THE EFFECT OF GREEN TEA (*Camelia sinensis*) EXTRACT ON THE GROWTH OF BACTERIA ISOLATED FROM MASTITIS INFECTED LOCAL MILKING COWS

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ABSTRACT

Nationally, many dairy farms suffered from tremendous economic losses due to mastitis. Laboratory investigation demonstrated that several bacteria, i.e., *Staphylococcus aureus, Staphylococcus epidermidis*, and *Streptococcus agalactiae* were responsible for this disease. This research was to promote the uses of traditional medicinal herb as an alternative way to cure mastitis. Green tea (*Camelia sinensis*) was a well-known herb that had antibacterial effects. So, the objective of this study was to assess the effects of Green tea extracts on the growth of isolates collected from Mastitis infected local milking cows. A 3 x 4 Factorial design was employed for this investigation. Three isolates of bacteria, i.e., *Staphylococcus aureus, Staphylococcus epidermidis*, and *Streptococcus agalactiae* were used in *in-vitro* tests. Fifteen microliters each of four concentrations of Green tea extract, i.e., 7.50, 5.00, 2.50, and 1.25 % were dropped in sterile paper disks. The disks were then laid on the MEU blood agar media previously inoculated with each of the three isolates and were incubated overnight at 37 degree C. The test results demonstrated that the higher the concentration of the Green tea extracts, the higher the bacterial growth inhibition effects obtained. Further tests pointed out that the growth inhibition effects of the Green tea extracts on *Staphylococcus aureus, Streptococcus agalactiae* and *Staphylococcus epidermidis* isolates were highly significantly different at P<.0001. Similar result was also found between the last two isolates. Therefore, the Green tea extracts were very effective in controlling the bacterial growth.

Key words: Green tea, Traditional medicine, Mastitis, *Staphylococcus aureus, Streptococcus agalactiae, Staphylococcus epidermidis*

INTRODUCTION

Mastitis was one of the common problems of animal health in dairy herds. It caused milk yield to decline and affected the milk quality and composition. Subclinical mastitis occurred more frequently and, as there were changes in the udder or milk, the causative agents could persist for a longer period, leading to considerable economic losses (Baldassi, *et al.*, 1995).

Herd surveys demonstrated that 95 percents of the clinical mastitis cases in dairy cows were associated with the gram-positive bacteria. However, the incidence of mastitis associated with *Coliform* bacteria was ncreasing (Clark, *et al.*, 1995).

From recent investigations, researchers concluded that the main bacteria involved in mastitis incidences were *Staphyloococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae* and *Coliform* (Calva, *et al.*, 1995; Obritzhauser, *et al.*, 1995; and Baldassi, *et al.*, 1995).

Veterinarians in many countries traditionally used the wide spectrum antibiotics to cure these bacterial incidences among dairy cows. This research, however, proposed a different way to deal with these problems, i.e., by using the Green tea extract (*Camelia sinensis*).

Research discovered that the Green tea contained organic phenollic compounds that had antibacterial activities. Tests accomplished show that these compounds were even better than Tetracycline antibiotic in stopping the human mouth diseases. Then researchers also found that the Black tea, the

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Oolong tea and the Green tea as well, could prevent caries by killing the *Streptococci* mutants, the main germs that caused the human tooth decay (Carper, 1996, p. 175).

In line with these findings, three hypotheses tested in this investigation were: (1) the type of bacteria observed would produce different diameter of the growth inhibition zones as their responses to the Green tea extract. (2) the higher the concentrations of the Green tea extract, the larger the diameter of the bacterial growth inhibition zones produced, and (3) the combined effects of type of bacteria and the concentrations of the Green tea extract would produce different diameter of the bacterial growth inhibition zones.

MATERIAL AND METHODS

Material

Methanol was used to make four concentrations of the Green tea 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*, *Staphylococcus epidermidis*, and *Streptococcus agalactiae* were collected from the udders of local dairy cows with clinical mastitis, raised by small farmers in Bogor, West Java. These specimens were later used for bacterial verification.

