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Antibacterial and antifungal activities of *Muntinga calabura* leaves extract as alternative to antibiotic in mastitis treatment

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Abstract

Mastitis is the most damaging worldwide disease of dairy industry. The aim of the study was to evaluate the antibacterial and antifungal effects of *Muntinga calabura* leaves extract against mastitis infection in dairy cattle. Experimental treatments were subclinical mastitis milk added with different levels of ethanol extract of *Muntinga calabura* leaves (EEMC) (6.25%, 12.5%, 25% and 50%) in a completely randomized design with five replications. The result showed that total bacterial *Staphylococcus aureus* and fungal counts were significantly decreased ($P < 0.01$) with increasing EEMC concentration. The EEMC concentration of 50% had the highest ($P < 0.01$) antimicrobial activity among the groups. The EEMC could reduce 87.2% – 94.8%, of total bacteria, 71.1- 95.8% of *Staphylococcus aureus*, and 64.4% - 97.8% of fungi in mastitis milk. Taken together, current findings suggested that *Muntinga calabura* leaves have antibacterial and antifungal activities and can be used as an alternative to synthetic antibiotic for mastitis treatment to prevent the antibiotic residue in milk.

Keywords: mastitis, antibacterial, antifungal, Muntinga calabura, antibiotic alternative

Introduction

Mastitis is an inflammation of the mammary gland caused by bacterial infection. In the previous research, it has been reported that fungi is another causative agent of mastitis (Pachauri et al., 2013). One of the challenge to treatments of bovine mastitis due to many of the causative agents, especially fungi do not respond to antibiotic therapy, however they use some antibiotics like tetracycline as their source of energy (Tarfarosh and Purohit, 2008). Moreover, the use of antibiotics to treatment the disease is limited due to public concerns for the antibiotic residue on milk and it will be resisten to the humans who consumed milk. Recently, numbers of studies focused to find alternatives to changes antibiotics with herbal plants. Plant-derived natural bioactive compound is very potential to be use as an alternative antibiotic for mastitis treatment. *Muntinga calabura* is one of the most common trees in Indonesia and have been used as a traditional medicine to treatment of fever, cold, liver, and to decrease gastritic. It also has anti-inflammatory activities (Balan et al., 2015). Therefore, this study was aimed to elucidate the potential of *Muntinga calabura* leaves extract as antimicrobial against bacteria and fungi in mastitis milk, as well as to evaluate the prevalence of mastitis in Central Java.

Methodology

Examination of mastitis prevalence was conducted in Semarang Regency, the center of dairy farming in Central Java. A total of 105 lactating cattle were tested by California Mastitis Test (CMT) (Bovivet, Kruuse Denmark). Milk samples from 20 quarters were aseptically collected and tested against *Muntinga calabura* leaves extract.

Muntinga calabura leaves were collected from the area of Diponegoro University in Semarang. Fresh leaves were oven-dried at 50°C for 24 h then grinded. The leaves powder was soaked in the 96% ethanol in the ratio of 1:10 (w/v) for 24 h. The supernatant was filtered and then evaporated using rotary evaporator at 40°C. The EEMC was dissolve in 10% DMSO just before used. The experimental groups were: mastitis milk added with either 6.25%, 12,5%, 25%, or 50% EEMC (M1, M2, M3, and M4 respectively), mastitis milk added with synthetic antibiotic (K+) and mastitis milk only (K-). The synthetic antibiotic was used TERREXINE (combination of Kanamycin and Cefalexine, Univet Ireland). Mastitis milk sample were analyzed for total bacterial count, gram staining, *Staphylococcus aureus* and fungal counts.

Results and Discussion

The positive results of CMT were categorized as clinical and sub-clinical mastitis. Mastitis prevalence was 67%, whereas the healthy udder was only 33% (Figure 1). In the current study, mastitis prevalence in Central Java was similar with in West Java, that is 67.5% (Susanti et al., 2017). The dominant cases were sub-clinical mastitis not sign of abnormality of mammary gland and milk, thus farmers could not recognize the symptoms and they were depend on the paramedic person to check the animal health status. Good milking practices may give contribution to the mastitis problem. Most of farmers applied traditional dairy practices. Our previous study reported that 80% of farmers used plastic bucket for milking rather than using stainless steel (Prihutomo et al., 2015) with improper disinfection procedure.

