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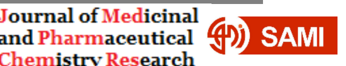

FULL PAPER

Analysis of phytochemical, mineral, total phenol, flavonoids and antimicrobial from ethanol extracts and fractions of *phyllanthus acidus* (L.) skeels fruit

Masfria Masfria[a,b] |Hafid Syahputra[a,*] |Laili Khairani[c] |Pani Reflesia Nainggolan[c] |Gitta Yohana[c] |Elda Lastry Marta Yanti[c]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia*

[b]*Department of Nanomedicine, Faculty of Pharmacy Universitas Sumatera Utara, Medan 20155, Indonesia*

[c]*Undergraduate Program, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia*

*** Corresponding Author:**
Hafid Syahputra
Email: hafid@usu.ac.id
Tel.: + 628117506033

Many chemical substances can act as drugs, so different plants can be grown to treat illnesses. *Phyllanthus acidus* (L.) Skeels is one of several plant species known for its medicinal properties. The fruit has been found to contain various minerals, including calcium, potassium, sodium, magnesium, and zinc. However, it has been observed that the levels of these minerals decrease after boiling, with percent decreases of 53.20%, 64.90%, 41.27%, 60.96%, and 35.40% for calcium, potassium, sodium, magnesium, and zinc, respectively. The total phenol levels in the ethanol extract and fraction (n-hexane, ethyl acetate, and water) were 39.27, 5.99, 48.01, and 25.1 mg GAE/g samples. The total levels of flavonoids, respectively, were 7.72, 0.57, 8.11, and 3.38 mg QE/g samples. The presence of phenolic and flavonoids compounds has antimicrobial activity in fruit against several fungi and bacteria. The antimicrobial activity of *Phyllanthus acidus* fruit was investigated using ethanol extract, n-hexane, ethyl acetate, and water fractions. The study found that these fractions exhibited antimicrobial activity against the growth of *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Microsporum canis*. The ethyl acetate fraction demonstrated the most potent antimicrobial activity, whereas the n-hexane fraction exhibited weaker activity.

KEYWORDS

Antimicrobial; flavonoids; mineral analysis; Phyllanthus acidus (L.) Skeels; phytochemical; total phenol.

Introduction

The fertile soil in Indonesia, attributed to its tropical climate, facilitates the growth of various plant species. Among these, the diverse array of plant species possesses medicinal properties. *Phyllanthus acidus* (L.) Skeels is a plant that has been observed to be utilized for medicinal purposes. The fruit of this specimen comprises a combination of saponins, flavonoids, tannins, and polyphenols as chemical compounds [1].

Phenolic compounds have a broad range of applications. As an antioxidant, this molecule can slow the aging process, protect against sickness, and improve the immune system.

The class of naturally occurring phenolic chemicals known as flavonoids is the largest. The compounds above are chromatic agents of red, purple, blue, and yellow hues naturally occurring in botanical specimens. Several botanical species that comprise flavonoids have been documented to exhibit antioxidant, antibacterial, and anticancer properties [2,3].

The fruit of *Phyllanthus acidus* is commonly utilized in the confectioneries production or as a flavoring agent in culinary preparations owing to its characteristic tartness. The utilization of *Phyllanthus acidus* fruit in Indonesia has been limited. Cooking traditionally can change nutrition quickly because it is affected by temperature, especially by boiling. The traditional method of cooking with heat is a factor in the loss of important nutrients [4].

This investigation aims to assess the concentrations of various minerals to determine their impact on the fruit's nutritional value. Furthermore, the objective of this study is to ascertain the concentrations of overall phenolic compounds and flavonoids and evaluate the antimicrobial efficacy of the extracted and fractionated *Phyllanthus acidus* fruit.

