

Comparison of Total Phenolic Content in Water, Ethyl Acetate, and n-Hexane Fractions of Cinnamon Bark (*Cinnamomum burmannii*) Ethanol Extract

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Received : August, 4th, 2025 ; **Accepted** : Month, date, year

ABSTRACT

According to previous research, the main antioxidant components found in cinnamon bark extract are polyphenols (tannins, flavonoids) and phenolic essential oils, making phenolic identification important for evaluating its antioxidant potential. The aim of this study was to determine the total phenol content in the ethanol extract fraction of cinnamon bark. Fractionation is carried out using two types of solvents that do not mix with each other and have different levels of polarity so that secondary metabolite compounds can be maximally attracted by the solvent. The solvent used namely water, ethyl acetate and n-Hexane. The total phenolic content of each fraction was determined using the Folin–Ciocalteu method analyzed by UV–Vis spectrophotometry, and all measurements were performed in triplicate. The cinnamon bark water fraction has a total phenol content of 65.089 mg GAE/g $\pm$ 0.968, the cinnamon bark ethyl acetate fraction has a total phenol content of 37.0908 mg GAE/g $\pm$ 0.182 and the cinnamon bark thick extract has a total phenol content of 48.8552 mg GAE/g $\pm$ 0.325. The results showed that the cinnamon bark water fraction had the highest total phenol content of 65.089 mg GAE/g $\pm$ 0.968 while the cinnamon bark ethyl acetate fraction had a total phenol content of 37.0908 mg GAE/g $\pm$ 0.182. It can be seen that phenol compounds are compounds that are included in the group of polar compounds, because phenol compounds are more abundant in the cinnamon bark water fraction.

Keywords: Cinnamon bark; fractionation; total phenolic; folin–ciocalteu; UV–Vis spectrophotometry.

INTRODUCTION

Cinnamon bark is a common spice used to enhance the flavor of food. Cinnamon bark was one of the first spices used by humans. In the food and beverage industry, cinnamon is also often used to make ice cream, candy, and other chewing gums (1). Apart from being used as a spice and flavoring, cinnamon is also useful as a medicine (2).

According to previous research, the main antioxidant components found in cinnamon bark extraction are polyphenols (tannins, flavonoids) and phenolic essential oils (3). GCMS analysis results (*Gas Chromatography–Mass Spectrometry*) of essential oil from cinnamon bark show cinnamaldehyde which is a phenolic group and the main component with a content of up to 92.02% (4). As antioxidants, phenolic compounds can prevent and treat degenerative diseases such as cancer, immune system disorders, and premature aging (5). Apart from being an antioxidant, Phenol also has benefits as a broad-spectrum antibacterial (6). Knowing the total phenol content is important for assessing the antioxidant potential of cinnamon and understanding how much phenol is contained in each extract fraction, so it is necessary to separate the fraction based on solvent polarity.

Fractionation is the process of separating and grouping the chemical components of an extract based on their polarity. This process involves two immiscible solvents of different polarities. Multi-stage fractionation with different solvents depending on their polarity produces different natural extracts, allowing maximum attraction of secondary metabolites to the solvent (7). Therefore, the fractionation method has advantages over other

methods because it can distinguish bioactive compounds based on their level of polarity. Polar compounds dissolve in polar solvents such as water, semi-polar compounds in semi-polar solvents such as ethyl acetate, and non-polar compounds in non-polar solvents such as n-hexane (8). Previous studies have focused on cinnamon in general, but specific focus on cinnamon bark and phenolic analysis per extract fraction remains limited. Accordingly, this study is expected to fill this gap and present new findings on the distribution of phenols in each fraction. Therefore, the aim of this study was to determine the total phenol compound content in the ethanol extract fraction of cinnamon bark. Where ethanol was selected as a universal solvent capable of extracting chemical compounds ranging from nonpolar to polar.

METHODS

Tool

The tool used in this study was an analytical scale (Fujitsu FSR-A320), oven (Mettler), grinder, mesh 60, crucible, macerator, stirring rod, funnel, rotary vacuum evaporator (RE-201D), water bath (B-One), steam cup (Pyrex), refrigerator (LG), separating funnel (Pyrex), stand, xlem, test tube (Pyrex), drop pipette, 10 mL measuring flask (Pyrex), micro pipette and UV-Vis spectrophotometer (Metash).

