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Evaluation of herbal antiseptic red teak leaf extract (*Tectona grandis* Linn. F.) as teat gel for dairy cow

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Abstract. Antiseptic application on cow's teat after milking is essential to prevent the udder infection during milking. This study aimed to evaluate the antibacterial effectiveness of *Tectona grandis* leaves extract as the main ingredient of antiseptic gel for cow's teat as the innovation from conventional antiseptic solutions. The effects of herbal gel antiseptic on milk quality and the level of mammary inflammation (mastitis) were also determined. The mammary inflammation level determined by California Mastitis Test (CMT). Total of four sub-clinical mastitis cows (sixteen teats) were used in the completely randomized design split plot in time (four treatments and four replications). The main plots were the red teak leaves extract with different levels: 0.10% (P1); 0.06% (P2); 0.02% (P3) and povidone iodine (P0) as positive control. The sub-plot was the duration of treatments (0, 5 and 10 days). The result showed that bacterial counts in milk from cows using herbal antiseptic gel (P1, P2, P3) were lower ($P<0.05$) than those using povidone iodine (P0). The bacterial counts in milk and the mammary gland's inflammation degree were significantly decreased ($P<0.05$) as the treatment duration increased. There were significantly difference on the level of mammary inflammation healing between cows using herbal antiseptic gel and povidone iodine. The decreased level of mammary inflammation was thought to have an effect on increasing the protein, fat, and lactose contents in milk of cows using herbal gel ($P<0.05$). Hence, the antiseptic gel with the lowest dose (0.02%) has the potential for use as anti-inflammatory agents.

1. Introduction

Dairy farms in Indonesia are generally small-scale public farms and means to conventional maintenance with poor management, especially to sanitation issues. Poor sanitation may affect livestock to be infected of mastitis. Mastitis is an inflammation of the udder caused by microbial activity. These microbes are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Streptococcus uberis* and *Coliform* which dominated by 80% [1]. The strike canal milking then it was in an open position, therefore microorganisms can enter through the udder, this can be prevented by dipping of an antiseptic [2]. Povidone iodine is a synthetic antiseptic that is often used as a teat dipping material. Povidone iodine is able to kill bacteria in 3-5 minutes, however it may leave chemical residues in milk [3]. Human health can be disrupted only if milk containing chemical residues enters the body. Teat dipping can be replaced by an udder gel by applying to the udder after milking and it is easier without a teat dipper. In addition, the gel formulation can better protect the udder longer from bacterial invasion.

Red teak leaves contain flavonoids, saponins, tannins and anthocyanins which function as antibacterial which can inhibit bacterial growth [4]. Anthocyanins are part of phenol compounds classified as flavonoids that cause purple colour on the leaves. The function of anthocyanin is as an antioxidant and it is safe for the udder tissue and prevents damage to the udder[5]. The problems need an innovation in handling the amount of bacterial contamination without causing any residues in milk. Steps need to be taken is to implement herbal antiseptics. The development of the potential for red teak leaves as a new alternative need to be used. The research objective was to identify alternative herbal antiseptics could be used by farmers. The hypothesis of this study was the effectiveness of red teak leaf extract (RTLE) in reducing the number of bacteria in milk and the level of inflammation in the udders of cows with sub-clinical mastitis, which antiseptic based on red teak leaf extract as a substitute for synthetic antiseptic for dairy cattle. The use of herbal antiseptics is expected to be able to support the productivity of dairy cattle and ensure food safety.

2. Materials and Methods

The research was conducted for 3 months and used 4 cows of Frisian Holstein lactation (FH) (16 udders). The tools implemented in this research were Vacuum Rotary Evaporator, Water bath, CMT paddle. While the materials were red teak leaves, filter paper, *California Mastitis Test* (CMT) reagent, povidone iodine with a concentration of 10%, alcohol, carbopol, TEA, methyl paraben, glycerin and aquadest.

2.1. Preparation Phase

2.1.1. Fabrication of Red Teak Leaf Extract (RTLE). The process was selecting the fit and disease-free red teak leaves by withering those then put it dried in the oven for 24 hours at a temperature of 50°C. Dried red teak leaves were mashed to mold the flour. 500 g of red teak leaf flour was soaked with 70% ethanol as much as 8 liters (until the flour is down), stirred it for 30 minutes and every other 6 hours in period of 24 hours. 24 hours after filtering process, the filtrate was formed, then the filtrate was evaporated by a Rotary Evaporator. The dense extraction from evaporation process becomes the raw material for producing the udder gel.