Experimental

Instrument

The laboratory equipment utilized in this study comprised a Hitachi Z-2000 Atomic Absorption Spectrophotometer, which was equipped with cathode lamps for calcium, potassium, sodium, and iron. In addition, an analytical balance (ANDGF-200), hot plate (BOECO Germany), furnace, glassware (Pyrex and Oberoi), microscope (Olympus), analytical balance (Sartorius), oven (Memmert), rotary evaporator (Stuart), UV-Vis spectrophotometer (Shimadzu), vortex (Boeco Germany), petri dish, incubator (Fibre Scientific), Laminar Air Flow Cabinet (Astec HLF I200L), refrigerator (Toshiba), drying cabinet, micropipette (Eppendorf), and autoclave (Fison).

Materials

The materials used are pro-analytical quality materials for E-Merck and sigma output, namely 65% w/v nitric acid, calcium standard solution, potassium standard solution, sodium standard solution, magnesium standard solution, zinc standard solution, methanol, gallic acid, aluminium (III) chloride, Folin-Ciocalteau reagent, 96% ethanol, ethyl acetate, quercetin, n-hexane, sodium bicarbonate (g), and Potato Dextrose Agar (PDA). *Phyllanthus acidus* fruit samples were obtained from Deli Serdang district, Indonesia. Test microbes from the pharmacy laboratory Universitas Sumatera Utara used included *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Microsporum canis*.

Mineral content analysis

Fresh *Phyllanthus acidus* fruit weighing 25 g into a porcelain crucible. Subsequently, they are subjected to a hotplate for 4 hours, followed by an ashing process in a kiln. The kiln is initially set at a temperature of 100 °C and gradually elevated to 500 °C, with an increment of 25 °C every 5 minutes. The process of ashing was conducted for approximately 72 hours, after which the sample was permitted to cool within a desiccator. The ash sample was hydrated with a total of 10 droplets of demineralized water, followed by the addition of 5 mL of hydrochloric acid (1:1). Subsequently, the surplus HNO_3 was subjected to evaporation on a hotplate, utilizing a temperature range of 100-120 °C, until complete desiccation was achieved [5].

The sample that underwent digestion was dissolved in a solution of 10 mL of nitric acid and water in a 1:1 ratio. The resulting

mixture was subsequently transferred into a volumetric flask with a capacity of 100 mL and was further diluted with demineralized water until the solution reached the marked line. Subsequently, the sample was filtrated using Whatman Paper No. 42. After discarding 5 mL of the initial filtrate to saturate the filter paper, the remaining filtrate was collected in a bottle. The methodology above was employed for both qualitative and quantitative analyses.

Mineral qualitative testing uses the calcium crystal test method with 1 N sulfuric acid, potassium crystal test with picric acid, sodium crystal test with picric acid, magnesium color test with 0.1% w/v titan yellow solution, zinc precipitation test with $K_2Hg(CNS)_4$ 1.3% solution.

Quantitative testing to determine mineral content is carried out with a standard solution (1000 g/mL) pipetted to a volume of 2.5 mL, placed in a 25 mL measuring flask, and finished with demineralized water (concentration 100 g/mL) (standard solution I). The solution for the calcium calibration curve was prepared by pipetting (1, 2, 3, 4, and 5) mL of 100 g/mL standard solution, each transferred to a 100 mL volumetric flask and made up to the mark with demineralized water (this solution contains (1, 2, 3, 4, and 5) g/mL and the absorbance was measured at specific wavelengths with an air-acetylene flame. For each mineral test solution, 1 mL of the sample solution is pipetted into a 100 mL volumetric bottle, and then filled with deionized water to the marked line. Next, each sample solution was measured at the mineral wavelength absorption, where calcium was 422.7 nm, potassium 766.5, sodium 589.0, magnesium 285.2, and zing 213.9 using an atomic absorption spectrophotometer that had been calibrated in air flame acetylene. The absorption value obtained must be within the range of the calibration curve of the respective standard mineral solution. The mineral concentration in the sample was confirmed using the calibration curve regression line equation to get precise and accurate concentrations [6-8].

