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Thin layer chromatography analysis and antibacterial activity of ecoenzyme solution

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Abstract

Ecoenzyme is known as fermented organic garbage solution that can be used for everyday purposes for example as organic liquid fertilizer and antiseptic. Until now, no information has been reported yet regarding further phytochemical tests and antibacterial activity of ecoenzyme solution. Based on these facts, it was deemed necessary to carry out this research in order to obtain scientific information regarding phytochemical activity using thin layer chromatography (TLC) as well as antibacterial activity contained in ecoenzyme solution. Initial screening for phytochemical content was carried out by testing alkaloids compound using the Wagner method, testing flavonoids and saponins compound using strong acid method. Advanced phytochemical tests were carried out using TLC using a silica gel plate with mobile phase, i.e., methanol: ammonia for alkaloid test, n-hexane: ethyl acetate: formic acid for flavonoid test, and chloroform: methanol for saponin test. Antibacterial activity test was carried out using disc method. Initial screening results on the phytochemical content of ecoenzyme solutions showed that three ecoenzyme solutions tested did not contain saponins. The TLC results showed that ecoenzyme solution derived from three types of fruit peel contained flavonoids and saponins, also this ecoenzyme has the best antibacterial activity against gram-positive and negative bacteria.

Keywords

Ecoenzyme, Thin layer chromatography, Antibacterial activity

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Introduction

One effort to utilize and process organic waste is to convert it into ecoenzymes. According to [1], ecoenzyme or in Indonesian called "EKOENZIM" is a solution of complex organic substances produced from the fermentation process of organic residues, sugar and water. If fermentation occurs completely, a dark brown ecoenzyme solution will be formed and a residue in the form of pellets will also be formed, which is leftover the vegetables and fruit. The residue in the form of pellets can then be used as organic fertilizer, while the ecoenzyme solution itself can be used for various household

needs, as reported by [2], that the ecoenzyme solution is useful as an all-purpose cleaner, biological fertilizer, and repellent for various plant pests.

The presence of active compounds dissolved in the ecoenzyme solution means that it can be used as organic liquid fertilizer. Initial screening results for the presence of alkaloid compounds in ecoenzyme solutions derived from vegetable and fruit peels was reported [3]. According to [4], alkaloids are compounds that have one or more nitrogen rings that are useful for plant growth development. Apart from that, the enzymes that contained in ecoenzyme solution such as protease, amylase and lipase, as well as acetic acid makes the ecoenzyme solution full of nutrients that are important for plant growth and development [5]. This is in accordance with research results on the positive influence of ecoenzyme solutions on the growth of various plants, for example *Impatiens balsamina* [6], cayenne pepper [7], and cassava [8].

Apart from that, ecoenzymes are also reported to have antibacterial activity. This is in line with research by [9] that there are phytochemical compounds contained in the ecoenzyme solution. According to [10–12], antibacterial activity are contained in many active compounds in the form of alkaloids, flavonoids, saponins, or other secondary metabolite compounds.

So far there have been no scientific reports yet regarding advanced tests to determine the phytochemical content in ecoenzyme solutions. By knowing the quality of phytochemical compounds contained in the ecoenzyme solution, it is possible to use it more widely, such as for its commercial purpose of ecoenzyme-derivative's

Methods

Initial screening of phytochemical compounds

Initial screening of phytochemical compounds was carried out by testing the ecoenzyme solution with alkaloid, flavonoid and saponin test solutions.

The alkaloid test was carried out by preparing 2 mL of ecoenzyme solution in reaction tube. Next, about 2 drops of 2N H_2SO_4 was added to it and then to be tested with Wagner's reagent. Wagner's reagent was made by dissolving 2.5 mL of iodine with 10 mL and diluting it to 200 mL. A positive test was indicated by the formation of a brown to yellow precipitate.

The flavonoid test was carried out by preparing a 2 mL ecoenzyme solution in reaction tube. This solution then to be heated until it boiled for 5 minutes. Next, a little Mg powder and 1 mL of HCl were added to the sample and shaken vigorously. A positive test was indicated by the formation of red, yellow or orange.

The saponin test was carried out by preparing 2 mL of ecoenzyme solution in reaction tube. Next about 10 drops of hot water and 3 drops of HCl was added in reaction tube containing ecoenzyme solution. A positive test was indicated by the formation of permanent foam in approximately 15 minutes.

Advanced screening of phytochemical compound using TLC

Advanced screening was carried out using the TLC method for qualitative indicator of alkaloids, flavonoids and saponins compound.

The alkaloids test was carried out by preparing10 mL of the ecoenzyme solution in reaction tube. This solution later to be evaporated, added 10% ammonia, and extracted using Chloroform solution. Next, the Chloroform phase was taken and 20 µl of the sample was spotted on the F254 silica gel plate. The plate was then put into a saturated chamber with the Methanol: ammonia (100:1.5) mobile phase, eluted it until the limit line, removed and dried. Finally, the plate was sprayed with Dragendorff's reagent and observed under UV and visible wavelength

The flavonoids test was carried out by preparing 100 mg of ecoenzyme solution (or equivalent) in reaction tube. This solution then to be added 1 mL of ethanol and to be homogenized using Vortex. The solution was then sonicated for 60 minutes and extracted by maceration for 24 hours. The solution was then centrifuged, the supernatant was taken, and a 20 µL sample was spotted on a silicagel plate. The plate was then put into a saturated chamber with the n-hexane: ethyl acetate: formic acid (6:4:0.1) mobile phase, eluted it until the limit line, and finally observed under UV wavelength.

