THE EFFECT OF RED GINGER (*Zingiber offinalle var. Rubra*) EXTRACT ON THE GROWTH OF *Escherichia coli* ISOLATED FROM NATIVE CHICKEN, CATTLE AND PIG

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ABSTRACT

Every year, many young farm animals died due to diarrhea or other gastro-intestinal disturbances. This of course was a tremendous loss to the national farm income. For long, Red ginger (*Zingiber officinale var. Rubra*), a traditional medicinal herb with good reputation, had been used by ancient doctors in many Asian cultures to treat these problems. Research show that *E coli* was the main responsible agent for diarrhea. In this conjunction, the objectives of this study were to examine the effects of Red ginger extracts on the growth of *Escherichia coli*. A 4 x 5 Factorial design was employed in this investigation. For this purpose, four *E coli* isolates collected from native chicken (C), young cattle (A), piglet (B1) and pig (B2) were used in *in vitro* tests. Fifteen microliters each of five concentrations of Red ginger extract, i.e., 10.00, 7.50, 5.00, 2.50, and 1.25 % were dropped in sterile paper disks. These disks were then laid on the MEU blood agar media previously inoculated with each of the four isolates and were incubated overnight at 37 degree C. The test results demonstrated that the higher the concentration of the Red ginger extracts, the higher the bacterial growth inhibition effects obtained. Further tests pointed out that the growth inhibition effects of the Red ginger extracts on *E coli* isolates C, A, B1, and B2 were significantly different at $P<.05$. So, it could be concluded that the Red ginger extracts were effective in controlling the *E coli* growth.

Key words: Red ginger, *Escherichia coli*, Traditional medicine, Native chicken, Cattle, Pig

INTRODUCTION

Colibacillosis incidences in cattle, pig and other farm animals were well documented in Indonesia. These bacterial incidences in young calves and piglets were reported in Bali (Hartaningsih and Hasan, 1985), Lampung (Suastama, 1983) and Central Java (Setiawan, *et al.*, 1982). Piglet neonatal diarrhea associated with enterotoxigenic (ETEC) *Escherichia coli* was commonly observed in intensive piggeries in Bogor and Kapok areas. Here diarrhea occurred at the rates of 13 to 40 percents within the first two weeks of life. The associated mortality rates were from 12 to 30 percents (Supar, *et al.*, 1989). In turn, this young animal mortality contributed considerable losses to the national farm income.

As an effort to control diarrhea and other gastro-intestinal disorders, farmers regularly added antibiotics to farm animal feeds, especially in poultry and swine rations. In the long run, this practice may damage the animal health. Supar, *et al.*, (1990) proved that several *E. coli* isolates were resistant to commonly use antibiotics, including Ampicillin, Streptomycin, Trimethoprim, and Sulphamethoxazole. Further observation show that 100 *E. coli* strains were resistant to at least one antibiotic. The highest percentages being attained for resistance was to Penicillin, Tetracycline and Cephalothin (Carvalho, *et al.*, 1992).

Considering the *E. coli* resistance to many commonly used antibiotics, this study investigated the potential use of Red ginger extract (*Zingiber officinale var Rubra*) to control the *E coli* growth. For long, ginger was well known for its ability to strengthen

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the gastric and the upper intestinal mucous and to protect them from peptic ulcers, to cure stomachache, infection, and to relieve pain. Ancient Chinese and Indian doctors had used ginger as one of their top remedy (Weil, 1996 and 1997).

In this study, three hypotheses were tested: (1) the four types of *E coli* isolate observed would produce different diameter of growth inhibition zones as their responses to the Red ginger extract, (2) the higher the concentration of the Red ginger extract, the larger the diameter of the bacterial growth inhibition zones produced, and (3) the combined effects of type of *E coli* isolates and the concentrations of the Red ginger extract would produce different diameter of the bacterial growth inhibition zones.

MATERIAL AND METHODS

Material

Methanol was used to make four concentrations of the Red ginger extract for this investigation. Then, Mueller Hinton blood agar and broth media was used as the growth media for the four bacterial isolates for this study. Additionally, the blood agar media was also used as a purification control.

Isolates of *Echerichia coli* were collected from native chicken, cattle, piglet,and pig raised by small farmers in Bogor, West Java. These specimens were later used for bacterial verification.

The obtained bacterial specimens were brought to BALITVET laboratory at Bogor. Here, they were cultivated in the blood agar media plates. The inoculated blood agar plates were incubated for 24 hours at 37 degree C. The bacterial isolates grown in the blood agar media plates were identified by employing Cowan and Steel methods (1973).

Extracting the Red Ginger

Dried Red ginger rhizomes were ground into powder. Methanol was then added to the Red ginger powder. To homogenize the mixture, the liquefied Red ginger was shaken for one hour. This agitation would accelerate the solution of the Red ginger active compounds in the methanol solvent. The mixture was kept for 24 hours. Then, the liquefied Red ginger was filtered by paper filter. The obtained methanol solution containing the Red ginger active compounds was poured into a Florentine tube. The tube was placed in a rotary evaporator to evaporate the methanol solvent at 40 degree Celsius at 140-160 rpm and at 15-20 lbs of pressure.