Simplicia characterization and phytochemical screening

Simplified characterization of macroscopic examination, microscopic examination, determination of water content, total ash content, acid insoluble ash content, water-soluble extract content, and ethanol soluble extract content were carried out [9].

Phytochemical screening was carried out in the form of the identification of alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenes/steroids [9].

Extraction of phyllanthus acidus fruit

The percolation method is employed in the extraction process. A quantity of 600 g of *Simplicia* powder was dampened with ethanol of 96% purity and allowed to rest for 3 hours. Subsequently, the *Simplicia* was introduced into the percolator, and the ethanol filter was added until complete submergence. The mixture was then left for 24 hours. Following this, the tap was opened, and the extract droplets were permitted to flow at a percolate speed of 1 mL/minute, and the percolate was suitably accommodated. The percolation process was stopped after observing that a small amount of percolate did not produce any residue on evaporation in a water bath, and then the percolate was concentrated with a rotary evaporator [10].

Fractionation of phyllanthus acidus fruit

In this study, 10 g of ethanolic extract derived from *Phyllanthus acidus* fruit was mixed with 10 mL of ethanol and 100 mL of distilled water. The resulting mixture was homogenized and subsequently transferred into a separating funnel, subjected to extraction with 200 mL of n-hexane. The extraction process was continued until the n-

hexane fraction no longer yielded positive results upon testing with the Liebermann-Burchard reagent. Moreover, 200 mL of ethyl acetate was utilized for extraction until the ethyl acetate fraction achieved a colorless state, producing both ethyl acetate and water fractions [11].

Determination of total phenol levels

Quantification of overall phenolic content was carried out using a colorimetric approach using Folin-Ciocalteu reagent and gallic acid (GAE) on a UV-Vis spectrophotometer. A total of 10 mg of standard gallic acid was dissolved in 10 mL (1000 g/mL) methanol (standard solution). A total of 2 mL of standard gallic acid solution was dissolved in 5 mL of methanol (400 g/mL). A 0.1 mL pipette was used to transfer the sample, which was then mixed with 7.9 mL of distilled water and 0.5 mL of Folin-Ciocalteau. The mixture was then vortexed for approximately 1 minute, and 1.5 mL of 20% sodium carbonate was added. The resulting solution was incubated for 90 minutes. UV-Vis spectrophotometry was used to measure solution absorption in the wavelength range 400-800 nm.

Samples were obtained from standard solutions with varying concentrations of 200-600 g/mL. 0.1 mL of each concentration was pipetted, followed by adding 7.9 mL of distilled water, 0.5 mL of Folin-Ciocalteau, and vortexed. Next, 1.5 mL of 20% sodium carbonate was added, and the mixture was incubated for 90 minutes. UV-Vis spectrophotometry was used to measure the absorbance of standard and blank solutions at various concentrations, with a maximum wavelength of 765 nm.

Phyllanthus acidus fruit was fractionated to obtain ethanol extract, n-hexane fraction, ethyl acetate fraction, and residue fraction. Each fraction weighing 25 mg was dissolved in 5 mL of methanol (5000 g/mL), and then pipetted 2 mL into a 10 mL flask filled with methanol (1000 g/mL). A volume of 0.1 mL of test solution was extracted and then mixed with 7.9 mL of distilled water. Then 0.5 mL of Folin-Ciocalteau was added and vortexed for approximately one minute. Next, 1.5 mL of 20% sodium carbonate was added, and then incubated for 90 minutes. UV-Vis spectrophotometry was used to measure the absorbance of the test solution towards gallic acid calibration at a maximum wavelength of 765 nm. The phenol concentration in the test solution was determined via a calibration plot. Quantification of the overall phenolic content is shown in GAE (Gallic Acid Equivalent), which is a unit of measurement that represents the number of milligrams of gallic acid present in one gram of sample.

