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Short Communication

Exploring the Potential of Citrus Fruit Peel Eco-Enzyme: A Natural Teat Dipping Solution for Dairy Cattle

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Abstract | The dairy industry is actively seeking natural alternatives to replace synthetic teat dipping solutions in order to prevent mastitis occurrences. One such natural compound is eco-enzyme, which can be extracted from citrus fruit peels, waste materials, and other sources. The objective of this research was to prepare an eco-enzyme using citrus fruit peel (Citrus reticulata) and analyze its organoleptic properties as well as its inhibitory effects against *Staphylococcus aureus* and *Escherichia coli* pathogens. Eco-enzyme was prepared from citrus peel waste, resulting in a solution with a typical fresh sour aroma. The pH of the eco-enzyme ranged from 3.7 to 4.5. Conducting the agar diffusion test revealed a dose-dependent inhibitory effect of the citrus fruit peel eco-enzyme against both *Staphylococcus aureus* and *Escherichia coli*. These findings underscore the antibacterial potential of the citrus fruit peel eco-enzyme solution and suggest its viability as a natural and environmentally friendly teat dipping solution for dairy cattle.

Keywords | *Escherichia coli*, dairy cattle, *Staphylococcus aureus*, natural antiseptic, synthetic antiseptic

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INTRODUCTION

Dairy cows are recognized as a vital livestock species, providing a valuable source of animal protein, with their primary product being milk. These remarkable animals play a significant role in meeting the global demand for milk production, contributing around 95% of the world's milk supply. Their importance cannot be overstated, as they fulfill a crucial role in providing a key commodity that is widely consumed and utilized in various dairy products worldwide. In order to ensure hygienic milk production, proper handling techniques during milking are crucial. One such practice is teat dipping, which involves immersing the nipples of dairy cattle in an antiseptic solution. This process aims to coat and safeguard the cattle's nipples and udders against contamination by pathogenic bacteria and microorganisms. By implementing teat dipping, the incidence of mastitis can be minimized, and the bacterial content in the milk can be reduced. This helps to maintain the quality and safety of the milk produced. Regular teat dipping treatment for cattle can effectively reduce the bacterial count and somatic cell count in milk (Swadayana et al., 2012).

Antiseptic ingredients commonly used for dipping teats are iodine, chlorhexidine, chlorine 4%, and alcohol 70% (Pisestyani et al., 2017). Aprilia et al. (2016) stated that administering a chemical antiseptic (povidone-iodine) has several disadvantages, namely causing burning, pain, itching, and redness and even leaving chemical residue in milk. Also, the use of synthetic antiseptic materials hurts human health and the environment (Muliarta and Darmawan, 2021). If humans consume milk containing chemical residues, it will be perilous for health. So, several innovations

are needed to deal with bacterial contamination without causing residue in the milk produced.

Eco-enzyme is an alternative natural cleaning agent that comes from fresh vegetable and fruit waste through a fermentation process (Rasit et al., 2019). The fermentation process of fruits and vegetables under anaerobic conditions can be carried out by yeast, such as *Saccharomyces cerevisiae*, which produces ethanol, and bacteria, such as *Lactobacillus* spp. and *Acetobacter* spp., which produce various organic acids. Meanwhile, mold acts as a decomposer of carbohydrates, cellulose, and hemicellulose found in fruit skin (Madigan et al., 2012). The eco enzymes have been widely used in the medical field, namely healing wounds caused by sharp objects or burns, burns, scratches, boils, relieving skin infections, etc (Riyanti et al., 2023). Vama and Cherekar (2020) stated that Orange (*Citrus reticulata*) is a fruit plant that is known throughout the world to contain active phytochemicals that can be used in health. Citrus fruit peel contains various secondary components with significant antioxidant activity compared to other parts of the fruit (Benny et al., 2023). Quoting Benny et al. (2023), The UN Food and Agriculture Organization stated that global orange production has increased significantly in recent years, reaching 144 million metric tons. Hence, the availability of citrus fruit peel has the potential to be used as a natural antiseptic for dairy cattle.

