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Research Article

The Effectiveness of Clove Leaf Oil Antibacterial against Sub-Clinical Mastitis-Inducing Bacteria in Dairy Cattle

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Abstract: The effectiveness of clove leaf oil antibacterial is tested by in-vitro at Laboratory of Bacteriology, Faculty of Agriculture, University of Brawijaya, aimed to obtain the antibacterial concentration of eugenol from clove leaf oil because this concentration is useful to prevent sub-clinical mastitis-inducing bacteria in dairy cattle. The test utilizes well-based diffusion method by measuring preventive zone diameter and bacteria colony total. Results indicate that treatments are not significantly influential ($p< 0.05$) to bacterial preventive zone because media and conditions required for incubation and also for bacterial growth are almost similar. Result of testing over bacterial colony total (CFU/ml) has shown the influence that is very obviously different between treatments ($p> 0.05$). Duncan Test is conducted and indicates that Treatment A1 has bacterial colony total that obviously differ from others ($p> 0.05$), and that is more or in higher level than Treatments A2, A3, A4, A5 and A6. Higher bacterial colony total at Treatment A1 is caused by the absence of eugenol of clove leaf oil which may prevent bacterial growth during incubation. It contrasts with what has been found from A2 to A6. It means that eugenol of clove leaf oil must be functional as antibacterial to prevent bacterial growth. This finding is persistent although Treatment A2 at concentration 7.5 % until Treatment A6 at concentration 37.5 % are less obviously different after comparison ($p< 0.05$), or having similar effect on bacterial colony total. Result of this research indicate that eugenol antibacterial of clove leaf oil at concentration 7.5 % is quite effective to prevent bacterial growth and to reduce bacterial colony total (CFU/ml) in the milk of dairy cattle infected by sub-clinical mastitis.
Keywords: clove leaf oil, bacteria, sub-clinical mastitis

INTRODUCTION

Mastitis is caused by infection induced by bacteria such as Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis and Streptococcus aureus. Bacterial infection usually begins by entering through nipple hole and then spreads over the udder tissue. The metabolism product of bacteria in this tissue will damage the udder tissue and disturbs alveoli epithelial cells [1].

According to Kirk, quoted by Sugiri and Anri [2], high level of bacterial infection within milk may signify the fact that the breeders have not applied milking management system and milking hygiene in proper way.

Bacterial count within milk is closely related with milk quality. Test over Total Plate Count (TPC) is a method to examine the accuracy of milk quality by understanding the microbial count within milk. The milk is considered as having good quality if the microbial count ranges within 500,000-1,000,000/ml milk or lower. Indonesia National Standard (SNI 3141.1:2011) has stated that minimum microbial requirement for fresh milk is $1x10^6$ CFU / ml [3].

Sub-clinical mastitis caused by bacteria is difficult to prevent by antiseptic or antibiotic because most these bacteria are already resistant. Dealing with this problem, the alternative solution may be using naturally secure antibacterial without risking from increasing resistance or residues within milk (Wahyuni, 2010) [4].

Sugiri and Anri [2] have reported that clinical and sub-clinical mastitis are problematic for breeders although they do not experience the direct effect of this infection. However, their ignorance to this infection may cause economic loss among breeders because such infection may reduce the production and quality of milk, or increase the risk from consumer rejection due to defected milk.

Sub-clinical mastitis may happen due to loosening supervision and this mastitis remains less recognized until it becomes acute. The repeated case of sub-clinical mastitis is only hampered through preventive measures [5]. These measures help to develop self-awareness among breeders to the impact of chemicals such as chemical residues and antibiotic resistance. Breeders must understand the importance of natural products to preserve the health (preventive measure) because natural products may be safer, cheaper and with few side effects. Kumala and Indriani [6] assert that breeders start to replace the antibiotic with efficacious herbs as antibacterial among other is clove (*Syzygium aromaticum (L) Merr & Perry*).

