Antibacterial Activity of Some Herbs Water Extract against *Escherichia coli*

Bondan Prakoso[1*], Eko Widodo[2], Edhy Sudjarwo[2]

[1]Graduate Program of Animal Husbandry, Faculty of Animal Husbandry, University of Brawijaya, Malang, Indonesia
[2]Faculty of Animal Husbandry, University of Brawijaya, Malang, Indonesia

Abstract

The research aims to select the highest diameter inhibition zone of several types of herb water extract against *Escherichia coli* and find the best herb water extract towards minimum inhibition concentration. It was divided into two parts, in the first stage was to evaluate antibacterial activity of herbs water extract and the second stage was to evaluate concentration of extract to effectively inhibit growth of *E. coli*. In the first experiment there were 6 treatments; P_0 (aquadest 100%), P_1 (antibiotic tetrachlor), P_2 (*Zingiber officinale* extract), P_3 (*Z. officinale* var. Rubrum extract), P_4 (*Kaempferia galanga* extract) and P_5 (*Z. zerumbet* L. extract) tested to find the best herbs on the basis of diameter inhibition zone. In the second experiment 6 treatments were used namely from 0%, 50%, 60%, 70%, 80%, 90% and 100% of *Z. officinale* var. Rubrum extract tested for Minimum Inhibition Concentration (MIC) against *E. coli*. The results showed that among the herbs, *Z. officinale* var. Rubrum extract had the strongest antibacterial activity based on diameter of inhibition zone against *E. coli*. While as low as 50% of *Z. officinale* var. Rubrum extract showed an MIC although the highest activity indicated when 100% of *Z. officinale* var. Rubrum extract used.

Keywords: *Z. officinale* var. Rubrum, diameter of inhibition zone, MIC and *E. coli*.

INTRODUCTION

Duck population is the fourth largest poultry, after broiler, layer and native chickens. There is a tendency that duck population increases as the management of raising duck changes from the traditional system toward more intensive system. Therefore, duck egg and meat is potentially to be source of cheap animal protein for Indonesian people. Therefore, as commodity, development of duck raising needs to be supported by the government [1].

Duck farmers often use several types of antibiotics to maintain good health and improve production performances. The use of antibiotics as antimicrobial growth promoter effectively enhance growth and improve feed efficiency. Implementative use of antibiotics has been banned particularly in European countries [2]. However, residual antibiotics in poultry products are of importance consumer awareness. The residue might also lead to the development of more resistance pathogenic microorganism [3].

Substitution of antibiotics is needed to get saver and healthier poultry products. Herbs which have been proposed by some researchers could be an alternative solution. Herbs have been found to improve metabolism of nutrients, act as antimicrobial, exhibit better immune response are among the mechanism by which active substances of herbs enhance poultry performances [4]. Ginger (*Zingiber officinale*), red ginger (*Z. officinale* var. Rubrum), galanga ginger (*Kaempferia galanga* L.) and zerumbet ginger (*Z. zerumbet* L.) are among the herbs which have been elaborated the use as feed additive.

Some herbs have been reported to show antibacterial effect against *Escherichia coli*, *Staphylococcus aureus* dan *Bacillus cereus* [5]. Red ginger (*Z. officinale* var. Rubrum) which contained essential oil 2.58 – 3.90% inhibited growth of pathogenic bacteria, higher than other kind of ginger [6] including galanga ginger (*K. galanga* L.).

Current experiment was intended to evaluate anti-bacterial of some herbs against *E. coli*. On the basis of Diameter of Inhibition Zone and Minimum Inhibition Concentration of some gingers, it is expected that a correct dose of implementation for feed additive for poultry to replace antibiotics can be decided.

MATERIALS AND METHODS

The materials used were equipments such as Petri dish, test tube, erlenmeyer, incubator, Ohaus balance, micropippet 1 ml, autoclave, waterbath and magnetic stirrer. The others were aquadest, tetrachlor, ginger, red ginger, galanga ginger and zerumbet ginger. Variables observed were Diameter of Inhibition Zone and Minimum Inhibition Concentration against *E. coli*.

* Correspondence author:
Bondan Prakoso
Email : bondan.ub89@yahoo.co.id
Address : Faculty of Animal Husbandry, University of Brawijaya, Jl. Veteran Malang, 65145

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The method of research was Completely Randomized Design. This experiment used 6 treatments, and each treatment was repeated 4 times. The treatments were P_0 = Aquadest; P_1 = Tetrachlor; P_2 = Ginger water extract; P_3 = Red ginger water extract; P_4 = Galanga ginger water extract; and P_5 = Zerumbet ginger water extract.

Diameter of Inhibition Zone and Minimum Inhibition Concentration

Water extract of gingers were prepared by shredding fresh gingers using traditional shred equipment, then manually squeezing the water out and filtering to avoid unnecessary debris. Procedure to test Diameter of Inhibition Zone was started by sterilizing equipments and media of Mueller Hinton Agar (MHA) using autoclave at temperature of 121°C for 30 minutes. Detail of measuring diameter of inhibition zone against *E. coli* was done in accordance with the agar diffusion method [7]. Firstly, a hole with diameter of 6 mm in the media was made, then drop 1 ml of *E. coli* from prepared inoculum containing 10^6 cells.ml^{-1}. Next, its were incubated for 24 hours at 37°C, cooling down for a while and measuring the final diameter. While Minimum Inhibition Concentration was conducted by testing only red ginger because it has the best Diameter of Inhibition Zone [8]. The concentration used 50-100% *Z. officinale* var. Rubrum extract with increasing concentration of 10%, compared with control (0%).

