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# EVALUATION OF THE BIOACTIVE COMPOSITION OF COCOA POD HUSK FROM SULAWESI ISLAND, INDONESIA, FOR HEALTH BENEFITS

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## ABSTRACT

**Background.** Indonesia is the world's third-largest cocoa producer. Processing cocoa beans into chocolate products generates a substantial amount of cocoa pod husk (CPH) waste. The island of Sulawesi is a major cocoa hub in Indonesia. However, there has been limited exploration of the phytochemical content of cocoa pod husks originating from this region.

**Materials and methods.** The proximate composition was determined using the official AOAC method. A spectrophotometer and GC-MS analysis were used to identify polyphenols, flavonoids, and tannins, as well as other chemical compounds in CPH.

**Results.** CPH has an average moisture content of 25.63%, ash content of 8.48%, fat content of 0.65%, protein content of 10.34%, and carbohydrate including fiber content of 54.91%. The total polyphenol content is 98.68 mg GAE/g, flavonoid content is 3.58 mg quercetin/g, and tannin content is 79.12 mg EGCG/g. A total of 23 compounds were identified, and based on their chemical structures, the compounds in CPH are derivatives of terpenoids, fatty acids, and steroids. The largest compound compositions are butyric acid (25.91%), Hexadecanoic acid, Methyl ester (24.35%), 13-Docosenoic acid, Methyl ester, (Z)-(CAS) (11.05%), and 2-Furancarboxaldehyde (8.14%). Butane-1,2,3,4-tetraol (5.44%), 9,12-octadecadienoic acid methyl ester (4.71%), and Methyl 2-hydroxy-3-butenoate (3.67%).

**Conclusion.** The CPH originating from the island of Sulawesi, Indonesia, possesses a bioactive composition that is nearly identical to CPH sourced from various other countries, as well as other regions within Indonesia, despite varying levels. These compounds exhibit beneficial effects on health. However, further evidence

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and development are still required to verify the health benefits of CPH, both in vitro and in pre-clinical settings, prior to actual human application.

**Keywords:** bioactive composition, cocoa pod husk, derivative compound, GC-MS, health benefits

## INTRODUCTION

Cocoa (*Theobroma* L. *cocoa*) is a food crop plant that naturally inhabits tropical climates (ICCO, 2022). Global cocoa production between 2022 and 2023 reached 4.9 million tons. Indonesia, as one of the tropical countries, is the world's third-largest cocoa producer after Ghana and the Ivory Coast. Estimations based on the double exponential smoothing model indicate that the average annual growth of cocoa production in Indonesia from 2022 to 2026 is expected to be positive at 1.97%, despite fluctuations in the increase. It is projected that cocoa availability in Indonesia will rise from 509 thousand tons in 2022 to 550 thousand tons in 2026 (Kementerian Pertanian, 2020).

The production of chocolate products from cocoa beans generates a considerable amount of cocoa bean husk waste. On average, 1 ton of dried cocoa beans produces about 10 tons of cocoa bean husk waste (Campos-Vega et al., 2018). Exploration surveys conducted among cocoa farmers in Indonesia reported that 57% of CPH is not used at all, 34% is discarded as fertilizer, 8% is utilized as livestock feed, and 8% is used as combined fertilizer.

Recent research developments in various countries have reported that CPH contains various bioactive compounds with the potential to yield pharmaceutical, medical, nutraceutical, novel functional food products, and even cosmetics (Nguyen and Nguyen, 2017; Campos-Vega et al., 2018; Vásquez et al., 2019). CPH serves as a source of flavonoids, phenolic acids, and various other active phytochemical compounds (Figueroa et al., 2020). Flavonoids and polyphenols are natural antioxidants that correlate with protection against the development of chronic diseases such as cardiovascular diseases, diabetes, cancer, and other chronic conditions related to inflammation and oxidative stress (Rebollo-Hernanz et al., 2019; Felice et al., 2020).

Although the chemical composition of CPH has been extensively studied in several countries, each of them has focused on specific chemical compositions and methods, and of course, the origin of the plant is different despite sharing a tropical climate. Similarly in Indonesia, several studies have been conducted to measure the antioxidant content of CPH, including the analysis of flavonoid content in methanol extract of CPH from West Java (Azizah et al., 2014), phenolic content in ethanol extract of CPH from Bali based on maceration duration (Pratyaksa et al., 2020), tannin content in methanol extract of CPH from South Sulawesi and West Sulawesi (Pappa et al., 2019), and total polyphenols in liquid volatile matter from pyrolysis of CPH from Southeast Sulawesi (Pallawagau et al., 2019). However, chemical compositions can vary due to the diverse climate, soil, topography, and environmental physical properties in Indonesia, both across regions and within a single region (Djaenudin et al., 2002). Climate factors such as temperature, rainfall, humidity, wind, and sunlight intensity directly influence enzyme activity in metabolic processes, ultimately impacting the chemical composition of cocoa (Tjahjana et al., 2008; Alam et al., 2010)

A renewed commitment to the reevaluation of cocoa bean husks may be significant for Indonesia, as it is one of the largest cocoa-producing countries in the world. In this country, cocoa is a prominent plantation commodity that plays a crucial role in the Indonesian economy, serving as a provider of employment, a source of income for farmers, and a foreign exchange earner for the nation (BPS, 2018; Kementerian Pertanian, 2020). However, information regarding proximate and other bioactive compounds in cocoa bean husks is still limited, especially those originating from Southeast Sulawesi as one of the main cocoa centers in Indonesia. Therefore, this study aims to focus on proximate content, polyphenols, flavonoids, and tannins, and qualitatively determine other bioactive compound compositions to add value to cocoa bean husk waste from this region and to explore new avenues for

utilizing cocoa waste as an abundant, cost-effective, and renewable resource, particularly for the development of functional foods, pharmaceuticals, and medical applications.

