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Research Article

Identification of Bioactive Compounds from Cocoa Fruit Skin (*Theobroma Cacao* L.) with Variations in Solvent Polarity Properties

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Abstract: Bioactive compounds are chemical compounds formed through metabolic processes in plants, contributing to their beneficial properties. Cocoa fruit, derived from 22 cultivated Theobroma species, contains a variety of bioactive compounds that offer several health benefits. Identifying these bioactive compounds in plants can be achieved through extraction using various solvents, depending on their polarity properties. This study aims to explore the content of bioactive compounds in dried cocoa fruit peel (Theobroma cacao L.) extract, focusing on variations in solvent polarity. The results of the study showed that the 96% ethanol extract (polar solvent) of cocoa fruit peel contained a range of bioactive compounds, including flavonoids, tannins, alkaloids, and saponins. Ethanol, being a polar solvent, effectively extracted a variety of bioactive compounds due to its ability to dissolve polar substances. This extract is significant due to its wide range of potential health benefits attributed to the presence of flavonoids, tannins, alkaloids, and saponins, all known for their antioxidant, antimicrobial, and anti-inflammatory properties. Additionally, the acetone extract (semi-polar solvent) of cocoa fruit peel also contained alkaloids, flavonoids, tannins, and saponins. Acetone, as a semi-polar solvent, was able to extract a variety of compounds with moderate polarity, making it a versatile solvent for bioactive compound extraction. The presence of alkaloids and saponins in this extract indicates its potential use for various medicinal purposes. On the other hand, the n-Hexane extract (non-polar solvent) of cocoa fruit peel produced primarily alkaloids and terpenoids. Hexane, being non-polar, tends to extract non-polar compounds, which include alkaloids and terpenoids known for their various biological activities. In conclusion, the study demonstrates the varying effectiveness of solvents with different polarities in extracting bioactive compounds from cocoa fruit peel, which can be utilized for health-promoting purposes.

Keywords: Bioactive, Cocoa Fruit Peel, Extraction, Polarity, Solvent.

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1. Introduction

Compound bioactive is results from the metabolic processes in plants that produce something compound will but its function No so important in growth and development in plants the (Julianto, 2019) . Compounds bioactive often called compound metabolit secondary . Function from metabolit secondary For prevent growth bacteria , fungi and animals eater plants on a plant as well as maintaining and developing ability reproductive organs owned by plants That himself (Justinus et al ., 2018). Plants are known as the main producers of secondary metabolite compounds that have different high bioactive potentials that are used in the drug and pharmaceutical industry (Jain et al., 2019) .

Plants that contain bioactive compounds include the cocoa plant, where the most widely used part is the fruit. Included in the 22 cultivated Theobroma species, this plant is known to have various benefits and advantages (Rocio, 2018). Cocoa fruit (Theobroma cacao L.) is usually processed into chocolate, but the cocoa fruit skin is only thrown away as waste in large quantities and is not utilized, therefore cocoa fruit skin waste becomes a problem if it is produced in large quantities and not handled properly (Rachmawaty, 2018).

Utilization of cocoa fruit skin is one alternative effort in reducing the amount of waste produced by identifying bioactive compounds contained in cocoa fruit skin which are expected to be used in the world of health, especially in pharmaceutical studies.

These compounds can be identified through a qualitative analysis process after obtaining extracts from a plant (Wakeel et al., 2019) . Extraction is a procedure for separating active compounds from plant materials carried out through an extraction process using a solvent selected based on its suitability to the properties of the compound and its level of polarity (Azwanida, 2015) .

The choice of a solvent depends on the plant or plant part to be extracted, the plant's bioactive compounds, and the availability of a solvent. The solvents used for extraction are generally Some solvents have different levels of polarity, such as n-hexane, ethyl acetate, and chloroform which are non-polar; n-butanol and acetone as semi-polar solvents; and water, methanol, and ethanol which are classified as polar solvents. (Abubakar, 2020). The solvent variation in this study used ethanol, a highly polar alcohol solvent capable of efficiently dissolving compounds in a relatively short time. (Putu et al., 2020). Acetone is a simple ketone used as a semi-polar solvent in the extraction process of various types of organic compounds (Melia, 2018). Due to its stability, n-hexane as a non-polar solvent is effective for extracting large quantities of non-polar compounds. (Habibi et al., 2018).

