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Phytochemical Test of Several Organic Fermentation Solutions

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ABSTRACT

Fermented organic materials usually contain active compounds that can be useful as antimicrobials. This study was conducted by fermenting organic materials such as fruits, vegetables, rhizomes, and flowers for 5 months. After that, the solution was filtered and analyzed for odor, pH value, and active compound content such as alkaloid, flavonoid, saponin, tannin, and triterpenoid tests. The results showed that all test solutions had a distinctive fresh pungent odor, with a pH value of around 2.5-3.5, and contained tested active compounds except for the saponin test. These results indicated that the fermented solution made has the potential to be used as an antibacterial or antifungal.

INTRODUCTION

Fermentation of organic materials with the addition of palm sugar or molasses has often been done by the general public, especially environmentalists. Organic materials that are usually fermented are usually vegetable and/or fruit waste. The results of the fermentation of vegetable and/or fruit waste are commonly known as ecoenzymes.

Ecoenzymes are known as beneficial solutions that can be used as plant fertilizers (1), natural preservatives (2), and can even be used as agents for killing bacteria and fungi (3). It has been reported that several secondary metabolites such as alkaloids and flavonoids (3) (4) are present in ecoenzyme solutions. These secondary metabolite compounds play a role in the effectiveness of ecoenzyme solutions.

Organic materials other than fruit and vegetable waste such as rhizomes and flowers also contain secondary metabolites. Several research reports state that ginger rhizomes contain secondary metabolites in the form of alkaloids (5) saponins (6), and phenolic compounds such as gingerol and shogaol (7). Lemongrass, which is also a member of the rhizome family, has been reported to contain terpenoids in the form of geranial compounds (8), phenolic compounds (9) and flavonoids and tannins (10). Aromatic ginger, turmeric, and galangal have been reported to contain secondary metabolites in the form of terpenoids in the form of cyperene (11), phenolic compounds in the form of curcuminoids (12) and alkaloids in the form of camptothecin (13). Secondary metabolites in the form of tannins, saponins, alkaloids, and terpenes have been reported to be contained in rose flowers (14) and alkaloids, flavonoids, saponins, and terpenes in jasmine flowers (15). Ylang-ylang flowers have also been reported to contain secondary metabolites in the form of terpenes, saponins, and phenols (16). Secondary metabolites contained in organic materials have positive benefits for humans, such as being used for medicines or other purposes. Secondary metabolites can be defined as active compounds produced from compounds resulting from primary metabolism. Secondary metabolite compounds can be categorized into several types, for example terpenoid, phenolic, and alkaloid groups. With the presence of secondary metabolite content in plants, plants can survive biotic and abiotic stresses because secondary metabolites act as protective agents or immunity in plants (17). Based on the above and based on literature searches that until now there has been no report related to phytochemical tests on rhizome and/or flower fermentation solutions, this study is deemed necessary to be conducted. The results of this study are expected to provide scientific information related to the phytochemical content contained in organic material fermentation solutions. Furthermore, the initial screening related to this phytochemical content is expected to be continued and can be used for the utilization of organic material fermentation solutions in order to improve the goodness for humans.

METHODS

Preparation of organic material fermentation solution

Organic materials with composition A (ginger, galangal, aromatic ginger, turmeric), composition B (rose flower, jasmine flower, ylang-ylang flower), composition C (lemongrass, ginger, galangal, ylang-ylang flower, rose flower, and aster flower), and composition D (durian skin, lemongrass, and water spinach) are mixed with molasses and water with a ratio of organic material: molasses: water of 3:1:10. The mixture is then put into a bottle and fermented for 5 months. The bottle cap is opened every day for 5 seconds in the first week of fermentation. Harvesting of the fermentation solution is done by filtering the solution so that a solution without pulp is obtained.

Odor and pH value test

The odor test is carried out on the fermentation solution without pulp by identifying the odor of the solution. The pH value test is carried out by inserting pH paper into the solution and then comparing the resulting color with the color standard on the pH paper packaging.