## MATERIALS AND METHODS

The preparation of CPH extract was conducted in the integrated laboratory of the Faculty of Pharmacy, Haluoleo University, Southeast Sulawesi. Compound analysis was conducted in the Biofarmaka laboratory of the Faculty of Pharmacy, Hasanuddin University, and the Chemistry laboratory of Ujung Pandang State Polytechnic.

### Materials

The raw material, mature cocoa pods without visible damage or defects on the skin, were collected from smallholder plantations in Kolaka Regency, Southeast Sulawesi Province, Indonesia (-4.040324, 121.549185). Ethanol P. (Medika), methanol P. (Jennychem), Folin-Ciocalteau reagent (Sigma-Aldrich), quercetin (Sigma-Aldrich), epigallocatechin gallate (EGCG) (Sigma-Aldrich), distilled water, NaOH (Merck KGaA, Germany), aluminum chloride (Merck KGaA, Germany), and sodium acetate (Merck KGaA, Germany) were used as materials.

### Methods

#### Preparation of CPH samples

Fresh CPH samples were obtained from ripe cocoa fruits, separated from the seeds, cleaned, and then cut into small pieces approximately 2.5 cm in size (Figueroa et al., 2020). These were air-dried in an open room for about a week and subsequently ground into a powder using a blender (Pappa et al., 2019). The extraction process employed the maceration method utilizing 96% ethanol as the solvent for 48 hours (Quiroz-Reyes et al., 2013; Pratyaksa et al., 2020). Subsequently, the solution was filtered using an aspirator device, followed by evaporation using a rotary evaporator. The utilization of ethanol as the solvent was due to its effectiveness, particularly for the extraction of various phenolic compounds, including flavonoids (Hernández-Hernández et al., 2019).

#### Determination of proximate content

The proximate composition was determined using the official AOAC method (1998). Moisture content was determined using a hot air oven at 100°C overnight. Ash content was measured by heating the CPH in a furnace at 600°C for 5 hours. The crude lipid content was determined using the Soxhlet extraction method after the extract was dried in an oven at 100°C for 30 minutes. The crude protein content was determined by measuring the nitrogen content using the Kjeldahl method, and then the protein content was calculated by multiplying the nitrogen content by a factor of 6.25. Carbohydrates, including crude fiber, were calculated using the equation: % Carbohydrate = 100 – (moisture + ash + lipid + protein).

#### Determination of total polyphenol content

Total polyphenols were determined using the Folin-Ciocolteau reduction method with gallic acid as the standard (Waterhouse, 2022). Approximately 0.2 g of extract was placed into an Erlenmeyer flask, followed by the addition of 25 mL of *methanol P*, and stirred for 30 minutes using a magnetic stirrer. The mixture was filtered into a 25 mL volumetric flask, and *methanol P* was added to the mark. Additionally, 10 mg of reference solution was added to another 25 mL volumetric flask, dissolved, and then topped up with *methanol P* to the mark. Dilutions were prepared with concentrations of 5, 15, 30, 50, 70, and 100 µg/mL. For each sample, 1 mL of the test solution and diluted reference solution was mixed with 5 mL of Folin-Ciocalteu reagent (7.5% in water) in a test tube. The mixture was incubated for 8 minutes, followed by the addition of 4 mL of 1% NaOH and further incubation for 1 hour. Incubate for 8 minutes, then add 4 mL of 1% NaOH and incubate for 1 hour (Kementerian Kasehatan, 2013). The sample concentration was 1000 ppm. Subsequently, the polyphenol content of CPH was calculated based on the calibration curve of standard gallic acid by comparing the retention time and peak area of the standard gallic acid with the extract of cocoa bean husk. Measurement is performed using a UV-Vis Spectrophotometer. The calibration curve of standard gallic acid is shown in Figure 1.

