Anti-bacterial activity of Dayak onions extract (*Eleutherine palmifolia*) against *Salmonella* spp and *Escherichia coli*

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Abstract. Dayak onions (*Eleutherine palmifolia*) is a plant from Kalimantan. Dayak onions contain high flavonoid and phenol compounds and can be used as an alternative feed additive for broilers to replace synthetic antibiotics that can cause resistance and residues. This study aims to determine how much the ability of Dayak onions extract to inhibit *Salmonella* spp and *Escherichia coli* bacteria by using factorial randomized design and Duncan's advanced test with three replications. The first factor was the maceration test consisting of control using tetracycline and replication of maceration I, maceration II, combined maceration I and II. The second factor is the concentration consisting of three concentrations of 0.50%, 1.00% and 1.50%. Antimicrobial activity is characterized by the presence of a clear zone that forms around a good hole. The results showed that the best antimicrobial activity of Dayak onion extract was obtained in K3M1 with inhibitory zone diameters of 15.47 mm for *Salmonella* spp and 13.40 mm for *Escherichia coli*. The value of the diameter of the inhibition zone shows a high sensitivity level as an antibacterial. The lowest diameter value of the inhibition zone was K1M2 which was 3.40 mm for *Salmonella* spp and 4.18 mm for *Escherichia coli*. It was concluded that the best replication factor of the antibacterial activity was at M1 and the higher the concentration of Dayak onion extract was used, the higher the antibacterial activity and could be used as an alternative feed additive.

1. Introduction

The increasing demand for broiler chicken has led to genetic evolution with a harvest period of 28-40 days. Improving feed quality is one of the steps takes by providing feed additives in the form of an Antibiotic Growth Promoter (AGP) to growth promoter but can harm livestock and consumers. Antibiotics can cause residues in chicken carcasses and visceral organs [1]. *Salmonella* spp and *Escherichia coli* are potentially large bacteria infecting chickens and are reported to have experienced resistance to some types of antibiotics. The negative impact caused some of Indonesia's most intended countries to begin banning the use of antibiotics in Article 15 and 16 of PERMENTAN No.14/2017. The prohibition led many people to start looking for antibiotic replacements that turned out to be contained by onion in Dayak.

Dayak onions (*Eleutherine palmifolia*) have much phytochemical content such as flavonoids [2]. Phenol compounds work by denaturing cell proteins through hydrogen bonds formed between phenols and bacterial proteins by causing macromolecules and ions in cells that bacteria need to become lysis. Flavonoid compounds inhibit bacterial growth by disrupting cell membrane permeability and inhibiting enzyme bonds such as ATPase and phospholipase [3]. This research aims to find out the ability of Dayak

onion extract in inhibiting the growth of *Salmonella* spp and *Escherichia coli* using the good method. The use of this research is expected to be a source of information to the public, especially farmers in utilizing onion bulbs as one of the alternative feed additives.

2. Materials and methods

The research was conducted in September-October 2018 at the Integrated Laboratory of the Faculty of Animal Husbandry, Phytochemical Laboratory of the Faculty of Pharmacy and Microbiology Laboratory of the Faculty of Medicine, Universitas Hasanuddin, Makassar.

The ingredients used in this study consisted of Dayak onions, *Salmonella* spp, *Escherichia coli*, nutrient agar (NA), ethanol, aquades and tetracycline.

The study used a factorial randomized design with two factors. The factor I is the number of macerations (M) consisting of 4 treatments. Factor II is the concentration of Dayak onion extract (K) consisting of 3 levels. In this study, there were 4x3 combinations or 12 combinations for *Salmonella sp* and *Escherichia coli*. The factor I namely M0= tetracycline as control, M1= Dayak onion extract resulting from maceration I, M2= Dayak onion extract resulting from maceration II and M3= Dayak onion extract resulting from maceration I and II. The second factor is the concentration of Dayak onion extract consisting of 3 treatments namely K1= 0.5% onion ethanol extract, K2= 1% Dayak onion ethanol extract and K3= 1.5% onion ethanol extract.

3. Results and discussions

3.1. Eleutherine palmifolia against Salmonella spp

The research that has been done, obtained results that different real different onion extracts ($P<0.05$) inhibit *Salmonella* spp bacteria by administering different levels at each maceration. The results of further research are further tested with a Duncan test which presented in table 1.

