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MORINGA LEAF ANTIBACTERIAL TEST IN LAYER CAKE PRODUCTION AGAINST ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS BACTERIA

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MORINGA LEAF ANTIBACTERIAL TEST IN LAYER CAKE PRODUCTION AGAINST *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* BACTERIA

I Ketut Budaraga[*], Dian Pramana Putra[1], Wellyalina[2]

[1]Faculty of Agriculture, Ekasakti University, Padang, Indonesia
[2]Faculty of Agriculture Technology, Andalas University, Padang, Indonesia

[*]Corresponding author
Email: budaraga1968@gmail.com[*], dian.pramana90@gmail.com, wilyalina.lia@gmail.com

Abstract. *The layer cake is one of the traditional cakes that are very popular with the community. The addition of Moringa seeds is expected to extend the storage period and the components of the nutrition can be increased. Moringa leaves indicate to contain an antibacterial compound that is the result of secondary metabolites. This compound consists of alkaloids, saponins, flavonoids, tannins, terpenoids, and others. The purpose of this study was to determine the antibacterial properties of Moringa leaves added to layer cake against pathogenic bacteria Escherichia Coli and Staphylococcus Aureus. Research has been implemented on April - May 2019. The testing of antibacterial activity by using Sumur method. The results showed that the layer cake with the addition of 4% Moringa leaves indicated the high inhibition zone on the bacteria E. Coli by 10.7 mm and S. Aureus by 9.7 mm when compared with the addition of 1%, 2%, and 3 % Moringa leaves. The result of bacterial pathogens that were tested in Moringa leaves showed that the bacteria E. Coli had resistance to more robust compared with S. Aureus. This is indicated by the inhibition zone of E. Coli that is greater than S. Aureus bacteria.*

Keywords: *moringa leaf; layer cake; escherichia coli; staphylococcus aureus bacteria.*

1. Introduction

The use of Moringa in Indonesia is still not widely known, generally, only it is known as one of the vegetable menus. In addition to direct consumption in the fresh form, Moringa can also be processed into the form of powder. Furthermore, it can be used as the material for fortificant ingredients to provide nutrients for various food products, such as processed pudding, cake, nuggets, biscuit, crackers, and other processed products [1].

Bioactive compounds in Moringa leaves cause Moringa to have pharmacological properties. Besides, it has been identified that Moringa leaves contain high antioxidant and antibacterial properties. Therefore, Moringa has the function as the natural preservative and extend its storage period. Snack foods sold at traditional markets are diverse, one of them is a layer cake. A layer cake is one type of traditional snacks that are known and circulated in the community [2].

In previous studies, Moringa leaves were used as the preservative catfish nugget. At the beginning of storage for all the concentration of Moringa leaves, there were no bacteria found. However, after being stored for two days, some bacteria grew. on the fourth day, it even tends to multiply. Furthermore, it can be seen that concentrations of 0 g, 25 g, and 30 g

are visible that the longer it is stored, the more of the total number of bacteria is increasing. However, at the concentration of Moringa leaves 35 g and 40 g on the fourth day, the total number of bacteria decreases [3]. Based on the data above, this study conducted the test of Moringa leaves antibacterial on layer cake production against the bacteria Escherichia coli and Staphylococcus aureus.

This research was conducted to determine the antibacterial properties of Moringa leaves added to layer cake against pathogenic bacteria Escherichia coli and Staphylococcus aureus with total concentrations of 0% -8% Moringa leaf powder.

2. Methods

Tools and Materials

The research has been carried out at the Agricultural Product Technology Laboratory of the Universitas Ekasakti and the Agricultural Product Microbiology and Biotechnology Laboratory of Universitas Andalas in April - May 2019. This research used the explorative study design by using the collection of Escherichia coli and Staphylococcus aureus bacterial isolates from the Agricultural Product Microbiology and Biotechnology Laboratory, Faculty of Agricultural Technology, Universitas Andalas.