2.1.1. Udder Gel Production. The available gel was carried out on a carbohydrate polymer basis by adding 20 ml of distilled water at 70° C and leave it for 1 × 24 hours. Red teak leaf extract was dissolved in glycerin, then the basis was gradually mixed to the extract solution. Finally, distilled water up to 100 ml was added and homogenized.

Table 1. Udder Gel Formula Design

Materials	Formula		
	T1	T2	T3
Red Teak Leaf Extract (g)	0.10	0.06	0.02
Carbopoly (g)	0.50	0.50	0.50
TEA (g)	0.05	0.05	0.05
Methyl Paraben (g)	0.50	0.50	0.50
Glyserin (g)	22.88	22.88	22.88
Aquadest up to (ml)	100.00	100.00	100.00

2.2. Implementation Phase

2.2.1. Teat Dipping. The teat dipping treatment was implemented for 10 days on every morning and evening after milking. This research was conducted by using a Complete Random Plot Design (CRD) Split Plot in Time with four treatments, each with four replications. A total of 16 lactating dairy cow udder were divided into four treatment groups dipping as follows:

P0 : Dipping with povidone iodine 10.00%
 P1 : Dipping with RTLE concentration 0.10%
 P2 : Dipping with RTLE concentration 0.06%
 P3 : Dipping with RTLE concentration 0.02%

2.2.2. Subclinical Mastitis of Milk Collection. Milk was collected from mastitis cows which have previously been treated by using the California Mastitis Test (CMT) method. The first milk sample from the four cow's udder (approximately 1-2 ml) was inserted into the paddle cup. Each cup was added with CMT reagents with the same amount to the volume of milk. After the reagent was added, the cup and contents were whipped for 15 seconds. After checking by corresponding to the CMT procedure, the CMT score was then interpreted into value [6].

2.2.3. Calculation of Total Bacteria. Palm sampling was executed before and after use, while milk sampling was conducted on the day before the treatment (H0), the 5th day (H5) and the 10th day (H10). Calculation of total bacteria was carried out based on SNI 2897: 2008, namely the Total Plate Count (TPC) method by counting bacterial colonies grown on an agar medium with the following formula [7]:

$$\text{Total Bacteria} = \text{number of colonies} \times \frac{1}{\text{Dilution Factor}}$$

2.2.4. Quality of Milk. Nutrient content of milk was measured by lacto scan which is implemented on the day before the treatment (H0), the 5th day (H5) and the 10th day (H10). Milk samples were taken from morning milking. The process of taking milk was executed by removing the flow of milk first, then milking it directly into a sterilized bottle and then close down. The bottle was placed in a cooling box around ice jelly. The observation of milk quality included fat, protein, and lactose.

3.2. Data Analysis

The obtained data were analyzed by t-test and General Linear Model (GLM) of the University Edition's Statistical Analysis System (SAS) program. If the parameters show a significant difference ($P < 0.05$) then it will proceed with the Duncan's New Multiple Range Test (MRT) [8].

3. Results and Discussion

3.1. California Mastitis Test Score (CMT)

Based on research to CMT score on milk detailed as pictured on Table 2.

Table 2. Means of CMT score during 10 days treatment

Test Type	Long Time Usage (Days)			Means
	0	5	10	
	CMT Score			
P0	2.00	1.75	1.25	1.67 ^a
P1	2.75	2.25	2.00	2.33 ^b
P2	2.25	2.00	2.00	2.08 ^b
P3	1.75	1.25	1.00	1.33 ^b
Means	2.19 ^a	1.81 ^{ab}	1.56 ^b	

^{a,b}Different superscripts on the same line or column showed significant differences ($P < 0.05$).

