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Antibacterial Activity *Moringa oleifera* Leaf and Red Ginger Extract as Natural Feed Additive

Sunarti Sunarti[a], Nancy Lahay[a], Muhammad Fadhlirrahman Latief[a], Reynard Gondipon[b]

[a]Department of Nutrition and Animal Feed, Faculty of Animal Science, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10 Tamalanrea, Makassar 90245, South Sulawesi, Indonesia
[b]Innovasi Sdn Bhd, Whalers Gate, New Plymouth, New Zealand

*Corresponding author: E-mail: lahaynancy@gmail.com

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ABSTRACT

Natural feed additives such as moringa leaf and red ginger used as an antibacterial is one of the solution in disease prevention and performance improvement in animal husbandry. The purpose of this study is to determine the inhibition zone strength of Moringa leaf and red ginger extracts combinations against *Escherichia coli* and *Staphylococcus aureus* bacteria. Complete Randomized Design, Duncan's test and T-test are used to determine the difference between each replication that consisted of 5 treatments and 4 replications consisting of, P0 (positive control, namely cotrimoxazole); P1 (40% Moringa leaves + 30% Red ginger); P2 (40% Moringa leaf + 35% Red ginger); P3 (40% Moringa leaf + 40% Red ginger); P4 (40% Moringa leaf + 45% Red ginger). The results indicated that the various combinations of moringa leaf and red ginger extracts concentrations have a significant effect on inhibition zone towards *Escherichia coli* and *Staphylococcus aureus*. The highest inhibition zones are in treatment P4 (40% Moringa leaves + 45% Red Ginger) *Escherichia coli* bacteria 23.50 mm ± 0.11 mm and *Staphylococus aureus* 23.60 mm ± 0.11 mm. This study concludes that the combined extract of Moringa leaf and Red ginger at a concentration level of 40% Moringa leaf + 45% red ginger has the strongest inhibition towards the growth of Gram-Positive and Gram-Negative bacteria.

Keywords: Moringa leaf, red ginger, feed additive, *Escherichia coli*, *Staphylococcus aureus*

INTRODUCTION

Feed additives such as antibiotics have been banned from being used in developed countries including Indonesia. This relates to the current global issue particularly in poultry

farming and the safety of animal feed in regards to the contamination and residues that can be harmful to consumers. Experts have started looking for substitutes and focus on natural ingredients [1]. *Natural feed additive* from natural ingredients is an alternative solution in preventing disease and can improve the performance of poultry [2]. Several types of plants that have the potential as natural feed additives are *Moringa oleifera* leaf [3] and red ginger [4].

Moringa oleifera leaves are used as a natural feed additive because they contain various beneficial nutrients [5]. The most superior content in this plant is a high protein, vitamins A, C, B, amino acids, calcium and high iron [6]. The content of secondary metabolites such as saponins, alkaloids, flavonoids, terpenoids, and tannins inhibit bacterial growth by damaging bacterial cell membranes [7]. In addition to Moringa leaves, red ginger is also used as an antibacterial because of its essential oil content [8].

Ginger Red is also used as a natural feed additive because of its essential oil content which acts as an antibacterial, improves the work of the digestive organs, stimulates pancreatic juice which contains the enzymes amylase, lipase, and protease [9]. However, excessive use of ginger will cause a negative (toxic) impact on the body chicken. The main components of ginger are zingiberen and zingerol which can stimulate the digestive system by controlling pH, enzyme activity and microbial activity. Red ginger is also a static bacteria that reduces pathogenic bacteria in digestion [10].

Moringa leaf and red ginger are combined because each ingredient has content contain antibacterial thereby inhibiting the activity of bacterial growth. Moringa leaves contain alkaloids, flavonoids and saponins that can inhibit *Escherichia coli* bacteria. Moringa leaf extract also has antibacterial activity against *Staphylococcus aureus*. Meanwhile, according to Widiastuti and Pramestuti [11], red ginger plants contains flavonoid, phenol, terpenoid and artisi oil groups that can inhibit the growth of harmful pathogens such as *Escherichia coli* bacteria. This study used the antibiotic Cotrimexasol as a control.