Sterile aquadest was added to the obtained extract to make four concentrations of the Red ginger extract, i.e. 1.25, 2.50, 5.00, 7.50 and 10.00 percents. Then, 15 microliters of each concentration was dropped at sterile paper disks. Each disk was laid on the MEU blood agar media that previously had been inoculated with each of the three bacterial isolates and were incubated for 24 hours at 37 degree Celsius.

The bacterial growth inhibition zones were observed and measured. The size of the growth inhibition zones would indicate the effectiveness of the Red ginger extracts in controlling the bacterial infection.

Table 1. The means of the bacterial inhibition growth zones (Mm) by treatment groups

Type of	The Concentration of The Red ginger Extract (%)					Average
E. coli Isolate	10.00	7.50	5.00	2.50	1.25	
Cattle (A)	17.00	13.00	12.00	11.00	9.00	12.40
Piglet (B1)	16.00	12.00	11.00	10.00	9.00	11.60
Pig (B2)	11.00	11.00	10.00	10.00	5.00	9.40
Chicken (C)	17.00	15.00	14.00	12.00	9.00	13.40
Average	15.25	12.75	11.75	10.75	8.00	11.70

Table 2. The main effects of type of *E. coli* isolates on the bacterial growth inhibition zones

Isolate	Diameter of Growth Inhibition Zone (mm)	Level of Significance*
Cattle (A)	12.40	b
Piglet (B1)	9.40	c
Pig (B2)	11.60	b
Chicken (C)	13.40	a

*Different alphabet code indicated a significant difference at P<.05 DMRT

Design

This study was designed as a 4 by 5 factorial experiment. The first factor observed in this *in vitro* test was the type of *Escherichia coli* isolate. There were four levels of this factor, i.e., *E coli* isolate taken from native chicken, piglets, matured pig, and cattle. The second factor was the concentrations of the Red ginger extracts. This factor had five levels, i.e., 10.00, 7.50, 5.00, 2.50, and 1.25 percents. The observed dependent variable of this investigation was the diameter of each bacterial growth inhibition zone.

Data Analysis

In this study, the Analysis of Variance was used to analyze the data about the diameters of the bacterial growth inhibition zones. Following this analysis, the Duncan Multiple Range Test (DMRT) procedure was used to determine further differences among the means of the diameters of the bacterial growth inhibition zones.

RESULTS AND DISCUSSION

Results

Research results about the effects of five concentrations of the Red ginger extracts on the bacterial growth inhibition zones of *E coli* isolates obtained from native chicken, piglet, matured pig, and cattle were presented in the following sections.

Table 1 above pointed out that the average growth inhibition zones of *E coli* isolates obtained from cattle, piglet, pig and chicken were different. Further, Table 1 also show that the average of the bacterial growth inhibition zones was getting lower with the lower concentrations of the Red ginger extract.

Analysis of Variance of the above data proved that the main effect of types *of E coli* isolate, the main effect of the Red ginger concentrations, and the interaction effect of types of *E coli* isolate and the Red ginger concentration on the diameters of the bacterial growth inhibition zones were all highly significant.

1. The Main Effect of Type of *E coli* isolates on the Growth Inhibition Zones

The Analysis of variance show that the main effect of type of *E. coli* isolates on the bacterial growth inhibition zones was highly significant. Further test results were presented in the Table 2.

The above Table 2 show the size of four bacterial growth inhibition zones at MEU blood agar media, after being treated with five different concentrations of the Red ginger extracts. In these *in vitro* tests, *E coli* isolate collected from native chicken produced the largest growth inhibition zone, followed by isolates collected from cattle, pig, and piglet. Statistically, these differences were significant at alpha equal to or less than 0.05.

2. The Main Effect of Five Red ginger Concentrations on the Bacterial Growth Inhibition Zones

Now, what about the main effect of the Red ginger concentration increase on the bacterial growth inhibition zones? As mentioned earlier, the Analysis of variance of this effect was highly significant, too. Further

Table 3. The main effect of the red ginger concentration increase on the bacterial growth inhibition zones

The Red ginger Extract Concentration (%)	Diameter of Bacterial Growth Inhibition Zones (mm)	Significance Level
10.00	15.25	a
7.50	12.75	b
5.00	11.75	b
2.50	10.75	c
1.25	8.00	d

Different alphabet indicated a significant difference at P<.05 DMRT

results of Duncan Multiple Range Tests were presented in the Table 3.

Table 3 above demonstrated that the first concentration of Red ginger extract, i.e., 10.00 percent, produced the largest growth inhibition zone, followed by 7.50 percent, 5.00 percent, 2.50 percent, and finally 1.25 percent. Statistically, the four growth inhibition zones were different at alpha equal to or less than 0.05. Therefore, it could be concluded that the higher the concentration of the anti bacterial agents in the Red ginger extracts, the larger the diameter of the bacterial growth inhibition zones obtained.

