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Antibacterial activity test combination of *Indigofera zollingeriana* and turmeric (*Curcuma domestica* val.) as alternative *Feed Additive* for poultry

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**Abstract.** *Indigofera zollingeriana* and turmeric are potentially antibacterial plants. Tannin and phenol in *Indigofera zollingeriana* and turmeric are able to block the formation of bacterial cells and lyse cell membranes. The purpose of this research was to examine the antibacterial effectiveness contained in *Indigofera zollingeriana* and turmeric flour against *Salmonella* sp., *Escherichia coli* and *Lactobacillus* sp. The research consisted of 5 treatments and 4 replications with T0 treatment: Tetracycline (Control), T1: *Indigofera zollingeriana* 5% T2: *Indigofera zollingeriana* 10%, T3: *Indigofera zollingeriana* 15%, T4: *Indigofera zollingeriana* 20% and addition of turmeric powder as much as 2.5% at the level of T1, T2, T3 and T4. The research designed according to the Completely Randomized Design and Duncan test was performed to defining the difference among treatments. The results showed that the inhibition zone around the wells of *Escherichia coli* bacteria was T0: 33.03, T1: 11.02, T2: 11.42, T3: 11.72, T4: 11.32; *Salmonella* sp. T0: 24.12, T1: 10.68, T2: 12.33, T3: 14.12, T4: 13.65 and *Lactobacillus* sp. T0: 33.61, T1: 25.67, T2: 25.93, T3: 26.65, T4: 27.16 mm, respectively. It was concluded that the inhibition zone was influenced by several factors such as the concentration of samples used and the properties of bacteria. The combination of *Indigofera zollingeriana* and turmeric can be used as an alternative feed additive for poultry.

1. Introduction

Poultry is one of livestock commodity which is currently being developed to meet the needs of animal protein in Indonesia. As an animal protein source, poultry has big potential because able to produce meat quickly and economically compared to other livestock. The high market demand for poultry makes this business much occupied, but besides the high demand for poultry it is very susceptible to disease so that many industries use feed additives in the form of antibiotics as drugs to prevent disease.

Antibiotics are widely used to prevent infection with pathogenic bacteria and increase productivity. Some countries currently prohibit the use of antibiotics because they can cause residues on products that can endanger consumers. So we need a feed additive that can prevent bacterial infections but does not have a negative impact on consumers. Natural feed additives can be obtained from active substances in plants. Another plant that have antibacterial potential is turmeric. Turmeric is believed to be a traditional medicine because turmeric contains various compounds including curcumin and essential oil. The essential oil can be used as antibacterial because they contain functional groups of hydroxyl and carbonyl which are phenol derivatives. Phenol derivatives interact with bacterial cell walls, then absorb
and penetrate into bacterial cells, causing precipitation and protein denaturation to lyse bacterial cell membranes [1].

The content of Indigofera zollingeriana and turmeric have the potential to be used as an antibacterial. Tannins and phenols contained in Indigofera zollingeriana and turmeric can block the formation of bacterial cells and lubricate cell membranes. The combination of Indigofera zollingeriana and turmeric is expected to be able to inhibit bacterial growth more optimally.

2. Materials and methods

2.1. Material

The study was conducted in October to November 2018 in Tamalanrea and the inhibitory test at the Microbiology Laboratory of the Faculty of Medicine, Hasanuddin University, Makassar. The material used by turmeric, Indigofera zollingeriana, Salmonella sp. Escherechia coli, and Lactobacillus bacteria.

2.2. Methods

The combination anti-bacterial inhibitory test method of Indigofera zollingeriana and turmeric using diffusion method was carried out by making a well with a certain diameter on the agar medium which had been planted with microbial tests. [2] the advantages of the well method are that it is easier to measure the extent of the inhibitory zone that is formed because isolates move not only on the surface of the nutrient agar but also to the bottom.

2.2.1. Making bacterial suspension. The bacteria are cultured on MHA media and incubated at 37°C for 24 hours. Four to five colonies from cultured bacteria are taken with a sterile ose and then put in a test tube containing 5 ml Phosphate Buffer Solution (PBS). Incubation at 37°C for two hours, then turbidity is formed which is equivalent to the standard from Mc Farland 1. The number of bacteria has met the requirements for the sensitivity test 1.5 x 10⁸/ml.

2.2.2. Inhibitory test. The parameters observed in the study were the formation of a bacterial growth inhibition area around the well in the form of a clear zone. Measurements have done several times on different sides because the inhibition zone is not perfectly circular. The obstacle zone formed in the form of a clear area is measured by the sorong term [3].