Cotrimexasol is an antibiotic that can treat various types of bacterial infections of the digestive tract. This aims of this study to determine the inhibition zone of the combined extract of Moringa leaves (*Moringa oliefera*) and red ginger (*Zingiber officinale* var*. rubrum*) against *Escherichia coli* and *Staphylococcus aureus* natural *feed additives*. This research is also aim as a reference and provide information to the public with regards to its potential as a natural feed additives and the the inhibition zone strength of the Moringa leaf (*Moringa oliefera*) and red ginger extract (*Zingiber officinale var rubrum*) combination against *Escherichia coli* and *Staphylococcus aureus* bacteria as natural additive.

MATERIALS AND METHODS

Research Design

The design used in this study was a completely randomized design (CRD) with 5 treatments and each treatment consisted of 4 replications. Antibacterial activity test using the following treatments: P0: Positive control was Antibiotic cotrimexasol, P1: 40% Moringa leaf extract + 30% Red ginger extract, P2:40% Moringa leaf extract+ 35% Red ginger extract, P3:40%

Moringa leaf extract + 40% Red ginger extract, P4:40% Moringa leaf extract + 45% Red ginger extract.

Treatment Stage

This research uses well diffusion method. In this method, wells are made on Mueller-Hilton Agar (MHA) media which has been planted with the test bacteria and the wells are given an antibacterial agent to be tested. 2.28 grams of MHA was dissolved in 60 ml of distilled water and then heated and homogenized using a heater and magnetic stirrer. The MHA media must be completely homogeneous as seen from the clear yellow color which indicates that the MHA has been mixed well with the distilled water. Prior to use, the media was sterilized using an autoclave at 121^{o}C with a pressure of 1 atm for 15 minutes. Bacterial suspension as much as 10 mL put into a petri dish and 10 mL of MHA solution homogenize and allowed to stand at room temperature until the media solidifies. Each petri dish made 5 wells. Moringa leaf powder extract suspension and red ginger were put into wells using a spoid, one well for contimexasol antibiotics as a positive control, then each well was filled with a 0.25 mm combination suspension of Moringa leaves and red ginger at each concentration of suspension, contimexasol antibiotics as much as 0.25 mm, then stored in an incubator for 24 hours at 37^{o}C. After 24 hours, the results of testing the inhibition of the combination of Moringa leaves and red ginger against *Escherichia coli* and *Stapylococcus aureus* by measuring the diameter of the inhibition zone formed using a calliper.

Resistance Measurement

According to Paliling *et al.* [12] the extract used is said to be effective if the area inhibited by the extract is seen. The inhibition area will look clearer than the surrounding area. The area of inhibition was measured using a calliper. The inhibition zone formed was measured in vertical and horizontal diameters and expressed in units (mm).

According to Davis and Stout [13] the strength of antibacterial activity is: 1. Very strong activity: Inhibitory Diameter (DDH) 20 mm; 2. Strong activity: DDH 10 – 20 mm; 3. Moderate activity: DDH 5 – 10 mm; 4. Weak activity: DDH < 5 mm.

RESULTS AND DISCUSSIONS

Zone Inhibition the Combination extract on *Escherichia coli*

Based on the results of the combination of Moringa leaf extract and red ginger with different concentrations, the inhibitory zone against *Escherichia coli* was obtained which is presented in Table 1.

Table 1 are displayed the results of variance of the combination of Moringa leaf extract and red ginger extract with different concentrations, showed a significant difference ($P<0.05$) against the inhibition zone of *Escherichia coli*. The control treatment (P0) had an inhibition zone of 20.35 mm ± 0.20 mm, P3 = 22.55 mm ± 0.12 mm, and P4 = 23.50 mm ± 0.11 mm with the

same activity strength, namely very strong, while for P1 = 19.55 mm ± 0.34 mm, and P2 = 19.85 mm ± 0.20 mm, they are the same, which is strong against Escherichia coli. This indicates that the higher the concentration given the strength of the antibacterial activity will be very strong against *Escherichia coli*. According to Dima *et al.* [14] stated that the concentration of Moringa leaf extract added with a higher concentration of red ginger extract inhibited the growth of *Escherichia coli*.