Materials

The materials used in this study were fresh cinnamon bark obtained from Pasiramis Village, RT/RW 001/001, Sukanagara Village, Jatinagara District, Ciamis Regency, 96% ethanol (DPH), filter paper (Whatman), distilled water (Amidis), n-Hexane (DPH), ethyl acetate (DPH), FeCl₃ 1% (DPH), Pb acetate 10% (DPH), reagent Millon, gallic acid, ethanol 96% pro analysis (pa), Na₂CO₃ 7.5% (DPH) and Folin-Ciocalteu reagent (Pallav).

Detailed Procedure

Sample Preparation

Cinnamon bark is cleaned using running water, then sliced into 5 cm lengthwise (split), then dried with indirect sunlight for 5 days. Then the sample was ground with a grinder and sieved with a 60-mesh sieve to produce cinnamon bark powder.

Cinnamon Bark Extraction

The maceration method was used to extract cinnamon bark. A total of 500 grams of cinnamon bark powder was soaked in 5 liters of 96% ethanol solvent at a ratio of 1:10. This was done for 3 x 24 hours, while stirring every 4 hours, and re-maceration was carried out every 24 hours. The macerate was filtered through filter paper, then concentrated in a rotary evaporator at 50°C and evaporated in a water bath until a thick extract was obtained. The extract was stored in a refrigerator at 4°C until use (9). The concentrated extract is then calculated for its yield. The requirement for a good yield value is not less than 10%. The yield value can be calculated based on the formula (2):

$$\% \text{ Yield} = \frac{\text{Weight of extract (g)}}{\text{Weight of raw material (g)}} \times 100\%$$

Cinnamon Bark Fractionation

A total of 5 grams of thick cinnamon bark extract was mixed with 100 mL of distilled water. The solution was then separated by adding 100 mL of n-hexane solvent, shaken in a separatory funnel, and left for 10 minutes until two layers formed (n-hexane on the top layer, distilled water on the bottom layer). The n-hexane layer was separated. The distilled water layer was then separated again by adding 100 mL of ethyl acetate solvent, shaken in a separatory funnel, and left for 10 minutes until two layers formed (ethyl acetate on the top layer, distilled

water on the bottom layer). The fractionation results were then separated in a container until each fraction was obtained, which was then subjected to a qualitative phenol test (10).

Qualitative Analysis of Phenol

1. Ferric Chloride Test

The sample was pipetted as much as 1 mL, then 3-4 drops of 1% FeCl_3 were added.. A positive result is indicated by the formation of a solution.green to blue-black (11).

2. Lead Acetate Test

A 1 mL sample was pipetted, then 3-4 drops of 10% lead acetate solution were added. A positive result is indicated by the formation of a white precipitate (12).

3. Millon Test

A 1 mL sample was pipetted into the sample, then 3-4 drops of Millon's reagent were added. A positive result is indicated by the formation of a red solution (11).

Quantitative Analysis of Phenol

Quantitative analysis of total phenol compounds was carried out using procedures as carried out in previous studies, namely by modifying the variation in gallic acid concentration (13). The total phenol compound content was analyzed using Folin-Ciocalteu reagent. Gallic acid was dissolved into a standard solution with varying concentrations of 6, 7, 8, 9 and 10 ppm. Gallic acid was pipetted as much as 3000 μL mixed with 1.2 mL of 7.5% Na_2CO_3 and 1.5 mL of Folin-Ciocalteu reagent. The extract and each cinnamon bark fraction were pipetted as much as 100 μL and dissolved in 10 mL of ethanol. Next, 1000 μL of the extract and fraction solution was added with 1.2 mL of Na_2CO_3 solution and 1.5 mL of Folin-Ciocalteu reagent. The incubation process was carried out for 10 minutes, then the absorbance value was measured at a wavelength of 750 nm using a UV-Vis spectrophotometer. The total phenolic content is expressed as milligrams of gallic acid equivalents per gram of fraction (mg GAE/g), meaning that each gram of fraction is equivalent to one milligram of gallic acid. The total phenolic content can be calculated using the following formula (14) :

$$\text{Total Phenol Content} = \frac{C \times V \times Fp}{M}$$

Notes :

