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Phytochemical Profiling of Ethanol Extract from Cocoa Leaves (*Theobroma Cacao* L.) using Gas Chromatography-Mass Spectrometry

Skrining Fitokimia Ekstrak Etanol Daun Kakao (Theobroma cacao L.) Menggunakan Gass Chromatography Mass Spectrometry

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Abstract. Cocoa (Theobroma cacao L.) is one of the highest plantation commodity crops in Indonesia. Previous research showed that cocoa leaves were used as a larvicide, antioxidant, antibacterial, and anticancer. This study aims to determine the phytochemical compounds of ethanol extract of cocoa leaves qualitatively and quantitatively. Extraction of cocoa leaves using maceration method and phytochemical screening using Gas Chromatography-Mass Spectrometry (GC-MS) method. Based on the qualitative phytochemical screening of this study, alkaloid, flavonoid, and saponin tests showed positive results. GC-MS test analysis results showed ethanol extract from cocoa leaves detected 20 compounds. The extract with ethanol solvent produced the most compounds namely glycerol 1,2,3-Propanetriol, 1-acetate (CAS) 1-Acetoxy-2,3-Dihydroxypropane; 1,2,3-Propanetriol, monoacetate; and 1,2,3-Propanetriol, triacetate, phenolic 1,4-Benzenediol (CAS) Hydroquinone, and alkaloid 1H-Indole (CAS) Indole. Therefore, cocoa leaves have many bioactive compounds that have potent pharmacological effects.
Keywords: alkaloid; extraction; glycerol; pharmacologist; phenolic

Abstrak. *Kakao (Theobroma cacao L.) merupakan salah satu tanaman komoditas perkebunan cukup tinggi di Indonesia. Penelitian terdahulu menyatakan pemanfaatan daun kakao digunakan sebagai biolarvasida, antioksidan, antibakteri, dan antikanker. Penelitian ini bertujuan guna mengetahui senyawa fitokimia ekstrak etanol daun kakao secara kualitatif dan kuantitatif. Ekstraksi daun kakao menggunakan metode maserasi dan dianalisis menggunakan metode Gass Chromatography Mass Spectrometry (GC-MS). Berdasarkan skrining fitokimia secara kualitatif ekstrak daun kakao teridentifikasi senyawa alkaloid, flavonoid, serta saponin. Hasil analisis uji GC-MS menunjukkan ekstrak etanol daun kakao terdeteksi 20 senyawa. Ekstrak dengan pelarut etanol menghasilkan senyawa terbanyak yakni gliserol 1,2,3-Propanetriol, 1-acetate (CAS) 1-Acetoxy-2,3-Dihydroxypropane; 1,2,3-Propanetriol, monoacetate; 1,2,3-Propanetriol, triacetate, fenolik 1,4-Benzenediol (CAS) Hydroquinone, dan alkaloid 1H-Indole (CAS) Indole. Oleh karena itu, banyaknya senyawa fitokimia yang teridentifikasi pada daun kakao berpotensi memiliki efek farmakologis.*
Kata kunci: *alkaloid; ekstraksi; farmakologis; fenolik; gliserol*

INTRODUCTION

Indonesia has a high biodiversity of flora that is known to be useful in medicine, cosmetics, food industry, and beverages. Cocoa plants belong to the Sterculiaceae family which is spread throughout the island of Indonesia as one of the national plantation commodities. (Manalu, 2018; Farhanandi dan Indah, 2022; Sartika, 2022; Kadju *et al.*, 2022). Indonesia is the world's fourth largest cocoa producer with a production volume of 767,280 tonnes in 2018. One of the coffee and cocoa producers is Jember Regency, marked by the Indonesian Coffee and Cocoa Research Centre (Puslitkoka) (Felicia *et al.*, 2016; Handayani *et al.*, 2022). According to the Central Bureau of Statistics (2023), the cocoa plantation production of Jember Regency in 2022 was 2,957 tonnes. Cocoa cultivation is located in a tropical region with rainfall, high humidity, and relatively the same temperature throughout the year. The five parts of the cacao tree are the roots, trunk, leaves, flowers, and fruit. (Riono, 2020). The most commonly utilised and processed part of the cacao tree is the fruit.

