

## The Effect of Garlic (*Allium Sativum*) Extract on The Growth of Bacteria Isolated From Uterus Dairy Cattle

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### ABSTRACT

Many dairy farms suffered from tremendous economic losses due to repeat breeding. Laboratory investigation demonstrated that several bacteria. i. e., *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas* sp., and *Klebsiella* sp. were responsible for this syndrome. This research was to promote the uses of traditional medicinal herb as an alternative way to cure repeat breeding. Garlic was as well known as a herb containing antibacterial effects. The objective of this study was to asses the effects of garlic extracts on the growth of bacteria isolate which were collected from repeat breeding infected of local milking cows. A factorial design 3 x 4 was employed for this investigation. Four isolates of bacteria from repeat breeding, i.e., *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas* sp., and *Klebsiella* sp. were used in *in-vitro* test. Fifteen micro liters each of three concentrations of garlic extract, i.e., 75, 50, and 25% were dropped in sterile paper disks. The disks were than laid on the MEU blood media previously inoculated with each of the four isolates and were incubated overnight at 37 degree C. The finding results showed that that the highest concentration of garlic extract inhibited bacterial growth In further results showed that the garlic extract significantly inhibited the growth of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas* sp., and *Klebsiella* sp. [p<.0001]. As conclusion, the Garlic (*Allium sativum*) extracts had bactericide effects, especially on *Staphylococcus aureus*, and *Streptococcus pyogenes*; the is positive correlation between the high concentration of garlic extract with inhibition zones; *Staphylococcus aureus* shown the most affected by the Garlic extracts at all concentrations and Garlic extract was able to use effectively for controlling the bacteria growth.

*Key words:* Garlic, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas* sp., *Klebsiella* sp.

### INTRODUCTION

Repeat breeder syndrome is a major source of economic disadvantage in dairy herds. Cows that fail to conceive after a defined number of inseminations with fertile semen (generally 3 or more) are classified as repeat breeder (Levine, 1999). Around 10%-15% of cows fail to conceive on time can be classified as repeat breeder (Hartigan *et al.*, 1972).

Bacterial infection is the most important among the various causes of the sub fertility (Dholakia *et al.*, 1987). Such a condition may cause cervicitis or endometritis of various degrees, which in turn may lead to embryonic death and repeat breeding problems (Elliot *et al.*, 1968). These infections effect fertility by altering the uterine environment resulting in impairment of sperm transport, sperm death and hostile environment to the subsequent development and maintenance of the conceptualizing to their death.

Early embryonic death (<42 days) is a major factor in reproduction failure, which in turn causes economic loss to the dairy industries (Rahman *et al.*, 1996).

The Garlic has important efficacy as antibacterial activity (Ali and Saoji, 2002). The effects of an aqueous extract of garlic and its active constituent allicin were tested against 40 drug resistant isolates of the strains of *Shigella dysenteries* type 1 and *Shigella flexneri*, enterotoxigenic *Escherichia coli* and *Vibrio cholerae*. The aqueous garlic extract and allicin had potential activities against all of the tested bacteria, while from the five standards of antibiotics only gentamycin was active. Both allicin and the aqueous extract had a broad spectrum as antibacterial agents. Allicin appeared to have the strongest activity compared with that of the extract and the standard antibiotics (Ahsan, *et al.*, 1996).

In addition, allicin, one of the sulfur-compounds responsible for garlic's characteristic odor, is a powerful antibacterial and antiviral agent that joins forces with vitamin C to help kill harmful microbes. In research studies, allicin has been shown to be effective not only against common infection like colds, flu, stomach viruses, and Candida yeast, but also against powerful pathogenic microbes including tuberculosis and botulism (The George Mateljan Foundation, 2008).

The objective of this study was to assess the effects of garlic extracts on the growth of bacteria isolate which were collected from repeat breeding infected of local milking cows.

## MATERIALS AND METHODS

### Material

Aqua sterile was used to make three concentrations of the garlic extract for this investigation. Then, Mueller Hinton blood agar and broth media was used as the growth media for the four bacteria isolates for this study. Additionally, the blood agar media was also used as a purification control.

Isolates of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas* sp., and *Klebsiella* sp. were collected from the uterus of local dairy cows with repeat breeding, raised by small farmers in Bogor, West Java. These specimens were later used for bacterial verification.

The obtained 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 method's (1973).

### Garlic Extract Procedure

In this procedure, the sterile aqueduct was added to obtain 75, 50, and 25% of garlic extract concentrations. Fifteen micro liter from each concentration was dropped onto sterile paper disk. Each disk was laid on the MEU blood agar media after it had been inoculated with four types of bacteria isolates. Those cultures were incubated for 24 hours at 37 degree Celsius.

The bacterial growth inhibition zones were observed and measured. The size of the zones

indicated the effectiveness of the garlic extracts for control the bacterial growth.

### Design

This study was designed as a 4 by 3 factorial experiments. The first factor observed was the type of bacteria for *in vitro* test i.e. *Staphylococcus aureus* and *Streptococcus pyogenes*. The second factor was the concentration of the Garlic extracts i.e., 75, 50, and 25%. The parameter observed was the diameter of inhibition zone of each bacteria. The analysis of *variance* was used to analyze the data of those inhibition zone diameters, and then Duncan Multiple Range Test (DMRT) procedure was used to determine further differences among the means of those diameters.