The main ingredients used in this study were dark green Moringa leaf 3600 g and other ingredients namely rice flour (Rose Brand) 75 g, starch (Pak Tani Gunung) 50 g. Materials used for antibacterial analysis are the growth test bacteria (Escherichia coli and Staphylococcus aureus) and jelly nutrient media (Merck, Darmstadt, Germany). The tools used for making layer cake are the baking pan, stirring spoon, basin, stove, steamer pot, napkin, measuring spoon, and measuring cup. The tools used for making Moringa leaf powder are the basin, drainer, blender (Vitara), 80 mesh sieve, and scale. Laminar airflow antibacterial test equipment (Telster BV-100 Spain), analytical balance (Kern ABJ 220-4 M), autoclave (Hiclave HVE - 50 Hirayama, Japan), oven (Memert, Germany), Petri dish, ose needle, Bunsen lamp, Erlenmeyer 250 ml (Iwaki TE-32 Pyrex, Japan), test tube (Iwaki TE-32 Pyrex, Japan), micropipette, vortex, incubator (Memmert Model 100-800 INE 600, Germany), hotplate stirrer (AREC Velp Scientifica, Europe), Colony Counter (Philip Harris, England), callipers, cotton, plastic wrap, aluminium foil and tweezers.

Research design

This research uses exploratory design through experiments in the laboratory with the addition of Moringa leaf powder in the layer cake, which are (1, 2, 3 and 4%). Data were collected using direct observation after the treatment is given to the research objects, and then

performing a series of tests.

Body Text

Procedure for obtaining Moringa leaf powder according to [4] (1) pick dark green Moringa leaf as much as 3600 g, (2) wash fresh old Moringa leaves using clean water and drain those leaves, (3) spread the thin fresh old Moringa leaves in the drying container, the drying process is completed at the room temperature. The final product must be very dry, (4) grind the fresh, dried Moringa leaves by using the blender and sieved with 80 mesh sieve, (5) Moringa leaf powder.

Making layer cakes by adding Moringa leaf powder

The procedure in making layer cakes by adding Moringa leaf powder as the natural preservative is as follows: (1) stir in 275 ml coconut milk with 50 g sugar and 2 g salt, until the sugar dissolves for 3-5 minutes, (2) mix in the container: 75 g rice flour and 50 g starch. Boil coconut milk little by little, stir until the dough mixed thoroughly, (3) add the powder according to the treatment, (4) heat the steamer pan until the water boils, spread the pan with cooking oil, (5) After steaming pan steams a lot, pour the mixture according to the desired layer with the thickness of 0.5 cm.

Testing the Inhibition Zone Activity of E. Coli and S. Aureus Bacteria [5]

Making the media

Slanted jelly media is made by way dissolving Nutrient Agar (NA) about 2 grams in 100 ml of distilled water using Erlenmeyer flask. After that, the mixture is homogenized with the stirrer on the hot plate stirrer until it boils. A total of 5 ml was poured each in 3 sterile test tubes and covered with aluminum foil. The media is sterilized in the autoclave at 121o C for 15 minutes, then left at room temperature for ± 30 minutes until the media solidifies at the slant of 30o. The slanted jelly media is used for bacterial inoculation.

Media base is made by weighing Nutrient order (NA) as much as 6 grams, then dissolved in 300 ml of distilled water using Erlenmeyer flask. After that, the media is homogenized with the stirrer on the hot plate stirrer until it boils. These homogeneous media are sterilized in an autoclave at 121oC for 15 minutes, then cooled to a temperature of ± 45-50oC. This media is ready for use by adding bacteria as much as 0.2 ml for every 100 ml of media.

Preparation of standard turbidity of solution (McFarland's solution)

99.5 ml of H2SO4 0.36 N solution was mixed with a solution of BaCl2.2H2O 1.175% as much as 0.5 ml in an Erlenmeyer flask. Then shake until the turbid solution is formed. This turbidity is used as the standard for standard turbidity suspension test bacteria.

Making Test bacteria suspension

Test bacteria that have been inoculated are taken with sterile ose wires and then suspended into a tube containing 2 ml of 0.9% NaCl solution until turbidity is obtained which is the same as the standard turbidity of Mc. Farland solution. The same treatment was carried out on each type of test bacteria.

Making testing med

The base layer is made by pouring 75 ml NA each and adding with bacterial suspense. Leave it until the media solidifies on laminar airflow. Furthermore, sumurs are made according to their sample amount by using sterile pipette bases until sumurs to be used in antibacterial testing are formed.

Anti-bacterial activity test In-vitro

Layer cake test solution with various additions of Moringa leaf powder (1%, 2%, 3%, and 4 %); distilled water solution as a negative control; amoxicillin solution as positive controls respectively dropped on different sumurs as much as 0, 2 ml. Then the petri dish was incubated at 37oC for 24 hours.

Observation and Measurement

Observations were made after 24 hours of the incubation period. A clear area is a sign of bacterial sensitivity to antibacterial material used as a test material expressed by the width of the inhibitory zone diameter. The inhibition zone diameter is measured in millimeters (mm) using calipers by the calculation of the total diameter minus the sumur diameter. Then the diameter of the inhibition zone is categorized based on the strength of the antibacterial power based on Davis and Stout classification.