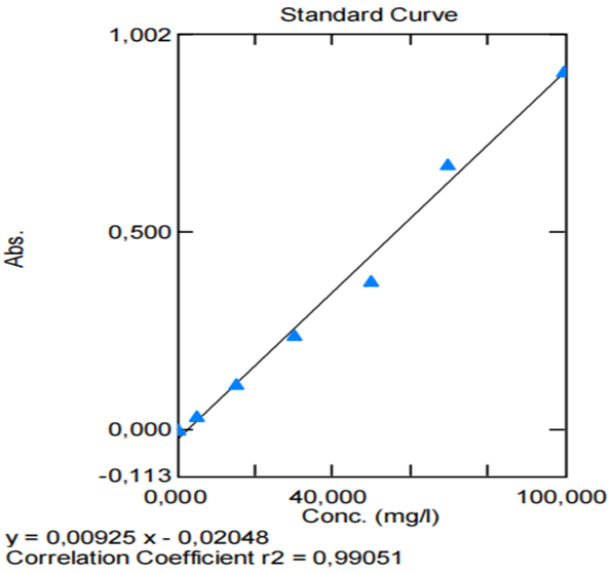


**Fig. 1.** Calibration curve of standard gallic acid

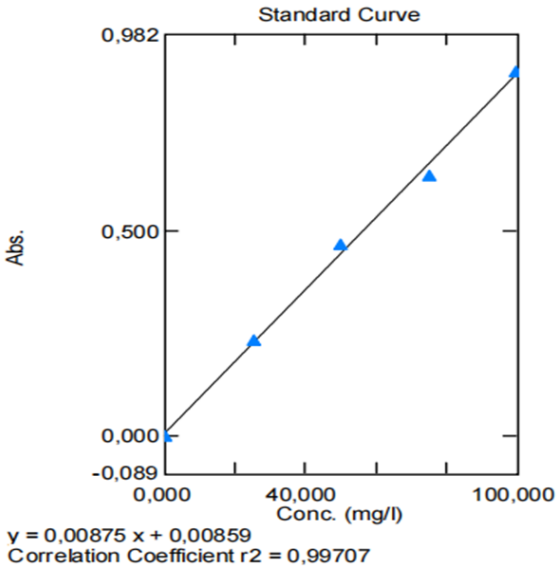


**Fig. 2.** Calibration curve of standard quercetin

### Determination of total flavonoid content

Total flavonoids were determined following the established method (Kementerian Kesehatan, 2013) with slight modifications. About 0.2 g of the extract was placed in an Erlenmeyer flask, and 25 mL of *ethanol P* was added. The mixture was stirred for 30 minutes using a magnetic stirrer. It was then filtered into a 25 mL volumetric flask, and *ethanol P* was added up to the mark. Additionally, 10 mg of quercetin was placed into a 25 mL volumetric flask, dissolved, and topped up with ethanol P to the mark. Quantitative dilutions were prepared with concentrations of 25, 50, 75, and 100 µg/mL. To carry out the measurement, 0.5 mL of the extract solution and the diluted quercetin solution were separately added to 1.5 mL of ethanol P, 0.1 mL of 10% aluminum chloride P, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. The mixture was shaken and allowed to stand for 30 minutes at room temperature. Blank measurement was performed in the same manner without adding aluminum chloride. The sample concentration was 8000 ppm. Subsequently, the content of flavonoids in CPH was calculated based on the calibration curve of standard quercetin (Pandey et al., 2020) by comparing the retention time and peak area of the standard quercetin with the extract from cocoa fruit peel. Measurement is conducted using a UV-Vis Spectrophotometer. The calibration curve of standard quercetin is shown in Figure 2.

### Determination of tannin content

A total of 0.1 g of extract is placed into an Erlenmeyer flask, followed by the addition of 25 ml of *methanol P.* Stir the mixture for 30 minutes using a magnetic stirrer. Filter it into a 25 ml volumetric flask, then add *methanol P* up to the mark. A reference solution of 10 mg is placed into a 25 ml volumetric flask, and methanol P is added up to the mark. Dilutions are prepared with concentrations of 5, 15, 30, 50, 70, and 100 µg/mL. For each 1 mL of the test solution and the diluted reference solution in separate test tubes, add 2.5 mL of Folin-Ciocalteu reagent (7.5% in water). Let it stand for 8 minutes, then add 2 mL of 1% NaOH and incubate for 60 minutes. Then, measure the absorbance of each solution at a maximum absorbance wavelength of approximately 730 nm. Perform blank measurements in the same manner, without adding the test solution. The sample concentration is 500 ppm. Subsequently, the tannin content of CPH is calculated based on the standard epigallocatechin gallate (EGCG) calibration curve by comparing the retention time and peak area of the standard EGCG with the cocoa fruit peel extract. Measurements are conducted using a UV-Vis

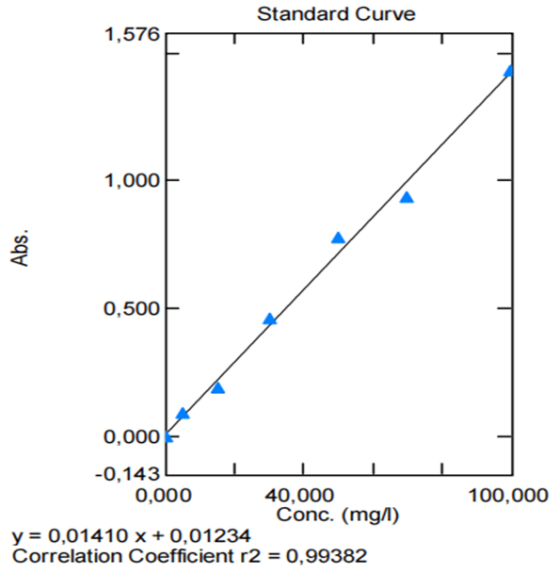


**Fig. 3.** Calibration curve of standard EGCG

Spectrophotometer. The calibration curve of the standard EGCG is depicted in Figure 3.

### Gas chromatography–mass spectrometry (GC-MS) analysis

The GC-MS analysis was conducted using a Shimadzu GC-MS Ultra QP 2010 instrument. Initially, approximately 0.2 g of the isolate sample was prepared, and 5 mL of methanol (p.a) was added. Extraction was carried out using a sonicator for 20 minutes at a temperature of 40°C. The resulting extract was then placed into GC-MS vials. The instrument conditions for GC-MS included an injector temperature of 250°C in Splitless mode, a pressure of 76.9 kPa, a flow rate of 14 mL/min, and a split ratio of 1:10. The ion source and interface temperature were set at 200°C and 280°C, respectively. Solvent cut time was 3 minutes, and the mass range was set from 400 to 700 m/z. The column used was SH-Rxi-5Sil MS, with a column length of 30 meters and an inner diameter of 0.25 mm. The initial column temperature was 70°C with a hold time of 2 minutes. The temperature was then raised to 200°C at a rate of 100°C/min, and finally to 280°C with a hold time of 9 minutes at a rate of 50°C/min, resulting in a total analysis time of 36 minutes. The chromatographic data obtained was interpreted using the NIST 17 and Wiley 9 libraries.

## RESULTS AND DISCUSSION

### Proximate composition of CPH

Table 1 presents the proximate composition of ethanol extract from CPH. The moisture content remains relatively high at approximately (25.63%). The fat content is relatively low (0.65%), while the protein content remains within the average normal range for CPH, at 10.34%.