The average value of onion extract resistance to *Salmonella* spp shows the higher the concentration, the stronger the resistance. Dayak onion extract with a higher content of flavonoids and phenols is thought to have the ability to inhibit *Salmonella* spp. Flavonoids and phenols can penetrate and disrupt bacterial cell walls, precipitation proteins in bacterial cells and inactivate important enzyme systems [4], flavonoids inhibit bacterial growth by causing damage to cell membranes and inhibiting the macromolecule synthesis of bacterial cells [5]. Changes in cytoplasmic membrane permeability allow for the disruption of the transportation of important organic ions into cells resulting in inhibited growth even to cell death [6].

In addition to concentration factors, the maceration replay factor also can inhibit *Salmonella* spp. The average resistance of onion extract to *Salmonella* spp at control (M0) resulted in a noticeable difference ($P<0.05$) higher compared to maceration 1, maceration 2 and combined maceration of both. The highest activity is then followed by maceration 1 (M1), this is because bioactive substances are attracted more that can be seen from the more concentrated color of the filtrate. The least bioactive content can be seen from the color sensitivity during the extraction. The color pigment produced by the Dayak onion is red, so on M1, it looks like an act red color. The second maceration (M2) appears clearer compared to M1 indicating that fewer flavonoids and phenols are contained, while in the combined of both maceration (M3) it shows a more concentrated color compared to M2 and clearer when compared to M1. Arifin and Ibrahim [7] stated that flavonoids are found in plants, which contribute to producing pigments in yellow, red, orange, blue and purple. High pigment contents characterized by the increasing sensitivity or lightness of color on the part of the plant.

Table 1. Inhibition of Dayak onion extract against *Salmonella* spp bacteria.

Parameter	Level	Average inhibition of Dayak onion (mm)
Concentration (A)	K1	6.38±3.73[c]
	K2	9.56±6.36[b]
	K3	14.87± 9.50[a]
Maceration (B)	M0	20.43±7.33[a]
	M1	10.05±4.44[b]
	M2	4.27±0.81[d]
	M3	6.32±2.41[c]
Concentration (K)* Maceration (M) (A*B)	K1M0	12.45±0.24[a]
	K1M1	5.30±0.80[b]
	K1M2	3.40±0.20[d]
	K1M3	4.40±0.16[c]
	K2M0	19.55±0.10[a]
	K2M1	9.40±0.17[b]
	K2M2	4.25±0.10[d]
	K2M3	5.03±0.10[c]
	K3M0	29.30±0.10[a]
	K3M1	15.47±0.17[b]
	K3M2	5.20±0.10[d]
	K3M3	9.51±0.19[c]

[abcd]Superscript with different letters in the same column shows a noticeable difference (P<0.05).

The average of the interaction on the M0 (control) with all concentrations shows a noticeable difference higher than all the maceration present in all concentrations. Followed by interactions of M1 with all different concentrations that were noticeable higher than M3 and M2 interactions at all concentrations. The interaction between M0 and K3 shows a noticeable different inhibition (P<0.05) than other interactions with a value of 29.30±0.10 mm, this is because tetracycline used in control is a broad-spectrum antibiotic group that can inhibit the growth of Gram-positive and Gram-negative bacteria [8]. The average value of the next interaction was followed by K3M1 with a value of 15.47±0.17 mm higher than K3M2 and K3M3, this is because in K3M1 bioactive substances flavonoids and phenols are extracted more than other concentration and maceration treatments. The higher the concentration and the earlier the maceration replay signifies more flavonoids and extracted phenols. The content in the remaining filtrate powder has largely dissolved in the solution of maceration solid I and when remastered the remaining powder filtrate of its polarity is no longer following the ethanol solvent so that the flavonoid and phenol compounds are only a small part that can be extracted. Flavonoids on Dayak onions will be extracted through soaking during maceration. Immersion causes pressure differences inside and outside cells resulting in the breakdown of walls and cell membranes resulting in the dissolved secondary metabolites in the solvent [9]. A compound will dissolve more in solvents that have the same polarity [10].

The supersede of each treatment is obtained by calculating the diameter of the clear zone formed around the good hole. The clear zone indicates that there is an antibacterial activity produced by Dayak onion extract. The antibacterial activity is caused by the content of phytochemical compounds in the form of phenols and flavonoids in Dayak onion ethanol extract which is a natural compound and can be found in various types of *Collisions*. Kumar and Abhay [11] stated that flavonoids are a component of

polyphenols that are widely found in plants. Flavonoids appear in the form of aglicon, glycosides, and alcohol derivatives. Artanti et al [12] state that several medicinal plants containing flavonoids have been reported to have antioxidant, antibacterial, antiviral, anti-inflammatory, antiallergic and anticancer activity. Oliver et al [13] stated that phenols have been studied extensively as a disinfectant that has broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria.