2.3. Statistical analysis

Data for inhibitory test were subjected to an analysis of variance. The treatments’ means with significant differences at P<0.05 were compared using duncan test. The experimental design used was Completely Randomized Design (CRD), 5 treatments with 4 replications [4]. Data processing used was the SPSS program version 16.0.

3. Result and discussion

Based on the results of research that has been done, antibacterial activity combination of Indigofera zollingeriana and turmeric by giving different levels can be seen in table 1.

3.1. Antibacterial activity test on Escherichia coli bacteria

Based on antibacterial tests on Escherichia coli bacteria had a very significant effect (p <0.01) by giving a combination of Indigofera zollingeriana and turmeric flour. Duncan test results showed that the treatment T0 was very different (p <0.01) higher than treatments T1, T2, T3 and T4. In the T0 treatment, the clear zone formed was 33.03 mm while the lowest result was T1 treatment with clear zone results formed 11.02 mm. The high difference between the results of T0 because the treatment of T0 using tetracycline antibiotics. Tetracycline can inhibit bacteria by inhibiting protein synthesis. [5] that tetracycline is a family of antibiotics that inhibit protein synthesis in cells.
Table 1. The inhibitory zone formed around the well in Escherichia coli, Salmonella sp. and Lactobacillus sp.

| Treatments | Parameter       | Escherichia coli | Salmonella sp. | Lactobacillus sp. |
|------------|-----------------|------------------|----------------|-------------------|
| T₀         |                 | 33.03 ± 0.60ₐ    | 24.12 ± 1.30ₐ  | 33.61 ± 1.98ₐ     |
| T₁         |                 | 11.02 ± 0.60ₐ    | 10.68 ± 0.40ₐ  | 25.67 ± 2.79ₐ     |
| T₂         |                 | 11.42 ± 1.13ₐ    | 12.33 ± 0.96ₐ  | 25.93 ± 3.05ₐ     |
| T₃         |                 | 11.72 ± 0.89ₐ    | 14.12 ± 1.09ₐ  | 26.65 ± 2.85ₐ     |
| T₄         |                 | 11.32 ± 0.79ₐ    | 13.65 ± 0.92ₐ  | 27.15 ± 2.75ₐ     |

ₐ,b,c,d: Different superscripts in the same column show very significant differences (p<0.01)

T₀: Tetracycline (Control); T₁: Indigofera zollingeriana powder 5%; T₂: Indigofera zollingeriana powder 10%; T₃: Indigofera zollingeriana powder 15%; T₄: Indigofera zollingeriana powder 20% with the addition of 2.5% turmeric powder in treatments T₁, T₂, T₃ and T₄.

Antibacterial inhibitory results using Tetracycline in Escherichia coli, Salmonella sp. and Lactobacillus sp. respectively 33.03 mm, 24.14 mm and 33.61 mm, from these results it can be seen that tetracycline is a very strong antibiotic category. [6] antibacterial strength criteria are as follows: 5 mm inhibition zone diameter or less categorized as weak, 5-10 inhibition zone are categorized as medium, 10-20 inhibition zone are categorized as strong and 20 mm inhibition zone or more are categorized as very strong.

The use of tetracycline as an antibiotic has been widely used because tetracycline is a high class antibiotic that can inhibit the growth of bacteria. The use of tetracycline can be used in humans and livestock. [7] that tetracycline is a broad-spectrum bacteriostatic antibiotic that inhibits protein synthesis. Tetracycline works actively against the number of Gram-positive and Gram-negative bacteria, including anaerobic bacteria, rickets, chlamydia, microplasma L form and there are several protozoa, such as amoeba. The results of research conducted by the Veterinary Research Institute Bogor shows that 71.43% of feed mills in West Java provide additional antibiotics such a tetracycline and sulfonamide in chicken feed products. The concentration of tetracycline antibiotic added in animal feed is a low dose which ranges from 2.5 to 12.5 mg/kg (ppm).

3.2. Antibacterial activity test on Salmonella sp. bacteria.
Based on the antibacterial test on Salmonella sp. bacteria had a very significant effect (p<0.01) by giving different levels. The Duncan test results showed that the T₀ treatment was very significantly different compared to treatments T₁, T₂, T₃ and T₄. The Duncan test results showed that the T₀ treatment was very significantly different compared treatments T₁, T₂, T₃ and T₄. The T₀ treatment results of clear zones formed were 24.12 mm while the lowest yield was T₁ treatment with clear zone results formed 10.68 mm. In numerically the highest combination results of Indigofera zollingeriana and turmeric in T₃ treatment with a clear zone area that is formed at 14.12 mm. [8] that the administration of an antimicrobial combination aims to achieve the widest possible antimicrobial spectrum. In addition, antimicrobial combinations are also used to achieve synergistic effects and to inhibit the emergence of resistance to the antimicrobial drugs used.