3. Results and Discussion

Antibacterial activity testing is carried out using the wells method by observing the resulting clear zone. Antibacterial testing with the diffusion sumur method was carried out using several additions of Moringa leaf powder, namely 1, 2, 3 and 4%. While the positive control used in the form of amoxicillin and negative control in the form of sterile aquades. The positive control serves as a comparison whether the layer cake with MMoringa leaf powder that is used is feasible or not. Data on the results of antibacterial testing of Escherichia coli and Staphylococcus aureus can be seen in Table 1.

Table 1. The results of the activity analysis of Moringa leaf layer cake toward E. Coli and S. Aureus

Treatment	Total diameter (mm) of *S. Aureus*	The diameter of the sumur (mm) *E. Coli*

A (0%)	0	0
B (1)%	6.2	0
C (2)%	7.8	4.6
D (3)%	9	6.9
E (4)%	10.7	9.7
Moringa leaf powder	16.6	15.1
Positive control	30.2	27.5
Negative control	0	0

Note: positive control with amoxicillin tablets and negative control with sterile distilled water

From the test results, it can be seen that the addition of Moringa leaf powder in different concentrations can become the antibacterial in processed cake layers. Antibacterial activity at different concentrations of Moringa leaf powder on layer cake against E. Coli bacteria resulted in diameters ranging from 0-9.7 mm, Moringa leaf powder 15.1 mm, and positive control 27.5 mm. Antibacterial activity against S. Aureus bacteria produced a diameter ranging from 0 - 10.7 mm, while Moringa leaf powder produced a diameter of 16.6 mm and positive control in the form of amoxicillin produced was 30.2 mm. This shows that the amount of addition of different Moringa leaf powder affects the clear zone produced where there is an increase in the active component of the making of layer cake which is marked by the clear zone produced at different levels of Moringa leaf powder addition. The antibacterial test results are in Figure 1

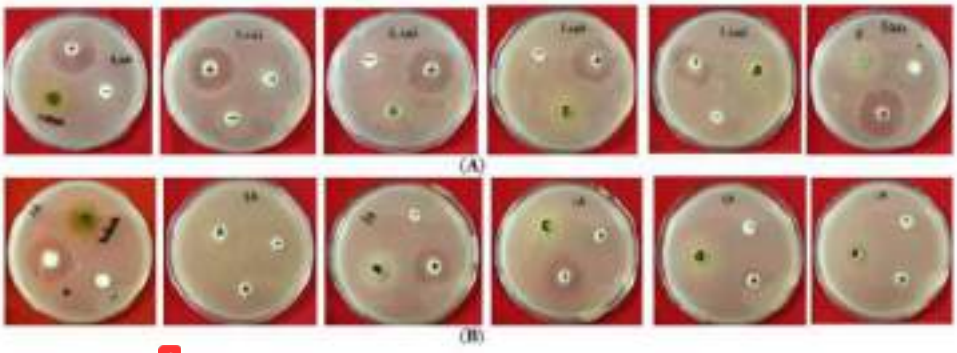

Figure 1 (A) Results of antimicrobial activity testing with sumuran diffusion method on several percentages of the addition of Moringa leaf powder (1, 2, 3 and 4%), negative control (-) and positive control (+) on the growth of E. Coli bacteria. (B) The results of the antimicrobial activity test with the sumuran diffusion method for several percentages of the addition of Moringa leaf powder (1, 2, 3 and 4%), negative control (-) and positive control (+) on the growth of S. Aureus bacteria.

The data obtained that Moringa leaf powder can inhibit the antibacterial activity of E. Coli with the addition of Moringa leaf powder 2 - 4% and is categorized as moderate, but the addition of 1% Moringa leaf powder has not been able to inhibit bacterial growth, whereas for S. Aureus bacteria the percentage of addition 1 - 3% can inhibit bacterial growth and is

classified as having moderate antibacterial activity, and on the addition of Moringa leaf powder the concentration of 4% is classified into strong antibacterial activity. The grouping of antibacterial power is based on the opinion of [6], the criteria for the strength of antibacterial power is inhibition zone diameter of 5 mm or less is categorized as weak, inhibition zone 5-10 mm is categorized as moderate. The inhibition zone of 10-20 mm is categorized as strong and the inhibition zone of 20 mm or more is categorized as very strong.