Determination of total flavonoids level

The quantification of overall flavonoids content pertains to the methodology with certain adjustments made to the colorimetric approach utilizing $AlCl_3$ and quercetin (QE) as benchmarks and gauged through the employment of a UV-Vis spectrophotometer. A standard amount of quercetin weighing 10 mg was measured and subsequently dissolved in methanol up to a volume of 100 mL in a volumetric flask. This resulted in forming a solution with a 100 g/mL concentration, considered the standard solution. The solution was prepared using a concentration of 400 g/mL. Dispense 2 mL of the solution using a pipette, followed by the addition of 0.1 mL of aluminum chloride ($AlCl_3$) with a concentration of 10% and 0.1 mL of sodium acetate (CH_3COONa) with a concentration of 1 molar. Subsequently, 2.8 mL of distilled water was introduced into the solution and it was kept to incubate for 30 minutes. UV-Vis spectrophotometry was employed to measure the absorbance of the solution within the wavelength range of 400-800 nm, using the reagent as a blank.

The standard solution was prepared with a concentration of 6-22 g/mL. Pipetted 2 mL of each concentration, 0.1 mL of 10%

aluminum chloride, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water were added, and then mixed and incubated for 40 minutes. UV-Vis spectrophotometry was used to measure the absorbance of quercetin standard solutions at various concentrations, using the reagent as a blank at a maximum wavelength of 427 nm.

Phyllanthus acidus fruit was extracted using ethanol, n-hexane, ethyl acetate, and water fractions. Each fraction weighing 25 mg was dissolved in methanol (1000 g/mL). Pipette 2 mL of the sample and add 0.1 mL of 10% aluminum chloride, 0.1 mL of sodium acetate, and 2.8 mL of distilled water to the solution. The test solution was incubated for 40 minutes, after which the absorbance was determined according to the quercetin calibration standard. The quercetin plot calibration standard was generated at the maximum absorption wavelength. Flavonoids concentrations in experimental specimens were determined using a calibration plot and then measured in QE (quercetin equivalent), which indicates the number of milligrams of quercetin in one gram of sample [12].

Antimicrobial activity test

In order to achieve an extract concentration of 500 mg/mL, 5 g of *Phyllanthus acidus* fruit ethanol extract, n-hexane fraction, ethyl acetate, and the remaining water were dissolved in 10 mL of dimethyl sulfoxide (DMSO). Following this, dilution procedures were executed to attain concentrations of 400, 300, 200, 100, 80, 60, and 40 mg/mL.

The microorganisms were collected sterilely using a hypodermic needle and then introduced onto slanted culture media for inoculation. The fungi were subjected to cultivation on Potato Dextrose Agar (PDA), whereas the bacteria were subjected to cultivation on Nutrient Agar. The process of injection entails scraping the microorganism onto the culture medium. The specimens were subjected to a regulated atmosphere with a temperature of 22 °C for 48 hours to promote the proliferation of fungi. In contrast, bacterial growth was encouraged by incubating them at 37 °C for 24 hours.

The agar diffusion method with disc paper was used for antimicrobial activity testing. A sterile petri dish was inoculated with 0.1 mL of microbial culture, followed by adding 15 mL of media. The mixture was then agitated until homogeneity was achieved and subsequently allowed to solidify. The extract solution and fruit fractions of *Phyllanthus acidus* fruit were dripped with various concentrations of 20 mL on disc paper. Then, pre-incubate for 15 minutes. After that, it was incubated at 22 °C for 48 hours for fungi and at 37 °C for *Phyllanthus acidus* fruit for 24 hours for bacteria. Furthermore, the inhibition area (clear zone) around the disc paper is measured [13].

Results and discussion

Simplicia characterization

Table 1 indicates the outcomes of the characterization of the *Simplicia* powder of *Phyllanthus acidus* fruit.

TABLE 1 Characterization results of Phyllanthus acidus (L.) Skeels fruit Simplicia powder

No.	Parameters	Results (%)
1.	Water content	3.33
2.	Water soluble extract content	23.32
3.	Ethanol soluble extract content	11.60
4.	Total ash content	5.99
5.	Acid insoluble ash content	0.45

The result of the water content test for *Phyllanthus acidus* fruit *Simplicia* was 3.33%, which indicates that the *Phyllanthus acidus* fruit *Simplicia* has met the standard requirements for water content of no more than 10% [14].

