

Phytochemical Analysis of Eco-Enzymes Combining Coffee Husks and Cocoa Husks

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Article Information	ABSTRACT
Article History Received : March 01, 2026 Revised : March 16, 2026 Published : April 10, 2026	Eco enzyme is a multipurpose fermentation liquid made from organic materials. The purpose of this study was to determine the qualitative phytochemical content in eco enzyme samples combining coffee husks and cocoa husks. The ratio used for the solid organic material:sugar:water was 3:1:10. The treatment focused on variations in water substitutes with solid organic materials in the form of coffee husks and cocoa husks, as well as molasses as a sugar substitute. There were four types of treatments, namely E1 (Control), E2 (Coconut Water), E3 (Coffee Pulping Water), and E4 (Rice Washing Water). The types of phytochemicals tested were phenols, alkaloids, flavonoids, tannins, saponins, and cardenolins. The test results were observed based on the color changes that occurred. The phytochemical content detected in the eco enzyme sample is phenols, alkaloids, flavonoids, tannins, and saponins, while the cardenoline content was detected negatively.
Keywords: <i>Eco enzyme;</i> <i>Coffee husks;</i> <i>Cocoa husks;</i> <i>Phytochemical.</i>	

INTRODUCTION

Eco enzyme is a liquid produced by fermenting certain organic materials with the addition of sugar or similar ingredients and water using a specific ratio. Eco enzyme products are also known as multipurpose liquids that have various benefits, including as plant-based pesticides, liquid fertilizers, disinfectants, carbolic acid, helping to neutralize water pollution, and can be used as soap ingredients. Several studies on eco enzyme have provided clear evidence regarding these benefits. One study, Sitawati et al (2022) , states that the application of eco enzyme from orange peel and pineapple peel has been proven to affect the growth of ornamental plants, namely the water lily (*Impatiens hawkeri* Bull). The study also shows differences in growth according to the different doses applied.

The benefits of eco enzymes as natural insecticides have been proven in a study Maryanti, et al (2024), which states that eco enzymes made from onion skins are 82.5% to 95% effective in controlling armyworms (*Spodoptera litura*). Based os research Safirah, et al, 2016 and Tampubolon, dkk 2018, is stated that phytochemical compounds such as phenols, flavonoids, alkaloids, tannins, and saponins have the potential to be used as pesticides. Based of research, Furthermore, the use of eco-enzymes as disinfectants was obtained from eco-enzymes made from rambutan peel, corn cobs, and pumpkin peel as substrates in various raw materials eko-enzim. Each treatment was added with 10% extract of kayu cendana kamboja. The results showed that the alcohol content of the eco-enzyme was 60-70% and the pH was <4, with an antibacterial activity against *Streptococcus aerus*, which was classified as strong at 31,85-34,41 mm (Rahayu and Situmeang, 2021).

Eco-enzymes also have benefits as biocatalysts for breaking down oil substances. Based on research by Wikaningrum and Pratamadina (2022), eco-enzymes made from orange waste have the potential to reduce the concentration of oil and fat in domestic wastewater. The samples used were artificial samples, namely domestic wastewater enriched with oil and fat to produce an initial concentration of 19.82 mg/L. In samples treated with 5% eco-enzyme, the oil and fat concentration decreased to 13.84 mg/L after a nine-day observation period, while in control samples without eco-enzyme addition, the final concentration was recorded at 15.93 mg/L. Based on the results of statistical analysis, the decrease in oil and fat concentration in both treatments was significant. However, the ANOVA test results showed that the difference in effectiveness between the two treatments was not statistically significant.

The various benefits above show that eco enzymes have considerable potential for human life and the environment. This indicates the vast opportunities for innovation to enrich knowledge and science, which are expected to benefit the wider community. There are various types of organic materials that can be used as raw materials for making eco enzymes, one of which is by utilizing coffee husks and cocoa husks to contribute knowledge to the community, especially coffee and cocoa farmers, so that they can optimize the resources available in their surroundings. Therefore, this study was conducted to determine the quality of eco enzymes made from a combination of coffee husks and cocoa husks through physiological characteristic tests.

