonesian banana (*Musa* spp.) against pathogenic bacteria

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Abstract. *Sa'diyah H, Hariyadi YDP, Ubaidillah M, Sari VK. 2024. Screening of the antimicrobial activity of Indonesian banana (*Musa *spp.) against pathogenic bacteria. Biodiversitas 25: 1368-1374.* Investigation of natural sources of antibacterials becomes increasingly relevant and necessary due to the prevalence of high antibiotic resistance. To mitigate this issue, banana, a consumable fruit, is gaining attention as a potential natural source of antibacterials, with some varieties showing potential as alternatives to conventional antibiotics against pathogens. Exploring the potential for non-commercial bananas is important to discovering their antibacterial properties. Therefore, this study aimed to evaluate the antibacterial activity of 23 explored banana varieties against *Escherichia coli, Salmonella typhi, Staphylococcus aureus* bacteria using the diffusion disc method. The results showed that all the different types of banana did not significantly affect the antibacterial activity. Kayu banana variety indicated the highest activity against *E. coli*. In contrast, Ijo banana showed potent activity against *S. aureus* with a non-significantly different inhibition zone than the effectiveness of ampicillin. Meanwhile, Kepok Makassar banana variety showed the highest antibacterial activity against *S. typhi*, which was lower than chloramphenicol's effectiveness. The Raja Nangka, Morosebo, Janten, Selendang, and Biji Seribu banana varieties had low antibacterial activity against all three bacteria tested. Therefore, the results showed opportunities for further study of various bacterial types with different banana varieties and their extracts.

Keywords: Antibacterial activity, banana, disc diffusion, *Escherichia coli, Salmonella typhi*, *Staphylococcus aureus*

Abbreviations: PCA: Principal component analysis; SVD: Singular value decomposition

INTRODUCTION

Bacterial infections, such as *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Salmonella typhi* (*S. typhi*) are important contributors to various health problems and diseases in humans. These three bacteria have commensal and facultative anaerobic characteristics (Aijuka and Buys 2019) and are responsible for several digestive, respiratory, and skin infections, including typhoid fever cases. *Escherichia coli* is gram-negative, bacillus-shaped, motile bacterial, while *S. aureus* is gram-positive, coccus-shaped, non-motile, and *S. typhi* is a gram-negative, bacillus-shaped, motile bacterial. To address these infections, bananas, widely consumed globally and traditionally used in medicine, have proven beneficial. In Indonesia, Mas banana is used as yellow fever and typhoid medicine, while the Klutuk variety effectively treats diarrhea. Furthermore, banana is used in Pakistan to treat inflammation, rheumatism, diabetes, hypertension, coughs, and bronchitis, including in Thailand for treating digestive disorders such as diarrhea and ulcers (Pereira and Maraschin 2015). This plant also has potential antibacterial properties (Jain et al. 2011), containing phytochemical compounds, such as polyphenols and lectins, capable of preventing the formation of bacterial biofilms (Wu and Zhou 2021; Ahmed et al. 2023). Previous investigations on bioactive components showed that bananas are a natural source rich in antibacterial properties beyond being a consumable fruit, as shown in their use in various health practices.