The saponins test was carried out by preparing 100 mg of ecoenzyme solution (or equivalent) in reaction tube. Into that solution, about 10 mL of 2N sulfuric acid was added, hydrolyzed using reverse cooling method for 30 minutes, cooled, and extracted with 5 mL of chloroform solution. Next, the chloroform phase was taken, evaporated with nitrogen, and added with chloroform up to 500 µl. Next, 10 µl of the solution was spotted on the F254 silicagel plate and the plate was then put into a chamber containing a saturated mobile phase of chloroform: methanol (95:5). Next, elution was carried out until the limit line, then the plate was removed and dried. Finally, the plate was sprayed with sulfuric acid anisaldehyde reagent, heated at a temperature of 1100C to the maximum spot, and observed under UV wavelength.

Antibacterial activity

The antibacterial activity test was carried out using the disc method. The eco-hand sanitizer solution was dripped onto filter disc paper, then the paper was placed on solid media that has been inoculated with *Escherichia coli* and *Staphylococcus aureus*. The incubation process was carried out under optimal conditions with a temperature of around 37°C for 18-24 hours in an inverted petri dish. The inhibition zone was then analyzed using the radical method, i.e., measuring the area around the disc that was not contaminated with bacteria.

Results and Discussion

Initial screening of phytochemical compound

Several ecoenzyme solutions obtained by fermenting various raw materials, i.e fruit peels and or vegetables, apparently contained active compounds that were different from each other. Based on the phytochemical tests carried out, i.e., the alkaloids, flavonoids and saponins tests, it turned out that the four ecoenzyme solutions tested were negative for saponins content (Table 1). These initial results indicated that perhaps the four ecoenzymes did not contain saponin, which is one of the active compounds that plays a role in antiviral [13] , antifungal [14], and antibacterial [15]. These initial screening results must be further confirmed using a more sensitive method for detecting active compounds, such as TLC.

Table 1. Initial screening of phytochemicals compound's results

No	Ecoenzyme composition	pH	Alkaloid's test	Flavonoid's test	Saponin's test
1	3 types of fruit peels	3	+	+	-
2	2 types of fruit peels + 1 vegetable	3	+	-	-
3	3 types of vegetables	3	-	-	-
4	2 types of vegetables + 1 type of fruit peel	3	+++	++	-

Advanced screening of phytochemical compound

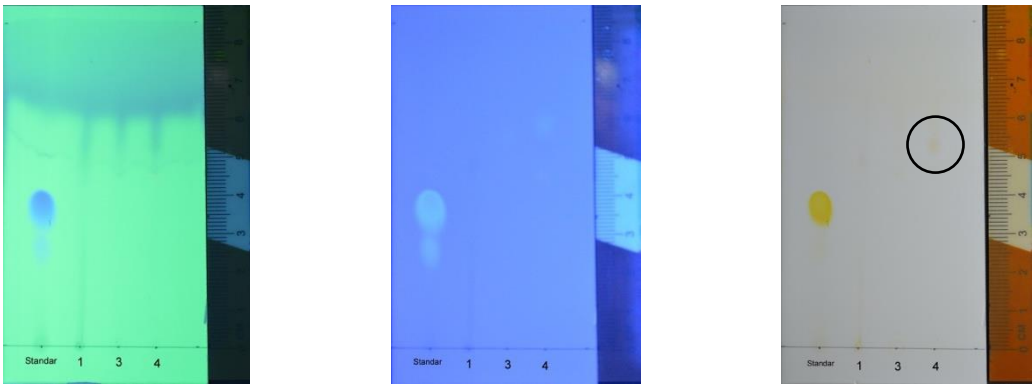
TLC analysis was carried out on 3 selected ecoenzyme solutions, i.e. ecoenzyme solutions derived from 3 types of fruit peels, 3 types of vegetables, and 2 vegetables 1 type of fruit peel. TLC analysis on alkaloids criteria showed the presence of this compound in the ecoenzyme solution derived from 2 types of vegetables and 1 type of fruit peel with 0.61 of Rf value (Table 2), with yellow spot indicator on the TLC plate (Figure 1C). The composition ecoenzyme solution derived from 2 types of vegetables and 2 types of fruit peels was lime, cabbage and long beans. With these results it can be said that those vegetables and fruits peels contained sufficient alkaloids after undergoing fermentation. Alkaloids content was reported to be contained in red cabbage, in addition to other active compounds such as glycosides, flavonoids, saponins, tannins, steroids, terpenes and phytosterols [16]. Other researchers reported that alkaloid content has also been contained in oranges [17] and long beans [18].