Table 1. Inhibition Zone Combination Extract of Moringa Leaf and Red Ginger Against *Escherichia coli*

	Bacterial Growth Inhibitory Zone (mm)	
Treatment	***Escherichia coli* (mm)**	**Category Strength of Bacterial Activity**
P0(+)	20.35 ± 0.20^{b}	Very Strong
P1	19.55 ± 0.34^{a}	Strong
P2	19.85 ± 0.20^{a}	Strong
P3	22.55 ± 0.12^{c}	Very strong
P4	23.50 ± 0.11^{d}	Very strong

Note: P0 = (Positive control, namely Cotrimexasol)
P1 = (Moringa Leaf Extract 40% + Red Ginger Extract 30%)
P2 = (Moringa Leaf Extract 40% + Red Ginger Extract 35%)
P3 = (Moringa Leaf Extract 40% + Red Ginger Extract 40%)
P4 = (Moringa Leaf Extract 40% + Red Ginger Extract 45%)
^{abcd} different superscripts in the same column showed significant differences ($P<0.05$).

Antibiotic *cotrimoxazole* are chosen as a positive control, because cotrimoxazole serves to treat various types of bacterial infections in the digestive tract. Cotrimoxazole is a combination of sulfa methoxazole and trimethoprim so that the combination will produce a synergistic effect to inhibit microbes that cause infectious diseases [15]. Cotrimoxazole works by inhibiting two stages of the synthesis of folic acid and protein which is essential for microorganisms [16]. Trimethoprimitself is bactericidal (kills bacteria) whereas sulfamethoxazole is a bacteriostatic (stop bacterial reproduction) [17].

Duncan`s test analysis of the inhibition of the combination of Moringa leaves and red ginger against *Escherichia coli* showed that there was a significant difference between treatments P1, P2, P3, and P4. The difference in the inhibition zone formed is influenced by the difference in the concentration given. Table 1 shows that the higher the concentration is given, the greater the inhibition zone formed because it contains bioactive compounds that can inhibit bacterial growth [7] moringa leaves contain alkaloids, flavonoids, terpenoids, and saponins that can inhibit bacterial growth.

Indonesian people, especially in rural areas, often use Moringa leaves as traditional medicine in the form of a decoction to treat various diseases [18]. Meanwhile, according to Widiastuti and Pramestuti [11] the content contained in red ginger plants, especially the flavonoids, phenols, terpenoids and essential oils can inhibit the growth of harmful pathogens.

Secondary metabolites found in Moringa leaves and red ginger that functions to inhibit bacterial growth are terpenoids. This is in accordance with the opinion of Retnowati [19] which states that secondary metabolites such as terpenoids can inhibit the growth of bacterial activity. The mechanism of action of terpenoid compounds as antibacterial substances involves membrane damage by lipophilic compounds. Terpenoids can react with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming strong polymeric bonds and damaging the porin, reducing the permeability of the bacterial cell wall so that the bacterial cell lacks nutrients, inhibits bacterial growth or dies.

Other secondary metabolites such as flavonoids, alkaloids, phenols can also inhibit bacterial activity. Saponin compounds belong to the glycoside group found in various types of plants that function to store carbohydrates and as protection from pests, with the mechanism of reducing the surface tension of the bacterial cell wall, resulting in increased permeability of cell leakage and resulting intracellular compounds will be released. Flavonoids function as antioxidants that are able to maintain the oxidation of body cells [20].

Zone Inhibition the combination extract on *Staphylococcus aureus*

The combination of Moringa leaf extract and red ginger with different concentrations was resulted an inhibition zone against *Staphylococcus aureus* was presented in Table 2.

Table 2. was displayed the effect of the combination of Moringa leaf extract and red ginger with different concentrations have a significant difference ($P<0.05$) on the growth of *Staphylococcus aureus*. Control treatment (P0) = 21.65 ± 0.12 mm, P2 = 20.15 ± 0.19 mm, P3 = 22.42 ± 0.17 mm and P4 = 23.60 ± 0.11 mm, which is very strong, while for the treatment P1 = 19.50 ± 0.00 mm which is strong against *Staphylococcus aureus*. This indicates that the higher the concentration of red ginger extract given, the greater the strength of its antibacterial activity.