Alkaloid test

The alkaloid test is carried out by inserting 2 mL of the test solution into a test tube. Next, 5 drops of Dragendorff solution were added and shaken. A positive test is indicated by the presence of a reddish-yellow precipitate (18).

Flavonoid test

The flavonoid test is carried out by inserting 2 mL of the test solution into a test tube and then adding 5 mL of ethanol. The mixture is then heated and added with 5 drops of 2 N HCl and 0.2 grams of magnesium powder and left for 3 minutes. A positive test is indicated by the presence of a dark red color in the test solution (18).

Saponin test

The saponin test is carried out by inserting 2 mL of the test solution and 5 mL of distilled water into a test tube. Vigorous shaking is carried out until foam forms. A positive test is indicated by the presence of stable foam for 30 seconds with a thickness of about 1-3 cm (18).

Tannin test

The tannin test is carried out by inserting 2 mL of the test solution into a test tube and heating it in boiling water for about 5 minutes. Next, 1 drop of 1% $FeCl_3$ solution is dropped into the solution. Positive tannin test is indicated by the presence of blackish blue or greenish black sediment (19)

Triterpenoid test

The triterpenoid test is carried out by inserting 2 mL of the test solution into a test tube, adding 2 mL of glacial acetic acid, and leaving it for about 15 minutes. After that, 6 drops of the solution are taken and placed in a test cup. 2-3 drops of H_2SO_4 solution are then dripped into the test cup containing the solution. A positive test is indicated by the presence of a greenish blue color (18).

RESULTS

Table 1 Odor and pH value check

No	Solution	Compotition	Odor	pH value
1	A	ginger, galangal, aromatic ginger, turmeric	pungent acidic odor	3.5
2	B	rose flower, jasmine flower, ylang-ylang flower	pungent acidic odor	2.5
3	C	lemongrass, ginger, galangal, ylang-ylang flower, rose flower, and aster flower	pungent acidic odor	3
4	D	durian skin, lemongrass, and water spinach	pungent acidic odor	3

Table 2 Results of phytochemical tests on test solutions

No	Solution	Alkaloid test	Flavonoid test	Saponin test	Tannin test	Triterpenoid test
1	A	+	+	-	+	+
2	B	+	-	-	+	+
3	C	-	+	-	-	-
4	D	-	+	-	+	+

DISCUSSION

Odor and pH value check

Organic materials fermented for 5 months produce a brown solution typical of molasses with a unique odor. The uniqueness of the odor is similar to the odor of the ecoenzyme solution. In general, the odor identified in all test solutions is a pungent acidic odor.

This pungent acidic odor is usually correlated with the environment in the solution (20) Acidic conditions are usually closely related to the presence of an acidic odor. Usually solutions that have a sour odor have a pH below 7. This is in accordance with checking the pH value of the test solution, namely that all test solutions have a pH in the range of 2.5-3. Similar studies have shown that ecoenzyme solutions from tomatoes, oranges, mangoes, and pineapples have a pH below 7, which is around 3.07 (21) and ecoenzymes from papaya have a pH of around 3.29 (22)

The acidic conditions that occur in all test solutions are likely due to the organic acid content. According to (23), organic acids have various types, such as citric acid and malic acid. Malic acid is abundant in pears (24) while citric acid is abundant in lemons (25). In this study, no fruits were used as raw materials for ecoenzymes. However, it turned out that the resulting ecoenzyme solution was acidic. This is in line with the results of the study which stated that in the fermentation of rhizomes, for example ginger, organic acids such as malic acid, succinic acid, oxalic acid, acetic acid, and citric acid are produced (26) (27).

