Identification phytochemical compound of ethanol and acetone extract of Cocoa Pods (*Theobroma cacao* L.) using GC-MS

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Abstract. Identification of phytochemical components of ethanol and acetone extract of cocoa pods (*Theobroma cacao* L) has been carried out. Cocoa pods samples were extracted with maceration method using two kind of solvent, ethanol 96% and acetone 80%. Phytochemical compound of extract then identified using qualitative and Gas Chromatography - Mass Spectrophotometry (GC-MS) method. From the results of identification, both ethanol and acetone extract contained flavonoid, alkaloid, tannin, and saponins. It was found that the ethanol extract of cocoa pods contained 36 types of compound, and acetone extract of cocoa pods contained 26 types of compounds. Three major component of ethanol extract were isopropyl palmitate, 2-furancarboxaldehyde, 5-(hydroxymethyl)-, and oleic acid. Three major component of acetone extract were n-Hexadecanoic acid, oleic acid, and octadecanoic acid,2-(2-hydroxyethoxy)ethyl ester.

1. Introduction

Cocoa pods (*Theobroma cacao* L.) are one of the plantation commodities that have bright prospects because of their relatively high prices, easy marketability and economic significance as a foreign exchange earner. In Indonesia, especially in Lampung Province, cocoa is a crop of economic value as an export commodity. The future of this commodity is bright enough because it is estimated that world demand for this commodity will continue to increase. However, until now, cocoa farmers only use the seeds, while cocoa shells are not used and are just thrown away as waste. Along with the increase in cocoa production, the resulting waste will increase [1].

Fresh cacao pods are known to contain tannins, polyphenols, alkaloids and flavonoids [2]. Flavonoids and alkaloids have activity as insecticides, nematicides, larvacides, anti-fungi, and anti-inflammatory [3]. Phytochemical components in plants are chemical bioactive compounds that are produced naturally through primary or secondary metabolic pathways. Phytochemical components generally have biological functions for plants, including defending attacks from predators such as insects, fungi, interference from competitors, protection from pollution, stress, and even drought. In addition, phytochemicals are expected to be beneficial in human health or against various diseases [4].

The use of solvents in the extraction process affects the types of compounds that are extracted. Ethanol is a universal solvent because it is able to extract polar and nonpolar compounds and ethanol is also non-toxic so it is safe to use [5]. The reason for there were so many scientists used 96% ethanol solvent is that it
is more selective, that is, it only attracts the desired nutritious substances, has good absorption, molds and yeasts are difficult to grow, evaporates easily and gets a thick extract faster than 70% ethanol solvent [6]. Otherwise, acetone is a solvent which has semi-polar properties so that it can bind polar and non-polar compounds [7]. Based on this, scientists need to determine the extract compound of each solvent to know the phytochemical compound was extracted on each solvent. The results will help the scientist to determine which solvent is best uses for their cocoa pods research purpose. It is also hoped that the identification results of the phytochemical components of the cocoa pods extract can be used as a basis for information on natural ingredients that have the potential to become new medicinal ingredients in the health sector.

2. Methods

2.1. Materials

The tools and materials used in this study were oven, rotary evaporator, analytical balance, blender, water bath, electric balance, simplicia of cocoa pods (Theobroma cacao L.), 96% ethanol, and 80% acetone.

2.2. Sample Preparation

The pods used in this study were ripe and yellowish pods. The peels of the cocoa pods that have been sorted are wet then washed with running water until they are clean and then chopped to make drying easier. Drying is done by being aerated at room temperature until dry. The dried cocoa shells are then powdered using a blender or grinding machine. The pod husks were extracted with acetone 80% by maceration method. Acetone extract is made by soaking 500 g of simplicia in 3 liters of solvent for 24 hours and stirring occasionally. Extraction was carried out by repetition three times with a new solvent. The resulting extract was then filtered and the solvent evaporated using a vacuum evaporator at a temperature of 40°C.

2.3. Qualitative Analysis of Phytochemical Content

2.3.1. Flavonoid Analysis

A total of 3 mL of cocoa pod husk ethanol extract was inserted into tube a, and 3 mL of acetone extract of cocoa pod husks was put into tube b, then added with 3 ml of HCl and Mg powder. Positive results when the solution changes color to red, orange red [8].