Citrus fruit peel also contains natural metabolites antimicrobials and antioxidants, such as vitamin C, citric acid, essential oils, bioflavonoids, polyphenols, coumarins, flavonoids, and volatile oils in the peel such as limonene (±70%), α-terpinene, α- pinene, β-pinene, as well as coumarin, and polyphenols (Krisnawan et al., 2017). According to the research findings by Anggraini (2021), citrus fruit peel extract contains flavonoids, which have antibacterial properties. Based on this information, the objective of the research is to develop an eco-enzyme from citrus fruit peel (specifically *Citrus reticulata*) as a natural teat dipping solution for dairy livestock. The aim is to explore the potential of using citrus peel eco-enzyme as an alternative to synthetic antiseptics, with the goal of reducing the residual effects typically associated with synthetic options. By harnessing the antibacterial properties of citrus peel eco-enzyme, it is anticipated that this natural solution can effectively replace synthetic antiseptics, offering a more sustainable and environmentally friendly approach in dairy farming.

MATERIAL AND METHODS

In this study citrus peel waste was collected from the market. This research was conducted at the Dairy Animal Production Laboratory of the Faculty of Animal Husbandry, Universitas Andalas, and the Microbiology Laboratory of the Faculty of Medicine, Universitas Andalas. The research methods used were descriptive and experimental methods using a completely randomized design (CRD), which consisted of 4 treatments and four replicates. The treatments applied in this study were:

Synthetic Antiseptic (control)
Eco-enzyme of Citrus Fruit Peel (30%)
Eco-enzyme of Citrus Fruit Peel (60%)
Eco-enzyme of Citrus Fruit Peel (100%)

Producing Eco-Enzyme

The eco-enzyme production was started by combining one-part sugar, three-part pealed *Citrus reticulata*, and 10-part aquades (distilled water). The mixture was placed in an air-tight bucket and then incubated for three months (Anaerobe fermentation). The fermentation succeeds if the pealed *Citrus reticulata* gravitate on the bottom of the bucket and has a citrus flavor. We filtered the pealed *Citrus reticulata* to harvest the eco-enzyme. The eco-enzyme was stored in a 1-liter bottle and kept at room temperature. The pictorial summary of whole process is shown in Figure 1.

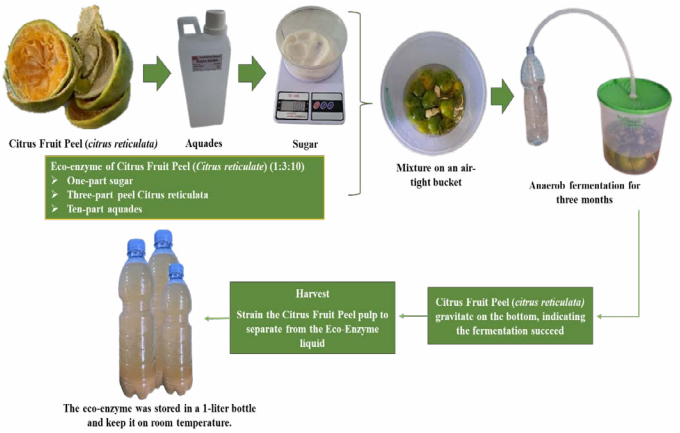

Figure 1: Process of preparation of eco-enzyme

Parameters

a) Organoleptic Test of eco-enzyme

Aroma: An aroma test of the eco-enzyme was conducted using an olfactory test, in which participants sniffed the odor and indicated its scent (Kartika et al., 1988).

pH: pH testing was performed using a pH meter to assess the acidity level of the sample. A pH below 4.0 indicates suitability for body parts such as medication and mouthwash, while a pH above 4.0 suggests it can be used as an antiseptic.

b) Inhibition Test of Citrus Fruit Peel eco-enzyme

The test was conducted to recognize the efficacy of eco-enzyme as an antiseptic to inhibit the growth of microorganisms. For this purpose both Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacteria were used (Daud et al., 2023). Both type of test organisms were

cultured in the bacteriological media. The turbidity was adjusted to Mac Farland 0.5 standard. Streaked the homogeneous indicator strain by sterile cotton to Mueller Hinton Agar media. A hole was made in the media (Mueller Hinton Agar) with a diameter of 6mm that has been smeared with bacteria. Then, a 50 µl of eco-enzyme sample was added into the hole using a micropipette. Incubated the media at 37^{0}C for 24 h, then the clear zone was observed.