The presence of secondary metabolites in both ingredients can inhibit bacterial growth, one of which is terpenoids. Monalisa *et al.* [21] states that secondary metabolites such as terpenoids were work by involving membrane damage by lipophilic compounds. Terpenoids can react with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming strong polymeric bonds and damaging the porin, and reducing the permeability of the bacterial cell wall. As a result, bacterial cells lack nutrients and their growth will be stunted or die. The mechanism of action of steroids as an antibacterial is by damaging the bacterial cell membrane.

Red ginger contains essential oils that play an active role in inhibiting antibacterial activity. The essential oil components contained in red ginger have an active role as antibacterial, they are sabinen, -mirsen, -pinene, -tuyan, trans-caryophyllene, -pinene. -pinene and -pinene are terpenoid compounds known to have antimicrobial effects [22].

Table 2. Inhibitory Zone of Moringa Leaf and Red Ginger Combination Against *Staphylococcus aureus*

Treatment	Bacterial Growth Inhibitory Zone (mm)	
	***Staphylococcus aureus* (mm)**	**Category Strength of Bacterial Activity**
P0(+)	21.65 ± 0.12 [c]	Very strong
P1	19.50 ± 0.00 [a]	Strong
P2	20.15 ± 0.19 [b]	Very strong
P3	22.42 ± 0.17 [d]	Very strong
P4	23.60 ± 0.11 [e]	Very strong

Note: P0 = (Positive control, namely *Cotrimexasol*)
P1 = (Moringa Leaf Extract 40% + Red Ginger Extract 30%)
P2 = (Moringa Leaf Extract 40% + Red Ginger Extract 35%)
P3 = (Moringa Leaf Extract 40% + Red Ginger Extract 40%)
P4 = (Moringa Leaf Extract 40% + Red Ginger Extract 45%)
[abcde]different superscripts in the same column showed significant differences (P<0.05).

Duncan`s test analysis of the combination of Moringa leaves and red ginger against *Staphylococcus aureus* resulted that there were significant differences between treatments P1, P2, P3, and P4. The difference in the inhibition zone formed due to the difference in the concentration level of each given treatment. Table 2 shows that the higher the concentration given, the larger the inhibition zone formed. That the higher the concentration of red ginger extract given, the higher the inhibition of the growth of *Staphylococcus aureus* [23].

The inhibition zone of *Staphylococcus aureus* bacteria on average are very strong this is because *Staphylococcus aureus* is a Gram-positive bacterium that has a simpler cell wall than *Escherichia coli.* One of the polar ingredients in Moringa leaf extract and red ginger is flavonoids. This is in accordance with the opinion of Sipayung [24] which states that the cell wall of gram-positive bacteria has a peptidoglycan and teichoic acid structure. Flavonoid compounds are polar compounds, so they penetrate the polar peptidoglycan layer more easily than non-polar lipids such as those in *Escherichia coli*.

Comparison of Inhibitory Zones between *Escherichia coli* and *Staphylococcus aureus*

Duncan's test which was strengthened by the results of the T-test, it was proven that the combination of Moringa leaf extract and red ginger with different concentrations showed significant differences (P< 0.05) on the growth of *Escherichia coli* and *Staphylococcus aureus*. The average inhibition zone formed in Duncan's test and T test was the same for *Staphylococcus aureus* of 21.46 ± 1.53 mm while *Escherichia coli* was 21.16 ± 1.62 mm. *Staphylococcus aureus* showed that the strength of its antibacterial activity was higher than *Escherichia coli bacteria* but the difference was not too much.

This is due to the cell wall structure between the two bacteria, where *Escherichia coli* have more complex cell wall structures while *Staphylococcus aureus* have simpler bacterial cell

walls. Handrianto [25] states that the difference in the diameter of the resulting inhibition zone is due to differences in the cell wall structure between the two bacteria.

