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The antibacterial activity of Kasumba turate *(Carthamus tintorius L.)* against *Salmonella pullorum* and *Escherichia coli* as an alternative feed additive for poultry

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Abstract. Kasumba turate *(Carthamus tinctorius Linn)* is a kind of traditional plant which has been widely used by the people of South Sulawesi to cure measles disease and chicken pox. Kasumba turate contains flavonoid and volatile oil. Some compounds have activity potential as antibacterial. This research aimed to determine the antibacterial activity of kasumba turate against *Salmonella pullorum* and *Escherichia coli* bacteria. This study was conducted based on a completely randomized design with 4 treatments and 5 replications. The treatment consisted of PO (tetracycline as control), P1 (0.5% kasumba turate extract), P2 (0.75% kasumba turate extract), P3 (1% kasumba turate extract), and P4 (1.25% kasumba turate extract), respectively. The results of this study showed that kasumba turate extract could inhibit the *Salmonella pullorum* and *Escherichia coli* growth with an inhibition zone of approximately 11-19 mm. Increasing the level of kasumba turate extract up to 1.25% did not show any differences of inhibition zone compared to the lower levels, however, its effectivenss was significantly lower than commercial antibiotic as the positive control. It concluded that a low level of Kasumba turate extract (0.5%) showed inhibition activity on bacterial growth so that it could be used as an alternative to feed additive poultry.

1. Introduction

Many types of poultry in Indonesia are used as a source of animal protein such as free-range chicken, broiler chicken, quail, ducks, and others. However, poultry farms are vulnerable to disease. One of them is colibacillosis, an infectious disease in poultry caused by *Escherichia coli* (*E. coli*) bacteria. *E. coli* infection can occur in broilers and laying hens of all age groups, as well as other poultry such as turkeys and ducks [1]. Another type of bacterial infectious disease that is susceptible to poultry is pullorum disease which is often found in various countries. This disease is caused by a bacterial infection of *Salmonella pullorum* [2].

The use of antibiotic growth promoters (AGPs) substances in feed is one solution to enhance chicken growth as well as to control microbial load in the chicken farming system. The use of antibiotics as feed additives not only can provide benefits but also reportedly has some negative sides that can harm livestock and consumers as its potential to left residues in chickens' carcasses or organs [3].

Raising the concern of the negative impact of AGPs, some countries, especially developed countries, restrict the use of antibiotics in animal feed. In 2018 the Government of Indonesia officially enacted a regulation restricting the use of antibiotics in accordance with Article 15 and 16 of Regulation No. 14

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of 2017 which contains a restriction on the use of veterinary medicines as antibiotics for animal origin products. Therefore, an alternative that can function as an antibacterial for poultry is needed.

One alternative plant that reported contains antibacterial substances is Kasumba turate (*Carthamus tinctorius* L). Kasumba turate is an endemic plant species from South Sulawesi, especially in the district of Bone. Kasumba turate is mostly contained a flavonoid compound i.e., chalcones, carthamin, carthamone, and lignin [4]. Another study reported the main compound of Kasumba Turate flower extract such as carthamine, carthamone, neo-chartamine, nona-cosane, yellow saflower, safflomin A, dipalmitine, adenoside, beta-sitosterol, and polysaccharides [5]. For essential oil i.e., thymol, carvacrol, linalool, and eugenol was found in kasumba turate flower or leaves [6]. Some of these compounds have the potential for antimicrobial activity.

Kasumba turate extract can inhibit the growth of three bacteria that cause typhoid fever, namely *Salmonella thyposa*, *Salmonella parathypi* A and B [7]. Based on the information that has been obtained, it can be seen that some bioactive substances in Kasumba turate could function as antimicrobial. However, the information on the use of Kasumba turate extract against *S. pullorum* and *E. coli* bacteria was scarce, so the objective of the study was to determine the effectiveness of Kasumba turate extract as a feed additive to inhibit the growth of *S. pullorum* and *E. coli.*

2. Material and method

2.1. Preparation and design

Kasumba turate flower used in this study was purchased in the local market, and preparation for its extract was conducted in a laboratory procedure. This procedure involving heating the distilled water (90^{o}C) for 15 minutes as the main medium to dissolve the Kasumba turate flower. The extract obtained was collected and used for the antibacterial inhibitory test.

Kasumba turate antibacterial inhibitory test uses wells (*Agar Diffusion*) technique. The design used in the study was a *Completely Randomized Design* (CRD) with 5 preparations and 5 replications. Each unit consists of 5 wells. The treatments applied were PO (tetracycline as control), P1 (0.5% Kasumba turate extract), P2 (0.75% Kasumba turate extract), P3 (1% Kasumba turate extract), and P4 (1.25% Kasumba turate extract).