Table 2. Results of alkaloid analysis using TLC

Sample number	Qualitative test for alkaloids content	
	Result	Rf
1	-	-
3	-	-
4	+	0.61

Testing for the presence of flavonoids content was also carried out using TLC. The results of the analysis showed that there was 1 ecoenzyme solution that was positive for flavonoids test, i.e. ecoenzyme solution derived from 3 types of fruit peels (Figure 2; Table 3). The presence of flavonoid content in this ecoenzyme was probably because of

flavonoids compound contained in it. Melon was reported to contain several active compounds, one of which was flavonoids [19]. Polyphenol content in the form of flavonoids was reported to be contained in mango skin [20], and bananas [21].



A B C

Figure 1. Result of alkaloids analysis using TLC observed at: UV 254 nm wavelength (A); UV 365 nm wavelength (B); and VIS wavelength (C). Black circle showed the positive test.

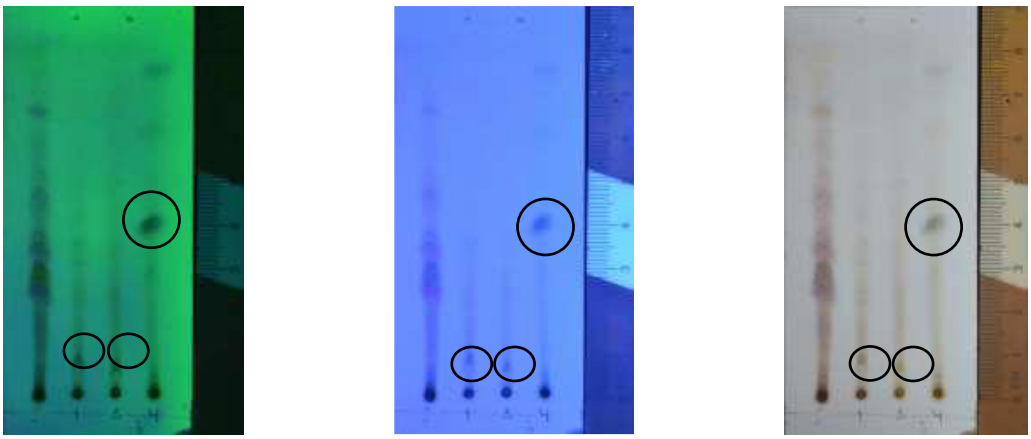
A B C

Figure 2. Result of flavonoids analysis using TLC observed at: UV 254 nm wavelength (A); UV 365 nm wavelength (B); and VIS wavelength (C). Black circle showed the positive test.

Table 3. Results of flavonoids analysis using TLC

Sample number	Qualitative test for flavonoids content	
	Result	Rf
1	+	0.32
3	-	-
4	-	-

Saponin was detected in all ecoenzyme solutions analyzed using TLC with a purplish-blue color at visible wavelengths (Figure 3). Saponins are a class of steroids and tritepen glycosides [22] and were reported to be found in bananas [23] and cabbage [24]. Saponins are reported to have biological activities such as antifungal [25] and antibacterial [26].



A B C

Figure 3. Result of saponins analysis using TLC observed at: UV 254 nm wavelength (A); UV 365 nm wavelength (B); and VIS wavelength (C). Black circle showed the positive test.

Antibacterial Activity

The gram-positive and gram-negative antibacterial tests of the three ecoenzyme solutions showed that the ecoenzyme solution containing the 3 types of fruit peels had the best gram-positive and gram-negative antibacterial activity (Table 4). Ecoenzyme solutions derived from 2 types of vegetables and 1 type of fruit peel apparently did not have gram-positive antibacterial activity. Thus, based on the results of this antibacterial analysis, ecoenzyme solutions derived from melon, banana and mango peels can be further investigated for its use as antibacterial agents. Antibacterial activity in melons has been reported [27] as well as in bananas [28] and in mangoes [29].

Based on alkaloids, flavonoids and saponin tests using TLC as well as antibacterial tests that have been carried out, it was known that the ecoenzyme solution derived from 3 types of fruit peels contained the most active compounds tested. This may be due to the compatibility of the sugar used for the fermentation process with the bacteria's found in 3 types of fruit peels which were used as basic ingredients for making ecoenzymes. Thus, in the future, it would be even better that further tests were to be carried out, such as testing the content of active compounds contained in ecoenzyme solution from 3 types of fruit peels, such as using GC-MS analysis.

Table 4. Diameter of inhibition zone of ecoenzyme solution

Number of samples	*Escherichia coli* (cm)	*Streptococcus aureus* (cm)
1	0.950	0.567
3	0.483	0.083
4	0.267	0

Conclusion

The ecoenzyme solution tested turned out to contain several active compounds. The TLC test for alkaloids content was detected in ecoenzyme solution derived from 2 types of vegetables and 1 type of fruit peel. Flavonoids and saponins content were detected in ecoenzyme solution derived from 3 types of fruit peels. Ecoenzyme solution derived

from 3 types of fruit peels was proven to be the best ecoenzyme solution contained gram-positive and gram-negative antibacterial activity.

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