Clove is unique because all parts of clove, including root, stem, leaf and flower, have contained *essential oil* (volatile). The content of essential oil varies with the parts of clove, such as flower with 10-20 %, stalk with 5-10 %, and leaf with 1-4%. The biggest component of clove oil is eugenol with 70-80 %. Eugenol is antiseptic [7]. Kumala and Indriani [6] have informed that the compound in the clove leaf oil is eugenol which is potential for antibacterial. Julia *et al*. [8] declare that clove leaf decoct is effective for antimicrobial.

The antibacterial in the clove leaf oil (eugenol) shall be important but the lack of information about how to use clove leaf oil as antibacterial or the limited utilization of this clove leaf oil are influencing research scope. Therefore, research is confined to the effectiveness of eugenol in clove leaf oil as natural antibacterial against sub-clinical mastitis-inducing bacteria in dairy cattle.

METHODOLOGY

Materials of research include bacterial isolate from the milk of sub-clinical mastitis infected dairy cattle (CMT Test), clove leaf oil with eugenol 64.24 % (HPLC Test), ethanol (90%), sterile aquades, NA Medium, MSA Medium and MRSA Medium. The equipments are Erlenmeyer, reaction tube, gauge glass, Petri dish, Bunsen burner, L-bar, Ose needle, micro pipette, laminar air flow (LAF), cork borner, sliding caliper, biological safety cabinet (BSC) and autoclave.

Research design is Nested Patterned Complete Random Plan [9]. Factor A is the concentrations of eugenol of clove leaf oil ($M_1.V_1 = M_2.V_2$) which includes A1 = 0%, A2 = 7.5%; A3 = 15%; A4 = 22.5%; A5 = 30%; and A6 = 37.5 %. These concentrations are nested into bacterial species, as Factor B, including B1 = Mixed Bacteria (without separation), B2 = *Staphylococcus sp*, B3 = *Staphylococcus sp* and *Streptococcus sp*, B4 = *Streptococcus sp*. Data are processed with *Statistical Program of Social Science* [10].

Preventive zone is measured by well-based diffusion method [11]. It begins with the stage of preparing the growth and sub-culture media for bacteria. Well test stage is arranged as follows. The prepared bacterial culture is added by ± 10 ml sterile aquades to produce bacterial suspension which contains 10^8 bacterial colonies. Sterile Petri dish is prepared (on demand) by pouring onto it with 100 µl bacterial suspense, and adding it with ± 20 ml growth media. Inoculum and media are blended and incubated to dry (± 30 minutes). Next step is the making of wells using cork borner (± 6 mm) to produce 3 holes. The pipette is used to bring along 50 µl eugenol of clove leaf oil into each well hole. The incubation takes for 24 hours. Preventive zone diameter is measured using slide caliper.

The count of bacterial colony total (CFU/ml) is made in the early stage, which is the preparation of growth and sub-culture media for bacteria (10^8). After bacterial media and culture are prepared, next activity is that each bacterial culture in Petri dish is mixed with 10 ml pure aquades and eugenol of clove leaf oil. The mixture is sent into glass tube until it reaches 5 ml. It is subjected to vortex force to obtain even blend and incubated for 24 hours.

Bacterial colony total is the difference between bacterial colony total during incubation at hour 0 and that during incubation after 24 hours. Total Plate Count (TPC) of the bacteria at hour 0 is conducted as follows. Before incubation, 100 µl bacterial suspense must be brought by pipette into Petri dish. It is mixed and incubated for 24 hours. Bacterial colony total is then counted. Bacterial TPC after incubation for 24 hours is estimated by bringing 100 µl bacterial suspense by pipette into Petri dish. It is mixed and incubated for 24 hours. The growing bacterial colony total is then counted.

RESULTS AND DISCUSSION

Results of measuring the preventive zone diameter of eugenol antibacterial of clove leaf oil against sub-clinical mastitis-inducing bacteria in dairy cattle are displayed in Fig. 1.

Result of the test over preventive zone diameter (Fig. 1) indicates that eugenol of clove leaf oil at concentration 7.5 % can prevent bacterial growth by establishing preventive zone at diameter 8.23 m. The order is arranged as follows. For concentration 15 %, the diameter of preventive zone is 8.61 mm, while for 22.5% is 8.88 mm, for 30 % is 7.17 mm, and for 37.5 % is 7.48 mm.

