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ANTIBACTERIAL ACTIVITY OF MORINGA LEAF LAYER CAKE AGAINST *S. AUREUS* AND *E. COLI*

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Abstract. *The layer cake is one of the traditional cakes that are very popular with the community. The addition of Moringa leafs is expected to extend the period of storage 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, tannins, flavonoids, terpenoids, saponins, and others. The purpose of this study was to determine the antibacterial properties of Moringa leaves added to layer cake against pathogenic bacteria S. aureus and E. coli. Research has been implemented on April - May 2019. The testing of antibacterial activity by using well 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, S. aureu, E. coli*

1. Introduction

Moringa oleifera Lam (Moringa oleifera plant) of Moringaceae tribe has been the object of many studies because of its various uses and is famous for its bactericidal potential (Rante *et al.*, 2017). 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 correspondingly 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 (Aminah, Ramdhan and Yanis, 2015).

Bioactive compounds in Moringa leaves cause Moringa to have pharmacological properties. Besides, it has been identified that Moringa leaves have 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 (Nuraya and Nindya, 2017).

In previous studies, Moringa leaves were used as the preservative catfish nugget. At

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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 the addition of Moringa leaves with concentrations of 0 g, 25 g, and 30 g is clearly visible the longer it is stored then the total number of bacteria increases, but at the concentration of the addition of Moringa leaves 35 g and 40 g on the fourth day, the total number of bacteria decreased, the total number of bacteria decreases (Putri, Yusra and Efendi, 2016).

This research was conducted to determine the antibacterial properties of Moringa leaves added to layer cake against pathogenic bacteria *S. aureus* and *E. coli* with total concentrations of 1% -4% Moringa leaf powder.

2. Methods

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 *S. aureus* and *E. coli* 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 and other ingredients namely rice flour, tapioca flour. Materials used for antibacterial analysis are the growth test bacteria (*E. coli* and *S. aureus*) and jelly nutrient media. The tools used for making layer cakes are baking sheets, stirring spoons, basins, stoves, steamed pans, napkins, measuring spoons, and measuring cups. The tools used for making Moringa leaf powder are the basin, drainer, blender, 80 mesh sieve. Antibacterial test equipment Laminar airflow (Telster BV-100), analytical balance (Kern ABJ 220-4 M), autoclave (Hiclave HVE - 50), oven (Memert), Petri dish, ose needle, Bunsen lamp, Erlenmeyer 250 ml, test tube (Iwaki), micropipette, vortex, incubator (Memmert), hotplate stirrer (AREC Velp Scientifica,), Colony Counter (Philip Harris), callipers, cotton, plastic wrap, aluminium foil and tweezers.

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.

Preparing 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.

Preparing 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.

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

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Making the media (Muljono, Fatimawali and 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 is made by weighing Nutrient Agar (NA) as much as 6 grams, then dissolved in 300 ml distilled water using erlenmeyer flask. After that, the media is homogenized on

the hot plate stirer 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 (Muljono, Fatimawali and Manampiring, 2016). Making this turbidity standard is based on Mc Farland's solution. 99.5 ml of H2SO4 0.36 N solution was mixed with 0.5 ml solution of BaCl2.2H2O 1.175% in an Erlenmeyer flask. Then shake until the turbid solution is formed. This turbidity is used as the standard for turbidity suspension test

Making Test bacteria suspension (Muljono, Fatimawali and 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.

Making testing media (Muljono, Fatimawali and 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, wells are made according to their sample amount by using sterile pipette bases until wells to be used in antibacterial testing are formed.

Anti-bacterial activity test In-vitro (Muljono, Fatimawali and 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 wells as much as 0, 2 ml. Then the petridish was incubated at 37^{o}C with the duration 24 hours.

Observation and Measurement (Muljono, Fatimawali and 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. 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 Moringa leaf powder that is used is feasible or not. Data on the results of antibacterial testing of E. *coli* and *S. aureus* can be viewed in Table 1.

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. According to (Nazzaro *et al.*, 2013), the ability and antibacterial effect is highly dependent on the concentration given. Besides (Reygaert, 2016), states that most bacterial cells have many potential components as targets of antimicrobial agent compounds; however, some bacteria can modify all target cells to be resistant to a compound or antibiotic.The antibacterial test results are in Figure 1.

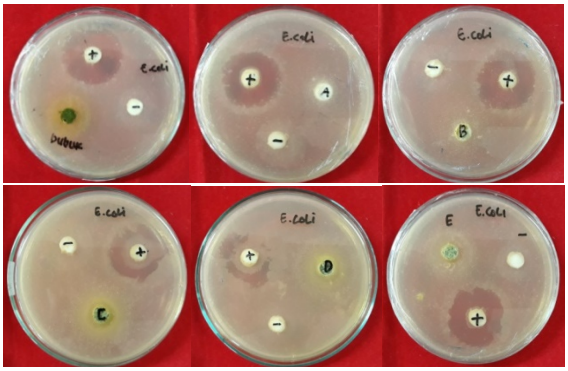
Table 1. The results of activity analysis on Moringa leaf layer cake toward *S. Aureus* and *E. Coli*

Treatment	Total diameter (mm) of *S. Aureus*	The diameter of well (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

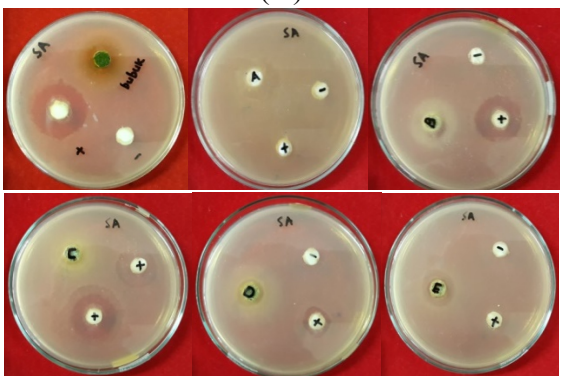
Note: positive control with amoxicillin tablets and negative control on sterile distilled water

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.



(A)



(B)

Figure 1. (A) Results of antimicrobial activity testing with well 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 *well* 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 antibacterial potential contained in extracts and antibiotics indicates that bacterial growth can be prevented. On test agar media, the expansion of bacterial colonies will be blocked by compounds contained in the test or treatment material. After incubation, the inhibition zone will be identified by the presence of a transparent area. This area shows that there is no bacterial colony (Ariza *et al.*, 2014).

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 *et al.*, 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, Budiani and Taniwiryono, 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 and 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 coli*, with inhibition zones between 7 - 9 mm, at concentrations of 50 mg/mL.

According to the results of research (Widowati, Efiyati and Wahyuningtyas, 2014) extract of kelor leaves with concentration 50% can be used as antibacteria for the composer *Pseudomonas aerugenosa* bacteria of fresh fish. this is consistent with the statement (Utami and Puspaningtyas, 2013) that Moringa leaves contain essential oils and flavonoids which can prevent fat peroxidation due to microbial growth. The *Moringa oleifera* etanol extract was higher inhibition than Moringa oleifera water extract which were able to inhibit the growth of *Streptococcus agalactiae* (Wulandari, Sarwiyono and Suryowardoyo, 2014). Several previous studies concluded that ethanol extracts from leaves, seeds and petiole of M. oleifera showed potential antimicrobial activity against gram-positive and gram-negative bacteria, and fungi (Alabaka *et al.*, 2011).

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*.

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