Banana is a highly diverse group of plants, with each cultivar characterized by a unique compound profile that contributes to its antibacterial properties. The different types of bananas that are currently consumed originated from two wild species, *Musa acuminata* and *Musa balbisiana* (Stover and Simmonds 1987). As the center of origin and diversity for bananas worldwide, Indonesia has played an important role in their development and distribution (Simmonds 1962). This phenomenon has led to the screening and evaluating bananas as medicinal plants for their therapeutic potential and antimicrobial properties (Jain et al. 2011). Many studies on the antibacterial activity of several parts of banana (including stem, leaves, and fruit peel) and using various extracts (including chloroform, ethyl acetate, ethanol, and water) have been conducted (Mokbel and Hashinaga 2005; Behiry et al. 2019; Subramaniam et al. 2020; Sivasamugham et al. 2021; Shaukat et al. 2023). Studies on antimicrobial properties of banana pulp have mainly focused on popular varieties. The antimicrobial activity in fruits varies among different species and cultivars (Sirajudin et al. 2014). Ananta et al. (2018) revealed that methanol extract from the peel of Susu banana, Mas banana, and Kayu banana inhibited the development of *E. coli* and *S. aureus*. Wahyuni et al. (2019) added that yellow Kepok banana peel, which contains flavonoids and phenolic, can also inhibit the development of bacteria. In general, plant secondary metabolites have been linked to antimicrobial properties. Banana peel contains secondary metabolites, namely terpenoids, glycosides, alkaloids, phlobatannins, and flavonoids. The antibacterial properties of banana peels may be due to these secondary metabolites and phytochemicals (Kapadia et al. 2015). The nutritional value of fruits varies greatly depending on various factors, including variety, climate, soil type, temperature, light intensity, and many more. Fruit phytochemical profiles are influenced by several factors, such as processing methods, cultivars, growing season, ripeness, and place of origin (Babu et al. 2012). The objective of this study was to assess the antimicrobial properties of banana cultivars explored in East Java, Indonesia. Furthermore, to enhance comprehension of the similarities/dissimilarities, relationships among cultivars in the antimicrobial characteristics of the examined extracts and the susceptibility of the evaluated microbial strains, we have employed multivariate statistical analysis (cluster analysis and Biplot PCA) on the activity data.

MATERIALS AND METHODS

Banana collection

Banana fruits were collected from markets in Jember and Lumajang, two of the major banana-producing cities in East Java Province (BPS 2023). The selected fruits were collected before reaching full ripeness and were not treated with ethylene. Subsequently, the fruits were stored to achieve level 6 of ripeness (Majaliwa et al. 2019), except for banana varieties that did not show a yellow color when ripe, such as Ijo and Raja Nangka banana (green) and Musang (red).

Extraction

A 10-gram pulp was ground and extracted using the maceration method, following the procedure by Bashmil et al. (2021) with modification. The sample was mixed with 30 mL of a 70% ethanol solvent, and 0.0008 g of sodium metabisulfite was added to prevent enzymatic browning. Subsequently, the sample was placed on a shaker at 20°C for 16 hours to separate the supernatant and pellet, then stored in a falcon tube at 4°C.

Preparation of bacterial media

In this study, the Nutrient Broth (NB) media was used, with a mixture of 2 g NB and 3.75 g bacterial agarose prepared by adding distilled water to an erlenmeyer flask to a volume of 250 mL. The mixture was homogenized using a magnetic stirrer and sterilized by autoclaving at 121°C for 30 minutes. After sterilization, the solution obtained was poured into 10 petri dishes, allowed to solidify, and sealed.

Bacterial rejuvenation

The three types of bacteria commonly encountered by humans were used to determine antibacterial activity, including *E. coli*, *S. aureus*, and *S. typhi*. Rejuvenation was achieved by taking a single dose of bacteria from the initial stock and inoculating it onto solid NB media using the streaking method. All activities were conducted near the Bunsen burner in Laminar Air Flow (LAF) to prevent contamination. Subsequently, the edges of the petri dish were heated with a Bunsen burner, sealed, and incubated until bacterial growth became apparent.

Making liquid bacterial cultures

The liquid bacterial culture was made by preparing liquid NB media and dissolving 2 grams of NB in 250 mL of distilled water. Subsequently, the solution was sterilized by pouring 6 mL into a screw tube and autoclaved it for 30 minutes. The successfully grown bacteria at the previous stage, namely the bacterial rejuvenation stage, were collected using a micropipette and transferred into a screw tube containing liquid NB. The tube was closed and shaken for 24 hours, and bacteria were obtained from a single colony.

Antibacterial activity assay

Antibacterial activity was tested using the paper disc diffusion method, following the Kirby-Bauer method, as described by Lai et al. (2010). The test was conducted with three replications, where each bacterial strain was grown overnight in liquid NB media and transferred onto the surface of solid NB media using a sterile cotton swab. Sterile filter paper discs, measuring 6 mm in diameter, were placed on the surface of solid NB media and incubated at 37°C for 24 hours. After incubation, the antibacterial activity was assessed by measuring the size of the inhibition zone formed in millimeters. Ampicillin, chloramphenicol, and 70% ethanol were used as positive controls, while aquadest was used as the negative control.