The organic acids contained in the test solution are products of the activity of anaerobic microorganisms. These anaerobic microorganisms utilize sugars, proteins, or lipids found in the skin of fruits and/or vegetables to be converted into smaller compounds such as glucose, amino acids, or short-chain fatty acids and finally obtain energy. Furthermore, according to (28) the results of fermentation can also be enzymes such as amylase, proteinase, catalase, cellulose, which are useful in biochemical reactions.

Fermentation in ecoenzyme solutions can generally occur in 2 main stages. The first stage is a process in which bacteria break down organic molecules into organic acids, carbon dioxide, hydrogen sulfide, and alcohol. At this time, bacteria use dissolved oxygen for this process, in addition nitrate and sulfate are reduced, resulting in a decrease in the acidity of the solution. In the second stage, metabolism occurs with raw materials in the form of first-stage products and produces methane gas, carbon dioxide, and mineral salts (29) Secondary metabolites are known as compounds found in plants that play a role in self-defense mechanisms. According to (30) secondary metabolites can be divided into 3 main groups, namely alkaloids, phenols, and terpenes. Alkaloids are organic compounds that have nitrogen, sulfur, oxygen, and may also contain phosphorus, chlorine, and bromine (31) and most have a bitter taste (32). Alkaloids can be divided into several groups such as true alkaloids, polyamine alkaloids, protoalkaloids, pseudoalkaloids, and peptide-cyclopeptide alkaloids. Alkaloids are reported to have a role as antifungals and antibacterials (33). The mechanism of antifungal and/or antibacterial agents in alkaloids is through binding of alkaloid molecules to DNA or topoisomerase enzymes which are related to DNA helix modification (34).

The results of the study showed that solutions A and B contained alkaloids, while solutions C and D did not contain alkaloids. The absence of alkaloids in solutions C and D is likely due to a special event in the fermentation process that occurred in both solutions or in fact both solutions contain alkaloids only in small quantities so that they are not detected by the simple phytochemical test that has been carried out. To ensure the presence of alkaloids in solutions C and D, further analysis is needed, such as alkaloid detection using chromatography. This assumption is supported by the results of studies (35), (36) which state that in rhizomes and flowers there are alkaloids that are detected using the thin layer chromatography (TLC) method (35). The same thing was also stated by (37) that thin layer chromatography (TLC) is a method to further confirm the results of phytochemical screening and can analyze small amounts of organic substances, including determining secondary metabolite particles. This method consists of a stationary phase and a mobile phase (eluent). The mobile phase or elution usually consists of a mixture of solvents that have good solubility so as to encourage elution and separation. The elution power and resolution are determined by the total polarity of the solvent, the polarity of the stationary phase, and the characteristics of the sample components.

Flavonoids are one of the secondary metabolite compounds that have antibacterial, antiviral, antioxidant, anti-inflammatory and antifungal properties

(38). Flavonoids can be grouped into several types such as flavones, flavanones, flavonols, catechins, flavanols, chalcones and anthocyanins (39). Flavonoids are classified as primary and secondary antioxidants (40), the mechanism of action as antioxidants by capturing free radicals through donation of hydrogen atoms to the free radicals (41)

The flavonoid test showed that solutions A, C, and D contained flavonoids, while solution B did not contain flavonoids. The absence of flavonoids in solution B is likely due to flavonoid degradation and the flavonoid levels in solution B are very low so that they cannot be detected using the simple phytochemical test that has been carried out. To ensure the presence of flavonoids in solution B, further research is needed such as flavonoid detection using the chromatography method. This assumption is supported by the results of research (41) which states that flowers contain flavonoids that are detected by the High Performance Liquid Chromatography (HPLC) method. The same thing was also stated by (42) that the High Performance Liquid Chromatography (HPLC) method was determined as the easiest method to use that allows the separation and identification of flavonoids.