2.3.2. Alkaloid Analysis

A total of 2 mL of ethanol extract of cocoa pod husks was put into tube a, and 2 mL of acetone extract of cocoa pod was put into tube b, then added a few mL of HCL and then filtered. The filtrate was then tested with Mayer's reagent (HgCl2 + KI). Positive results if there is a lit brass-colored precipitate, a white precipitate [9].

2.3.3. Tannin Analysis

A total of 2 mL of cocoa pod husk ethanol extract was put into tube a, and 2 mL of acetone extract of the cocoa pods were put into tube b, then added with 1% FeCl. A positive result when the solution turns blue or blackish green [8].

2.3.4. Saponins Analysis

A total of 2 mL of ethanol extract of cocoa pod skins was put into tube a, and 2 mL of acetone extract of cocoa pods was put into tube b, then added with enough distilled water and heated for 5 minutes, then shaken vigorously for 10 seconds. A positive result will be formed foam or foam that is stable for ± 10 minutes, as high as 1-10 cm [9].
2.4. Phytochemical Content Analysis by GC-MS

The analysis of the phytochemical content of the extracts used the GC-MS (Shimadzu GCMS-QP2010) Mass Spectrofotometry Gas Chromatography instrument. Helium is used as a carrier gas (0.85 mL / min) in the capillary column Rtx-5MS (60 mm x 0.25 mm x 0.25 μm) and is operated by electron impact mode (EI) at 70 eV and the ion source temperature at 200°C. The chromatography conditions were set at 50oC for the column oven temperature and 280°C for the injection temperature. The injection was carried out in split mode, i.e., isothermal at 50°C for 5 minutes and then increased the temperature to 280°C for 30 minutes then maintained at this temperature until 60 minute. Active compound components were identified based on the retention time and MS analysis.

3. Results and Discussion

The extraction process of the cocoa pod (Theobroma cacao L.) was carried out by maceration method. The maceration process was carried out with 96% ethanol and 80% acetone as a solvent to obtain a thick extract. The extraction results were analyzed qualitatively including the identification of flavonoids, alkaloids, tannins and saponins. The results of the analysis are presented in table 1. The ethanol extract and acetone extract of the cocoa pod husk showed positive results for flavonoids, alkaloids, tannins, and saponins. Cocoa beans are a rich source of flavonoids, especially epicatechin, and catechin and its polymer forms, the monomer of which is procyanidin. Different plant varieties and parts yield different compounds [10]. Polyphenols compound was known can affect the process of carcinogenesis through several mechanisms [11]. In particular, polyphenols are involved in counteracting the occurrence of oxidative stress, related to the prevention of cancer onset and development. Furthermore, polyphenol modulation of oxidative stress in cancer cells affects signal transduction, activation of redox-sensitive TFs, and the expression of specific genes that influence cell proliferation and apoptosis.

Table 1. Phytochemical Analysis of Cocoa Pod Extract.

| Phytochemical compound | Ethanol Extract | Acetone extract |
|------------------------|----------------|-----------------|
| Flavonoid              | +              | +               |
| Alkaloid               | +              | +               |
| Tannin                 | +              | +               |
| Saponins               | +              | +               |

Note:

+ = positive result, contained the phytochemical compound
-
= negative result, not contained the phytochemical compound

The results of the phytochemical analysis of the ethanol extract of cocoa pod using the GC-MS instrument are presented in Table 2, and the chromatogram is presented in Figure 1. Based on the results of the analysis by GC-MS, the ethanol extract of the cocoa pod contains 36 types of compounds (see table 2). Among the 36 types of compounds, there are 16 types of compounds in the fatty acid methyl ester group. Three major component of ethanol extract were isopropyl palmitate, 2-furancarboxaldehyde,5-(hydroxymethyl)-, and oleic acid.

The results of the analysis of the phytochemical content of the acetone extract of cocoa pods using the GC-MS instrument are presented in table 3, and the chromatogram is presented in Figure 2. The results of the phytochemical analysis of acetone extract of cocoa pods using the GC-MS instrument showed that there were 26 types of compounds that showed.
Figure 1. Chromatogram GC-MS Ethanol Extract of Cocoa Pod. X axes defines time (in minute), and Y axes defines amount of compounds.