Data analysis

In this study, three replicates of each sample were used for statistical analysis, and their average values were obtained. The original data obtained were analyzed using one-way analysis of variance (ANOVA), while the post hoc test applied Tukey's HSD method. After ANOVA, clustering was carried out by the average linkage method. A dendrogram, including all the cultivars, was created using the Euclidean distance of the original data (variables had similar scales, and had not been transformed before clustering). The Euclidean distance was defined as the square root of the sum of the squares of the differences between individuals i and i' for each variable. A biplot analysis was conducted to visualize data patterns using PCA (Principal Component Analysis) on the variance-covariance matrix. Subsequently, SVD (Singular Value Decomposition) was used to calculate the principal component. Biplot and cluster analysis were calculated using PAST 3.25 (Hammer et al. 2001).

RESULTS AND DISCUSSION

The morphological variation of 23 banana cultivars in Indonesia is presented in Figure 1. Antibacterial activity test against three types of bacteria are shown in Table 1 and Figure 2. The mean of inhibition zone against *E. coli* was 11.42 mm, with a range of 0.0-16.33 mm, and the CV was 39.2%. At the same time, the mean of inhibition zone against *S. typhi* and *S. aureus* were 9.46 mm and 14.87 mm, with a range of 0.0-13.06 mm and 5.37-22.59 mm, and the CV was 33.13 and 34.58%, respectively. The inhibition zone of banana cultivars showed considerable variability (the CV was greater than 30%). This meant that the cultivars collected were moderately varied. The calculation of the average antibacterial activity separately between dessert and cooking banana resulted that the mean of the inhibition zone of dessert banana was higher than cooking ones, against those three bacteria (Table 1). A two-sample t-test was conducted to investigate whether those differences were significant (the "dual" types were excluded), resulting in insignificant mean differences. It means that the antibacterial activity did not affected by the banana types.

The diameter of inhibition zone shows the susceptibility of the test bacteria, where a wider diameter represents better antibacterial activity (Bhargav et al. 2016). The results showed that banana cultivars, including Janten and Raja Nangka for *E. coli*, and Raja Nangka and Morosebo for *S. typhi*, were characterized by smaller inhibition zone values significantly different from 70% ethanol. Meanwhile, cultivars with lower values were not significantly different from 70% ethanol were four for *E. coli* and *S. aureus* and six for *S. typhi*, as shown by a smaller mean value and the same grouping letter. These cultivars had very low or zero antibacterial properties, indicating that not all banana varieties show significant antibacterial properties against the three tested bacteria. The variation in antibacterial activity among different banana cultivars is attributed to their genetic differences. According to Hoban et al. (2022), diversity exists at both the bioregional and genetic levels, with each cultivar possessing a unique genetic profile capable of affecting chemical compounds.

Table 1 showed that ampicillin, used as a positive control, indicated the highest antibacterial activity, with an inhibition zone of 17.11 mm against *E. coli* and 22.95 mm against *S. aureus*. This phenomenon occurred because ampicillin is an antibiotic, capable of inhibiting the growth of the two bacteria (Li et al. 2019; Ramon-Curay et al. 2023). Furthermore, Kayu, Raja, Mas, Sri Ayu, Agung, Cavendish, Kepok Sumatra, Susu, and Sebulan banana varieties had high antibacterial inhibition zone values against *E. coli*. The values obtained varied significantly from 70% ethanol and were not substantially different from ampicillin, where the largest inhibition zone of 16.33 mm, was observed in Kayu Banana. Moreover, in the antibacterial inhibition zone value against *S. aureus*, only one cultivar, the Ijo banana, had the highest value. Ijo banana cultivar showed a significant average inhibition zone of 22.59 mm, comparable to ampicillin's effectiveness. This suggested that the Ijo cultivar has significant potential as a natural source of antibacterial agents against *S. aureus*.