Through the explanation above, researchers are encouraged to conduct studies to identify the content of secondary metabolite compounds in dried cocoa pod waste by utilizing various types of solvents as variations.

This study aims to analyze the variation in bioactive compound content in dried cocoa pod skin extracted with 96% ethanol, acetone, and n-hexane solvents.

2. Research Methods

Tools and materials

The equipment used in this study includes a stirring rod, funnel, Erlenmeyer flask, measuring cup, watch glass, filter paper, measuring flask, oven, dropper pipette, and test tube complete with support rack. analytical balance, water bath and maceration jar. While the materials used include: thick extract of cocoa fruit peel, Aquades, Acetic Acid, Hydrochloric Acid (HCl), Sulfuric Acid (H 2 SO 4), Magnesium Powder (Mg), Liebermann-burchard, chloroform (CHCl 3), Ammonia (NH 3), Mayer reagent reagent, reagent Dragendroff, Wagner reagent, FeCl 3 and solvents n - Hexane, Acetone, Ethanol 96%.

Research Procedures

Sample Preparation

Cocoa pods were used as sample material in this study, harvested in Mopusi village, Lolayan sub-district, Bolaang Mongondow regency, North Sulawesi province. The cocoa pods were separated from the pods, which were collected at 9:00-10:00 a.m. After wet sorting, the cocoa pods were cleaned by washing with running water until they reached an optimal level of cleanliness. They were then chopped to speed up the drying process. After that, the samples were placed in a container with small holes and dried. by covering it directly with plastic The samples were dried by keeping them away from direct sunlight. After drying, they were sorted to select high-quality fruit skins. The crude extracts were weighed and then placed in glass containers for maceration.

Extraction Process

Dried Cocoa Fruit Peel Extract

Simplicia powder was weighed 750 g each used for the extraction process using 3 types of solvents put into 3 glass containers, then measured as much as 1.875 L of n-Hexane, acetone and 96% ethanol solvents using a ratio of 1: 7.5, then soaked for 5 x 24 hours while stirring occasionally. After obtaining the macerate, it was filtered and then poured into a smaller container. The extract was concentrated in a water bath at a temperature of 40 o - 60 o C until a thick extract was obtained (Waraney et al., 2020)

Alkaloid Test

The testing process begins with the preparation of a blank, which involves taking 3 mL of sample extract and placing it in a test tube. Next, 2 mL of chloroform ($CHCl_3$) and 2 mL of ammonia (NH_3) are added, followed by 3–5 drops of H_2SO_4 to form two layers. The mixture is then divided evenly into three test tubes, each containing 1 mL. 3–5 drops of a different reagent are added to each tube. The presence of alkaloids is indicated by the formation of an orange precipitate after the addition of Dragendorff's reagent, a white or yellow precipitate with Mayer's reagent, and a brown precipitate when Wagner's reagent is added (Herman et al., 2020).

Flavonoid Test

The testing process begins with taking 1 mL of sample extract, which is then transferred to a test tube. Approximately 0.05 grams of magnesium powder and 4 to 5 drops of concentrated HCl are added. A positive indication of the presence of flavonoids is indicated by the appearance of a red or orange color in the solution. (Suryadi, 2019).

Tannin Test

Tannin test The test is carried out by adding 2 to 3 drops of $FeCl_3$ solution into a test tube containing 1 mL of extract. A positive reaction to tannin compounds is indicated by a color change to green or bluish-black (Khotimah, 2016).

Saponin Test

The test is performed by adding 1 mL of sample extract into a test tube, then adding 5 mL of distilled water (aquades) and shaking vigorously for approximately 10 seconds. A positive result is indicated by the appearance of a stable foam 1–10 cm high that persists for 10 minutes. To maintain the stability of the foam, one drop of HCl solution is added (Herman et al. , 2018).