**Table 1.** Proximate content in CPH

| Parameters | (%) ±SD |
|---|---|
| Moisture | 25.63 ±0.30 |
| Ash | 8.48 ±0.12 |
| Fat | 0.65 ±0.02 |
| Protein | 10.34 ±0.04 |
| Carbohydrates (including crude fiber) | 54.91 ±0.41 |

### Polyphenol, flavonoid, and tannin content

Table 2. The total polyphenol content contained in this research is 9.868% w/w, equivalent to 98.68 mg GAE/g. The total flavonoid content is 0.358% w/w, corresponding to 3.58 mg quercetin/g. The total tannin content is 7.912% w/w, corresponding to 79.12 mg EGCG E/gram sample/CPH.

**Table 2.** Content of polyphenols, flavonoids, and tannins in CPH

| Parameter | Total sample concentration (ppm) | Absorbance | Total sample concentration % w/w |
|---|---|---|---|
| Polyphenol | 98.669 ±1.342 | 0.892 ±0.012 | 9.868 ±0.135 |
| Flavonoid | 28.637 ±0.922 | 0.259 ±0.008 | 0.358 ±0.011 |
| Tannin | 39.561 ±1.223 | 0.570 ±0.017 | 7.912 ±0.244 |

### Chemical compound composition using GC-MS

There are 23 peaks indicating the identified compounds in the cocoa pod husk (CPH) using GC-MS (Fig. 4). Among these 23 compounds, the CPHE contains four major components with the highest peak intensities

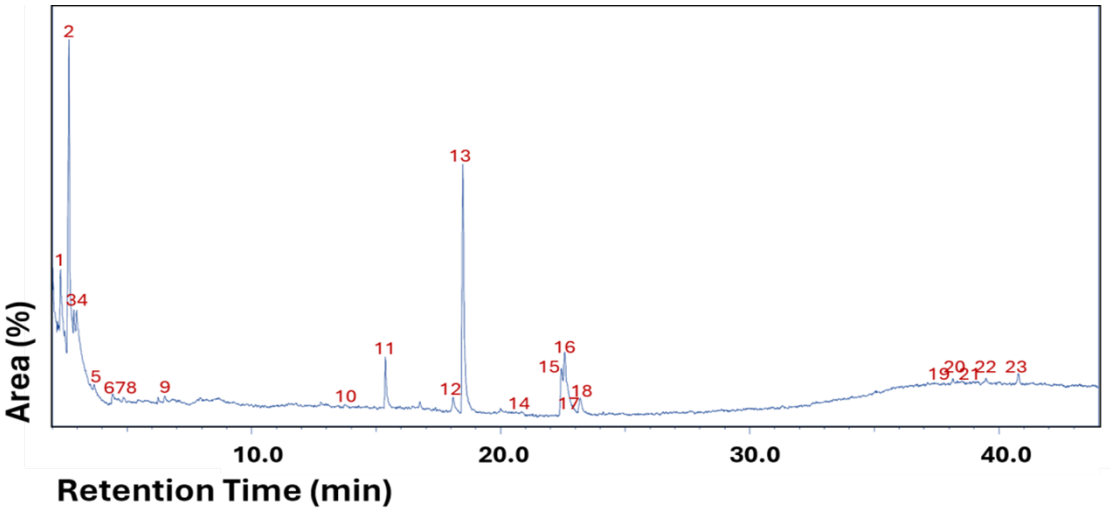


**Fig. 4.** Composition of compounds identified by GC-MS analysis

and area percentages: butyric acid (25.91%), Hexadecanoic acid, Methyl ester (24.35%), 13-Docosenoic acid, Methyl ester, (Z)-(CAS) (11.05%), and 2-Furancarboxaldehyde (CAS) (8.14%). Subsequently, there are three other compounds with notable concentrations as well, namely Butane-1,2,3,4-tetraol, 9,12-octadecadienoic acid methyl ester (E,E), and Methyl 2-hydroxy-3-butenoate, with respective area percentages of 5.44%, 4.71%, and 3.67% (Table 3).

**Table 3.** Peak data of compounds 1–23 identified in CPH

| Peak | RT | Compound | Area % |
|---|---|---|---|
| 1 | 2 | 3 | 4 |
| 1 | 2.328 | Butane-1,2,3,4-Tetraol | 5.44 |
| 2 | 2.674 | Butyric acid | 25.91 |
| 3 | 2.868 | Methyl 2-hydroxy-3-butenoate | 3.67 |
| 4 | 2.977 | 2-Furancarboxaldehyde (CAS) | 8.14 |
| 5 | 3.681 | Acetic acid, Heptyl Ester | 0.41 |
| 6 | 4.443 | Dimethylmalonic acid, 2-phenethyl tridecyl ester | 1.04 |
| 7 | 4.650 | 3-ethyl-3-pentyl methylphosphonofluoridate | 0.93 |
| 8 | 4.859 | Decane | 0.70 |
| 9 | 6.504 | Linalool oxide | 0.48 |
| 10 | 13.751 | Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester | 0.50 |
| 11 | 16.450 | (Z)-Ethyl 3-(4- methoxyphenyl)acrylate | 4.64 |