The banana cultivar with the highest average resistance zone value was Kepok Makassar, measuring 13.06 mm, and was categorized into group b according to the Tukey test. However, the value was lower and significantly different from chloramphenicol, a powerful antibiotic with the largest inhibition zone of 21.68 mm. This showed that Kepok Makassar had a relatively high antibacterial potential compared to other cultivars in inhibiting *S. typhi* bacteria. Banana cultivars with a high inhibition zone can be used as antibacterials, particularly against *E. coli*. Several cultivars have high scores against *E. coli* and are not significantly different from ampicillin, which a strong antibacterial properties (Zyryanov et al. 2021). Therefore, further chemical analysis tests to identify the specific compounds responsible for antibacterial activity are expected to yield numerous benefits in the pharmaceutical field.

Figure 1. Diversity of banana morphology. 1. Kepok Sumatra, 2. Cavendish, 3. Ambon, 4. Selendang, 5. Kepok Makassar, 6. Musang, 7. Ijo, 8. Mas, 9. Sebulan, 10. Rajamala, 11. Sri Ayu, 12. Agung, 13. Morosebo, 14. Susu, 15. Candi, 16. Kepok, 17. Kepok manurun, 18. Lilin, 19. Biji Seribu, 20. Janten, 21. Raja Nangka, 22. Kayu, 23. Raja

Figure 2. Antibacterial test of 23 banana cultivars on 6 mm diameter paper discs

Table 1. Antibacterial activity of 23 banana cultivars against *E. coli*, *S. aureus*, and *S. typhi*

Cultivars	Types	*E. coli*	*S. typhi*	*S. aureus*
Kayu	Dual	16.327 ± 0.863^{ab}	11.923 ± 1.678^{bcd}	17.21 ± 1.83^{abc}
Raja	Dual	15.627 ± 0.521^{abc}	11.733 ± 0.812^{bcd}	18.2 ± 4.69^{abc}
Mas	Dessert	15.003 ± 0.648^{abcd}	12.3 ± 2.05^{bc}	20.32 ± 6.57^{ab}
Sri Ayu	Cooking	13.993 ± 0.295^{abcd}	11.033 ± 0.911^{bcd}	15.78 ± 3.17^{abc}
Agung	Cooking	13.903 ± 0.966^{abcd}	8.54 ± 0.242^{bcd}	10.88 ± 9.43^{abcd}
Cavendish	Dessert	13.687 ± 0.525^{abcd}	11.32 ± 1.91^{bcd}	16.87 ± 3^{abc}
Kepok Sumatra	Cooking	14.34 ± 0.1353^{abcd}	11.38 ± 2.17^{bcd}	16.537 ± 1.193^{abc}
Susu	Dessert	14.273 ± 1.04^{abcd}	11.61 ± 0.918^{bcd}	10.68 ± 9.27^{abcd}
Sebulan	Dessert	14.23 ± 3.27^{abcd}	11.073 ± 0.886^{bcd}	18.54 ± 6.23^{abc}
Kepok Manurun	Dual	13.19 ± 0.494^{abcde}	10.497 ± 0.336^{bcd}	20.31 ± 8.27^{ab}
Candi	Cooking	13.04 ± 0.771^{abcde}	11.42 ± 1.75^{bcd}	15.467 ± 0.703^{abc}
Ijo	Dessert	12.72 ± 1.04^{abcdef}	10.19 ± 0.987^{bcd}	22.59 ± 3.01^{a}
Musang	Dessert	12.137 ± 0.853^{cdef}	8.61 ± 1.055^{bcd}	18.43 ± 0.551^{abc}
Kepok Makassar	Cooking	11.293 ± 0.273^{defg}	13.06 ± 0.911^{b}	19.03 ± 2.42^{abc}
Ambon	Dessert	8.92 ± 0.474^{fgh}	7.783 ± 0.575^{cd}	12.387 ± 0.533^{abcd}
Lilin	Dual	12.677 ± 1.368^{bcdef}	11.673 ± 1.259^{bcd}	17.52 ± 3.03^{abc}
Kepok	Cooking	12.647 ± 1.308^{bcdef}	11.76 ± 1.98^{bcd}	18.25 ± 2.75^{abc}
Rajamala	Dessert	12.163 ± 0.818^{cdef}	10.897 ± 1.348^{bcd}	14.38 ± 3.28^{abc}
Biji Seribu	Dessert	7.45 ± 1.466^{gh}	7.37 ± 0.904^{d}	10.027 ± 0.965^{abcd}
Selendang	Dessert	7.33 ± 0.384^{gh}	7.977 ± 0.76^{cd}	6.04 ± 5.24^{cd}
Morosebo	Cooking	6.17 ± 0.235^{h}	0^{e}	5.48 ± 4.97^{cd}
Janten	Cooking	0^{i}	7.517 ± 0.837^{d}	8.15 ± 2.14^{bcd}
Raja Nangka	Cooking	0^{i}	2.09 ± 3.63^{e}	5.37 ± 4.69^{cd}
Ethanol (70%)	-	9.48 ± 3.493^{efgh}	9.35 ± 1.243^{bcd}	8.567 ± 1.206^{bcd}
Aquadest	-	0^{i}	0^{e}	0^{d}
Ampicillin	-	17.11 ± 2.58^{a}	Not observed	22.95 ± 1.393^{a}
Chloramphenicol	-	Not observed	21.68 ± 3.07^{a}	Not observed
CV		39.2%	33.13%	34.58%