RESEARCH METHODS

Materials and equipment

The tools used to make eco-enzymes were 12 5-liter bottles, scales, knives, cutting boards, and buckets, while the materials used were molasses, coconut water, water, and vegetable and fruit waste. The tools used in the analysis are glass beakers, measuring cups, measuring pipettes and bulbs, measuring flasks (100 mL, 50 mL, 10 mL, and 5 mL), glass stirrers, dropper pipettes, test tubes, test tube racks, and UV-Vis spectrophotometers. The analysis materials used included concentrated HCl, dilute HCl, AlCl_3 , concentrated H_2SO_4 , P.A ethanol, FeCl_3 , distilled water, and Wagner.

Production of Eco Enzyme

The stages of the eco enzyme production process begin with the preparation of raw materials, the shredding of cocoa shells, the weighing and measuring of raw materials, the addition of all raw materials into a container and stirring, and finally, sealing the container tightly. Referring to the research Septinar, et al. (2024), which states that the ratio for making eco enzyme is 3:1:10 for the composition of solid organic matter: molasses :liquid material. There are four types of treatments focused on replacing the water raw material, namely E1 (Control), E2 (Coconut Water), E3 (Coffee Pulp Water), and E4 (Rice Washing Water). Each part (solid organic matter: brown sugar:water) uses a volume ratio of 360 mL :120 mL :1200 mL.

The eco-enzyme production process begins with weighing the ingredients according to the appropriate ratio. After weighing or measuring, all ingredients are then placed in a container and tightly sealed. During the first one to two weeks of fermentation, regular gassing is necessary if ventilation is not provided. The eco-enzyme volume is made up to 84% of the fermentation container volume. Fermentation process is carried out for 3 months.

Qualitative Phytochemical Analysis

The qualitative phytochemical analysis method refers to the research Widjanarko et al (2023), as follows:

a. Phenol test (Ferric Chloride Test)

Add 2 mL and then place it in a test tube. Add 5 drops of FeCl_3 to the test tube containing the sample and observe the color change.

b. Flavonoid Test (Alkaline Reagent Test)

Take a 2 mL sample, then add 3-5 drops of 20% NaOH. After a few seconds, a color change will occur. Then add 3-5 drops of concentrated HCl and observe the color change that occurs.

c. Alkaloid Test (Wagner's Test)

The Wagner test is performed by taking 2 mL of eco enzyme sample and adding 3-5 drops of Wagner reagent, then observe the color change.

d. Saponin

Test Take 2 mL of eco enzyme sample into a test tube, then add 6 mL of hot water, shake for 10 seconds until foam forms. Next, add 1 drop of 2 N HCl to observe the foam stability. A positive result is indicated by the stability of the foam formed, reaching a height of ± 1 cm for 30 seconds (Marjoni, 2016).

e. Quinones Test

The quinone test is performed by adding an alkali, in this case the researcher used 40% NaOH, to the eco enzyme sample (5 drops), then observe the color change that occurs.

f. Kardenolin Test (Keller Killiani Test)

The qualitative kardenolin test was performed by taking 2 mL of sample, then adding 2 mL of glacial acetic acid and 5 drops of FeCl_3 and homogenizing it. The final step was to slowly add 1 mL of concentrated H_2SO_4 and observe.

RESULTS AND DISCUSSION

Phytochemicals are bioactive compounds that occur naturally in plants. These compounds have various benefits, including antioxidant, antimicrobial, and anti inflammatory properties. Some types of phytochemicals include polyphenols (flavonoids, tannins, resveratrol), terpenoids (carotenoids, phytosterols), alkaloids, saponins, and glycosides (Devi dan Stiadi, 2025; Khasvarof, dkk, 2026). Qualitative phytochemical testing aims to determine the types of compounds contained in eco enzyme samples. The data presented are the average results of each treatment. Each qualitative test result for phytochemical content is discussed further in the discussion below.

Table 1. Results of qualitative phytochemical content tests of eco enzyme samples.