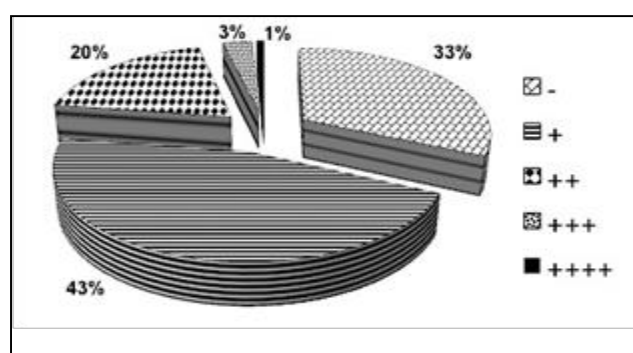


Figure 1. CMT result from each quarter

The ECMC at the concentration of 50% showed to possess the highest antibacterial and antifungal activity among the treatment groups (Table 1). The antimicrobial activity of synthetic antibiotic (K+) has shown the best among others, however the possibility of antibiotic residue in milk should be considered. Bacterial and fungal counts were significantly decreased ($P < 0.01$) with increasing extract concentration. The number of total bacteria reduction in milk was varied from 87.2% to 91.4%, whereas the fungi reduction in milk was varied from 64.4% to 97.8% (Table 2). Since this is a preliminary screening for the presence of antimicrobial properties in the *Muntinga calabura* leaves extract, at the moment, the identification of chemical constituents or bioactive compounds is not part of the objective from this study. Based on previous studies on the antibacterial activities of *Muntinga calabura* leaves, the antimicrobial activity is suggested to be due to the presence of phytochemical compound such as sterol, flavonoid, alkaloid, saponin, glycoside and tannin (Buhian et al., 2016). Gram staining of the individual colony has showed gram-positive cocci and gram-negative bacilli spore-forming bacteria (Figure 2). The mechanisms by which microorganism survive the action of antimicrobial agents still poorly understood. However, the present data suggest that ethanol extract of *Muntinga calabura* leaves is very potential used as an alternative to synthetic antibiotic due to high antibacterial and antifungal activity.

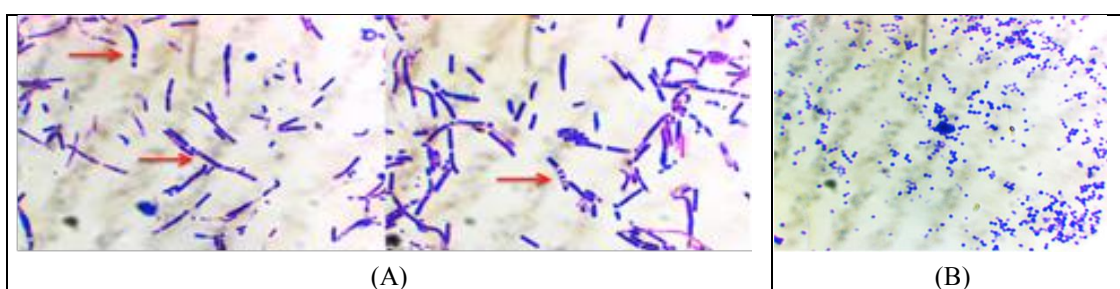
Table 1. The number of total bacteria, *Staphylococcus aureus* and fungi in mastitis milk added with ethanol extract of *Muntingia calabura* leaves (EEMC)

Microbes (cfu/ml)	K-	M1	M2	M3	M4	K+	National standard
Total bacteria	3.711.299 ^A	472.800 ^B	395.200 ^B	191.200 ^C	131.200 ^C	0 ^D	Max 10 ⁶
<i>S. aureus</i>	18.850 ^A	5.450 ^B	2.750 ^B	2.100 ^C	800 ^D	0 ^D	Max 100
Fungi *	450 ^A	160 ^B	110 ^B	40 ^C	10 ^C	0 ^D	-

*The maximum tolerated amount of fungi contamination in milk is not available at the Indonesian National Milk Quality Standard SNI 3141.1:2011. Fungi is defined as yeast and mold; ^{ABCD}Different superscript letters in the same row denotes significance (P<0.01)

Table 2. Percent decrease in the number of bacteria and fungi in milk

Parameters	M1	M2	M3	M4
Total bacteria (%)	87.2	89.4	91.4	94.8
<i>Staphylococcus aureus</i> (%)	71.1	85.4	88.9	95.8
Fungi (%)	64.4	75.6	91.11	97.8

**Figure 2.** Gram-negative bacilli spore-forming bacteria (red arrow) (A), and Gram-positive cocci bacteria (B)

Conclusion

A *Muntingia calabura* leaf has antibacterial and antifungal activities and can be use as an alternative to synthetic antibiotic for mastitis treatment to prevent the antibiotic residue on milk.

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