Antimicrobial compounds in plants are the result of secondary metabolites, these compounds consist of alkaloids, phenols, and others [7]. One of the contents in Moringa leaves is phenol compound. The content of phenol fresh Moringa leaves is 3,4%. As it is known that phenolic compounds are one of the compounds that can inhibit bacterial growth. [8], Moringa leaves contain phenols in large quantities which can be used as antibacterial. Phenolic compounds have glycoside bonds. Phenolic compounds will interact with bacterial cell membrane proteins through the process of adsorption by binding to the hydrophilic part of the cell membrane. Phenolic compounds will then enter the cell membrane and cause cell protein precipitation. This disturbs the permeability of cell membranes, so cell membranes can undergo lysis [9].

In the processing of layer cake, during the process of cooking layer cake added with Moringa leaf powder, it will be a little greasy. The more addition of Moringa leaf powder, the more oil will be formed in the layer cake. According to the results of research [10], leaf oil and Moringa seeds are the best natural ingredients that play an important role in water treatment to inhibit the growth of E. Coli bacteria. This means that the oil produced from the baking process layer serves as an antibacterial ingredient. [11] reported that M. Oleifera extract had antibacterial activity against Bacillus Cereus, Enterococcus Faecalis and Escherichia Voli, with inhibition zones between 7 - 9 mm,at concentrations of 50 mg/mL.

4. Conclusions

The results showed that a layer cake with the addition of Moringa leaves as much as 4% showed inhibition zone on the bacteria S. Aureus by 10.7 mm and E. Coli by 9.7 mm high if compared with the addition of Moringa leaves 1%, 2%, 3%. On both bacterial pathogens were tested on Moringa leaf powder that the bacterium E. Coli showed resistance to more robust compared with S. Aureus. This is demonstrated by the inhibition zone E.coli much smaller than bacteria S. Aureus.

References

Aminah S, Ramdhan T, and Yanis M 2015 Nutrient content and functional properties of Moringa plants (Moringa oleifera). Bul. Pertan. Perkota., 5 (2) 35–44.

Nuraya A D dan Nindya T S 2017 Relationship of traders' hygiene practices with the presence of Escherichia Coli bacteria in layer cake at the Surabaya City Flower Market. Media gizi Indones., 12 (1) 7–13.

Putri R E, Yusra dan Efendi Y 2016 Utilization of Moringa (oleifera leaves) as preservatives for catfish nuggets (clarias sp). J. Agric. Food Technol., 9 (2) 1–14.

Broin A 2010 Growing and processing moringa leaves. 1–70.

Muljono P, Fatimawali dan Manampiring A E 2016 The antibacterial activity test of male mayana leaf extract (Coleus astropurpureus Benth) on the growth of Streptococcus Sp and Pseudomonas Sp. J. e-Biomedik, 4 (1) 164–172.

Davis W W dan Stout T R 1971 Disc plate method of microbiological antibiotic assay. Appl. Microbiol 22 (4) 659–665.

Rahman M M, Sheikh I M, Sharmin S A, Islam S M, Rahman A.M., Rahman M M, dan Alam M F 2009 Antibacterial activity of leaves juice and extracts of Moringa antibacterial activity of leaf juice and extracts of Moringa oleifera Lam against some human pathogenic bacteria. J Nat Sci, 8 (2) 219–227.

Verma A R, Vijayakumar M, Mathela C S, dan Rao C 2009 In vitro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves. Food Chem. Toxicol, 47 2196–2201.

Mulyatri A S, Budiani A, dan Taniwiryono D 2012 Antibacterial activity of cocoa (Theobroma cacao L.) skin extract against Escherichia coli, Bacillus subtilis, and Staphylococcus aureus. Menara Perkeb., 80 (2) 77–84.

Anwar F dan Rashid U 2007 Physiochemical characteristics of Moringa oleifera seeds and seed oil from a wild provenance of Pakistan. Pak. J. Bot 39 (5) 1443–1453.

Shailemo D.H.P., Kwaambwa H M, Kandawa-Schulz M, dan Msagati T A M 2016 Antibacterial activity of Moringa ovalifolia and Moringa oleifera methanol, N-Hexane and water seeds and bark extracts against pathogens that are implicated in water-borne diseases. Green Sustain. Chem., 6 (6) 71–77.

