THE EFFECT OF CLOVE STEM OIL (*Oleum caryophylli*) ON THE GROWTH OF ESCHERICHIA COLI ISOLATED FROM NATIVE CHICKEN, CATTLE AND PIG

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ABSTRACT

The objective of this study was to examine the effects of Clove Stem Oil (*Oleum caryophylli*) or CSO extracts on the growth of *Escherichia coli*. A 4 x 3 Factorial Design was applied in this study. The first factor was 4 types of *E. coli* isolates that collected from native chicken (C), young cattle (A), piglet (B1) and pig (B2), while the second factor was 3 concentrations of CSO extract, that was 50.00, 25.00 and 12.50 percents. Fifteen micro liters of CSO extract was dropped in sterile paper disks. These disk were laid on the MEU agar media previously inoculated with each of the four isolates and were incubated overnight at 37^0C. The bacterial growth inhibition zones were observed and measured. The results demonstrated that the higher concentration of the CSO extracts, the higher bacterial growth inhibition effects obtained. Fhe growth inhibition effects of the CSO extracts on *E. coli* isolates C, A, B1 and B2 were significantly different at $P<0.05$. It could be concluded that the CSO extracts were effective to control the *E. coli* growth.

Keywords: Clove Stem Oil (*Oleum caryophylli*), traditional medicine, native chicken, cattle, pig.

INTRODUCTION

Clove (*Eugenia caryophyllata* Thunb.) is widely cultivated in Madagaskar, Sri Lanka, Indonesia and the South of China (Bureau of Drug Administration of China, 1989). *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 (Tzipori, 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 used antibiotics including *Ampicillin, Streptomycin, Trimethoprim*, and *Sulphamethoxazole*. Further observation showed that 100 *E. coli* strains were resistant to at least one antibiotic. The highest percentages being attained for resistance was 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 Clove Stem Oil (*Oleum cryophilic*) extract to control the *E. coli* growth. 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 CSO extract. (2) The higher concentration of the CSO extract, the larger diameter of the bacterial growth inhibition zones produced, and (3) the combined effects of type of *E. coli* isolates and the concentrations of the CSO extract would produce different diameter of the bacterial growth inhibition zones.

MATERIALS AND METHODS

Materials

Concentrations of the CSO obtained from PT. Phytochemindo extract used in this experiment. Mueller Hinton agar and broth media

was used as the growth media for the four bacterial isolates.

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

Experimental Procedures

The obtained bacterial specimens were brought to BBALITVET laboratory at Bogor and cultivated in the blood agar media plates. The inoculated blood agar plates were incubated for 24 hours at 37^0C. The bacterial isolates grown in the blood agar media plates were identified according to Cowan and Steel methods (1973).

Sterile oil palm was added to the obtained extract to make three concentrations of the CSO extract i.e. 50.0, 25.0 and 12.5 percents. Then, 15 micro liters of each concentration was dropped at sterile paper disks. Each disk was laid on the MEU blood agar media that previously had been inoculated by each of the four bacterial isolates and were incubated for 24 hours at 37^0 C.

The bacterial growth inhibition zones were observed and measured. The size of the growth zones would indicate the effectiveness of the CSO extracts to control the bacterial infection.

Design

This study was designed as a 4 x 3 factorial experiment. The first factor observed in this in vitro test was the type of *Escherichia coli* isolate. There were four types 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 CSO with three levels, that was 5.00, 25.0, and 12.50 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 data about the diameters of the bacterial growth inhibition zones. Following this analysis, the inhibition zones were determined by The Duncan's Multiple Range Test (DMRT) procedure, in order to know further differences among the means of the diameters of the bacterial growth inhibition zones.

RESULTS AND DISCUSSION

Results

Research results about the effects of three concentrations of the Clove Stem Oil (*Oleum caryophylli*) 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 pointed out that the average growth inhibition zones of *E. coli* isolates obtained from cattle, piglet, pig and chicken were different. In addition, Table 1 also showed that the average of the bacterial growth inhibition zones was getting lower with the lower concentrations of the CSO extract.