**Table 3 – cont.**

| 1 | 2 | 3 | 4 |
|---|---|---|---|
| 12 | 18.090 | 9-Octadecenoic acid (Z),-, Methyl ester | 1.53 |
| 13 | 18.478 | Hexadecanoic acid, Methyl ester | 24.35 |
| 14 | 20.833 | Octadecanoic acid (Z),-, Methyl ester (CAS) | 0.39 |
| 15 | 22.441 | 9,12-Octadecadienoic acid (Z),-Methyl ester, (E,E)- | 4.71 |
| 16 | 22.570 | 13-Docosenoic acid, Methyl ester, (Z)-(CAS) | 11.05 |
| 17 | 22.892 | Ethyl iso-allocholate | 0.63 |
| 18 | 23.187 | Nonadecanoid acid, Methyl ester | 1.87 |
| 19 | 38.151 | Cholesterol 3-O-((2-acetoxy)ethyl)- | 0.46 |
| 20 | 38.308 | Benzene, 1-[[2-Cyclohexylidene-2-(2-Propenyloxy) Ethyl] Sulfonyl] -4-Methyl | 0.72 |
| 21 | 38.502 | 1-Monolinoleoylglycerol trimethylsilyl ether | 0.39 |
| 22 | 38.961 | Cholest-5-en-3-yl (9Z)-9- Octadecenoate | 0.45 |
| 23 | 40.779 | Cholest-5-en-3-ol (3.beta.)-, tetradecanoate | 1.15 |
| Total | | | 100 |

The analysis results also indicate that the chemical structure of all identified compounds falls within the groups of terpenoid and aromatic derivatives (Fig. 5), fatty acids (Fig. 6), and steroids (Fig. 7). The most dominant terpenoid compounds found in this research are butyric acid, 2-Furancarboxaldehyde, Butane-1,2,3,4-Tetraol, and Methyl 2-hydroxy--3-butenoate, with respective peak areas of 25.91%, 8.14%, 5.44%, and 3.67%. The fatty acid derivative compounds with the highest percentage identified in this research are Hexadecanoic acid Methyl ester, 13-Docosenoic acid Methyl ester, and