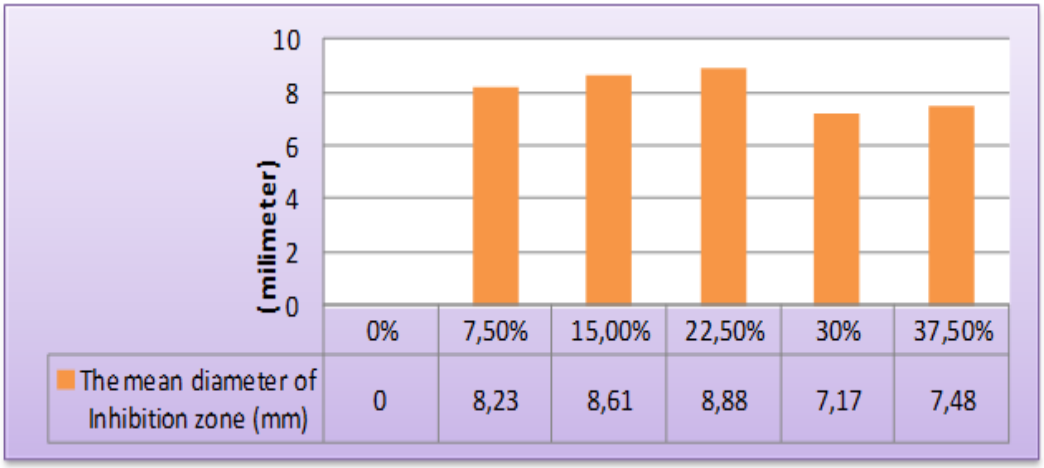

Fig. 1: The Average Results of Measurement of Preventive Zone Diameter (mm) of Sub-Clinical Mastitis-Inducing Bacteria

Result of analysis of variance using output from SPSS shows that treatments have produced insignificant influence ($p < 0.05$) on bacterial preventive zone. Therefore, bacterial preventive zones are less obviously different because media and incubation conditions used for bacterial growth are almost similar. Prescott [12] explains that some factors are influencing the diameter of preventive zone, such as sensitivity of the tested organism, culture media, and incubation conditions (temperature, timing and pH), diffusion speed of the antibacterial compounds to penetrate into agar medium and antibacterial compound concentration.

Result of the test over eugenol of clove leaf oil at concentration 7.5 % has shown that preventive zone diameter at 8.23 mm (moderate category) is strong enough to prevent the growth of sub-clinical mastitis-inducing bacteria in dairy cattle. Pelczar and Chan [13] add that main group with antibacterial activity comprises of phenolic, phenolat and alcohol compounds. Preventive zone in the test sample means that eugenol of clove leaf oil may contain active compounds.

The preventive power of eugenol antibacterial of clove leaf oil against bacterial growth is strictly clear by preventive zone diameter of 7.17 – 8.88 mm. If compared to the method of measuring antibacterial activity used by Davis and Stout as quoted in Ambarwati [14], the diameter shown in current research is classified into moderate category of bacterial prevention (preventive zone diameter of 5-10 mm). Suriawiria [15] declares that Davis and Stout distinguish preventive capacity into some categories such as: very strong (preventive zone diameter of 20 mm or more), strong (preventive zone diameter of 10-20 mm), moderate (preventive zone diameter of 5-10 mm), and weak (preventive zone diameter smaller than 5 mm).The average count of bacterial colony total (TPC) is the difference between bacterial colony total during incubation at hour 0 and that during incubation for 24 hours. The comparison is shown in Fig. 2.