3.2. Eleutherine palmifolia Against Escherichia coli

The research obtained results that different real onion Dayak extract ($P<0.05$) inhibits *Escherichia coli* by administering different levels at each maceration. The results of further research are further tested with a Duncan test which presented in table 2.

The average value of onion extract resistance to *Escherichia coli* (table 2) shows the higher the concentration of the resistance is also stronger. Dayak onion extract with a higher content of flavonoids and phenols is thought to have the ability to inhibit *Escherichia coli*. High concentrations indicate that more and more dissolved substances are in a solution [14]. The solvents used in this study are ethanol and dissolved substances are flavonoids and phenols, so if many flavonoids and phenols are dissolved then the antibacterial activity will be higher given that flavonoids and phenols can be antibacterial. The higher the concentration of the extract shows the greater the total flavonoid level and the stronger its reducing power capability, so it can be used as a base to make it a natural antibacterial [15]. The greater the concentration, the more active ingredients are used so that the value of the resulting slave zone is greater [16].

The concentration factor is only one of the factors that affect the antimicrobial ability of Dayak onions because other factors also give a real influence namely the maceration factor. The flattening of the onion extract resistance zone against *Escherichia coli* at control (M0) results in a noticeable difference ($P<0.05$) higher compared to maceration 1, maceration 2 and combined of both. The highest endurance is then followed by maceration 1 (M1), this is because the earlier the maceration replay then the contact time of the sample with solvent is also shorter. It is known that in maceration I is carried out immersion for 3 x 24 hours which then the remaining filtrate is re-macerated for 1 x 24. After maceration I is complete, the flavonoid and phenol content is not optimal for re-extraction because the optimal time to bind flavonoids and phenols into the material is 48 hours and contamination has occurred between the remaining onion extract filtrate and the environment when filtering. Yulianingtyas and Kusmartono [17] stated that the maceration time above 48 hours is no longer effective for increasing the weight of extracted flavonoids because the rate of flavonoid diffusion from the solid surface to the solvent is equal to the rate of flavonoid diffusion from the solvent to the solid surface so that the concentration of flavonoids in the solvent has reached equilibrium. Besides, in the sample, there has been a degradation of flavonoids due to exposure to heat, light and oxygen when filtrate screening was performed at previous levels of immersion.

The average of the interaction of the slave power in M0 (control) with all concentrations shows a noticeable difference higher than all the maceration present in all concentrations. Followed by interactions of M1 with all different concentrations that were noticeable higher than M3 and M2 interactions at all concentrations. The interaction between M0 and K3 shows a noticeable different inhibition ($P<0.05$) higher than other interactions with a value of 18.20 ± 0.10 mm, this is because the tetracycline used in the control is a bacteriostatic antibiotic and works by inhibiting bacterial protein synthesis [18]. The average value of the next servant is followed by K3M1 with a value of 15.47 ± 0.17 higher than K3M2 and K3M3, this is because in K3M1 there are the most flavonoids and phenols obtained from maceration I by contributing enough flavonoids extracted plus higher concentrations so that more dissolved substances can be utilized as antibacterials. Prawata and Dewi [19] stated that the effectiveness of an antibacterial substance is influenced by the concentration of such substances. The increased concentration of substances leads to the increased content of active compounds as antibacterial, so its ability to kill bacteria is also greater.

Table 2. Inhibition of Dayak onion extract (*Eleutherine palmifolia*) against *Escherichia coli*

Parameter	Level	Average inhibition of Dayak onion (mm)
Concentration (A)	K1	5.46±0.87[c]
	K2	8.24±1.68[b]
	K3	12.02±4.40[a]
Maceration (B)	M0	11.50±5.32[a]
	M1	9.31±3.50[b]
	M2	5.82±1.32[d]
	M3	7.63±1.34[c]
Concentration (K)* Maceration (M) (A*B)	K1M0	6.11±0.02[a]
	K1M1	5.30±0.23[a]
	K1M2	4.18±0.11[c]
	K1M3	6.25±0.03[b]
	K2M0	10.22±0.13[a]
	K2M1	9.26±0.12[b]
	K2M2	6.11±0.10[d]
	K2M3	7.34±0.16[c]
	K3M0	18.20±0.10[a]
	K3M1	13.40±0.19[b]
	K3M2	7.19±0.10[d]
	K3M3	9.30±0.11[c]

[abcd]Different superscript on the same column shows the difference (P<0.05).

4. Conclusion

Maceration replay factor has the best antibacterial activity in maceration I and the higher the concentration of Dayak onion extract (*Eleutherine palmifolia*) used then antibacterial activity against *Salmonella* spp and *Escherichia coli* is also higher.

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