## RESULTS AND DISCUSSION

### 1. Effect of Bacteria Isolate Types and Concentration of Garlic Extract on The Inhibition Zone

The growth of bacteria was known through diameter measurement of inhibition zone on the plate. The finding results of inhibition zones diameter of bacterial isolates showed at Tabel 1.

Table 1. Diameter of bacterial growth inhibition zone (mm) as effect of differences level of garlic extract concentration and bacteria isolate types/species from dairy uterus

Type of Bacteria	Concentration			Average
	25%	50%	75%	
<i>Pseudomonas</i> sp.	7	9	11	9 <sup>d</sup>
<i>Klebsiella</i> sp.	9	11	13	11 <sup>c</sup>
<i>Staphylococcus aureus</i>	15	18	21	18 <sup>a</sup>
<i>Streptococcus pyogenes</i>	13	15	19	15.7 <sup>b</sup>
Average	11 <sup>c</sup>	13.25 <sup>b</sup>	16 <sup>a</sup>	

\* Different alphabet code indicated a significant difference at p < 0.05 DMRT

The average of diameter was 9 mm on the *Pseudomonas* sp. and 18 mm on the *Staphylococcus aureus*. The diameter growth inhibition zones of *Staphylococcus aureus* was greater than *Streptococcus pyogenes*, and *Klebsiella* sp. was greater than *Pseudomonas* sp. The analysis of variance showed that the main effect of type of bacteria, i.e., *Staphylococcus*

*aureus*, and *Streptococcus pyogenes* isolates on the bacterial growth inhibition zones was highly significant. Further test, results were presented in the following Table 1.

Table 1. shown the size of four bacterial growth inhibition zones at MEU blood agar media, after being treated with three different concentration of garlic extracts. In these *in vitro* test, *Staphylococcus aureus* produced the largest growth inhibition zone., followed by *Streptococcus pyogenes*. The test also indicated that the species of *Staphylococcus* produced larger growth inhibition than *Streptococcus pyogenes*. Statistically, these differences were significant at alpha equal to or less than 0.01.

Furthermore, Table 2 also show that the average of the bacterial growth inhibition zones was getting lower with the lower concentrations of the garlic extract. Now, what about the main effect of the garlic concentration increase on the bacterial growth inhibition zones? As presented earlier, the Analysis of variance of this effect was highly significant too. Further test were presented in Table 2.

Table 2. demonstrated that the first concentration of the garlic extract, i.e., 75 percent, produced the largest growth inhibition zone, followed by 50 percent, and 25 percent. Statistically, the three growth inhibition zones were different at alpha equal to or less than 0.05. Therefore, it could be concluded that the higher concentration of the antibacterial agents in the garlic extracts make the larger diameter of the bacterial growth inhibition zones obtained.

Table 2. pointed out that combination of the garlic extract concentrations and the type of bacteria produced different growth inhibition zones of each bacteria studied. Further, Table 3 demonstrated that produced the *Staphylococcus aureus* largest growth inhibition zones and differed significantly with *Streptococcus pyogenes* at all concentrations of Garlic Extract.

Table 2. The main effect of the garlic concentration increase on the bacterial growth inhibition zones

The Garlic Extract	Diameter of Growth Inhibition Zone (mm)	Level of Significance*
25%	11	c
50%	13.25	b
75%	16	a

\* Different alphabet code indicated a significant difference at  $p < 0.05$  DMRT

## 2. The Combined Effects of the Garlic Extract Concentrations and the Type of Bacteria on the Bacterial Growth Inhibition Zones

As presented earlier, the combined effects of the garlic extract concentrations and the type of bacteria 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 at all findings. The result was presented in Table 2.

The explanation to the above finding was likely found in each species tolerance to surface tension reducer agents. Generally, bacteria in nature had a three-layer cell wall structured bond (Volk and Wheeler, 1988).

This simple structured bond was made of: (1) cytoplasmic membrane, (2) thicker peptidoglycan 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.

This allicin compound, could destroy the protein content of the bacterial cell wall. In this case, the allicine 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. *Staphylococcus aureus* and *Streptococcus pyogenes* would loose their pathogenic abilities and died (Volk and Wheeler, 1988). The Garlic (*Allium sativum*) extracts had bactericide effects (garlic extract as agent that destroys bacteria), especially on *Staphylococcus aureus*, and *Streptococcus pyogenes*.

Secondly, the allicin in the garlic extract would inactivate a number of the bacterial enzymatic activities. This too, would deactivate the *Staphylococcus aureus*, and *Streptococcus pyogenes* abilities and then killed them. Aliicin can obstruct growth a metabolism of bacteria with obstruct two enzyme: sistein proteinase and alcohol dehydrogenase.

## CONCLUSIONS

From all of research findings and interpretation, the conclusion were:

1. The Garlic (*Allium sativum*) extracts had bactericide effects, especially on *Staphylococcus aureus*, and *Streptococcus pyogenes*.
2. There is positive correlation between the high concentration of garlic extract with inhibition zones.

3. *Staphylococcus aureus* was shown the most affected by the Garlic extracts at all concentrations.
4. Garlic extract was able to use effectively for controlling the bacteria growth.

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