The phytochemical content of the acetone extract of the cacao pods was dominated by the fatty acid methyl ester group, which was 15 compounds with a total abundance percentage of 93.94%. Three major component of acetone extract were n-Hexadecanoic acid, Oleic acid, and Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester. Cocoa pods contain components of alcohol, aldehyde, ketones, esters, and terpenoids. The alcohol class component acts as a unique aroma agent for the cocoa pods [12].

The results of phytochemical analysis of both the ethanol and acetone extracts were dominated by the fatty acid and ester compounds. The methyl ester fatty acid group is known to have many roles in the human body. The unsaturated fatty acid group such as octadecadienoic acid is a type of essential fatty acid that is not synthesized by the human body [13]. Octadecadienoic acid is also known to have an important role in cell growth by acting as cyclooxygenase-2 (COX-2) which catalyzes the biosynthesis of prostaglandin inhibitors [13].
Table 2. Results of Phytochemical Compound Analysis of Ethanol Extract of Cocoa Pods.

| No | Compound name                                                                 | % Area  |
|----|-----------------------------------------------------------------------------|---------|
| 1  | 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-                           | 8,79    |
| 2  | 4H-Pyran-4-one,3,5-dihydroxy-2-methyl-                                       | 2,79    |
| 3  | 1,2-Benzenediol                                                             | 1,40    |
| 4  | Cyclohexanol,2,3-dimethyl-                                                   | 1,78    |
| 5  | 2-Furancarboxaldehyde,5-(hydroxymethyl)-                                     | 16,90   |
| 6  | 4-Hydroxy-2-methylacetophenone                                               | 1,06    |
| 7  | Phenol,2-methoxy-6-(2-propenyl)-                                             | 0,46    |
| 8  | 3-Allyl-6-methoxyphenol                                                      | 0,21    |
| 9  | Phenol,2-methoxy-6-(2-propenyl)-                                             | 0,39    |
| 10 | Phenol,2-methoxy-5-(1-propenyl)-                                            | 0,18    |
| 11 | 3-Hydroxy-4-methoxybenzoic acid                                             | 2,90    |
| 12 | 1-Propanone,3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-                         | 2,38    |
| 13 | Methyl-(2-hydroxy-3-ethoxy-benzyl)ether                                      | 0,32    |
| 14 | 3,4-Dihydroxyacetophenone                                                   | 0,08    |
| 15 | Phenol,2,6-dimethoxy-4-(2-propenyl)-                                        | 1,78    |
| 16 | Tetradecanoic acid                                                           | 0,32    |
| 17 | 2-Hydroxy-4-isopropyl-7-methoxytropane                                      | 0,23    |
| 18 | 2-Propenoic acid,3-(4-hydroxy-3-methoxyphenyl)-                              | 0,12    |
| 19 | D-Glucose,4-O-.alpha.-D-glucopyranosyl                                       | 1,38    |
| 20 | .alpha.-D-Glucopyranose,.alpha.-D-glucopyranosyl                             | 4,09    |
| 21 | Hexadecanoic acid, methyl ester                                              | 0,34    |
| 22 | Pentadecanoic acid,14-methyl-,methyl ester                                  | 4,54    |
| 23 | Hexadecanoic acid, Z-11-                                                     | 0,37    |
| 24 | Isopropyl Palmitate                                                         | 18,81   |
| 25 | Octadecanoic acid,2-(2-hydroxyethoxy)ethyl ester                           | 0,14    |
| 26 | Hexadecanoic acid, ethyl ester                                               | 0,15    |
| 27 | 8,11-Octadecadienoic acid, methyl ester                                     | 2,36    |
| 28 | 6-Octadecenoic acid, methyl ester                                           | 1,08    |
| 29 | 9-Octadecenoic acid (Z)-,methyl ester                                       | 0,41    |
| 30 | Heptadecanoic acid, 16-methyl-,methyl ester                                 | 1,03    |
| 31 | 9,12-Octadecadienoic acid (Z,Z)-                                            | 9,53    |
| 32 | Oleic acid                                                                  | 9,77    |
| 33 | Octadecanoic acid,2-(2-hydroxyethoxy)ethyl ester                           | 2,52    |
| 34 | 9-Octadecenoic acid,12-hydroxy-,methyl ester,(Z)-                           | 0,43    |
| 35 | Ricinoleic acid                                                             | 0,41    |
| 36 | 2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-,(all E)         | 0,54    |
|    | Total                                                                       | 100     |
Figure 2. Chromatogram GC-MS Acetone Extract of Cocoa Pods
X axes defines time (in minute), and Y axes defines amount of compounds.