The obtained milk 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 Green Tea

Dried Green tea leaves were ground into powder. Methanol was then added to the Green tea powder. To homogenize the mixture, the liquefied Green tea was shaken for one hour. This agitation would accelerate the solution of the Green tea active compounds in the methanol solvent. The mixture was kept for 24 hours. Then, the liquefied Green tea was filtered by paper filter. The obtained methanol solution containing the Green tea 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 Green tea extract, i.e. 1.25, 2.50, 5.00, and 7.50 %. 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 bacteria 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 Green tea extracts in controlling the bacterial infection.

Design

This study was designed as a 4 by 3 factorial experiment. The first factor observed in the study was the type of bacteria used in this *in vitro* test. There were three levels of this factor, i.e., *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus agalactiae*. The second factor was the concentrations of the Green tea extracts. This factor had four levels, i.e., 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 furthers differences among the means of the diameters of the bacterial growth inhibition zones.

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

The Bacteria	The Concentration of The Green Tea Extract (%)				Average
	7.50	5.00	2.50	1.25	
S. aureus	17.30	14.00	11.00	9.00	12.83
S. epidermidis	19.00	16.30	14.00	11.30	15.15
Str. agalactiae	15.00	13.00	10.00	7.00	11.25
Average	17.10	14.43	11.67	9.10	13.08

RESULTS AND DISCUSSION

Research results about the effects of four concentrations of the Green tea extracts on the bacterial growth inhibition zones of isolates of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus agalactiae* were presented in the following sections.

Table 1 above pointed out that the average growth inhibition zones of *S. Epidermidis* was greater than *S aureus* and *Str. agalactiae*, and *S aureus* was greater than *Str. agalactiae*. Further, Table 1 also show that the average of the bacterial growth inhibition zones was getting lower with the lower concentrations of the Green tea extract.

Analysis of Variance of the above data produced results as listed in the Table 2. Table 2 clearly show that the main effect of the Green tea concentrations, the bacteria and the interaction of the Green tea concentration and the bacteria on the diameters of the bacterial growth inhibition zone were all very highly significant. The respective F showed these values that was significant at alpha equal to 0.0007 or higher.

1. The Main Effect of Type of Bacteria on the Growth Inhibition Zones

The Analysis of variance show that that the main effect of type of bacteria, i.e., *S. aureus*, *S. epidermidis*, and *Str. agalactiae* isolates on the bacterial growth inhibition zones was highly significant. Further test results were presented in the following Table 3.

Table 3 show the size of three bacterial growth inhibition zones at MEU blood agar media, after being treated with four different concentrations of the Green tea extracts. In these *invitro* tests, *S. epidermidis* produced the largest growth inhibition zone, followed by *S. aureus*, and *Str. agalactiae*. The test also indicated that the two species of *Staphylococcus* produced larger growth inhibition zones than *Str. Agalactiae*. Statistically, these differences were significant at alpha equal to or less than 0.05.

2. The Main Effect of Four Green Tea Concentrations on the Bacterial Growth Inhibition Zones

Now, what about the main effect of the Green tea concentration increase on the

Table 2. Analysis of Variance of the Effects of Four Concentrations of the Green Tea Extract on Three Bacterial Growth Inhibition Zones

Source	DF	SS	MS	F Value	Pr > F
CONC	3	322.7500000	107.5833333	430.33	0.0001
BAC	2	93.1666667	46.5833333	186.33	0.0001
CONC*BAC	6	8.8333333	1.4722222	5.89	0.0007

Table 3. The Main Effects Of Type Of Bacteria, I.E., *Staphylococcus aureus, Staphylococcus epidermidis*, And *Streptococcus agalactiae* On The Bacterial Growth Inhibition Zones

Bacteria	Diameter of Growth Inhibition Zone (mm)	Level of Significance*
S. aureus	12.83	b
S. epidermidis	15.15	a
Str. agalactiae	11.25	c

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

bacterial growth inhibition zones? As presented earlier, the Analysis of variance of this effect was highly significant too. Further tests were presented in Table 4.