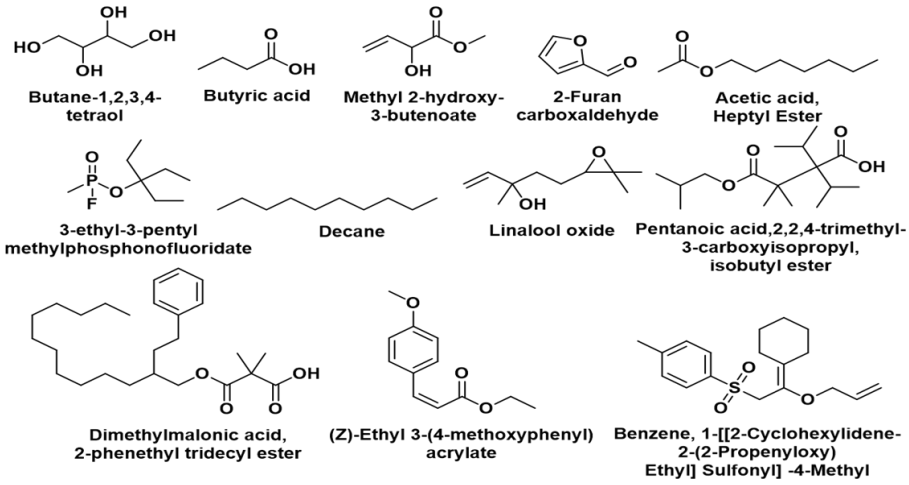


**Fig. 5.** Structure of terpenoid and aromatic derivative compounds in CPH

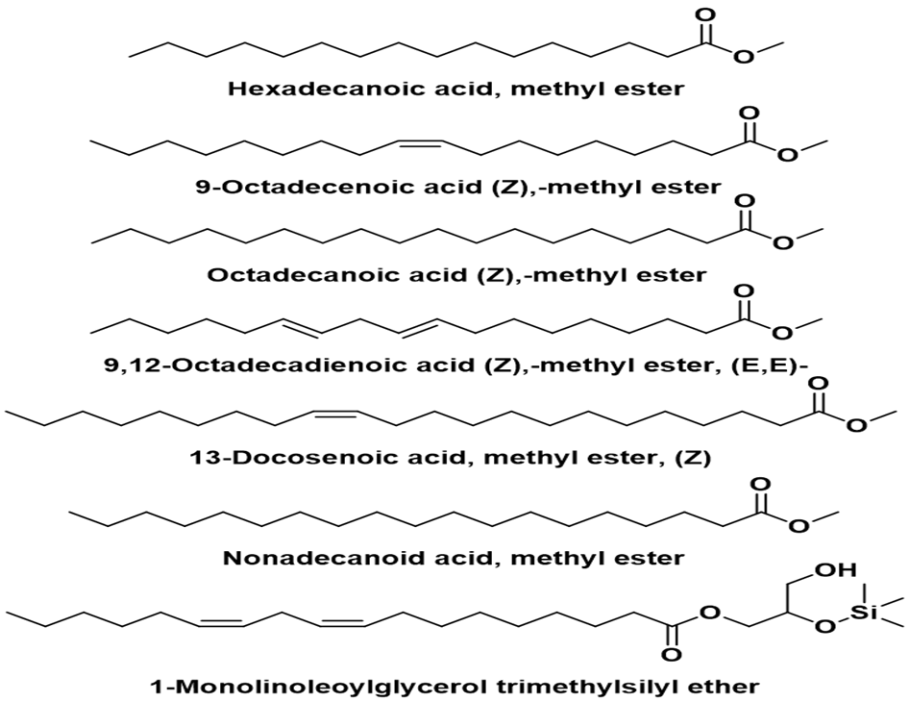


**Fig. 6.** Structure of fatty acid derivative compounds in CPH

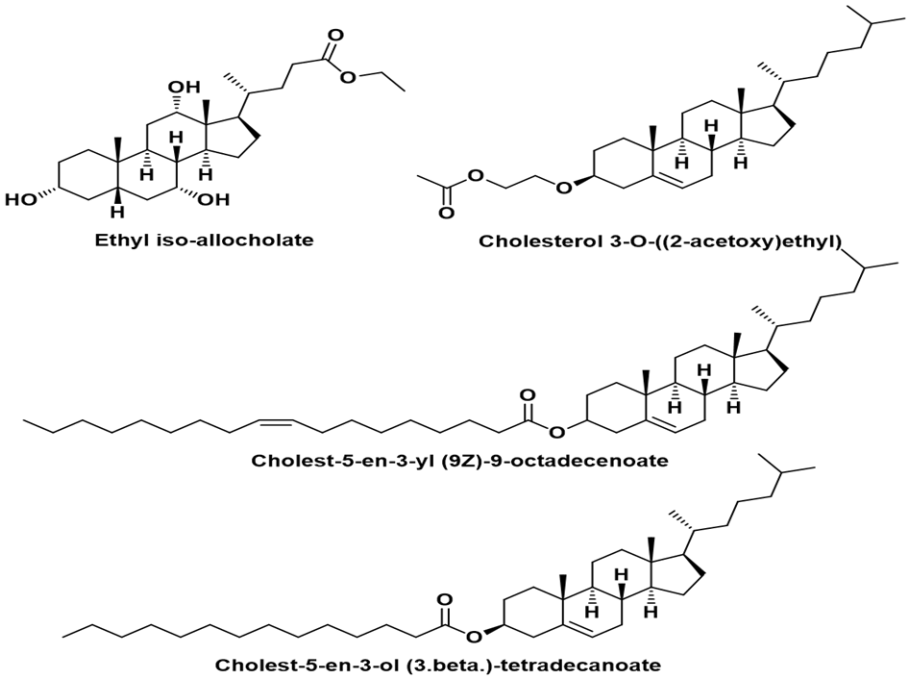


**Fig. 7.** Structure of steroid derivative compounds in CPH

9,12-octadecadienoic acid methyl ester, with peak areas of 24.35%, 11.05%, and 4.71%, respectively. The identified steroid derivatives in this research are Ethyl iso-allocholate, Cholesterol 3-O-((2-acetoxy) ethyl)-, Cholest-5-en-3-yl (9Z)-9-Octadecenoate, and Cholest-5-en-3-ol (3.beta.)-tetradecanoate, albeit in low percentages of 0.63%, 0.46%, 0.45%, and 1.15%, respectively (Table 3).

The results and main outcomes of this research are as follows:

1. The proximate content examined includes moisture; CPH in the form of ethanol extract exhibits higher moisture content compared to sliced and dried CPH, which is subsequently pelletized and dried, leading to a moisture reduction of up to 10% from the original 90% moisture content of fresh CPH (Figueroa et al., 2020). Several drying methods including microwave drying, forced-air drying, and forced-air-drying-extrusion yield even lower moisture contents, respectively, at 8.3%, 6.4%, and 6.6%.(Nieto-Figueroa et al., 2020) The persistently high moisture content may be attributed to the maceration method used in CPH extraction. The maceration process necessitates polar solvents such as ethanol, acetone, or distilled water to dissolve the active compounds within the material (Kurniawati et al., 2016) Contrastingly, the ash content found in this research is lower than that of CPH originating from the Perak plantations in Malaysia, which records an ash content of 9.02% (Chun et al., 2016), However, it falls within the range CPH sourced from Valle del Cauca, Colombia, processed into flour and frozen, with an ash content of 8.9% (Delgado-Ospina et al., 2021). The ash content of CPH from Dak Lak province, Vietnam, in fresh weight, is also lower at 1.48%. However, in dried weight, CPH has a higher ash content of 11.44% (Nguyen and Nguyen, 2017). The ash content of CPH has been extensively researched, primarily due to its potential use as a substitute or in combination with fertilizers, as well as its application in biofuel production (Dias, 2014). Nearly three-quarters of the total ash weight in the cocoa pod husk (CPH) cultivated in Ghana consists of potassium minerals (Donkoh et al., 1991).

The fat content of the cocoa pod husk (CPH) found in this research is lower than that of Malaysian-origin CPH, which is 1.53% (Chun et al., 2016). However, in line with several previous reports, the fat content of Ekona-origin cocoa pod husk (CPH) flour is 0.6% (Esong et al., 2015), while the fresh and dried CPH have fat contents of 0.12% and 0.93%, respectively (Nguyen and Nguyen, 2017). Drying methods such as microwave drying, forced-air drying, and forced-air-drying-extrusion yield fat contents of 0.2%, 0.6%, and 0.7%, respectively (Nieto-Figueroa et al., 2020), However, the fat content is significantly higher at 4.7% and 8.83% in CPH flour (Ozung et al., 2017) (Lateef et al., 2008). The fat content in cocoa is influenced by cocoa plant variety and seasonal factors. Cocoa that undergoes fertilization during the rainy season generally exhibits higher fat content compared to fertilization during the dry season. Furthermore, the fat-forming components and fatty acid composition are influenced by the elevation of the cultivation area for cocoa (Lipp and Enklam, 1998).

The protein content in this outcome is still consistent with the typical protein content of CPH, which ranges from 10% to 15% (Nieto-Figueroa et al., 2020); however, these outcomes are significantly higher than CPH from Perak, Malaysia, which has a protein content of 2.09% (Chun et al., 2016). On the other hand, frozen CPH flour, when placed in boiling water and kept at room temperature for 3 hours, contains protein contents of 5.71%, 4.85%, and 6.48%, respectively (Delgado-Ospina et al., 2021).

The carbohydrate content, including crude fiber, found in this research is still higher than that of wet CPH, while it is lower than that of dried CPH (Nguyen and Nguyen, 2017). The carbohydrate content in both untreated and heat-treated CPH flour is 49.3% and 50%, respectively (Delgado-Ospina et al., 2021). The total carbohydrate content of CPH without treatment is 20.6% (Laconi and Jayanegara, 2015).