Cocoa leaves are rarely processed into something useful. People use it as fertiliser but it is less than optimal and not many people know that cocoa leaves contain phytochemical compounds, which are antibacterial agents, antioxidants, biolarvicides, and anticancer agents (Priyanka dan Rani, 2018; Mandhaki *et al.*, 2021; Rani *et al.*, 2022; Sartika, 2022). Previous research on the exploration of the pharmacological effects of cacao plants has mostly been on the exploration of cacao fruit (Santos *et al.*, 2014; Indrianingsih *et al.*, 2021). Exploration of cacao leaves as pharmacological agents such as antioxidant and antiproliferative, antimicrobial, and antimalarial has been done (Osman *et al.*, 2004; Baharum *et al.*¸2014; Singh *et al.*, 2015; Komlaga *et al.*, 2021). However, quantitative identification or screening of phytochemical compounds from cocoa leaves has never been conducted.

Phytochemical compounds, also known as secondary metabolites, are sourced from plants and produced in flower, root, and leaf tissues. The most important preliminary procedure for obtaining phytochemical compounds is the efficient extraction of secondary metabolites with specialized biological activities for industrial processing of various medicinal products (Ozyigit *et al.*, 2023). Screening studies of phytochemical compounds are very important to determine specific pharmacological effects and can also be used for metabolomics studies.

Phytochemical screening of cocoa leaves using color reagents or qualitatively has often been done, while quantitative screening has never been done at all. One method of quantitative phytochemical screening uses the *Gas Chromatography Mass Spectrometry* (GC-MS) method. Hotmian et al. (2021) stated that the *Gas Chromatography-Mass Spectrometry* (GC-MS) test is a gas chromatography process used in conjunction with mass spectrometry to detect volatile compounds at high vacuum and low pressure when heated, determine molecular formula, and molecular weight, and obtain charged molecules. Based on the background, this study aimed to identify phytochemical compounds of ethanol extract from cocoa leaves qualitatively and quantitatively using GCMS. The potential of the chemical compounds found can be studied for their usefulness in the field of pharmacology.

MATERIALS AND METHODS

The extraction and phytochemical screening stages using cocoa leaf (*Theobroma cacao* L.) color reagents were carried out in June-August at the Toxicology Laboratory of Biology Education, University of Jember. The GC-MS analysis stage was conducted in August-September at the Bioscience Laboratory, Jember State Polytechnic. 1,000 g of dark green cocoa leaves were collected from Jember Regency, East Java. The extraction procedure of cocoa leaves followed the procedure of Rani et al (2022) with slight modifications. The leaves were cleaned with water and cut, then dried for 7 days. The dried 700 g cocoa leaf simplisia was ground using a grinding machine to obtain 500 g of powder which was then macerated as much as 2,000 mL of 96% ethanol solvent (1:4 ratio) in a jar and then tightly closed for 3×24 hours occasionally stirred using a glass stirrer. The results of soaking the liquid extract simplisia as much as 1,000 mL were evaporated using a rotary evaporator to get 1 g of thick cocoa leaf extract.

Pure compounds were tested qualitatively for secondary metabolites using the color reagent method. 1 g of thick extract was divided into three parts to test for the presence of alkaloid, flavonoid, and saponin compounds. The alkaloid test was carried out on 0.3 g which was dripped with 10 drops of HCl and distilled water solution, immersed in hot water for 3 minutes. 10 drops of Meyer's reagent were given, a brown precipitate appeared signaling a positive result (Najmah *et al.*, 2023). The flavonoid test was by mixing 0.3 g of the extract with 10 drops of H2SO4 solution, a yellow, red, or brown color appears, indicating the presence of flavonoid compounds. (Munadi, 2020). The saponin test used 0.3 g of extract dissolved in 10 mL of hot water and then shaken for 10 minutes until foam appears (Iskandar, 2020).