Statistical Analysis

Data of Diameter of Inhibition Zone tabulated and analyzed for ANOVA based on Completely Randomized Design. If significant responses appeared, then it is followed by Duncan's Multiple Range Test [9], while Minimum Inhibition Concentration data were descriptively analyzed.

RESULT AND DISCUSSION

Diameter of Inhibition Zone

The result showed red ginger had the largest diameter among the herbs, which was 12.01±0.03 mm and significantly different (P<0.01) ability inhibit growth of *E. coli* (Table 1). Although, the ability of red ginger to inhibit the bacteria was still significantly lower as compared with positive control (tetrachlor/P_1). The result may also indicate that bioactive substances in the red ginger potentially inhibit growth of pathogenic bacteria.

Table 1. Diameter of Inhibition Zone of some Herbs against *E. coli*

Treatment	Average Diameter of Inhibition Zone (mm)	SD
Aquadest (P_0)	6.01^a	0.01
Tetrachlor (P_1)	16.98^f	0.03
Ginger (P_2)	10.04^d	0.06
Red ginger (P_3)	**12.01^e**	0.03
Galanga ginger (P_4)	6.43^b	0.01
Zerumbet ginger (P_5)	6.93^c	0.10

*Superscript at the same column indicated significant different (P<0.01)

Red ginger contains phenylpropanoid, gingerol dan shogaol as active substances which would be able to act as anti-bacteria [10]. High contents of essential oil in the red ginger may also play an important role as anti-bacteria. This finding on Diameter of Inhibition Zone for red ginger is categorized as strong. Diameter of Inhibition Zone 10-20 mm, 5-10 mm and <5 mm can be categorized as strong, intermediate and low, respectively [11]. The pothograph is showed in Figure 1.

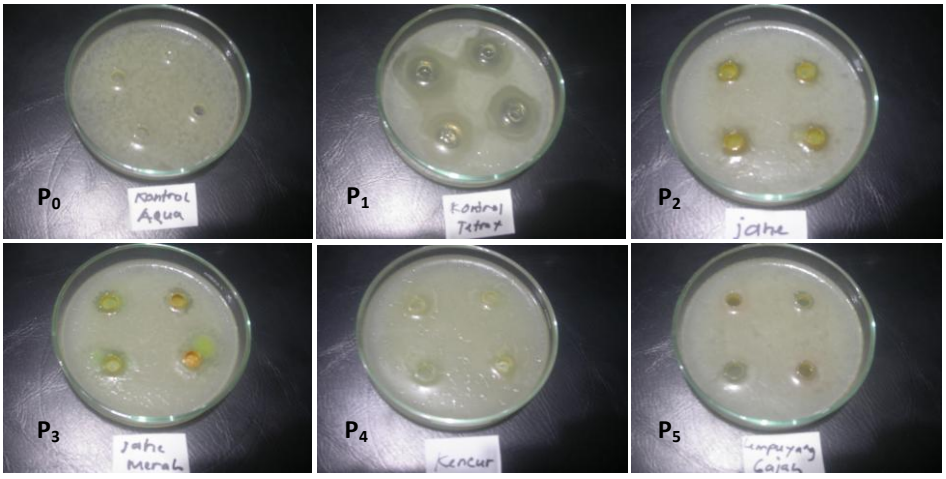

Figure 1. Diameter of Inhibition Zone

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Test for Minimum Inhibition Concentration

As the best result of Diameter of Inhibition Zone was obtained for red ginger, further test is needed whether diluting red ginger with water will show similar anti-bacterial effect. The result showed that decrease concentration of *E. coli* is indicated even red ginger was diluted 50% with water. The result was summarized in Table 2.

Tren of the result showed that the higher the concentration of red ginger the lower population of *E. coli* was obtained. Therefore, the use of 100% of red ginger extract is recommended. Similar result was reported by previous study which tested Minimum Inhibition Concentration for turmeric extract [8]. Red ginger contains essential oil 2.58 – 3.90%, while giant ginger was 0.82 – 1.66% and small emprit ginger was 1.50 – 3.50% [6]. Essential oil acts as anti-bacteria by the mechanism of inhibiting growth or killing the bacteria by disrupting membrane and cell wall formation [12]. Damage in cell membrane will disturb supply of nutrients so that the cell deficient on those nutrient, finally causing dead cell [13].

Table 2. Red Ginger Extract Concentration and Minimum Inhibition Concentration against *E. coli*

Red Ginger (%)	Average Minimum Inhibition Concentration	CFU per Plate
0	309.605	$3.1.10^7$
50	269.605	$2.7.10^7$
60	230.604	$2.3.10^6$
70	223.854	$2.2.10^6$
80	142.103	$1.4.10^5$
90	92.602	$9.3.10^4$
100	45.852	$4.6.10^3$

CONCLUSION

It can be concluded that red ginger has the highest Diameter of Inhibition Zone, and therefore the use 100% water extract of red ginger might be implemented in poultry farm to replace the use of antibiotics tetrachlor.

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