Note: Values are means ± S.D. of three replications; Superscript was cultivar grouping using Tukey's test with a 0.05 significance level

Kepok banana inhibition zone against *S. aureus* was not significantly different from ampicillin as a positive control. According to Maryati et al. (2021), the Kepok banana was effective as an antibacterial agent against *S. aureus*, but with a significantly different bacterial inhibition zone compared to chloramphenicol. Ambon banana has a relatively high inhibition zone against *S. aureus*, not significantly different from ampicillin. Another study also showed that some concentrations of leaf sheath extract from Ambon banana effectively heal burns infected by *S. aureus* (Samsuar et al. 2016). The observed variability in antibacterial effectiveness among banana cultivars could be attributed to genetic variation. The results showed that each cultivar possessed a distinct genetic composition capable of influencing chemical compound composition, including antibacterial properties. Environmental factors such as soil type, climate, and agricultural practices, can also influence the composition of compounds in banana fruits.

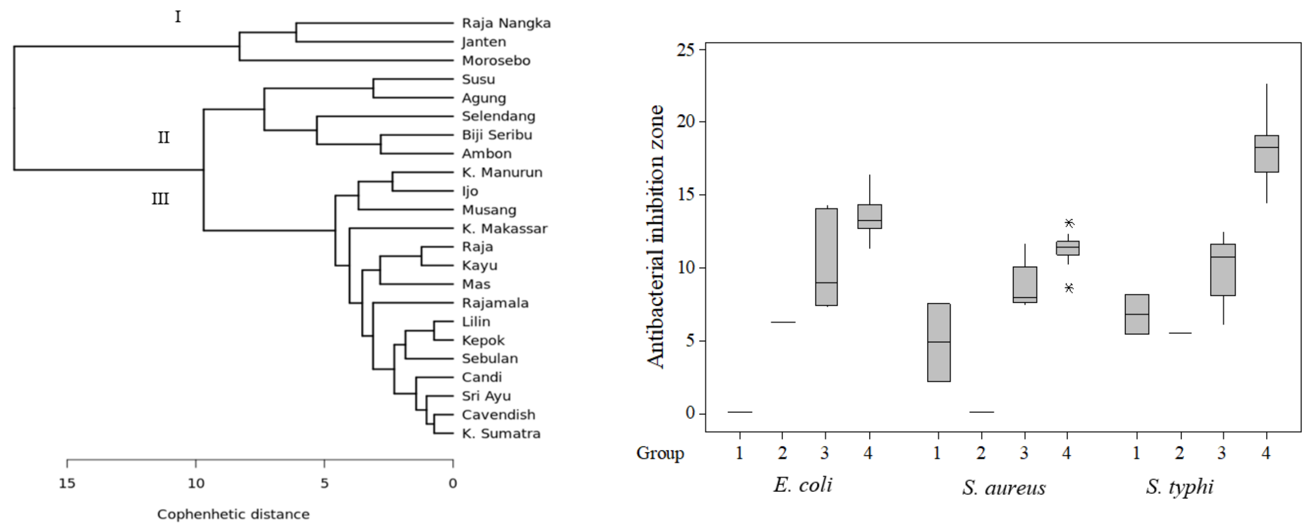
Almost all the inhibition zones of bananas against *S. aureus* were larger than *S. typhi* and *E. coli*, and only four (17.4 %) cultivars have different patterns, i.e. Agung, Susu, Selendang, and Morosebo. The type of bacteria possibly influences it. As a gram-positive type bacteria, *S aureus* contains lots of peptidoglycan which is easily soluble in water, making its cell walls more polar, and the antibacterial compounds of bananas can more easily enter the cells. So, the inhibition zone was larger than *S. typhi* and *E. coli* which are gram-negative bacteria. Therefore, to inhibit bacteria, the test material must penetrate the cell wall (Pelczar et al. 1993; Suhartati and Virgianti 2015). This is in line with the results of Rita et al. (2023) on Susu banana peel methanol extract. Mokbel and Hashinaga (2005) also found a similar on Cavendish banana green peel using EtOAc solvent, where the inhibitory zone against *S. aureus* was always higher than *E. coli* at all concentrations tested with an average of 12 mm and 9 mm for *S aureus* and *E. coli*, respectively. The antibacterial activity of a compound is related to its ability to influence the structure and function of bacterial cells. According to Efenberger-Szmechtyk et al. (2021), antibacterial compounds can induce morphological changes in microorganisms, damage bacterial cell walls, prevent biofilm formation, disrupt protein synthesis, alter bacterial cell metabolism, and inhibit ATP and