Table 4 demonstrated that the first concentration of the Green tea extract, i.e., 7.50 percent, produced the largest growth inhibition zone, followed by 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 antibacterial agents in the Green tea extracts the larger the diameter of the bacterial growth inhibition zones obtained.

3. The Combined Effects of the Green Tea Extract Concentrations and the Types of Bacteria on the Bacterial Growth Inhibition Zones

As presented earlier, the combined effect of the Green tea 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 results were presented in Table 5.

Table 5 pointed out that combination of the Green tea extract concentration and the type of bacteria produced different growth inhibition zones of each bacteria studied. Further, Table 5 demonstrated that *Staphylococcus epidermidis* produced the largest growth inhibition zones and differed significantly with *Staphylococcus aureus* and *Streptococcus agalactiae* at all concentrations of the Green tea extracts.

DISCUSSION

The explanation to the above findings 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 peptidoglicant membrane, and (3) varied outer membrane. According to Volk

Table 4. The main effect of the green tea concentration Increase on the bacterial growth inhibition zones

The Green Tea Extract Concentration (%)	Diameter of Bacterial Growth Inhibition Zones (mm)	Level of Significance*
7.50	17.11	a
5.00	14.44	b
2.50	11.67	c
1.25	9.11	d

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

Table 5. The combined effects of the green tea Extract concentrations and the types of bacteria on the bacterial growth inhibition zones

Extract concentration (%)	Type of Bacteria	Diameter of Growth Inhibition Zones (mm)	Significancy Level*
7.50	*S. aureus*	17.3	b
	S. epidermidis	19.0	a
	Str. agalactiae	15.0	cd
5.00	*S. aureus*	14.0	d
	S. epidermidis	16.3	bc
	Str. agalactiae	13.0	c
2.50	*S. aureus*	11.0	fg
	S. epidermidis	14.0	d
	Str. agalactiae	10.0	g
1.25	*S. aureus*	9.0	h
	S. epidermidis	11.3	f
	Str. agalactiae	7.0	i

*Different alphabet indicated a significant difference at P<.01 DMRT

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

In this relation, Katekine found in the Green tea extract was identified as one of the poly phenol compounds. It had a strong anti bacterial activity. Researchers believed that the Green tea, which was actually made from the unfermented, fresh dried young tea leaves, kept most of its katekine -- the powerful phenollic compound content (Penebar Swadaya, 1993).

Further more, this organic compound, according to Volk and Wheeler (1988) could destroy the protein content of the bacteria cell wall. In this case, the phenollic compound would dissolve the cytoplasmic membrane. In turn, these leaks would cause the loss of important metabolites from the bacterial cell. Therefore, the bacteria would loose their pathogenic abilities and then died.

Secondly, the phenollic compound in the Green tea extract would inactivate a number of the bacterial enzymatic activities. In *Staphylococcus aureus,* for example, the phenollic compound would deactivate the glucosiltransferase enzyme. This too, would deactivate the bacterial pathogenic abilities and then killed them. Finally, the phenollic compound in the Green tea extract could also precipitate proteins. In this case, the bacteria would be precipitated, clotted and then destroyed by the phenollic compounds (Volk and Wheeler, 1988).

CONCLUSIONS

From the above research findings and interpretations, it could be concluded that:

1. The Green tea (*Camelia sinensis*) extracts had bactericide effects, especially on *Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus agalactiae.*
2. The higher the concentration of the Green tea extracts, the larger the diameter of the bacterial growth inhibition zones obtained.
3. Of the three bacteria tested, *Staphylococcus epidermidis* was the most affected by the Green tea extracts at all concentrations.

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