Type of compounds	Analysis Method	Treatment Sample				Indicator of Posistif Response
		E1	E2	E3	E4	
Phenolic	Ferric chloride analysis	+	+	+	+	Color change to black.
Flavonoids	Alkaline reagen analysis	+	+	+	+	The intense color caused by the addition of 20% NaOH faded after the addition of concentrated HCl.
Alkaloids	Wagner's reagen analysis	+	+	+	+	Formation of a dark brown precipitat
Saponin	Foam analysis	+	+	+	+	Stable foam formed for 10 minutes.
Quinone	Quinones analysis	+	+	+	+	Color change to dark
Cardenoline	Keller Killiani analysis	-	-	-	-	No rings formed

*Note: E1 (Control), E2 (Coconut Water), E3 (Coffee Pulping Water), E4 (Rice Washing Water)

Phenols and Flavonoids

The Ferric Chloride Test is applied to eco enzymes to determine the presence of phenolic compounds. The dark color shown in the results of the is due to the absorption of light by the negative Fe^{3+} ion complex formed from phenolic compounds in the sample reacting with Fe^{3+}

compounds. The more negative Fe^{3+} ions that are formed, the more light is absorbed, resulting in a black color. According to Godlewska (2023), the ferric chloride test can detect phenol content with a positive result indicated by the formation of a dark-colored complex (dark green, bluish black, or blackish). This is due to the multi-group coordination of phenolates in polyphenol molecules with Fe^{3+} , resulting in a complex with an electron structure that absorbs many wavelengths of light, making it appear very dark.

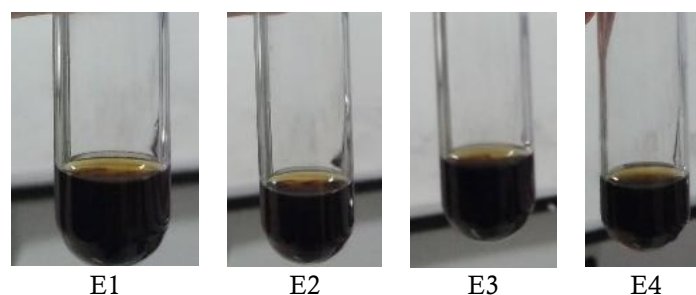


Figure 1. Color change of sample in the ferric chloride test.

Based on Table 1. above, it can be seen that the eco enzyme sample contains flavonoids. One of the benefits of flavonoids is have the ability to inhibit and prevent free radicals. . Flavonoids is one of polyphenol compounds, so quantity of fenol is determined of flavonoid contents (Baharuddin, 2018). Based on the research of Sioriki, et al (2021), it is stated that the addition of alkaline compounds, in this case NaOH solution, causes an increase in pH, resulting in a darkening process due to the oxidation of polyphenols into quinones. H^{+} ions from OH ions will decrease after the addition of NaOH solution, causing the conjugated double bond to widen. This widening of the conjugated double bond results in an increase in light absorption in the UV-vis region, causing the color of the eco enzyme sample to become darker. Next, to prove the presence or absence of polyphenol content, concentrated HCl solution is added. A positive result is indicated by the return of the dark color of the eco enzyme due to the addition of NaOH to a light color or back to its original color (He and Giusti, 2010). The addition of acid (HCl) causes the flavonoids to return to their original structure. The return of the dark color to its original color proves that the previous color change was due to the presence of flavonoid phenol groups that are sensitive to alkaline pH. This statement is supported by research by Hostnik et al (2022) that the UV-Vis spectrum of phenolic compounds such as gallic and ellagic acid changes systematically depending on the pH of the solution, which is related to its ionization form and molecular structure, this is relevant to color changes due to deprotonation by NaOH and protonation by HCl. The color changes that occur can be seen in the image bellow.

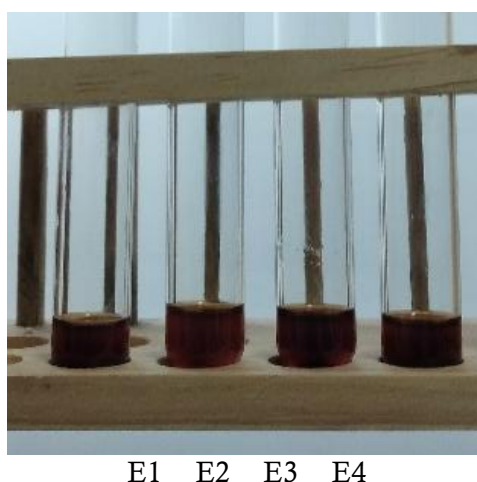


Figure 2. The intense color caused by the addition of 20% NaOH.