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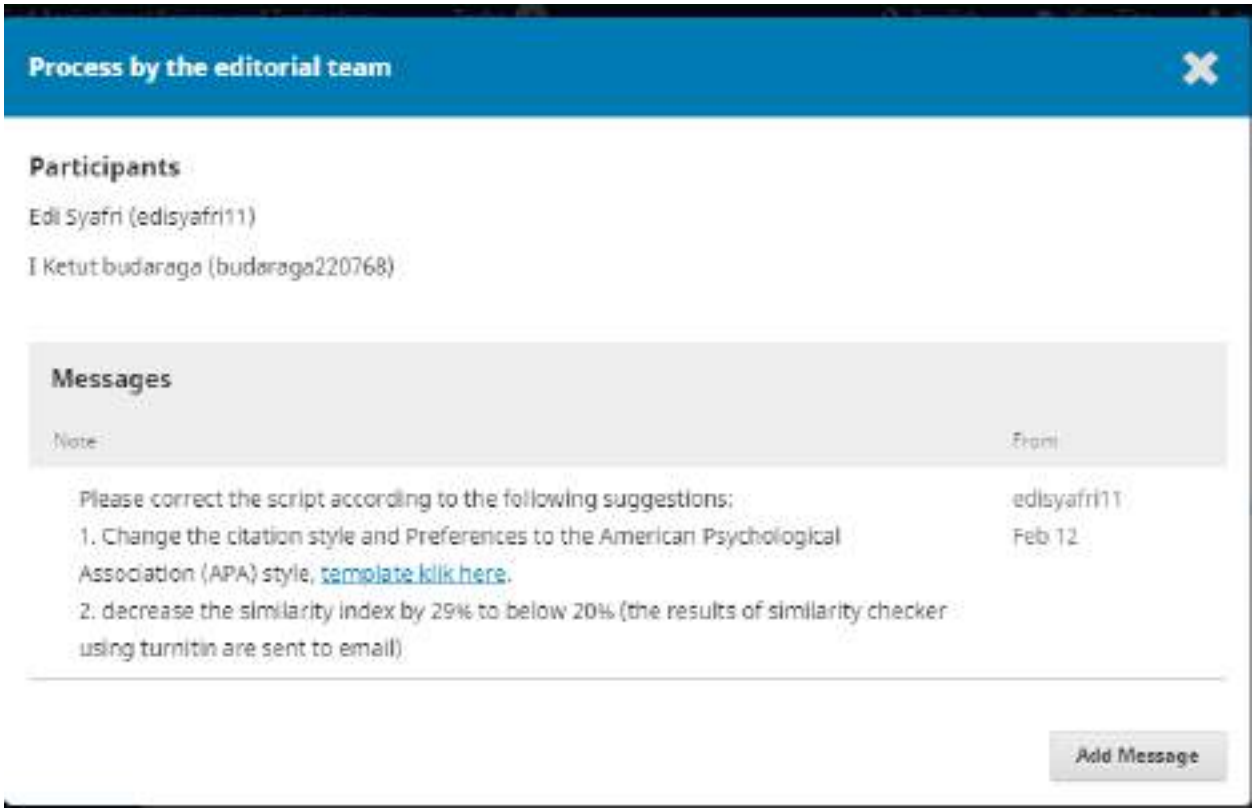
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2. Material and Methods

2.1. Tools and Materials

The research has been performed at the Agricultural Product Technology Laboratory of the Universitas Ekasakti and the Agricultural Product Microbiology and Biotechnology Laboratory of Universitas Andalas in April - May 2019. This research used the explorative study design by using the collection of *Staphylococcus aureus* and *Escherichia coli* bacterial isolates from the Agricultural Product Microbiology and Biotechnology Laboratory, Faculty of Agricultural Technology, Universitas Andalas

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2.2. Research Design

This study used an exploratory design through experiments in a laboratory with the addition of Moringa leaf powder to layer cakes (1, 2, 3 and 4%). Data were collected by direct observation after the research object was given treatment and repeated three times, then conducted a series of tests.

2.3. Making Moringa Leaf Powder

Procedure for obtaining Moringa leaf powder according to Broin (2010): (1) pick

dark green Moringa leaf as much as 3600 g, (2) wash fresh old Moringa leaves using clean water and drain those leaves, (3) spread the thin fresh fresh moringa leaves in the drying container, drying at room temperature of 27 degrees Celsius for three days. The final product must be very dry, (4) grind the fresh, dried Moringa leaves by using the blender and sieved with 80 mesh sieve, (5) Moringa leaf powder.

