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Research Article

Color Test and Screening of Alkaloid, Saponin Levels of Citrus Fruit Peel Eco-Enzyme (*Citrus reticulata*) Solution as a Candidate for Teat Dipping in Dairy Cattle

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ABSTRACT

Teat dipping is the activity of dipping the nipples of dairy cattle into an antiseptic solution that serves to coat and protect the nipples of livestock from harmful bacterial contamination so as not to cause disease in dairy cattle. This study aimed to obtain preliminary information about the color and screening of alkaloid and saponin content in the eco-enzyme solution, which will later be used as one of the natural antiseptic candidates for teat dipping solution in dairy cattle. This research uses descriptive analysis with the parameters observed, namely color and screening of alkaloid and saponin content in eco-enzyme solutions that have been diluted previously with the following percentages: A: 30% eco-enzyme solution + 70% aquades, B: 60% eco-enzyme solution + 30% aquades, and C: 100% eco-enzyme solution. From the results, it was found that the color of eco-enzyme solution A (transparent yellow), B (transparent yellow), and C (intense yellow and translucent color) while the qualitative test obtained A: positive, B: positive, C: positive (alkaloid content); A: negative, B: positive, and C: positive (saponin content). The results of color tests and screening of alkaloid and saponin content showed that good results were obtained in 100% eco-enzyme solution (C), which can be used as a candidate for natural teat dipping solution in dairy cows.

Keywords: alkaloid, color test, eco-enzyme, saponin, teat dipping

INTRODUCTION

Teat dipping is dipping the nipples of dairy cattle into an antiseptic solution that aims to coat and protect the nipples of cattle from bacterial contamination and pathogenic microorganisms after milking. The implementation of dipping in dairy cattle is widely reported in livestock development countries to minimize the occurrence of mastitis and reduce the number of pathogenic bacteria in the milk of dairy cows. Teat dipping treatment of cattle nipples can reduce the number of pathogenic bacteria entering the udder because the post-milking nipple hole will be open, so bacteria can quickly enter the udder. One of the efforts that can be done as a preventive measure to minimize this is teat dipping.

Implementing teat dipping in dairy cattle generally uses synthetic antiseptic solutions with iodine, chlorhexidine, or chlorine, which can suppress the growth of pathogenic microorganisms (Siregar, 2010). However, synthetic antiseptics will leave chemical residues in the milk produced (Abni *et al*., 2021). One of the solutions that can be used as a substitute for synthetic antiseptic solutions is the eco-enzyme solution, which from several studies shows promising results for use

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as a natural and environmentally friendly antiseptic that is safe for health, but not many farmers or researchers have used and researched this material as a teat dipping solution.

Eco-enzyme solution is a versatile solution produced from the fermentation of organic materials, such as fruit pulp, vegetables, sugar, and water. Yeasts, such as Saccharomyces cerevisiae, which produces ethanol, and bacteria, such as Lactobacillus sp. and Acetobacter sp., can carry out this fermentation process under anaerobic conditions, producing various organic acids. At the same time, molds decompose carbohydrates, cellulose, and hemicellulose in fruit peels. Eco-enzyme solutions have been widely used in the medical field to heal wounds caused by sharp objects or burns, scratches, boils, relieve skin infections, and so on (Riyanti *et al*., 2023). Vama and Cherekar (2020) added that eco-enzymes can be utilized as anti-fungal, anti-bacterial, insecticidal, and cleaning agents. Meanwhile, the results of research by Muliarta and Darmawan (2021) stated that the content of acetic acid (CH_3COOH) contained in eco-enzymes can kill germs, viruses, and pathogenic bacteria.

One of the materials that can be used in the manufacture of eco-enzyme is orange peel (*Citrus reticulata*) which contains many natural 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-pine, β-pine, as well as coumarins, and polyphenols (Krinawan *et al*., 2017). It is expected that the citrus fruit peel eco-enzyme (*Citrus reticulata*) can be used as a natural antiseptic candidate as a teat dipping solution in dairy cattle; this is by the research objectives of obtaining preliminary information about color, screening of alkaloid content, saponins in eco-enzyme solution which will later be used as one of the natural antiseptic candidates for teat dipping solution in dairy cattle.

MATERIAL AND METHODS

The method used in this research was descriptive, with the parameters observed as color test and qualitative test (alkaloid and saponin levels) on the eco-enzyme solution that has been diluted previously with the following percentages into three treatments below:

A: 30% eco-enzyme solution + 70% Aquades,
B: 60% eco-enzyme solution + 40% Aquades
C: 100% eco-enzyme solution.

Preparation of Citrus Fruit Peel Eco-Enzyme (*Citrus reticulata*)

Preparation of eco-enzyme based on citrus fruit peel with a capacity of 1 liter with a ratio of 1 : 3 : 10 with the following information; 1 = Sugar (100 grams); 3 = citrus fruit peel (*Citrus reticulata*) (300 grams); 10 = Aquades (1000 ml) all of these materials were put into an airtight container (anaerobic) to do fermentation for 3 months (Figure 1).