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

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

The analysis of variance showed that the main effect of type of *E. coli* isolates on the

Table 1. The Means of the Bacterial Inhibition Growth Zones (mm) by Treatment Groups

Type of E. coli isolate	The Concentration of the Clove Stem Oil (*Oleum caryophylli*) (%)			Average
	50.0	25.0	12.5	
Cattle (A)	15	11	10	12
Piglet (B1)	9	9	8	8.66
Pig (B2)	14	10	9	11
Chicken (C)	15	13	12	13.33
Average	13.25	10.75	9.75	

bacterial growth inhibition zones was highly significant. Further test results were presented in the Table 2. Table 2 showed that the size of four bacterial growth inhibition zones at MEU blood agar media, after being treated by three different concentrations of the CSO 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.

Table 2. The Main Effects of Type of *E. coli* Isolates on the Bacterial Growth Inhibition Zones

Isolate from	Diameter of Growth Inhibition Zone (mm) *
Cattle (A)	12.00^{b}
Piglet (B1)	8.66^{c}
Pig (B2)	11.00^{b}
Chicken (C)	13.33^{a}

* Different superscript at the same column indicated a significant difference at P<0.05

The Main Effect of Three CSO Concentrations on the Bacterial Growth Inhibition Zones

Analysis of variance of this effect was highly significant. Result of Duncan's Multiple Range Tests is presented in the Table 3. Table 3 demonstrated that the first concentration of CSO extract (50 percent) produced the largest inhibition zone, followed by 25 percent and 12.5 percent. The four growth inhibition zones were significantly different (P<0.05). Therefore it could be said that the higher concentration of the anti bacterial agents in the CSO extracts, the larger diameter of the bacterial growth inhibition zones obtained.

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

The combined effect of types of *E. coli* isolate and the CSO extract concentrations on the bacterial growth inhibition zones were highly significant (Table 4). It can be seen in Table 4 that the combination of types of *E. coli* isolate and the CSO extract concentrations produced different growth inhibition zones. *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 CSO extracts.

Table 3. The Main Effect of the CSO Concentration Increase on the Bacterial Growth Inhibition Zones

The CSO Concentration (%)	Diameter of Bacterial Growth Inhibition Zone (mm) *
50	13.25^{a}
25	10.75^{b}
12.5	9.75^{b}

* Different superscript at the same column indicated a significant difference at P<0.05

Discussion

The above findings proved the antibacterial effects of the Clove Stem Oil (*Oleum caryophylli*) extract. Clove bud oils have biological activities, such as as antibacterial, antifungal, insecticidal and antioxidant properties, and are used traditionally as flavoring agent and antimicrobial material in food (Huang *et al*., 2002, Lee and Shibamoto, 2001 and Velluti *et al*., 2003). For example, clove oil was effective against *L. monocytogenes* and *S. enteritidis* in tryptone soya broth (TSB) and cheese (Smith-Palmer *et al*., 1998 and Smith-Palmer *et al*., 2001). The high levels of eugenol contained in clove essential oil give it strong biological activity and antimicrobial activity. This phenolic compound can denature proteins and reacts with cell membrane phospholipids changing their permeability (Briozzo, 1989 and Deans and Ritchie, 1987).

The CSO was reported contain eugenol, oleanolat asid, kariofilin, resin and Gom. Clove oil is highly toxic to microbes, altering the cell membranes of yeast and bacteria (Marin Municipal, 2008).

So, how was the CSO 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

Table 4. The Combined Effects of Types of *E. coli* Isolate and the CSO Extract Concentrations on the Bacterial Growth Inhibition Zones

Extract Concentration (%)	*E. coli* Isolate from	Diameter of Growth Inhibition Zones Growth*
50	Cattle (A)	15.00^{a}
	Piglet (B I)	9.00^{ef}
	Pig (B2)	14.00^{ab}
	Chicken (C)	15.00^{a}
25	Cattle (A)	11.00^{de}
	Piglet (B I)	9.00^{fg}
	Pig (32)	10.00^{ef}
	Chicken (C)	13.00 ^{be}
12.5	Cattle (A)	10.00^{ef}
	Piglet (B I)	8.00^{gh}
	Pig (B2)	9.00^{fg}
	Chicken (C)	12.00^{cd}

* Different superscript at the same column indicated a significant difference at P<0.05

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. In this conjunction, eugenol the CSO probably was the responsible agents for the CSO anti bacterial property.

CONCLUSIONS

Based on the results, it may be concluded that the CSO extracts had the bactericide effects on four *Escherichia coli* isolates. The *E. coli* isolates collected from the native chicken and cattle were the most affected by the CSO. The higher concentration of the CSO extract, the larger diameter of the bacterial growth inhibition zones produced.

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