Data Analyses

Data was analyzed by Minitab 14 through Analysis of Variance (ANOVA). The significance level was adjusted at 5% ($p \leq 0.05$).

RESULT AND DISCUSSION

pH and Aroma

The pH and aroma test results of the citrus fruit peel eco-enzyme solution were presented in Table 1.

The aroma of the obtained eco-enzyme solution has a typical fresh sour aroma (Table 1). The finished eco-enzyme liquid has a pungent and fresh orange peel sour aroma. The sour aroma produced comes from the acetic acid contained in the eco-enzyme product liquid. Acetic acid generally give a sour aroma to the liquid. Acetic acid is produced from the metabolic process of bacteria naturally found in the orange peel used as the material for making eco-enzymes. The fermentation process is the process of decomposing organic compounds to produce energy produced by microorganisms. Microbes that perform fermentation require energy, which is generally obtained from glucose. Sugar added in making eco enzymes acts as an energy source for microbes in the fermentation process. The reactions during the fermentation process are CO2+N2O+O2 = O3+NO3+CO3 (Larasati et al., 2020). Dewi et al. (2021) added that the fermentation process is an attempt by bacteria to obtain energy from carbohydrates under anaerobic conditions by producing by-products in the form of alcohol or acetic acid. The alcohol or acetic acid produced can be a disinfectant (Larasati et al., 2020), used for cleaning or teat dipping in dairy cattle. With the formation of acetic acid in the fermentation process, it will get a fresh aroma and not smell foul.

The pH level of the eco-enzyme solution with a concentration of 30%, 60%, and 100%, respectively, has a pH of 4.5, 4, and 3.7 (Table 1). According to Rusdianasari (2021), a good eco-enzyme has a pH ≤4. 100% concentration of citrus fruit peel eco-enzyme exhibited significantly lower ($p<0.05$) pH as compared to other concentrations of eco-enzymes and synthetic antiseptic. The lower the pH, the better the eco-enzyme produced. The pH of eco-enzymes can vary depending on the materials used. The pH of eco-enzymes from fruit waste is usually slightly acidic (Benny et al., 2023). The pH of the eco-enzyme also depend on the amount of water added and the fermentation time. Generally, the longer the fermentation time, the lower the pH. Eco-enzyme is a microbial-based product made by fermenting vegetable waste with the resulting products in the form of beneficial bacteria and enzymes (Benny et al., 2023). Eco-enzyme solutions can be cleaners, eliminate unpleasant odors, and reduce environmental pollution (Putra et al., 2023).

Bacterial Inhibitory effect

In Figure 2, it can be seen that the best result of the inhibition test is at a concentration of 100%, where the size of the clear zone formed against *Staphylococcus aureus* (3.88 mm) and *Escherichia coli* (2.48 mm) was directly proportional to the pH level obtained. The zone of inhibition produced by control and 100% concentration of eco-enzyme was significantly higher ($p<0.05$) as compared to other concentration levels of eco-enzyme against both *Staphylococcus aureus* and *Escherichia coli* pathogens. *Staphylococcus aureus* and *Escherichia coli* are bacteria that often found in the udders of dairy cattle, and these two bacteria have the potential to infect udder tissue causing mastitis in dairy cattle (Artdita et al., 2020; Fesseha et al., 2021). The increasing concentration of eco-enzyme solution resulting high concentration of antibacterial compounds that diffuse in the agar medium and produced enlarged inhibition zone; otherwise, the decrease in the diameter of the inhibition zone is caused by the reduced effectiveness of antibacterial compounds from the level of eco-enzyme solution used (Welfalini et al., 2023; Rahma et al., 2017).