CPH is a good source of dietary fiber as it contains higher fiber content, compared to other fiber sources such as oranges, apples, and bananas, although it is lower than coconut husk, pea hulls, and passion fruit peel. Crude fiber constituents such as lignin, cellulose, and hemicellulose present in oven-dried and ground CPH at 80°C sequentially accounted for 24.2%, 26.4%, and 8.7% (Figueroa et al., 2020).

2. The polyphenol and flavonoid contents from this outcome differ from the methanol extract of CPH sourced from Pujapitiya, Sri Lanka, which had total polyphenol and flavonoid contents ranging from 3–17

GAE/g and 117–725 QE/g, respectively (Abeywansha and Karunaratne, 2014). A mixed extract of cocoa fruit peels from Costa Rica and Madagascar exhibited a total polyphenol content of 7105 mg GAE/100 g FW (Felice et al., 2020).

There were no differences in the total phenolic compound content, flavonoid content, and tannin content, each approximately at ~26.3 mg GAE, ~1.2 mg quercetin/g, and ~8.5 mg/g, respectively, in CPH subjected to various drying methods (Nieto-Figueroa et al., 2020). However, these outcomes are also consistent with the report by (Pratyaksa et al., 2020), which demonstrated the polyphenol content of ethanol extract from CPH in Bali, Indonesia, with maceration periods of 24–48 hours showing levels between 67.99–148.09 mg/GAE/g. The flavonoid content of methanol extract from CPH originating in West Java showed a value of 0.2371% (Azizah et al., 2014). Meanwhile, the research outcomes previously reported by (Pappa et al., 2019) indicated that the tannin content in the methanol extract of CPH from South Sulawesi, Indonesia, was higher at 12.68%, whereas the tannin content in CPH from West Sulawesi, Indonesia, was lower at 4.98%.

Although the CPH obtained from this research also originates from Sulawesi Island, it is evident that their contents are not consistent. This can be influenced by various factors, including sample origin, variety, soil conditions, weather, temperature, humidity, and processing methods such as drying, roasting, and storage, which can alter the phytochemical content and functional value of CPH (Valadez-Carmona et al., 2017; Figueroa et al., 2020; Upadhyay et al., 2022). Additionally, the composition and content of cocoa compounds can also be influenced by cocoa genotype and environmental conditions (Anita-Sari et al., 2023). In the Southeast Sulawesi region itself, there are various types of local superior cocoa genotypes (Izzah et al., 2018). These genotypes can influence enzyme activity, protein content, carbohydrates, and polyphenols. (Caligiani et al., 2007; De Vuyst and Weckx, 2016).

Phenolic acid, flavonoid, lignin, tannin, and stilbene are subgroups of phenolic compounds categorized based on the number of attached hydroxyl phenolic groups and the structural elements connecting the benzene rings (Singh et al., 2016). Phenolic compounds are bioactive secondary metabolites widely present in nature, primarily in plants, fruits, and vegetables. Cocoa and cocoa fruit peel are abundant sources of phenolic compounds. The majority of phenolic compounds found in cocoa include flavonols (37%), proanthocyanidins (tannins) (58%), and anthocyanins (4%) (Miller et al., 2006; Payne et al., 2010). (Rachmawaty et al., 2018) reported that CPH possesses a total phenolic activity content and has potential as a fungicide to inhibit the growth of pathogenic fungi. Polyphenols possess an antioxidant capacity that protects cells from oxidative stress by reducing the production of reactive oxygen species (ROS) and inhibiting caspase-3 activation. Epidemiological studies and related meta-analyses have demonstrated that long-term consumption of polyphenols correlates with protection against the development of chronic diseases such as cardiovascular diseases, diabetes, cancer, osteoporosis, and neurodegenerative disorders (Felice et al., 2020).

3. The compounds identified in CPH using GC-MS are fewer than the compounds identified in CPH from Peru, which totaled 49 compounds (De La Luz Cádiz-Gurrea et al., 2020). Peaks with different retention times indicate the multitude of components present in the cocoa fruit peel extract, while the peak area indicates the abundance of a composition at each retention time (Yodha et al., 2023).

4. The dominant terpenoid compound in this outcome is Butyric acid, also known as butanoic acid, which plays a crucial role in the chemical, food, pharmaceutical, and livestock feed industries (Brändle et al., 2016). Butyric acid holds potential as a substitute to antibiotics. (Andoh et al.,1999) reported that butyric acid has been proven to exert strong anti-inflammatory effects both in vitro and in vivo. Its immunoregulatory and anti-inflammatory activities are based on inhibiting topical inflammatory mediators in the epithelium and its ability to reduce the concentration of pro-inflammatory cytokines such as IL-8 and TNF-α. 2-Furancarboxaldehyde (methylfurfural) is a furan and aldehyde compound that plays a role in Maillard reaction products, human metabolites, and as an inhibitor of EC 2.2.1.6 (acetolactate synthase), as well as a flavoring agent (NIH National Library of Medicine, 2023). Furancarboxaldehyde is also a compound found at the third-highest concentration in palm fruit husk waste (4.44%) (Majid et al., 2022).

The National Library of Medicine describes butane-1,2,3,4-tetraol as a tetrathiol with butane substituted by hydroxyl groups at positions 1,2,3,4, and it is a natural product found in Salacia chinensis and Roccela phycopsis. Salacia chinensis is a traditional herbal medicine that has been reported to exhibit functions such as antidiabetic, anti-obesity, and cardiovascular disease therapeutic effects (Deokate and Khadabadi, 2012). The compound Methyl 2-hydroxy-3-butenoate, or methyl vinyl glycolate (MVG), is a small molecule with a simple structure yet possesses multiple functional groups, allowing for various chemical transformations and serving as a promising biobased platform molecule (Sølvhøj et al., 2016). One of the molecules produced from the isomerization of MVG is maple furanone – a food flavoring compound (Stach et al., 1987). However, the applications of this compound have not been extensively explored.