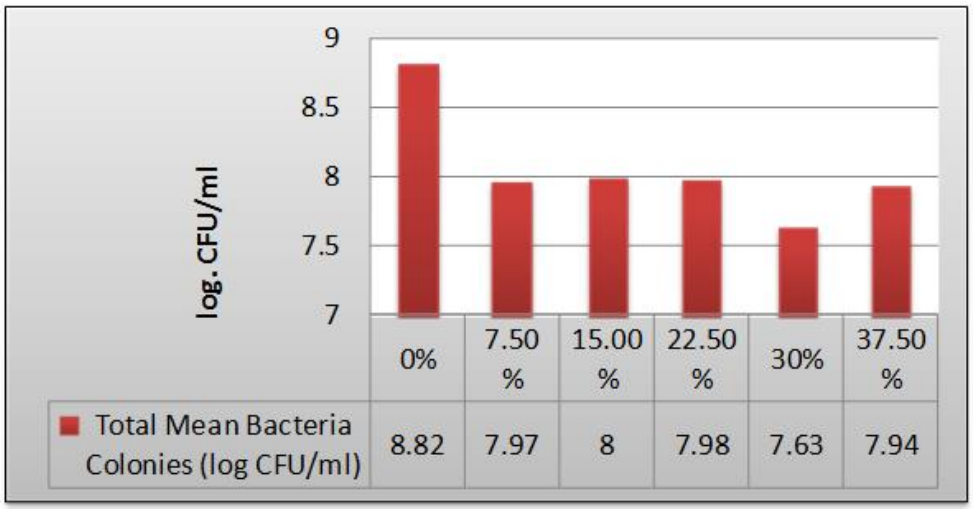

Fig. 2: The Average Count of Bacterial Colony Total (log CFU/ml)

Result of the test over bacterial colony total (Fig. 2) as shown by Colony Form Unit (CFU/ml) has indicated that bacterial colony total is influential, in very obviously different way ($p > 0.05$), to the sub-clinical mastitis-inducing bacteria in dairy cattle. Result of Duncan test exposes that Treatment A1 has bacterial colony total that obviously differ from others ($p > 0.05$) or that is more or in higher level than Treatments A2, A3, A4, A5 and A6.

Higher bacterial colony total at Treatment A1 is caused by the absence of eugenol of clove leaf oil which prevents bacterial growth during incubation. Other treatments involve the presence of eugenol of clove leaf oil, and the result is that eugenol of clove leaf oil is functional as antibacterial to prevent bacterial growth. This finding is still persistent although Treatment A2 at concentration 7.5 % until Treatment A6 at concentration 37.5 % are less obviously different after comparison ($p < 0.05$), or having similar effect on bacterial colony total.

This result means that the concentration of eugenol antibacterial of clove leaf oil at concentration 7.5 % is quite effective to prevent the growth of sub-clinical mastitis-inducing bacteria in dairy cattle. In other words, the reduction of bacterial colony total is closely related to the role played by eugenol of clove leaf oil as the antibacterial. It is supported by Parwata and Dewi [16] who say that eugenol component in the essential oil is useful as the antibacterial because it contains hydroxyl (-OH) and carbonyl clusters. Phenol reduction may integrate with bacterial cells through adsorption which involves hydrogen bonding. At lower degree, it produces phenol protein complex with loose bonding. It is easily resolved and followed by penetration of phenol into cells causing the precipitation and de-naturation of protein at bacterial cells. Similar findings are revealed by Dwidjoseputro [17] that the antibacterial mechanism of eugenol of clove leaf oil is by disturbing the peptidoglycan components of bacterial cells such that cellular wall layer will be damaged, thus eliminating the capacity of bacteria to produce colony, and finally, be followed by the death of bacterial cells.

Burt [18] insists that the ability of clove oil to prevent bacterial growth is related to the presence of eugenol. Eugenol may be quite important as the antibacterial due to its *hydrophobicity* character. Eugenol can enter into lipposaccharyde inside the membrane of bacterial cells and destroy cellular structure. The damaged cellular membranes of the bacteria can reduce protein synthesis such that it will disturb the function and growth of bacterial cells.

CONCLUSION

Result of this current research indicates that eugenol antibacterial of clove leaf oil at concentration 7.5 % is quite effective to prevent bacterial growth and to reduce bacterial colony total (CFU/ml) in the milk of dairy cattle infected by sub-clinical mastitis.

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