Unsaturated fatty acids in the human body are known to play many roles in health, including in cardiovascular metabolism [14]. Fatty acids are found in many plants and play an important role in improving brain function. As much as 60% of human brain tissue is composed of fatty acid groups [15]. Fatty acids also regulate lymphocyte metabolism and proliferation which induces T cell death, as well as enhancing autoimmune mechanisms. Fatty acids also play a role in anti-cancer mechanisms [16].
Table 3. Results of Phytochemical Compound Analysis of Acetone Extract of Cocoa Pod.

| No | Compound Name                                      | % Area |
|----|---------------------------------------------------|--------|
| 1  | Octanoic acid, methyl ester                       | 0.55   |
| 2  | Cyclohexanol                                      | 0.21   |
| 3  | 2,6-Octadiene,2,6-dimethyl-                       | 0.16   |
| 4  | Decanoic acid, methyl ester                       | 0.43   |
| 5  | Trifluoroacetyl-epiisoborneol                     | 0.16   |
| 6  | Phenol,2,4-bis(1,1-dimethylethyl)                 | 1.10   |
| 7  | Undecanoic acid, 10-methyl-,methyl ester          | 2.88   |
| 8  | Dodecanoic acid                                   | 0.30   |
| 9  | 1-Heptadecanol                                    | 0.30   |
| 10 | Pentadecanal-                                     | 0.43   |
| 11 | Tridecanoic acid,12-methyl-,methyl ester          | 1.74   |
| 12 | Tetradecanoic acid                                | 0.13   |
| 13 | 10-Heneicosene(c,t)                               | 0.20   |
| 14 | Pentadecanoic acid, methyl ester                  | 0.34   |
| 15 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol            | 1.68   |
| 16 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol            | 0.52   |
| 17 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol            | 0.97   |
| 18 | 7,9-Di-tert-butyl-1-oxaspiro(4,50deca-6,9-diene-2,8-dione | 0.34   |
| 19 | Hexadecanoic acid, methyl ester                   | 11.92  |
| 20 | n-Hexadecanoic acid                               | 31.02  |
| 21 | 8,11-Octadecadienoic acid, methyl ester           | 1.63   |
| 22 | 9-Octadecanoic acid (Z),methyl ester             | 3.38   |
| 23 | Heptadecanoic acid,15-methyl-,methyl ester       | 3.36   |
| 24 | 9,12-Octadecadienoic acid (Z,Z)-                 | 3.75   |
| 25 | Oleic acid                                        | 20.27  |
| 26 | Octadecanoic acid,2-(2-hydroxyethoxy)ethyl ester | 12.24  |
|    | Total                                             | 100    |

Based on Zhu et al., [17], fatty acids are very important repellents and significantly inhibit the attack of insects, including mosquitoes. In fact, the capacity of fatty acids as repellents is better than DEET. Dodecanoic acid, which is also found in tobacco smoke, is known to prevent mosquito bites, but in high concentrations (10x the DEET concentration). Based on [18], a mixture of octanoic, nonanoic and decanoic acid has repellent activity and prevents flies from landing. In addition, Hexadecanoic acid which is widely present in extracts is also reported to act as a repellent. Hexadecanoic acid is a carboxylic acid with a distinctive aroma. The carboxylic acid and ester compounds that dominate the extract of the cocoa pods cause an odor which is thought to act as a deterrent to mosquitoes [19]. The compounds of the fatty acid group are non-toxic, and have been widely used in the food and cosmetic industries.
4. Conclusion
The conclusion of this study is that the ethanol extract of the cocoa pods contains 36 types of compounds. Three major component of ethanol extract were isopropyl palmitate, 2-furancarboxaldehyde,5-(hydroxymethyl)-, and oleic acid. The acetone extract of the cacao pods contains 26 types of compounds. Three major component of acetone extract were n-Hexadecanoic acid, Oleic acid, and Octadecanoic acid,2-(2-hydroxyethoxy)ethyl ester.

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