About 1 g of thick cocoa leaf extract was incubated at 300ºC for 54 minutes. Transfer into GC-MS injector with split injector 260ºC. High-purity helium (P = 70.0 kPa) was used as carrier gas (1.16 mL/min). All data was generated according to the WILEY7.LIB library and metabolite functional data were obtained through the PubChem database, as well as literature studies.

RESULT

The results of the phytochemical compound screening of cocoa leaves obtained by the method of using color reagents can be seen in Table 1. These results show that cocoa leaf extract contains alkaloid and flavonoid compounds. Flavonoid compounds detected are less strong because the color formed is light brown. In addition, screening of saponin compounds showed weak results with a little foam.

This research is not only qualitative phytochemical screening with reagents but also quantitative screening with GC-MS. The results of the GC-MS chromatogram of cocoa leaf extract can be seen in

Figure 1 and the results of the relative percentage of compounds detected in cocoa leaf extract can be seen in Table 2.

Table 1. Qualitative phytochemical screening results of cocoa leaf (*Theobroma cacao* L.) extracts

Phytochemical test	Reagents	Description	Results
Alkaloid	Extract+HCl + Aquadest	A little sediment and color changes into light brown	++
Saponin	Extract+hot water+Chloric acid 2N	A slight foaminess	+
Flavonoid	Extract+H_2SO_4	Color changes light brown	+

Notes: contain many secondary metabolites (++), contain few secondary metabolites (+)

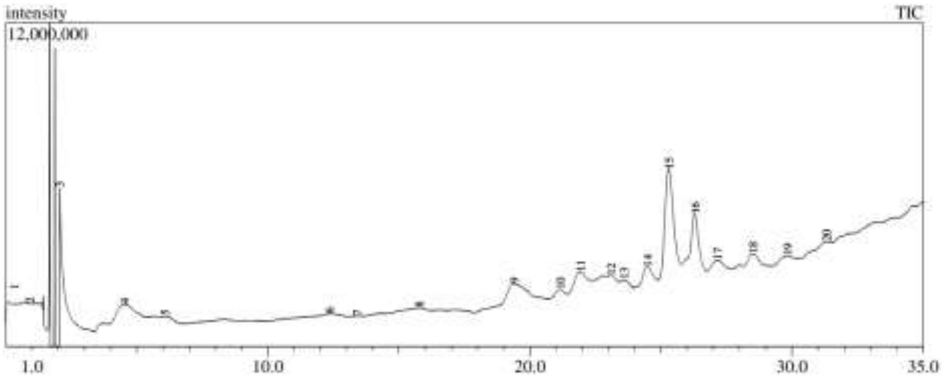

Figure 1. GC-MS chromatogram results of cocoa leaves (*Theobroma cacao* L.)

Table 2. The relative percentage of compounds detected in cocoa (*Theobroma cacao L.*) leaf extracts