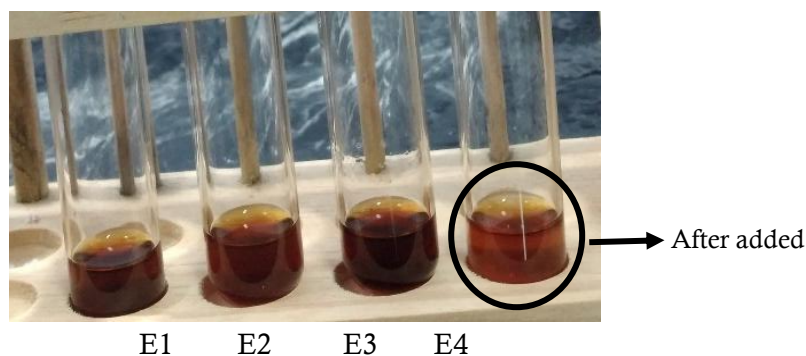


Figure 3. The color changes become faded due to addition.

Alkaloids

The alkaloid test results were positive, as indicated by the formation of a brownish precipitate. This color is characteristic of the reaction between eco enzyme and Wagner's reagent. Wagner's reagent contains metal ions in the form of iodine (I) and potassium iodide (KI). The principle of the reaction is the formation of a double salt (potassium-alkaloid complex) due to the metal ion bond from the Wagner reagent and nitrogen in the alkaloid structure. The potassium-alkaloid complex bond that forms is insoluble in water, resulting in a precipitate. This statement is supported by the theory in the journal Marlina and Suryanti (2005) which states that the precipitate formed in the phytochemical screening of eco enzymes is potassium-alkaloid. The brown color in Wagner's reagent is due to the presence of metal ions I_3^- . The potassium iodide (KI) content in Wagner's reagent reacts with nitrogen in the alkaloid structure to form a potassium-alkaloid complex, resulting in the formation of a precipitate. The color changes of alkaloid can be seen in the image bellow.

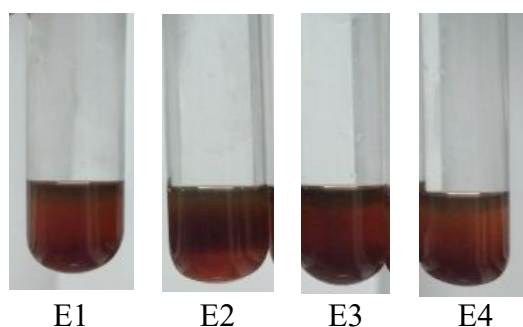


Figure 4. The result of Wagner's reagen test

Saponin

Based on Table 1, the saponin test for all treatments was positive, indicated by the constant formation of bubbles for 10 minutes due to shaking. The content of the eco-enzyme sample indicates antimicrobial properties. According to Irianto et al. (2024), the saponin content in the eco-enzyme *M. paradisiaca* var. *bluggoe* can inhibit bacterial growth. The following is a visual display of the saponin test results.