Terpenoid Test

The terpenoid content test is performed by placing 1 mL of sample extract into a test tube and adding 0.5 mL of acetic acid. Afterward, 1–2 mL of Liebermann-Burchard reagent is slowly added along the wall of the tube. A positive result is indicated by a color change to purplish purple or reddish brown, indicating the presence of terpenoid compounds (Ikalinus et al., 2015).

Steroid Test

The test is similar to the terpenoid test. A 1 mL sample extract is placed in a test tube and 0.5 mL of HCl is added. Then, another 1-2 mL of Liebermann-Burchard is added through the wall of the test tube. A positive steroid result is indicated by the formation of a bluish-green color (Ikalinus et al ., 2015).

3. Results And Discussion

Extraction Results

The extraction results obtained an orange or red n-Hexane extract of 3.627 g, an extract from a brownish acetone solvent obtained of 4.836 g, an extract obtained from a 96% ethanol solvent of blackish brown as much as 5.237 g. Yield is a comparison of the weight of the simplicia that produces the extract, the yield value will indicate the amount of a bioactive compound contained in the plant (Waraney et al., 2020). The results of the extract yield for solvent variations can be seen in table .1.

Table 1 Extract Results

Types of Solvents	Dry sample (g)	Extract (g)	Yield (%)
n-Hexane	250	3,627	1.30
Acetone	250	4,838	1.93
Ethanol 96%	250	5,237	2.09

Based on the table above, it is stated that the results of each extract yield obtained for each extract are different. The increase in the average yield value appears to be in line with the increasing level of solvent polarity. The extract using 96% ethanol showed the highest yield compared to acetone and n-hexane solvents, because ethanol has a higher polarity than both solvents. This finding indicates that the bioactive compounds in cocoa pod skin have higher solubility in polar solvents than in semi-polar or non-polar solvents. (Hidayah, 2016). The percent yield of a sample is very necessary to determine the amount obtained during the extraction process. According to the Indonesian Herbal Pharmacopoeia, a good percent yield is not <7.2%, in this study the yield produced was small because one of the factors influenced by the speed and length of stirring time in the extraction process (Chandra, 2014).

Table 2. Results of Identification of Bioactive Compounds from Cocoa Fruit Peel (Theobroma Cacao L.) with Variations in Solvent Polarity Properties

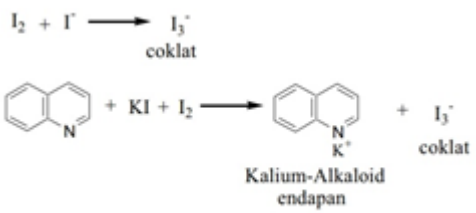
Compound Bioactive	Reagent	Test Results	Variation solvent		
			Ethanol 96%	Acetone	n-Hexane
Alkaloid	H_2SO_4P + Reagent Dragendorff	Sediment colored orange	-	-	-
	H_2SO_4P + Reagent Mayer	White or yellow sediment	-	-	+
	H_2SO_4P + Wagner Reagent	Sediment colored chocolate	+	+	-
Flavonoid	powder + HCl	Orange or red	+	+	-
Saponin	Aquadest	Stable foam	+	+	-
Tannin	$FeCl_3$ 10%	Blackish green	+	+	-
Terpenoid	Liebermann-burchard + acid acetate	Red or purple	-	-	+
Steroid	Liebermann-burchard + HCl	Green	-	-	-

Information :
a. (+) Contains compound chemistry
b. (-) Does not contain compound chemistry

Alkaloid testing of dried cocoa fruit peel extract (Theobroma cacao L.) using 96% ethanol, acetone, and n-Hexane solvents in this study was carried out using Dragendroff, Mayer, and Wagner reagents. The n-Hexane extract produced a yellowish white precipitate using Mayer's reagent, which means the cocoa fruit peel sample was positive for containing alkaloids.

test of the acetone extract obtained positive results using Wagner's reagent which was indicated by a brown precipitate, this indicated that the dried cocoa fruit peel extract (Theobroma cacao L.) using acetone solvent contained alkaloid compounds.