2.4. Making Layer Cakes by Adding Moringa Leaf Powder

Making dough is separated based on Moringa leaf powder. one part does not use Moringa leaves and one part uses Moringa leaves according to concentration. The procedure in making layer cakes by adding Moringa leaf powder as the natural preservative is as follows: (1) stir in 275 ml coconut milk with 50 g sugar and 2 g salt, until the sugar dissolves for 3-5 minutes, (2) mix in the container: 75 g rice flour and 50 g starch. Boil coconut milk little by little, stir until the dough mixed thoroughly, (3) add the powder Moringa leaves according to the treatment, (4) heat the steamer pan until the water boils, spread the pan with cooking oil, (5) After steaming pan steams a lot, pour the mixture according to the desired layer with the thickness of 0.5 cm for 25 minutes.

2.5. Testing the Inhibition Zone Activity of *S. Aureus* and *E. Coli* Bacteria (Muljono, Fatimawali &Manampiring, 2016)

The research has been performed at the Agricultural Product Technology Laboratory of the Universitas Ekasakti and the Agricultural Product Microbiology and Biotechnology Laboratory of Universitas Andalas in April - May 2019. This research used the explorative study design by using the collection of Staphylococcus aureus and Escherichia coli bacterial isolates from the Agricultural Product Microbiology and Biotechnology Laboratory, Faculty of Agricultural Technology, Universitas Andalas

a) Making the media (Muljono, Fatimawali &Manampiring, 2016)

Slanted jelly media is made by way dissolving Nutrient Agar (NA) about 2 grams in 100 ml distilled water using Erlenmeyer flask. After that, the mixture is homogenized with the stirrer on the hot plate stirrer until it boils. A total of 5 ml was poured each in 3 sterile test tubes and covered with aluminum foil. The media is sterilized in the autoclave at 121^{o} C for 15 minutes, then left at room temperature for ± 30 minutes until the media solidifies at the slant of 30^{o} C. The slanted jelly media is used for bacterial inoculation.

Media base is made by weighing Nutrient order (NA) as much as 6 grams, then dissolved in 300 ml distilled water using Erlenmeyer flask. After

that, the media is homogenized with the stirrer on the hot plate stirrer until it boils. These homogeneous media are sterilized in an autoclave at 121oC for 15 minutes, then cooled to a temperature of ± 45-50oC. This media is ready for use by adding bacteria as much as 0.2 ml for every 100 ml of media.

b) Preparation of standard turbidity of solution (Muljono, Fatimawali &Manampiring, 2016)

Making This turbidity standard is based on Mc Farland's solution. 99.5 ml of H_2SO_4 0.36 N solution was mixed with 0.5 ml solution of $BaCl_2.2H_2O$ 1.175% in an Erlenmeyer flask. Then shake until the turbid solution is formed. This turbidity is used as the standard for standard turbidity suspension test bacteria.

c) Making Test bacteria suspension (Muljono, Fatimawali &Manampiring, 2016)

Test bacteria that have been inoculated are taken with sterile ose wires and then suspended into a tube containing 2 ml of 0.9% NaCl solution until turbidity is obtained which is the same as the standard turbidity of Mc. Farland solution. The same treatment was carried out on each type of test bacteria.

d) Making testing med (Muljono, Fatimawali &Manampiring, 2016)

The base layer is made by pouring 75 ml NA each and adding with bacterial suspense. Leave it until the media solidifies on laminar airflow. Furthermore, sumurs are made according to their sample amount by using sterile pipette bases until sumurs to be used in antibacterial testing are formed.

e) Anti-bacterial activity test In-vitro (Muljono, Fatimawali &Manampiring, 2016)

Layer cake test solution with various additions of Moringa powder (1%, 2%, 3%, and 4 %); distilled water solution as a negative control; amoxicillin solution as positive controls respectively dropped on different sumurs as much as 0, 2 ml. Then the petri dish was incubated at 37oC with the duration 24 hours.

f) Observation and Measurement (Muljono, Fatimawali &Manampiring, 2016)

Observations were made after 24 hours of the incubation period. A clear area is a sign of bacterial sensitivity to antibacterial material used as a test

material expressed by the diameter of inhibitory zone. The inhibition zone diameter is measured in millimeters (mm) using calipers by means of the overall diameter minus the diameter of the well. Then the diameter of the inhibition zone is categorized by the strength of the antibacterial power based on Davis and Stout classification.