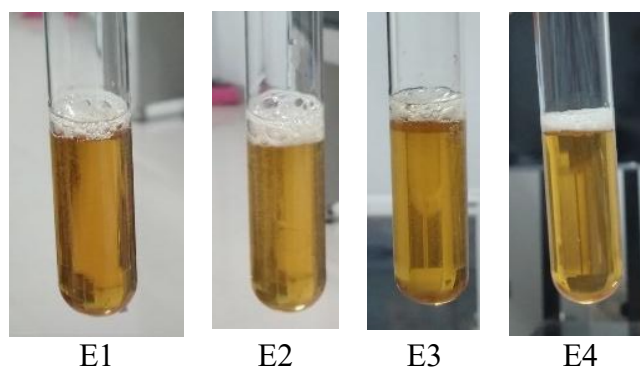


Figure 5. Stability of saponin test bubbles after 10 minutes

Quinone

The quinone test was conducted by adding an alkali in the form of NaOH. The concentration of NaOH used, which was 40%, was modified by the researcher. In general, the color change that occurs in the quinone test is yellow to purple, but due to the initial color of the eco enzyme sample produced by the fermentation process, the addition of 40% NaOH caused the color to become darker. According to Satria, et al (2025), the color produced from eco enzyme fermentation is influenced by the differences in the organic materials used. The color changes that occur can be seen in the image below.

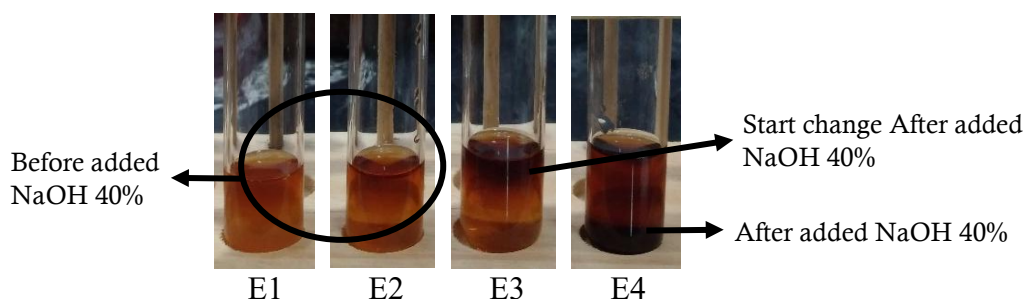


Figure 6. Gradual color change in the Quinon test due to the addition of 40% NaOH.

Cardenoline

The Keller-Killiani test is used to detect cardenoline content by the formation of a dark red-purple ring. In theory, this specific purple color is the result of a condensation product involving a dehydrated deoxy sugar group. This deoxy sugar group is a characteristic of cardenolides. This is evidence that the eco enzyme product contains secondary metabolites with a structure similar to cardiac glycosides (cardenolides). According to Albalawi (2016), the Keller-Killiani test process occurs through the hydrolysis of deoxy sugars in cardiac glycosides, resulting in the addition of a specific color that can be visually observed at the interface between glacial acetic acid and concentrated sulfuric acid. This color can be brown, purple, or blue depending on the specific compound and reagent conditions. However, based on the analysis results, a negative result is indicated by the absence of a ring with a specific color as explained above. This indicates that the eco enzyme sample does not contain cardenoline. The condition of the sample after the addition of glacial acetic acid and FeCl_3 can be seen in the image below.

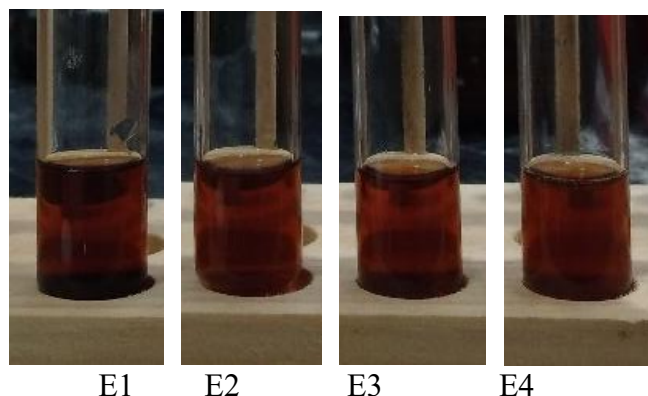


Figure 7. The Result of Keller-Killiani test.

CONCLUSION

Based on the results of qualitative phytochemical testing of eco enzyme samples treated with E1 (Control), E2 (Coconut Water), E3 (Coffee Pulp Water), and E4 (Rice Washing Water), all sample contained phenols, alkaloids, flavonoids, tannins, and saponins, while cardenoline was detected negatively. These results also provide scientific evidence that eco enzyme combinations of coffee husks and cocoa husks have good potential for further use.

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