Alkaloid test by adding Drangerdroff, Mayer, Wagner reagents from dried cocoa fruit skin extract (Theobroma cacao L.) extracted using 96% ethanol solvent, the formation of brown precipitate occurred only in the reagents. Wagner showed positive results, this is in line with research conducted by Herman, (2020) alkaloids are generally found in plants in the form of salts that are only soluble in organic solvents, so they have the property of being difficult to dissolve in water but soluble in chloroform, acetone and alcohol.



Picture 1

The results of the flavonoid test of cocoa fruit peel extract (Theobroma cacao L.) using three different solvents, showed a positive result from the 96% ethanol extract, a red color change. This proves that the polar 96% ethanol solvent can attract polar flavonoid compounds as well (Nuryanti, 2014). The acetone extract also experienced a red color change in the flavonoid test based on this change, acetone, which has semi-polar properties, can attract polar and non-polar compounds, this means that the acetone extract positively contains flavonoids (Melia, 2018). While the flavonoid test using n-Hexane solvent did not produce a red or orange color change, so the negative result was not attracted to the flavonoid compounds contained in the dried cocoa fruit peel extract (Theobroma cacao L.). The n-Hexane solvent is non-polar so it cannot attract polar flavonoid compounds which can only be attracted by polar solvents (Habibi, 2018).

The results of the identification of saponins in dried cocoa fruit peel extract using 96% ethanol solvent obtained positive results, indicated by the appearance of foam. This refers to research conducted by Herman (2020) which used cocoa fruit peel extract extracted with ethanol solvent. Saponin testing for extracts using acetone solvents obtained positive results, indicated by the presence of stable foam for a period of 10 minutes which did not disappear. This shows that extraction with acetone solvents can extract saponin compounds contained in cocoa fruit skin extract .

The n-Hexane extract did not show any foam in the saponin test, indicating that the n-Hexane extract did not contain saponins because the foam formed after shaking did not last long, only a few seconds. Based on research conducted by Habibi, (2018) the absence of saponin compounds in the extract with n-Hexane solvent is non-polar, while saponins have glycosyl as a polar group that can be attracted by polar solvents .

Based on the results of testing tannin compounds with different solvent variations from dried cocoa fruit peel extract (Theobroma cacao L.), different results were obtained. The 96% ethanol extract showed positive results containing condensed tannins which were indicated by a blackish green color change. This is in line with research conducted by Mutmainnah (2017) that 96% ethanol solvent is polar and can attract tannin compounds. In the tannin test, the acetone extract showed a green color change, this indicates that the acetone solvent can attract tannin compounds contained in dried cocoa fruit peel.

Rachmawaty, (2018) stated that cocoa pod skin extracted with acetone has a high phenol content. While in the n-Hexane extract there is no green color change after the addition of FeCl 3 but instead becomes orange, it can be said that the dry cocoa pod skin extract extracted using n-Hexane solvent does not contain tannin compounds. The polar nature of tannin as a polyphenol compound allows its solubility in solvents that are also polar. (Romadanu, 2014).

The results of the identification of the presence of terpenoid and steroid compounds in each extraction result obtained different results, namely in the 96% ethanol extract in the test terpenoids produce an orange color and the steroid test produces a red color so it does not show a positive result Terpenoid and steroid compounds were detected in the extract, while the extract obtained using acetone was also analyzed to determine the content of both compounds. produces a brown color, there is no purple or green color change, indicating that 96% ethanol and acetone cannot extract terpenoid and steroid compounds. The cause is because the two types of solvents used are classified as polar and semi-polar so they cannot perfectly extract terpenoids and steroids. However, the terpenoid test on the n-Hexane extract sample obtained a positive result with a purple color change but there was no green color change for steroid compounds. Due to their non-polar characteristics, steroid compounds can be extracted effectively using non-polar solvents.

4. Conclusion

Identification of bioactive compounds in cocoa pods (Theobroma cacao L.) using different solvents based on their polarity showed varying results. Extracts using 96% ethanol, which is polar, contain alkaloids, flavonoids, saponins, and tannins. Acetone, a semi-polar solvent, is also capable of extracting alkaloids, flavonoids, saponins, and tannins. Meanwhile, non-polar solvents such as n-hexane only extract alkaloids and terpenoids.

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