DNA synthesis. This study used ampicillin and chloramphenicol as positive controls due to their common usage as antibiotics with proven antibacterial activity against *E. coli* and *S. aureus*. In contrast, chloramphenicol showed high activity against *S. typhi* (Zyryanov et al. 2021). *E. coli* is a bacteria commonly present on human skin, serving as a normal part of the human body's flora and opportunistic pathogen. As the primary cause of digestive tract infections, *E. coli* shows genetic adaptations that allow colonization and survival in different environments, including the natural and the host body (Budiarti et al. 2022). A previous study has identified *S. typhi* as the main cause of typhoid fever in humans, primarily spreading through consuming contaminated food and drinks. According to Oludairo et al. (2022), *S. typhi* is a facultative anaerobe with motility characterized by a rod-shaped gram-negative bacterial size of approximately 2-3 x 0.4-0.6 µm, lacks spore formation and possesses several characteristics, including negative results for oxidase, indole, urease, lactose, and sucrose tests. Moreover, *S. aureus* is a gram-positive bacterial widely recognized as a nosocomial pathogen, causing several diseases, including mild skin infections and more severe conditions such as life-threatening infective endocarditis and toxic shock syndrome (Che Hamzah et al. 2019).

Some biochemical compounds, namely alkaloids and tannins are known to damage bacterial cell walls which are also found in bananas. Alkaloids work as antibacterial agents by reacting with the peptidoglycan component to prevent the bacterial cell wall layer from forming. This mechanism damages the bacterial cell wall, prevents bacterial growth, resulting in cell death (Kusumastuti et al. 2021). Tannins are phenolic chemicals that have the ability to block cell wall protein activity, leading to cell lysis and a loss of physiological activity. Strong antibacterial effects can be obtained by disrupting the permeability of bacterial cell walls through the astringent qualities of tannins, which cause cell walls or cell membranes to shrink (Tyler et al. 1976).

Many food-borne bacteria cause disease and, in some cases, death. The food business continues to face the challenge of eliminating germs from food without affecting the product's beneficial features (Panda et al. 2019). The current study's findings may pave the way for appropriate and natural preservatives in the food-processing industry to control food-borne infections. However, more research is needed to evaluate these extracts in food matrixes and identify the beneficial components using bioassay-guided purification.