Saponins are secondary metabolites and are included in the triterpenoid glycoside or steroid aglycone group, which consists of one or more sugar groups bound to aglycone or sapogenin (43). The structure of this saponin is like soap or detergent, so saponin is known as a natural surfactant (44), (45). Saponins play a role in plant defense mechanisms, especially because of their antifungal, antimicrobial, antiparasitic and molluscicidal activities (46), (47). The mechanism of action of saponins can be antifungal because their surface active substances are similar to detergents, which function to reduce the surface tension of the sterol membrane of the fungal cell wall, thereby increasing its permeability (48).

The saponin test showed that solutions A, B, C and D did not contain saponin. The absence of saponin in solutions A, B, C and D is likely due to the saponin levels in the solution being very low so that they cannot be detected using a simple phytochemical test or solutions A, B, C and D do not contain saponin. To ensure the presence of saponin in solutions A, B, C and D, further research may be required using several methods, such as TLC Scanner, UV-Vis Spectrophotometry, High Performance Liquid Chromatography (HPLC) and gravimetric methods (49), (50).

Tannin is a secondary metabolite in plants that has the ability to bind and precipitate proteins (51). Tannin is an organic compound consisting of a mixture of complex polyphenol compounds, which contain carbon, hydrogen, oxygen elements and often form large molecules with a higher molecular weight. Its chemical structure is divided into hydrolyzed tannins and condensed tannins (52). Tannin plays a role in antifungal defense, the way it works as an antifungal is by inhibiting the biosynthesis of ergosterol which is the main sterol produced by fungi as a component of the fungal cell wall (53). The tannin test showed that solutions A, B and D contained tannins while solution C did not contain tannins. The absence of tannins in solution C is likely due to the tannin levels being too low so that they cannot be detected using a simple phytochemical test. To analyze the tannin levels in solution C, further analysis is needed such as tannin detection

using phytochemical screening methods and chromatography (54). This assumption is supported by the results of research (54) (55) which states that in flowers and rhizomes there are tannins that are detected using phytochemical screening and thin layer chromatography (TLC) (54) The same thing was also stated by (56) that phytochemical screening is a method to identify the concentration of secondary metabolite compounds in natural products. While thin layer chromatography (TLC) is a method to further confirm the results of phytochemical screening and can analyze a small amount of organic matter, including determining secondary metabolite particles (37). Triterpenoids are secondary metabolites in plants (57) which have pharmacological activities such as antifungal, antiviral, antibacterial and anti-inflammatory (58). Triterpenoids are bioactive compounds that function as antifungals. These compounds can inhibit fungal growth, either through the cytoplasmic membrane or interfere with the growth and development of fungal spores (59) The mechanism as an antifungal by playing a role in producing an inhibition zone due to the toxic properties of the triterpenoid compounds in the extract, so that when the active compound is absorbed by pathogenic fungi it can cause damage to cell organelles, inhibit the work of enzymes in cells, and ultimately inhibit the growth of pathogenic fungi (60)

The triterpenoid test showed that solutions A, B and D contained triterpenoids while solution C did not contain triterpenoids. The absence of triterpenoids in solution C is likely due to the triterpenoid levels in the solution being very low so that they cannot be detected using a simple phytochemical test. To ensure the presence of titerpenoids in solution C, further analysis is needed such as triterpenoid detection using the chromatography method. This assumption is supported by the results of research (61) which states that rhizomes and flowers contain triterpenoids which are detected using the thin layer chromatography (TLC) method. This is in line with what was stated by (62) that thin layer chromatography (TLC) can also be used as phytochemical screening to determine terpenoid compounds in extracted samples.

CONCLUSIONS

all tested fermentation solutions have a distinctive odor like ecoenzyme solution, which is fresh and pungent. In the pH value indicator, all tested fermentation solutions show a pH value indicator of 2.5-3.5 and this value is included in the good category in the ecoenzyme pH value. Finally, all tested fermentation solutions have the activity of the active compounds tested except for saponin activity.

ADVANCED RESEARCH

It is hoped that this research can be continued with further phytochemical analysis using TLC or GC-MS to determine more convincing phytochemical content.

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