C: Gallic Acid Concentration

V: Volume Fraction

M: Fraction Weight

Fp: Dilution Factor

Data analysis

Microsoft Excel is the program used to analyze the collected data. The absorbance value (y) of the cinnamon bark fraction solution sample at the maximum wavelength was entered into the linear regression equation $y = ax + b$, which was obtained from the gallic acid calibration curve, and then the x value was substituted into the formula to calculate the total phenol content. The total phenol content measurement was carried out with three repetitions and the average was taken (15).

RESULT AND DISCUSSION

Cinnamon Bark Extraction

The manufacture of cinnamon bark extract uses the maceration method using 96% ethanol solvent. The yield of the resulting thick extract is then calculated. The yield value is the ratio of the weight of the thick extract to the amount of raw material. The yield value correlates with the number of compounds contained; a higher yield value indicates a higher content of active compounds attracted to the raw material (16). The requirement for a good yield value is not less than 10% (2). The results of the cinnamon bark extract yield can be seen in Table 1.

Table 1. Yield Value of Ethanol Extract of Cinnamon Bark

Simple Weight (g)	Extract Weight (g)	Extract Yield (%)
500	162.352	32.470

Cinnamon Bark Fractionation

Fractionation is performed using the liquid-liquid extraction method with the aim of separating compounds based on their polarity. The solvent n-hexane is used to dissolve non-polar compounds, ethyl acetate to dissolve semi-polar compounds, and water to dissolve polar compounds. The principle of fractionation is the extraction of compounds using immiscible solvents. In fractionation, a shaking process is carried out to maximize the extraction of compounds (17). Fractionation results cinnamon bark ethanol extract can be seen in Table 2.

Table 2. Fractionation Results of Ethanol Extract of Cinnamon Bark

Types of Fractions	Volume Fraction
Water Fraction	87 mL
Ethyl Acetate Fraction	90 mL
n-Hexane Fraction	77 mL

Qualitative Analysis of Phenol

Qualitative tests were conducted on the simplex powder, thick extract, and each cinnamon bark fraction. The qualitative test for phenol compounds was conducted using three methods: the 1% Ferric Chloride test, which indicates a positive result when a green to blue-black color appears, and the formation of a blackish-green color in the sample after the addition of FeCl_3 , as the phenol compound reacts with Fe^{3+} ions to form a blackish-green complex compound (18).

Furthermore, a positive result for the 10% Lead Acetate test is indicated by the formation of a white precipitate. The precipitate formed after the addition of Pb acetate is the result of a complex reaction between the Pb^{2+} ion and the hydroxyl functional group (-OH) in the phenol molecule (19). Then the last Millon test with a positive result is indicated by the formation of a red color, which confirms the presence of phenolic compounds containing a phenolic hydroxyl group (11). The results of qualitative tests of phenol compounds in the simple powder, thick extract and each cinnamon bark fraction can be seen in Table 3.

Table 3. Qualitative Test Results of Phenol Compounds

No	Test Name	Sample				
		Simple powder	Thick extract	Faction water	Ethyl acetate fraction	Faction n-Hexane
1	Ferric Chloride	+	+	+	+	-
2	Lead Acetate	+	+	+	+	-
3	Millon	+	+	+	+	-

Based on the qualitative test results shown in Table 3, only the n-Hexane fraction did not test positive for phenolic compounds. This is consistent with previous research, which found that phenolic compounds, as bioactive compounds, are polar compounds. Therefore, the n-Hexane fraction did not contain phenolic compounds because n-Hexane is a non-polar solvent (20).

