Antimicrobial Clove Bud Oil for Inhibiting *Salmonella* sp. Isolated from Broiler Carcass Samples

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ABSTRACT

The aim of this study was to determine the inhibition effect of clove bud oil against the growth of *Salmonella* sp. isolated by in vitro method. Three isolates of *Salmonella* sp. I, II and III were obtained from the broiler carcasses in Central Java used in this study. Every isolates was tested against several concentrations (25, 20, 15, 10 and 6% of c*love bud oil.* The results indicated that the higher of oil concentration the higher the growth inhibition of *Salmonella enteritidis* obtained (P<05). The growth inhibition produced by 25, 20, 15, 10 and 5% oil concentrations respectively were 18.7, 15.7, 14.3, 9.3 and 6.7 mm. The most sensitive *Salmonella* sp. isolate to clove bud oil was *Salmonella* sp. isolate II (P<05). Growth inhibitions of *Salmonella* sp. I and III and I respectively were 7.2, 11.6 and 13.4 mm respectively. It was concluded that clove bud oil could inhibit the growth of *Salmonella* sp.isolated from broiler carcass samples.

Key words: *antimicrobial, clove, Salmonella sp.*

INTRODUCTION

Clove bud oils have biological activities, such 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; 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; Deans and Ritchie, 1987).

The development of antimicrobial resistance among pathogenic bacteria has emerged as a major public health concern, which has led to an intensification of discussion about the prudent use of antimicrobial agents, especially in veterinary medicine, nutrition and agriculture (Caprioli *et al.*, 2000).

The utilization of antimicrobial drugs has played an important role in animal husbandry, since they are used in prophylaxis, treatment and growth promotion. Overall, there are largest quantities of antimicrobials that are used as regular supplements for prophylaxis or growth promotion in the feed of animal herds and poultry flocks. This result in the exposure of a large number of animals, irrespective of their health, has shown frequently sub therapeutic concentrations of antimicrobials (Dupont and Steele, 1987 and Franco *et al.*, 1990). Furthermore, antibiotics given to animals and closely related compounds used in human therapy have been exerting selective pressure on their target bacteria for decades (Witte, 1998), and can generate a reservoir of antimicrobial resistant bacteria (Endtz *et al.*, 1991 and Smith *et al.*, 1999).

Antimicrobial-resistant bacteria in food animals threaten the efficacy of human drugs if antimicrobial-resistant bacteria or antimicrobial-resistance genes become incorporated into human bacterial populations (Smith *et al.*, 2002). Agricultural antibiotic use increases the frequency of antibiotic resistant zoonotic pathogens such as Salmonella (Smith *et al*., 2002). Most antimicrobial-resistant Salmonella infections are acquired from eating contaminated foods of animal origin (Angulo *et al.*, 2000). The husbandry practices used in poultry industry and the widespread use of medicated feeds in broiler and layer operations made poultry a major reservoir of antimicrobial-resistant Salmonella (D'Aoust *et al*., *1992*). Resistance in Salmonella limits the therapeutic options available to veterinarians and physicians in the treatment of certain human cases of salmonellosis (Witte, 1998). Furthermore, if there is a confection with HIV, it may result in rapid disease progression in the infected individual and has a potential multiplier effect on the dissemination of the resistant pathogen to the rest of the population (WHO, 2001).

Therefore, the aim of this study was to determine the inhibition effect of clove bud oil against the growth of *Salmonella* sp. isolated by *in vitro* method. Three isolates of *Salmonella* sp. I, II and III were obtained from the broiler carcasses in Central Java used in this study.

MATERIALS AND METHODS

This study was assigned to 3 x 5 factorial arrangements of treatments to examine the effect of three isolates of *Salmonella* sp. and five concentrations of clove bud oil. Each treatment was replicated three times. Duncan's Multiple Range Test was used to estimate the differences between treatment means.

The *Salmonella* sp. isolates (I. II and III) used in this experiment were *Salmonella sp.* isolates that are collections of Balitvet-Bogor. Clove bud oil (*Oleum caryophylli*) was collected from PT. Phytochemindo extract, Central Java. Concentrations of the clove bud oil (*Oleum caryophylli*) for this investigation were 25, 20, 15, 10 and 5%. Mueller Hinton agar and broth media were used as the growth media for the three bacterial isolates for this study.

Anti bacterial study was done at Balitvet-Bogor. Fifteen microlitres of clove bud oil were dropped into sterile disk. The disks then were laid on Mueller-Hinton-Agar Medium that had been inoculated with *Salmonella* sp. isolates and incubated at 37^0C overnight. The next day, the growth inhibition on every plate was observed.

RESULTS AND DISCUSSION

Clove bud oil concentration

Increasing clove bud oil concentrations resulted in significantly increased growth inhibition of *Salmonella* sp. isolates (P <.05) (Table 1). The result indicated that the higher concentrations of clove bud oil, the higher its abilities to inhibit growth of *Salmonella* sp. isolates (P<0.5). This might be due to the increase in concentration of usnat acid as bacteriostatic component in the clove bud oil. This result agrees with Windholz *et al*. (1983) who showed that the clove bud oil contains usnat acid as antibiotic and bacteriostatic.

Table 1. The effect of *Usnea* spp. extract concentration on growth inhibition of *Salmonella* sp. isolates

Extract concentration (%)	Growth inhibition (mm)
25.0	18.65[a]
20.0	15.65[b]
15.0	13.31[c]
10.0	9.31[d]
5.0	6.65[e]

Note: means within column with different superscripts are significantly different (P<0.5).

Table 2. Growth inhibition of three *Moraxella* isolates

Salmonella sp. isolates	Growth inhibition (mm)
I	9,18[c]
II	15,38[a]
III	13,58[b]

Note: means within column with different superscripts are significantly different (P<0,5).

Table 3. The effect of the clove bud oil on growth inhibition of three *Salmonella* sp. isolates

Extract concentration (%)	Type of isolate	Growth inhibition (mm)
25	I	16
20	III	18
15	II	11
10	I	8
5	III	11
25	II	16
20	I	4
15	III	24
10	II	16
5	I	12
25	III	16
20	II	8
15	I	6
10	III	8
5	II	17

Note: means within column with different superscripts are significantly different (P<0,5).

Salmonella sp. Isolates

Three *Salmonella* sp. isolates (I, II and III) used in this study had significantly different sensitivity (P<0.5) to clove bud oil (Table 2). Growth of *Salmonella* sp. isolate II was the most inhibited by clove bud oil (P<0.5), while growth of *Salmonella* sp. isolate I was the least inhibited. These results have indicated that *Salmonella* sp. isolate II was the most sensitive to the clove bud oil. The sensitivity differences among three

Salmonella sp. isolates used in this study might be due to the effect of antibiotic that was applied previously to the affected animal. Poeloengan *et al*. (1992) showed that continuity of antibiotic application resulted in resistency. Table 3 shows that *Salmonella* sp. isolate II was the most sensitive isolate to clove bud oil especially at high concentrations (25 and 20%), while the isolate I of *Salmonella* sp was the least sensitive to the clove bud oil at all concentrations (P<0.5). This result suggested that to inhibit the growth of *Salmonella* sp isolate II would be more effective at high concentration of the clove bud oil.

CONCLUSIONS

Increasing clove bud oil concentrations resulted in increasing the growth inhibition of *Salmonella* sp isolates significantly (P<0.5). Isolate II of *Salmonella* sp was the most sensitive to clove bud oil at 25% and 20% concentration.

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