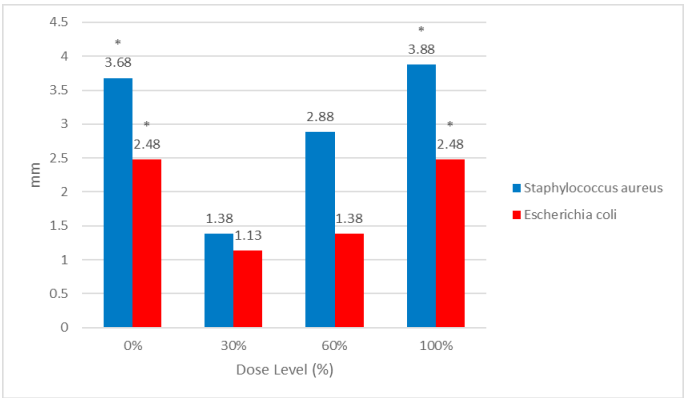

Figure 2: Inhibition test of citrus fruit peel eco-enzyme against *Staphylococcus aureus* and *Escherichia coli* bacteria. * Significantly higher at ($p<0.05$)

The clear zone formed at a concentration of 100% is almost the same ($p>0.05$) as the results on synthetic antiseptic solution. This is due to the acetic acid content (H3COOH) in the eco-enzyme solution, which can kill germs, viruses, and bacteria. In contrast, the enzyme content is lipase, trypsin, and amylase, which can kill/prevent pathogenic

Table 1: pH and Aroma of Eco-enzyme

	Concentration of eco-enzyme			
Parameter	**A. 0%**	**B. 30%**	**C. 60%**	**D. 100%**
Aroma	Specific Aroma Antiseptic (Sintetik®)	Specific aroma of fresh citrus	Specific aroma of fresh citrus	Specific aroma of fresh citrus
pH	4.00[b]	4.50[b]	4.00[b]	3.70[a]

bacteria (Nurdin et al., 2021). The acetic acid content in the eco-enzyme solution diffuses into the bacterial cells, where the low pH content of the eco-enzyme solution and the high pH in the bacteria cause the cytoplasm of the bacteria to become more acidic and its cell activity becomes disrupted so that damage to DNA and protein denaturation occurs which causes the bacteria to die. From the results of the inhibition test, the best candidate for eco-enzyme solution obtained was 100% concentration level (treatment D), which is expected to be a natural ingredient-based teat dipping solution for dairy cattle with environmentally friendly and readily available ingredients. Teat dipping solution works by coating the nipple, especially the nipple hole, to prevent outside bacteria from entering the udder, which can infect the udder of dairy cattle (Hartanto et al., 2021). Figure 3 shows the results of the inhibition test against *Staphylococcus aureus* and *Escherichia coli* bacteria.

Figure 3: Inhibition test of Eco-enzyme solution against *Staphylococcus aureus* (a) and *Escherichia coli* (b)

CONCLUSION

From the results of the study, it is concluded that the citrus fruit peel (*Citrus reticulata*) eco-enzyme solution may potentially be used as a natural and environmentally friendly teat dipping solution for dairy cattle, thus can reduce residues of synthetic antiseptics in milk.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

NOVELTY STATEMENT

This study succeeded in the best dose of citrus fruit peel eco-enzyme solution (100%) as teat dipping in dairy cattle, which provided an inhibition zone on Staphylococcus aureus and Escherichia coli bacteria.

AUTHORS' CONTRIBUTIONS

The manuscript has been compiled and written by author and co-authors Rizqan, as a writer and correspondence, has contributed to Data curation, Writing original draft - reviews, and project administration. Hilda Susanty, as a co-author, has contributed to Resources and supervision. Linda Suhartati, as a co-author, has contributed to analysis, software, and writing. Azhar, as a co-author, has contributed to data curation. Arief, as a co-author, has contributed to methodology and supervision. Elly Roza, as a co-author, has also contributed to methodology and supervision.

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