Multivariate analysis

In this cluster analysis, the diameter inhibitory zones of all three bacteria served as variables, whereas banana cultivars were used as observations. The resulting dendrogram is given in Figure 3.A. According to cluster analysis, the banana cultivars were divided into 4 groups. The number of cultivars per group for the four groups ranged from 1 in Group 2 to 15 in Group 4, and the distance between groups was approximately 7.3 (Figure 3.A). Raja Nangka and Janten belong to Group 1, Morosebo belong to Group 2, Susu, Agung, Selendang, Biji Seribu and Ambon belong to Group 3, and the rest belong to Group 4. The characteristics of each group were explained using a boxplot (Figure 3.B). The dendrogram showed that Group 1 was characterized by having the lowest mean bacterial activity against *E. coli* (0.0 mm). Group 2 was characterized by the lowest mean against *S. aureus* (0.0 mm) and *S. typhi* (5.48 mm). Group 3 had relatively moderate antibacterial activity but the highest variance for the three bacteria. Group 4 has the highest number of members, and is characterized by having the highest antibacterial activity values for all tested bacteria. There were two outliers for *S. aureus*. i.e., Musang with the smallest value, and Kepok Makassar with the highest value.



A **B**

Figure 3. A. Dendrogram representing antibacterial activity (diameters of growth inhibition zones) against *E. coli*, *S. aureus*, and *S. typhi* using average linkage cluster analysis and Euclidean distance on the unstandardized-variable matrix; B. Boxplot of three variables included in cluster analysis

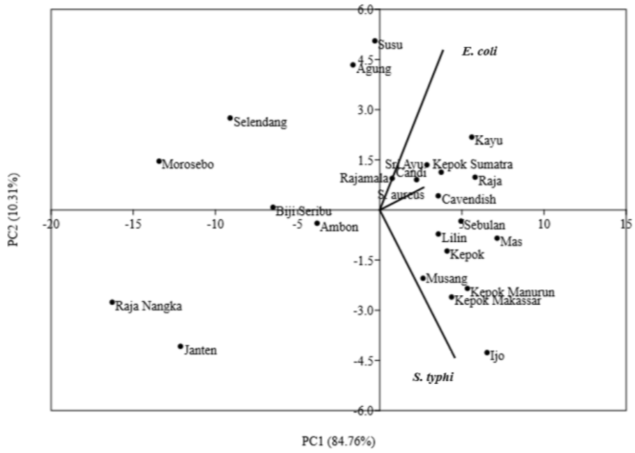


Figure 4. Biplot analysis of the antibacterial activity of 23 bananas against *E. coli*, *S. aureus*, and *S. typh*i based on the diameter of the inhibition zone

Biplot analysis is a valuable tool for visualizing high-dimensional data using a two-dimensional graph, illustrating the relationships between variables and observations in a dataset (Fukuyama 2022). This study used Biplot analysis to examine the relationship between various banana cultivars as antibacterial agents and their efficacy against different bacterial strains. The first two principal components (PC1 and PC2) explained 95.07% of the variance in the data, effectively visualizing the observations through projections onto the span of the first two PCs.

The Biplot result showed that Raja Nangka, Morosebo, Janten, Selendang, and Biji Seribu banana had the lowest antibacterial properties for the three bacteria tested. The results showed that their positions on Biplot were in different quadrants from the three bacteria and distanced from other cultivars (Figure 4). Several banana cultivars were positioned close to each other, namely between Sebulan, Lilin, and Kepok; Susu and Agung; Kayu and Raja; as well as Sri Ayu, Kepok Sumatra, and Candi. Furthermore, the variation of antibacterial abilities of all banana varieties observed was lowest in *S. aureus*, as evident in its short line length in Biplot compared to the others.

Moreover, to our knowledge, this is the first detailed study that explores many Indonesian bananas including commercial and noncommercial and contributes to the understanding of their antibacterial activity. This study successfully identified several types of bananas with high antibacterial potential for certain bacteria. The antibacterial activity for dessert banana and plantain was not significantly different. The results showed that Kayu banana showed antibacterial activity against *E. coli* bacteria, Kepok Makassar for *S. typhi*, and Ijo banana against *S. aureus* bacteria. Moreover, these results presented opportunities for further study of other bacterial types with different banana varieties and their extracts.

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