Quantitative Analysis of Phenol

Quantitative analysis of total phenols aims to determine the amount of phenols contained in the ethanol extract of cinnamon bark and each cinnamon bark fraction. Total phenol content was determined using UV-Vis spectrophotometry. This method is based on a reduction-oxidation reaction using Folin-Ciocalteu reagent as an oxidizing agent. Gallic acid was used as a reference solution (20). With an incubation time of 10 minutes, the maximum wavelength is 750 nm. The Folin-Ciocalteu reagent, (molybdo tungsto phosphotungstate heteropolyanion $3\text{H}_2\text{O}-\text{P}_2\text{O}_5-13\text{WO}_3-5\text{MoO}_3-10\text{H}_2\text{O}$) reacts with phenolic compounds to form a blue complex $[(\text{PMoW}_{11}\text{O}_4)_4]^-$. The blue color in the solution is caused by the reduction of molybdenum metal (Mo(VI)) in the Folin-Ciocalteu complex to Mo(V), and electron donors from phenolic compounds that act as antioxidants (20).

Gallic acid as a standard solution was prepared at varying concentrations from 6 ppm to 10 ppm. A standard curve was created based on the absorbance value versus gallic acid concentration. The calibration curve data can be seen in Table 4.

Table 4. Gallic Acid Absorbance Value

Concentration (ppm)	Absorbance	Linear Regression Equation
6	0.286	$y = 0.0099x + 0.222R^2$ $= 0.9399$
7	0.288	
8	0.298	
9	0.309	
10	0.325	

The results of the measurement of the absorbance of the standard gallic acid solution were then used to create a calibration curve which is the relationship between the concentration of gallic acid and absorbance and a linear equation was obtained. From the calibration curve, a linear regression equation was obtained, namely y

= $0.0099x + 0.222$ with a correlation coefficient (r) of 0.9399 which meets the feasibility requirements of the analysis method. The gallic acid calibration curve can be seen in Figure 1.

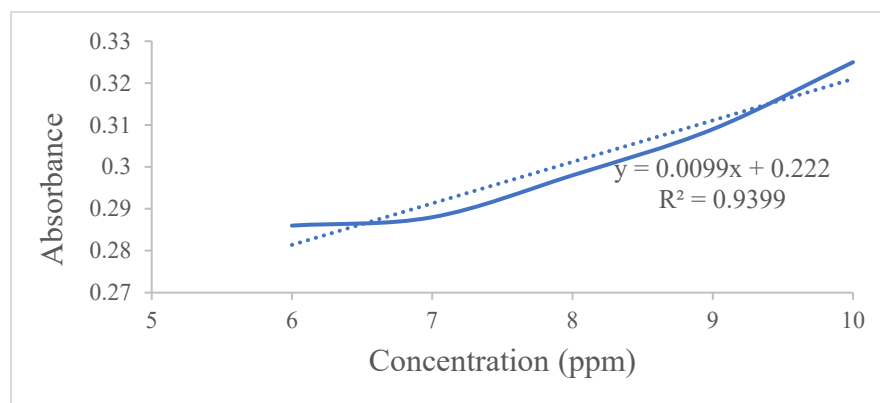


Figure 1. Gallic Acid Calibration Curve

Based on the research results, it was found that the cinnamon bark water fraction had the highest total phenol content, namely 65.089 mg GAE/g, meaning that each gram of fraction is equivalent to 65.089 mg of gallic acid. The data from the determination of total phenol content can be seen in Table 5.

Table 5. Results of Determination of Total Phenol Content

Sample	Replicate 1 (mg GAE/g)	Replicate 2 (mg GAE/g)	Replicate 3 (mg GAE/g)	Mean (mg GAE/g) ± SD
Thick Extract	48.9898	49.0909	48.4848	48.8552 ± 0.325
Water Fraction	66.085	65.030	64.152	65.089 ± 0.968
Ethyl Acetate Fraction	37.2726	37.0908	36.909	37.0908 ± 0.182

Judging from the total phenol content produced, it can be seen that phenol compounds are included in the polar compound group, as phenol compounds are more abundant in the water fraction of cinnamon bark. This is because the phenol group in these compounds is polar and can form hydrogen bonds with water. As a result, phenol compounds tend to be less soluble in semi-polar fractions such as ethyl acetate (14).

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that the cinnamon bark fractions that contain phenol compounds are the water fraction and the ethyl acetate fraction. The cinnamon bark water fraction had a total phenol content of 65.089 mg GAE/g ± 0.968 while the cinnamon bark ethyl acetate fraction had a total phenol content of 37.0908 mg GAE/g ± 0.182.

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