Statistical data on the antibacterial activity test on Salmonella sp. there was a difference in each treatment, the difference in inhibitory power is influenced by differences in the treatment given. The addition of the concentration of antibacterial compounds is thought to increase the penetration of antibacterial compounds into the interior of microbial cells thereby damaging the cell metabolism system and resulting in cell death. The combination of Indigofera zollingeriana and turmeric can be used as an antibacterial because the content of the Indigofera zollingeriana in the form of tannins can interfere with the enzyme's work system so that it disrupts the growth of microorganisms and turmeric contains the essential oil which is a phenol derivative with an antibacterial mechanism which denatures cell proteins.
3.3. Antibacterial activity test on Lactobacillus sp.
Based on the variety of antibacterial tests on Lactobacillus sp. by giving different levels to get the results very real difference (P<0.01). The Duncan test results showed that the T0 treatment was very significantly different (P<0.01) with treatments T1, T2, T3 and T4. In the T0 treatment, the clear zone formed was 33.61 mm, whereas in T1, T2, T3 and T4, they were 25.67 mm, 25.93 mm, 26.65 mm and 27.15 mm respectively. The results of this study are not in accordance with the research conducted by [9] the results of the antibacterial activity test against Lactobacillus sp, the lowest inhibition diameter zone at 2.5% turmeric concentration was 6.13 mm, and the biggest inhibition zone at 1.5% concentration, was 6.25 mm. The effectiveness of turmeric extract test on Lactobacillus sp at concentrations of 1.0%, 1.5%, 2.0%, and 2.5% found inhibition zones with diameter barriers of 6.25 mm, 6.50 mm, 6.25 mm and 6.13 mm.

The difference in results obtained can be influenced by several factors including the presence of active substances in Indigofera zollingeriana and turmeric. The difference in the inhibitory power combination of the two plants can be said to have a synergistic effect because it is able to reach a fairly broad spectrum. Turmeric inhibitory test results against Lactobacillus sp. is 29.4 mm and in Indigofera zollingeriana 5%, 10%, 15% and 20% respectively are 28.3 mm, 29 mm, 29.05 mm and 29.7 mm. From these results a single use is higher than a combination, this can be caused by differences in the effectiveness of the active compounds contained. One other factor is the fertility of the bacteria used determines how bacteria survive antibacterial. [10] the factors that influence the test of inhibition are the concentration of the active compound, the sensitivity of the microbial growth test, the thickness and viscosity of the medium and the reaction between the active substance with the medium and the incubation temperature. Besides that, in each microbe test there are differences in the composition of cell walls between Gram-positive and Gram-negative bacteria. The Gram-positive bacterial cell wall generally consists of one peptidoglycan layer so that it is more easily penetrated by antibacterial substances contained in the extract. While Gram-negative bacteria, the cell wall consists of two layers, the outer layer of lipopolysaccharide and protein and the inner layer of peptidoglycan.

3.4. Antibacterial activity in Gram-negative bacteria and Gram-positive bacteria
Antibacterial activity tests were carried out with three different bacterial parameters, Escherichia coli, Salmonella sp. and Lactobacillus sp. different results for each bacteria. Inhibition of Lactobacillus sp. which is a larger Gram-positive bacteria compared to Escherichia coli and Salmonella sp. which is Gram-negative bacteria because of differences in the nature of bacteria where Gram-positive bacteria have antibacterial sensitivity compared to Gram-negative bacteria. Some differences between Gram-negative and Gram-positive bacteria cause differences in antibacterial sensitivity. The content of tannins in Indigofera zollingeriana has the potential as an antibacterial that can interfere with the growth of microorganisms.

4. Conclusion
Based on the results of the research test the combination of antibacterial activity of Indigofera zollingeriana and turmeric as an alternative feed additive for poultry it can be concluded that the inhibition zone is influenced by several factors including the concentration of the sample used and the nature of the bacteria. The combination of Indigofera zollingeriana and turmeric can be used as an antibacterial alternative for poultry that can inhibit the bacterium Escherichia coli, Salmonella sp. and Lactobacillus sp.

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