Peak#	RT (minute)	Compounds	% Area
Alkaloids			
1	0.333	2-Heptanamine (CAS) Heptin	0,69
2	0.923	METHYL-PENT-4-ENYL-AMINE	0,71
10	21.171	1-Azabicyclo[2.2.2]octan-3-ol (CAS)	2,12
18	28.521	**1H-Indole (CAS) Indole**	7,99
Glycerole			
12	23.109	**1,2,3-Propanetriol, 1-acetate (CAS)**	6,33
15	25.318	**1,2,3-Propanetriol, monoacetate**	15,32
16	26.312	**1,2,3-Propanetriol, triacetate**	10,73
Phenolics			
6	12.364	Heptanal (CAS) n-Heptanal	1,79
8	15.794	2-Decen-1-ol, (Z)- (CAS) cis-DEC-2-ENOL	1,14
9	19.405	**Benzenesulfonic acid, 4-hydroxy- (CAS)**	7,97
11	21.939	Benzenepropanoic acid, 3,4-dimethoxy-	5,15
13	23.607	Phenol, 2,4-dimethyl- (CAS) 2,4-Xylenol	1,87
14	24.499	Phenol, 4-ethyl- (CAS) p-Ethylphenol	4,30
17	27.174	Phenol, 2-methyl-5-(1-methylethyl)-	5,04
19	29.844	1,5:2,4-Dimethanopentalene-3,6-diol	5,41
20	31.327	**1,4-Benzenediol (CAS) Hydroquinone**	8,77
Others			
3	2.060	Ethanol (CAS) Ethyl alcohol	8,42
4	4.542	Acetic acid (CAS) Ethylic acid	5,00

DISCUSSION

Based on the GC-MS results in Table 1, 20 compounds were detected from the cocoa leaf extract. There are nine phenolic group compounds detected. Besides phenolics, the most compounds detected were glycerol compounds (1,2,3-Propanetriol, 1 acetate; 1,2,3-Propanetriol, monoacetate; 1,2,3-Propanetriol, triacetate). The compound 1,2,3-Propanetriol, monoacetate was the highest compound detected at 15.32%. This compound has potential as an antimicrobial, anti-inflammatory, and anticancer (Casuga *et al.*, 2016). Two other propanetriol compounds; 1,2,3-Propanetriol, 1 acetate from Punica granatum have antimicrobial potential, and 1,2,3-Propanetriol, triacetate from *Bacillus amyloliquefaciens* AMM has potential as an anti-*Salmonella* agent (Harini *et al*., 2018; Omar *et al*., 2021).

The most abundant phenolic compound was 1,4-Benzenediol (CAS) Hydroquinone which was detected at 8.77%. This compound has clinically proven and relevant antimicrobial activity on several pathogens (Lana *et al*., 2019). Benzenesulfonic acid, 4-hydroxy compounds including phenolic compounds were detected as much as 7.97%. This compound has not done much research on pharmacological effects. In addition to phenolics, there are five alkaloid compounds detected in cocoa leaf extract. The most alkaloid compound was 1H-Indole (CAS) Indole, detected at 7.99%. This compound has larvicidal activity against *Aedes aegypty* larvae (Diaz *et al*., 2022).

Quantitative phytochemical screening research on cocoa leaf extract has never been done, but qualitative phytochemical screening has been done by Parbuntari *et al* (2018). The study showed that the ethanol extract of cocoa leaves identified alkaloids through 3 types of tests. In addition, flavonoids, terpenoids, and saponins were also identified in the ethanol extract of cocoa leaves (Parbuntari *et al*, 2018). Abulude *et al* (2022) research also showed the results of qualitative phytochemical screening of cocoa leaf extracts with different types of extraction solvents. The results showed that the ethanol extract of cocoa leaves identified carbohydrate compounds, tannins, resins, flobatanins, and alkaloids. Flavonoids and saponins were not identified in the ethanol extract of cocoa leaves (Abulude *et al*., 2022).

Based on the results of qualitative phytochemical screening and quantitative screening using GC-MS, there is a correlation that there are alkaloid compounds in cocoa leaf extract. In addition to alkaloids, flavonoids and saponins compounds detected in qualitative screening were not detected in GC-MS. This proves that the results of qualitative phytochemical screening are less accurate than phytochemical screening using GC-MS. Future research can be carried out to isolate phenolic compounds or alkaloids followed by pharmacological tests both in vitro and in vitro to evaluate antioxidant, antimicrobial, biolarvicidal, or hepatoprotective effects.

CONCLUSION

The results of phytochemical screening proved that cocoa leaf extract has several secondary metabolite compounds of alkaloid, phenolic, and glycerol groups quantitatively through GC-MS analysis.

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