Effect of teat dipping red teak leaf extract (RTLE) on the reduction percentage in mastitis scores of California Mastitis Test (CMT) was shown on Table 2. CMT is an indicator of mastitis disease and it has been used as a tool for genetic selection of dairy cattle having resistant properties to mastitis. The test statistically done with a 5% level was not significantly different from P1, P2 and P3 treatments on the decrease in CMT scores, but significantly different from P0. These results indicate that RTLE was better than povidone iodine as antibacterial. Bacteria that enter the udder gland through it, will cause an inflammatory reaction and trigger an active immune system, it is an increase in the number of inflammatory cells (leukocytes) in the blood and the collapse of the udder's internal cells. Leukocyte cells and udder cells are referred to as somatic cells [9]. Milk with high bacterial content will be rejected by the milk processing industry because it produces processed products of low sensory quality [10].

3.2. Total of Bacteria on milk

Table 3. Means of Total Bacteria on milk during 10 days treatment

Test Type	Long Time Usage (Days)			Means
	0	5	10	
	------(10 ³ CFU/ml)-----			
P0	134.50	84.50	99.75	106.25 ^a
P1	103.50	66.75	24.75	65.00 ^b
P2	62.50	27.25	23.50	37.75 ^b
P3	97.25	37.50	19.67	51.58 ^b
Means	99.44 ^a	54.06 ^b	42.00 ^b	

^{a,b}Different superscripts in the same line or column showed significant differences ($P < 0.05$).

In this research, the treatment of udder gel after milking with different antiseptic concentrations of P1, P2 and P3 did not have a significant effect on decreasing the number of bacteria in milk, but slightly different from P0. The average number of bacteria showed different concentrations in 10 consecutive days as 106.25x10³ CFU / ml (P0), 65.0x10³ CFU / ml (P1), 37.75x10³ CFU / ml (P2) and 51.58x10³ CFU / ml (P3). The average difference of the decrease in the number of bacteria was significant, therefore it can be seen that RTLE has a better ability than 10% povidone iodine in decreasing the number of bacteria. RTLE contains antibacterial compounds such as saponins, anthocyanins, and flavonoids. The active ingredient played as an antibacterial with a different mechanism of action. Anthocyanin can damage the cell wall of the bacterial cell hence nucleic acid material exits the cell nucleus [11]. The enzymatic activity of the bacteria becomes disrupted and the bacterial TCA cycle was inhibited which results in inhibition of bacterial growth and reproduction activity. Anthocyanin has the ability as an anti-inflammatory which is able to cure inflammation with the same ability as the antibiotic ciprofloxacin [12]. Flavonoids in RTLE were able to stop bacterial energy metabolism by inhibiting bacterial oxygen used [13], whereas saponins can bind to cholesterol in bacterial cells and make complex bonds of saponin-cholesterol that cause bacterial cells to rupture [14].

3.3. Nutritional Content of Milk

According to the research on nutritional content of milk as presented on Figure 1.

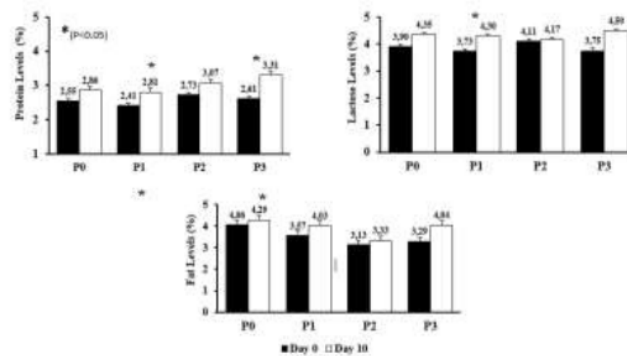


Figure 1. Nutrient levels of milk (protein, fat and lactose) after treatment for 10 days

Picture 1. showed that there was a significant effect ($P < 0.05$) of RTLE treatment on protein, fat and milk lactose content. Mastitis can reduce the amount of milk production up to 9 - 45.5%, and reduce milk nutrient levels [15]. This shows that the EDJM herbal antiseptic was able to maintain the productivity of dairy cows. Milk produced from this research is in accordance with SNI 3141.1-2011 standards regarding Fresh Milk, which contains a minimum of 3% fat; 2.8% protein and 4% lactose [16].

4. Conclusion

Red teak leaf extract had a level of effectiveness in reducing the number of bacteria in milk and the level of inflammation in udders of mastitis patients with sub-clinical mastitis and it can be used to maintain the quantity and quality of milk. The recommended concentration was 0.02% after each milking process has been finished.

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