3. Results and Discussion

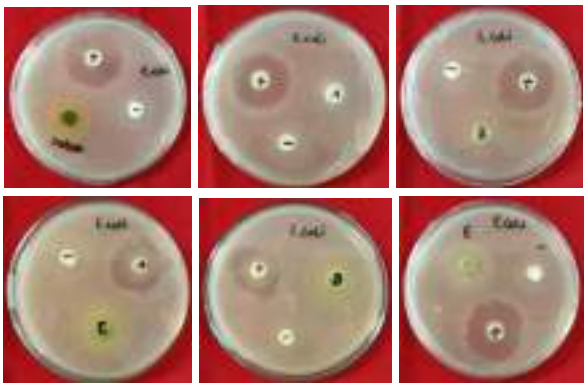
Antibacterial activity testing is performed by using the wells method by observing the resulting clear zone. Antibacterial testing with the well diffusion method was carried out using several additions of Moringa leaf powder, namely 1, 2, 3 and 4%. While the positive control used in amoxicillin form and negative control in sterile aquades form. The positive control serves as a comparison whether the layer cake with MMoringa leaf powder that is used is feasible or not. Data on the results of antibacterial testing of Escherichia coli and Staphylococcus aureus can be viewed in Table 1.

Table 1. The results of activity analysis on Moringa leaf layer cake toward *S. Aureus* and *E. Coli*

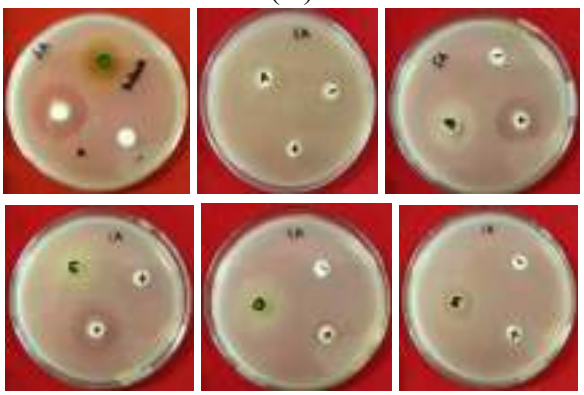
Treatment	Total diameter (mm) of S. Aureus	The diameter of sumur (mm) E. Coli
A (0%)	0	0
B (1)%	6.2	0
C (2)%	7.8	4,6
D (3)%	9	6.9
E (4)%	10.7	9.7
Moringa leaf powder	16.6	15.1
Positive control	30.2	27.5
Negative control	0	0

Note: positive control with amoxicillin tablets and negative control on sterile distilled water

From the test results, it can be seen that the addition of Moringa powder in different concentrations can become the antibacterial in processed cake layers. Antibacterial activity at different concentrations of Moringa powder on layer cake against E. Coli bacteria resulted in diameters ranging from 0-9.7 mm, Moringa leaf powder 15.1 mm, and positive control 27.5 mm. Antibacterial activity against S. Aureus bacteria produced a diameter ranging from 0 - 10.7 mm, while Moringa leaf powder produced a diameter of 16.6 mm and positive control in amoxicillin form produced was 30.2 mm. This shows that the amount of addition of different Moringa leaf powder affects the clear zone produced where there is an increase in the active component of the making of layer cake which is marked by the clear zone produced at different levels of Moringa powder addition. The antibacterial test results are in Figure 1



(A)



(B)

Figure 1. (A) Results of antimicrobial activity testing with *sumuran* diffusion method on several percentages of the addition of Moringa powder (1, 2, 3 and 4%), negative control (-) and positive control (+) on the *E. Coli* bacteria growth. (B) The results of antimicrobial activity test with the *sumuran* diffusion method for several percentages of the addition of Moringa powder (1, 2, 3 and 4%), negative control (-) and positive control (+) on the growth of *S. Aureus* bacteria.

The data obtained that Moringa leaf powder can inhibit the E. Coli antibacterial activity with the addition of Moringa powder 2 - 4% and is categorized as moderate, but the addition of 1% Moringa leaf powders have not been capable to inhibit bacterial growth, whereas for S. Aureus bacteria the percentage of addition 1 - 3% can inhibit bacterial growth and is classified as having moderate antibacterial activity, and on the Moringa powder addition of the concentration of 4% is classified into strong antibacterial activity. The grouping of antibacterial power is based on the opinion of Davis and Stout (1971), the criteria for the strength of antibacterial power is 5 mm inhibition zone diameter or less is categorized as weak, inhibition zone 5-10 mm is categorized as moderate. The inhibition zone of 10-20 mm can be categorized as strong, and the inhibition zone of 20 mm and more is categorized as very strong.