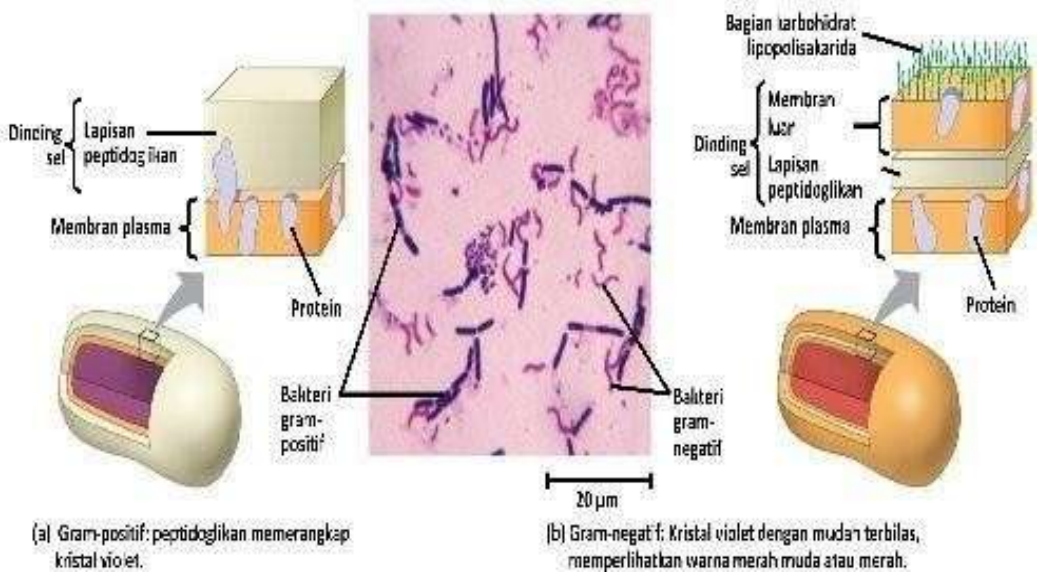

Figure 1. Cell Wall Structure of Gram-Positive Bacteria and Gram-Negative Bacteria[26]

The cell wall of the bacterium *Staphylococcus aureus* (Gram-positive) has a simpler structure than the cell wall of the bacterium *Escherichia coli* (Gram-negative) whose cell wall is more complex. *Staphylococcus aureus* bacteria are only composed of a thick layer of peptidoglycan and teichoic acid. This layer consists of a water-soluble polymer, making it easier for polar antibacterial compounds, such as phenolic compounds (flavonoids and tannins) to more easily penetrate the polar peptidoglycan layer [26].

Escherichia coli have cell walls with a peptidoglycan composition of about 10% of the dry weight of cells equipped with lipopolysaccharides and proteins (fatty acids coupled with polysaccharides), no teichoic acid, high lipid content. about 11-22% and has a cell wall arrangement that is not compact but more complex [27]. Gram-negative bacteria have a layer of peptidoglycan located in the periplasmic membrane, which is a membrane between the plasma membrane (inside) and the outer membrane. The outside is composed of a thick layer of lipopolysaccharide (LPS) which is aa form of defence of Gram-negative bacteria against foreign substances, including antibacterial compounds.

Secondary metabolite compounds such as terpenoids can inhibit the growth of Escherichia coli bacteria activity. The mechanism of action of terpenoid compounds as antibacterial agents against *Escherichia coli* involves membrane damage by lipophilic compounds. Terpenoids can react with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming strong polymer bonds and damaging the porin, reducing the permeability of the bacterial cell wall so that bacterial cells lack nutrients, inhibiting bacterial growth or dying [19].

CONCLUSIONS

The results showed that the combination of Moringa leaf extract and red ginger could inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*. In addition, giving a combination of Moringa leaf extract and red ginger with a concentration of 40% Moringa leaves and 45% red ginger can inhibit as much as 23.50 ± 0.11 mm for *Escherichia coli* and 23.60 ± 0.11 mm for *Staphylococus aureus* with a very strong bacterial inhibition zone strength category. The combined of these two plants can be used as a substitue for natural feed.

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