3. The Combined Effects of Types of *E coli* Isolate and the Red ginger Extract Concentrations on the Bacterial Growth Inhibition Zones

As presented earlier, the combined effect of types of *E. coli* isolate and the Red ginger extract concentrations on the bacterial growth inhibition zones were highly significant too. To determine further differences of the combined effects, the Duncan Multiple Range Tests were performed on all findings. The results were presented in the Table 4.

The following Table 4 pointed out that the combination of types of *E coli* isolate and the Red ginger extract concentrations produced different growth inhibition zones. Further, Table 4 demonstrated that *E coli* isolate collected from the native chicken and cattle produced the largest growth inhibition zones and differed significantly from *E coli* isolates collected from piglet and pig at least at three concentrations of the Red ginger extracts.

Discussion

The above findings uncovered more facts about the antibacterial property of ginger extract. For long, ginger, both fresh and dried, had been used for curing various ailments in Asia. Ginger could heal wound, infection and inflammation (Weil, 1996; 1997). In China, fresh ginger had been reported highly effective in the clinical treatment of acute bacterial dysentery; whereas dried ginger had been used for thousand of years to treat stomach ache, diarrhea, nausea, cholera, and bleeding (Leung, 1996, p.185).

Ginger was reported to contain oleoresin that contained gingerols, shogaols and zingerone. Shogaols and zingerone were dehydration and degradation products of gingerol. Shogaols were twice as pungent as gingerols. Additionally, ginger also contained a protease (Leung, 1980, p. 184).

So, how was the Red ginger extract actually deactivating the *E coli* isolates in these *in vitro* tests? The explanation was likely found in each *E coli* isolate tolerance to surface tension reducer agents. Naturally, bacteria had a three layer cell wall structured bond (Volk and Wheeler, 1988). This simple structured bond was made of: (1) cytoplasmic membrane, (2) thicker peptidoglicant membrane, and (3) varied outer membrane.

According to Volk and Wheeler (1988), the cytoplasmic membrane was mainly made of proteins and lipids that were vulnerable to surface tension reducer agents.

Table 4. The combined effects of types of *E. coli* isolate and the red ginger extract concentrations on the bacterial growth inhibition zones

Extract concentration (%)	*E coli* Isolate	Diameter of Growth Inhibition Zones (mm)	Significance Level*
10.00	Cattle (A)	17.00	a
	Piglet (B1)	11.00	ef
	Pig (B2)	16.00	ab
	Chicken (C)	17.00	a
7.50	Cattle (A)	13.00	de
	Piglet (B1)	11.00	fg
	Pig (B2)	12.00	ef
	Chicken (C)	15.00	bc
5.00	Cattle (A)	12.00	ef
	Piglet (B1)	10.00	gh
	Pig (B2)	11.00	fg
	Chicken (C)	14.00	cd
2.50	Cattle (A)	11.00	fg
	Piglet (B1)	10.00	gh
	Pig (B2)	10.00	gh
	Chicken (C)	12.00	ef
1.25	Cattle (A)	9.00	h
	Piglet (B1)	5.00	i
	Pig (B2)	9.00	ih
	Chicken (C)	9.00	h

* Different alphabet indicated a significant difference at P<.05 DMRT

In this conjunction, gingerol and shogaol -- the pungent principles of ginger oleoresin probably were the responsible agents for the ginger anti bacterial property. In this case, gingerol was one of the major phenollic compounds found in the ginger oil (Budaveri, *et al.*, 1996, p. 751) that might have surface tension reducer activity.

This organic phenollic compound, according to Volk and Wheeler (1988) could destroy the protein content of the bacterial cell wall. In this case, the phenollic compound would dissolve the cytoplasmic membrane and create leaks at the cell wall. In turn, these leaks would cause losses of important metabolites from the bacterial cell. So, the *E coli* would loose their pathogenic abilities and died.

Secondly, the phenollic compound in the Red ginger extract would inactivate a number of the bacterial enzymatic activities. This too, would deactivate the *E coli* pathogenic abilities and then killed them.

Finally, the phenollic compound and the protease enzyme could also precipitate the outer protein membrane of the *E. coli.* So, the bacteria would be precipitated, clotted and then destroyed by the phenollic compound and the enzyme (Volk and Wheeler, 1988).

CONCLUSIONS

The following conclusions were derived from the above research findings and interpretations:

1. The Red ginger (*Zingiber officinale var Rubra*) extracts had the bactericide effects on four *Escherichia coli* isolates.
2. The higher the concentration of the Red ginger extracts, the larger the diameter of

the bacterial growth inhibition zones obtained.

3. Of the four *E. coli* isolates tested, those collected from the native chicken and cattle were most affected by the Red ginger extracts, at least at four concentrations.

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