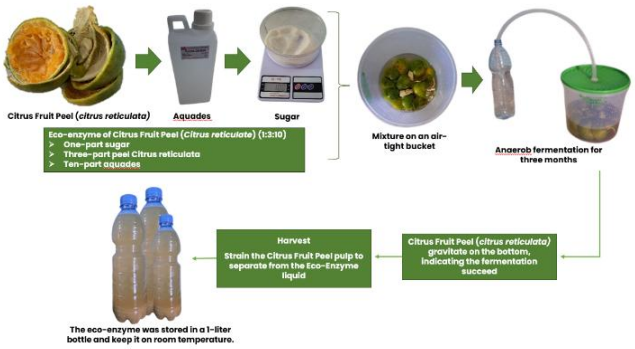


Figure 1. Preparation of citrus fruit peel eco-enzyme (*Citrus reticulata*) (Rizqan *et al*., 2023)

Parameter Testing Method

1. Color test

Color testing is one of the organoleptic tests on samples using the five senses of vision directly to determine changes in each sample.

2. Alkaloid Screening

Citrus fruit peel eco-enzyme (*Citrus reticulata*) was taken as much as 2 mL added with two drops of Dragendorff reagent and homogenized. After that, observe the color changes that occur for ± 30 minutes. The test result is positive if an orange/orange color is formed (Irianto *et al*., 2023).

3. Saponin Screening

Citrus fruit peel eco-enzyme (*Citrus reticulata*) was taken as much as 2 mL with two drops of 2N HCl solution. Hot water was added and homogenized by shaking the tube for 10 seconds. The test result is positive if the foam is formed as high as 1-10 cm (not less than 10 minutes) (Irianto *et al*., 2023).

Data Analysis

Color test data, Alkaloid screening, and Saponin screening were performed qualitatively and presented in tabular form using Ms. Excel 2010 application.

RESULTS AND DISCUSSION

The results of the color test and screening of alkaloid and saponin levels in the citrus fruit peel eco-enzyme (*Citrus reticulata*) are in Table 1.

Tabel 1. Color test results, screening alkaloid and saponin levels

No.	Sampel	Results		
		Color test	Alkaloid screening	Saponin screening
1.	A: 30% eco-enzyme solution + 70% Aquades	Transparent yellow	Positive	Negative
2.	B: 60% eco-enzyme solution + 40% Aquades	Transparent yellow	Positive	Positive
3.	C: 100% eco-enzyme solution	Intense yellow and translucent color	Positive	Positive

Based on the color test that has been done (Table 1), it was found that the color of the orange peel eco-enzyme in dilution A (transparent yellow), B (transparent yellow), and C (Intense yellow and translucent color) (Figure 2). This color is caused by the materials used, especially the use of granulated sugar so that the color of the eco-enzyme obtained is yellowish; this is different from the use of molasses, where the eco-enzyme produced is cloudy brown (Suprayogi *et al*., 2022). Sugar is used to manufacture eco-enzymes to serve as an energy source for microorganisms during fermentation. In addition, the screening test of phytochemical content in the eco-enzyme solution showed diverse results (Table 1). Still, it was found that

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sample C (100% eco-enzyme solution) showed good results, whereas the screening results of alkaloids and saponins showed positive results (+). Fajrina *et al*. (2019) believe eco-enzyme solution contains chemical compounds, including alkaloids and saponins, that function as inhibitors of bacterial growth.



Figure 2. Citrus fruit peel eco-enzyme (*Citrus reticulata*) based on dilution percentage

Alkaloids themselves are secondary metabolite compounds with alkaline nitrogen atoms (Maisarah *et al*., 2023). Alkaloid compounds are antifungal because these compounds work by disrupting the components that make up the peptidoglycan in fungal cells, causing the failure of the cell wall formation process intact and causing the cell to die (Sari *et al*., 2022). Alkaloids are also reported to have antifungal activity by inhibiting DNA esterase and RNA polymerase (Fatma *et al*., 2021). In addition, the alkaloid content can also be used as an antibacterial (Lase, 2019).

In this citrus fruit peel eco-enzyme (*Citrus reticulata*) solution, positive results were also obtained for saponin levels; this is supported by research from Shetty *et al*. (2016), which showed that the water fraction of *Citrus sinensis* fruit peel contains alkaloids and saponins as antibacterial. Saponins are complex glycoside compounds that have properties similar to detergents. According to Yanuartono (2017), saponins have a variety of biological properties, including antibacterial. According to research by Putri *et al*. (2023), saponin compounds have antibacterial abilities with a mechanism of action to denature proteins. This is also supported by the surface of saponins similar to detergents where the surface tension of the bacterial cell wall will be lowered and the permeability of the bacterial membrane is damaged, which results in disruption of bacterial survival due to damage to the cell membrane (Sani, 2013; Putri *et al*., 2023). From the description above, it is believed that the content contained in eco-enzyme can be used as a natural antiseptic candidate in the teat dipping process in dairy cattle.

CONCLUSION

Based on the color test and screening of alkaloid and saponin levels contained in the eco-enzyme solution of citrus fruit peel (*Citrus reticulata*), it can be concluded that the eco-enzyme solution (C) can be used as a natural antiseptic candidate for the dipping solution in dairy cattle.

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