Terpenoids have been widely recognized, both conceptually and empirically, as playing a crucial role in herbal medicine, biomaterials, and biofuels (Tong, 2013). Terpenoids exhibit anti-diabetic activity and improve diabetic conditions through pancreatic β-cell regeneration (Jasmine et al., 2018; Singh et al., 2022), anti-inflammatory effects (Hortelano et al., 2020; Truong et al., 2021), anti-cancer effects by modulating the immune system such as NF-KB signaling, acting as chemopreventive agents, and holding potential in the therapy of various diseases. (Huang et al., 2012).

This research also demonstrates the presence of aromatic compounds within the CPH from Sulawesi Island. Although CPH from Sulawesi is categorized as a non-aromatic cocoa genotype, there is no significant difference in cocoa terpenoids between aromatic and non-aromatic groups (Anita-Sari et al., 2023). The formation of aromatics is not solely determined by a single compound but involves several compounds collectively creating a distinct aroma. Aromatic groups consist of various compounds including aldehydes, acids, esters, and furans, which are generally more abundant than non-aromatic compounds. Conversely, non-aromatic groups contain alkaloid compounds that are absent in aromatics.

5. Compound derivatives of fatty acids such as Hexadecanoic acid, Methyl ester (palmitic acid) are long-chain saturated fatty acids that function as antibiotics. It has the highest antimicrobial effect against clinical pathogenic bacteria, (Shaaban et al., 2021), exhibits bactericidal properties against Salmonella typhi, and shows fungistatic activity against Aspergillus flavus (Sjafaraenan et al., 2021). 13-Docosenoic acid, Methyl ester, (Z)-(CAS), as mentioned by (NIST Chemistry WebBook, 2023), is known by other names such as Methyl erucate or Erucic acid methyl ester, primarily serving as a methyl ester of fatty acid and a component of triacylglycerol. Erucic acid (EA) is commonly found in oils and seeds of plants, primarily in seeds of Brassicaceae species like radish, mustard, and cabbage. It is also present in fish, albeit in low concentrations (Knutsen et al., 2016). EA and its erucamide analog have been reported to modulate signaling molecules associated with memory and cholinergic pathways to enhance cognitive function in animals. Moreover, erucamide also exhibits functions such as antidepressant and anxiolytic effects in animals. However, further preclinical investigation is still required to verify their potential benefits in ameliorating cognitive disorders (Kumar and Sharma, 2020). On the other hand, the compound 9,12-octadecadienoic acid methyl ester (E,E), also known as linolelaidic acid, is a polyunsaturated fatty acid found extensively within plant glycosides. It serves as an essential fatty acid in mammalian nutrition and is utilized in the biosynthesis of prostaglandins and cell membranes.

6. The steroid derivative compounds in this outcome align with previous reports indicating that cocoa waste (*Theobroma cacao* L. sweatings) contains a group of steroid compounds (Balladares et al., 2016). Steroids are terpenoid lipids characterized by distinct carbon frameworks with four basal rings. The majority of steroid derivative compounds in this discovery belong to the cholesterol or sterol group, which are most commonly found in the cell membranes of animals and red blood cells, although steroids also exist in plants. Their physiological functions primarily involve sexual hormones, corticosteroids (cortisol, aldosterone), and neurosteroids. These compounds serve as agents that enhance protein synthesis, regulate androgenic effects in males and virilization in females, and promote muscle and bone synthesis (Tong, 2013).

Based on previous review studies, cocoa waste, especially CPH, has potential health benefits, including as an antioxidant that can overcome oxidative stress and anti-inflammatory. It also has a hypolipidemic

effect, increases high density lipoprotein (HDL), lowers blood pressure as an endothelial vasodilator and vasorelaxant, is anti-diabetic, antibacterial, can maintain stable body weight, reduce stomach fat and waist circumference, and can increase serum protein (Irma et al., 2023).

This research is still limited to testing the bioactive composition which provides markers of the presence of potential compounds in CPH from the island of Sulawesi, Indonesia which are beneficial for human health, and has not further investigated its antioxidant capacity or effectiveness on health in vitro. Apart from that, the extraction method used is still conventional. Even though it has limitations, this study also has the advantage of providing an overview of the bioactive compounds in CPH originating from archipelagic region, which results in cocoa being cultivated at higher elevations above sea level compared to other countries or regions. This study also describes the grouping of terpenoid-aromatic derivative compounds, fatty acids, and steroids in the CPH.

## CONCLUSION

CPH from Sulawesi Island, Indonesia, has a similar composition of bioactive compounds (proximate, polyphenols, flavonoids, tannins) to CPH originating from various other countries as well as other regions in Indonesia, despite varying levels. The main compounds identified from the GCMS analysis with the highest peak areas are *butyric acid*, *hexadecanoic acid methyl ester*, *13-docosenoic acid methyl ester*, and *2-furancarboxaldehyde.* Based on their chemical structures, these compounds are derivatives of terpenoids, fatty acids, and steroids, and they are known to have positive effects on health. However, further evidence and development are still needed to verify the health benefits of CPH, both in vitro and in preclinical studies, before it can be applied to humans. The utilization of CPH in health products can also help mitigate the environmental impact of CPH waste.

## DECLARATIONS

### Data statement

All data supporting this study has been included in this manuscript.

### Ethical Approval

Not applicable.

### Competing Interests

The authors declare that they have no conflicts of interest.

## OPEN ACCESS



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