2.2. Bacterial suspension preparation

The bacteria were first cultured on the Blood Agar medium and incubated at 37°C for 24 hours. Four to five colonies of bacteria from culture were taken with sterile ose and then put in a test tube containing five milliliters of Phosphate Buffer Solution (PBS). Incubation at 37°C for two hours, turbidity was formed which is equivalent to Mc Farland 1 standard with a bacterial concentration of 3 x 10^{8}/ ml. The number of bacteria has fulfilled the requirements for sensitivity testing, namely: 10^{5} - 10^{8}/ ml [8].

2.3. Antibacterial test procedure

The method of making a wellbore is to pour the MHA into a petri dish until it hardens and then put five sterile borers in each cup. Then a mixture of MHA with bacterial suspension *Salmonella pullorum* and *Escherichia coli* is inserted into the cup then wait until it hardens and then remove the borer from the petri dish to form a pit, then each hole is filled with substances to be tested namely tetracycline and Kasumba turate. Finally, the petri dishes were put in the incubator for 24 hours. The formation of bacterial growth inhibition areas around the well (clear zone) was measured in mm unit.

2.4. Data analysis

The data obtained were analyzed based on a general linear model of a completely randomized design for 5 treatments and 5 replications. The significant differences of mean were separated using multiple ranges of Duncan test [9].

3. Results and discussion

The inhibitory results of using Kasumba turate extract on *Salmonella pullorum* and *Escherichia coli* bacteria growth are listed in table 1.

Based on of bacterial inhibition test by giving different concentrations of Kasumba turate showed a significant effect (P <0.01). The P0 treatment showed the widest clear zone area of 35.51 mm, and significantly different compared to other treatments, while no differences were observed among the Kasumba turate unit. The P0 treatment used as a positive control in this study was tetracycline which is known as a broad-spectrum antibiotic either for Gram-positive or Gram-negative bacteria. The mode of action of this antibiotic was to inhibit the synthesis of germ protein [10].

Table 1. The antibacterial inhibition of Kasumba turate against *Salmonella pullorum* and *Escherichia coli* bacteria.

Treatment	Inhibition zone (mm)	
	Salmonella pullorum	*Escherichia coli*
P0 (Tetracycline)	35.51±2.46[a]	36.00±1.58[a]
P1 (0.5%)	13.14±1.30[b]	13.89±0.96[b]
P2 (0.75%)	13.24±0.97[b]	13.50±1.83[b]
P3 (1%)	13.97±0.96[b]	14.34±2.54[b]
P4 (1.25%)	15.02±1.10[b]	14.68±3.65[b]

[ab]Different superscripts in the same column show a significant difference (P<0.01).

The clear zone formed in all levels of the Kasumba turate extract treatment indicated that active substance activity in Kasumba turate has the mode of action as an antimicrobial against both *S. pullorum* and *E. coli*. The bioactive compound of Kasumba turate extract i.e., flavonoids, glycosides, sterols, and serotonin derivatives were reported to have a different mode of action in inhibiting the growth of bacteria such as: inhibit protein and cell membrane synthesis [5,11], interfere and inhibit the binding of enzymes such as ATP-ase [12], inhibit the use of oxygen by bacteria [13], and denaturing bacterial cell proteins and damaging the cytoplasmic membrane [14]. All of these activities caused bacterial death.

The inhibition action of Kasumba turate in this study is classified as strong according to Davis and Stout (1971) criteria (11-19 mm) [15]. This measure was weaker than that of commercial antibiotic action of the control group in this study which shown an inhibition zone of more than 20 mm (very strong).

The study also revealed that although statistically the difference between the two bacteria was not performed, the average value of the inhibition zone of both bacteria was quite similar. This is indicated that the mode of action of antimicrobial properties for these bacteria was similar, and proven the action on either gram-negative or gram-positive bacteria. The hydrophobic components of essential oils contained in Kasumba turate extract were reported effective against both gram-positive and gram-negative bacteria [16,17]. However, a three-layer of cell membrane of gram-negative bacteria caused it difficult to be penetrated by antibacterial [18].

The use of 0.5% Kasumba turate extract as the lowest level of experiment unit in this study revealed that even at a low level of this extract, it showed the effective inhibition activity on the bacterial growth. The low level of feed additives is strongly correlated with the feed cost production.

4. Conclusion

The concentration of 0.5% Kasumba turate *(Carthsamus tinctorius L.)* was identified could inhibit the growth of *Salmonella pullorum* and *Escherichia coli* bacteria in laboratory tests. This information could be used in the formulation of an alternative feed additive for poultry.

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