Antimicrobial compounds in plants are the result of secondary metabolites, these compounds consist of alkaloids, phenols, and others (Rahman et al, 2009). One of the

contents in Moringa leaves is phenol compound. The content of phenol fresh Moringa leaves is 3.4%. As it is known that phenolic compounds are the compounds that can inhibit bacterial growth (Verma, 2009). Moringa leaves contain phenols in large quantities which can be used as antibacterial. Phenolic compounds have glycoside bonds. Phenolic compounds will interact with bacterial cell membrane proteins through the process of adsorption by binding to the hydrophilic part of the cell membrane. Phenolic compounds will then enter cell membrane to cause cell protein precipitation. This disturbs the permeability of cell membranes, so cell membranes can undergo lysis (Mulyatni et al, 2012).

In the processing of layer cake, during the process of cooking layer cake added with Moringa leaf powder, it will be a little greasy. The more Moringa powder addition, the more oil will be formed in the layer cake. According to the results of research (Anwar & Rashid, 2007), leaf oil and Moringa seeds are the best natural ingredients that play an important role in water treatment to inhibit E. Coli bacteria growth. This means that the oil produced from the baking process layer serves as an antibacterial ingredient. Shailemo et al (2016) reported that M. Oleifera extract had antibacterial activity toward Bacillus Cereus, Enterococcus Faecalis and Escherichia Voli, with inhibition zones between 7 - 9 mm, at concentrations of 50 mg/mL.

4. Conclusions

The results showed that a layer cake with the addition of Moringa leaves as much as 4% showed inhibition zone on the bacteria S. Aureus by 10.7 mm and the bacteria of E. Coli by 9.7 mm high if compared with the addition of Moringa leaves 1%, 2%, 3%. On both bacterial pathogens were tested on Moringa leaf powder that the bacterium E. Coli showed resistance to more robust compared with S. Aureus. This is demonstrated by the inhibition zone E.coli much smaller than bacteria S. Aureus.

References

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Anwar, F. & Rashid, U. (2007). Physiochemical characteristics of Moringa oleifera seeds and seed oil from a wild provenance of Pakistan. *Pak. J. Bot.* 39 (5): 1443-1453.

Aminah, S., Ramdhan, T. & Yanis, M. (2015). Nutrient content and functional properties of Moringa plants (Moringa oleifera). *Bul. Pertan. Perkota.* 5 (2): 35-44.

Broin, A. (2010). Growing and processing moringa leaves. 1–70.

Davis, W.W. & Stout, T.R. (1971). Disc plate method of microbiological antibiotic assay. *Appl. Microbiol.* 22 (4): 659-665.

Muljono, P., Fatimawali, & Manampiring, A.E. (2016). The antibacterial activity test of male mayana leaf extract (Coleus astropurpureus Benth) on the growth of Streptococcus Sp and Pseudomonas Sp. *J. e-Biomedik*. 4 (1): 164-172.

Mulyatni, A.S., Budiani, A., & Taniwiryono, D. (2012) Antibacterial activity of cocoa (Theobroma cacao L.) skin extract against Escherichia coli, Bacillus subtilis, and Staphylococcus aureus. *Menara Perkeb.* 80 (2): 77-84.
Nuraya, A.D. & Nindya, T.S. (2017). Relationship of traders' hygiene practices with the presence of Escherichia Coli bacteria in layer cake at the Surabaya City Flower Market. *Media gizi Indones.* 12 (1): 7-13.
Putri, R.E., Yusra, dan Efendi, Y. (2016). Utilization of Moringa (oleifera leaves) as preservatives for catfish nuggets (clarias sp). *J. Agric. Food Technol.* 9 (2): 1-14.
Rahman, M.M., Sheikh, I.M., Sharmin, S.A., Islam, S.M., Rahman, A.M., Rahman, M.M., & Alam, M.F. (2009). Antibacterial activity of leaves juice and extracts of Moringa antibacterial activity of leaf juice and extracts of Moringa oleifera Lam against some human pathogenic bacteria. *J Nat Sci*. 8 (2): 219-227.
Shailemo, D.H.P., Kwaambwa, H.M., Kandawa-Schulz, M., & Msagati, T.A.M. (2016). Antibacterial activity of Moringa ovalifolia and Moringa oleifera methanol, N-Hexane and water seeds and bark extracts against pathogens that are implicated in water-borne diseases. *Green Sustain. Chem.* 6 (6): 71-77.
Verma, A.R., Vijayakumar, M., Mathela, C.S., & Rao, C. (2009). In vitro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves. *Food Chem. Toxicol*. 47: 2196-2201.

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