The content of water-soluble extract and ethanol was an indicator of the concentration of active compounds extracted both by water and ethanol solvents. The test results indicate a water-soluble extract content of 23.32% and an ethanol-soluble extract of 11.60%. The results indicate that the solubility of polar compounds in water exceeded that of semi-polar and non-polar compounds in ethanol [14].

The overall ash content is linked to minerals, encompassing organic and inorganic compounds acquired obtained from both internal and external sources. In contrast, the acid-insoluble ash content is intended to gauge the quantity of ash procured from exogenous factors derived from sand or silicate soil contaminants. Based on the test findings, it can be inferred that the *Phyllanthus acidus* fruit satisfies the standard specifications for total ash content and insoluble ash content, with a total ash content of 5.99% and an acid insoluble ash content of 0.45% [14].

Phytochemical screenings

Table 2 presents the outcomes of the phytochemical analysis conducted on Simplicia powder, extract, and fraction of Phyllanthus acidus fruit.

TABLE 2 Phyllanthus acidus (L.) Skeels fruit phytochemical screening results

No.	Compound Group	*Simplicia* Powder	Ethanol Extract	n-hexane Fraction	Ethyl Acetate Fraction	Residual Fraction
1.	Alkaloids	-	-	-	-	-
2.	Flavonoids	+	+	-	+	+
3.	Glycosides	+	+	-	+	+
4.	Saponins	+	+	-	+	+
5.	Tannins	+	+	-	+	-
6.	Triterpenes/ Steroids	+	+	+	-	-

= (+)Positive (compound groups were identified)
(-) = Negative (compound groups were not identified)

Flavonoids, glycosides, saponins and tannins were identified in *Simplicia* powder, ethanol extract, and ethyl acetate fraction from *Phyllanthus acidus* fruit.
Triterpenes/steroids were identified in *Simplicia* powder, ethanol extract and n-hexane fraction, but not in the ethyl acetate fraction. Triterpenes/steroid is the only compound group identified in n-hexane fraction of *Phyllanthus acidus* fruit. Flavonoids, glycosides and saponins were identified in a residual fraction of *Phyllanthus acidus* fruit. All of the phytochemical screening tests were done in qualitative methods. Positive of flavonoid is indicated if there is red, yellow, or orange color on the amyl alcohol layer, saponin compounds that presence with a lot of foams 10 cm and did not disappear with the addition of HCl 2N, and tannins are positive with the blackish green color after adding 1% $FeCl_3$ reagent. The group of tannins compounds in the residual fraction did not give positive results in a qualitative test with $FeCl_3$ because they had low levels. This was related to the determination of the total phenol content in the residual fraction, which gives lower levels than the total phenol content in the ethanol extract and ethyl acetate fraction of *Phyllanthus acidus* fruit [15].

Mineral content analysis

The results of qualitative testing of *Phyllanthus acidus* fruit on all minerals showed positive results, which can be seen in Table 3.

TABLE 3 Qualitative analysis of samples from Phyllanthus acidus fruit

No.	Mineral	Reaction Result	Results
1.	Calcium	Fine needle crystal	+
2.	Potassium	Large needle crystal	+
3.	Sodium	Small needle crystal	+
4.	Magnesium	Red colour	+
5.	Zinc	White precipitated and fern crystals	+

The above table shows that *Phyllanthus acidus* fruit contains the minerals calcium, potassium, sodium, magnesium, and zinc. The results of the quantitative test of *Phyllanthus acidus* fruit for all minerals start from determining the calibration curve using Atomic Absorption Spectrophotometry. Regression value of the calcium calibration curve for potassium, sodium, magnesium, and zinc, respectively, are 0.9993, 0.9992, 0.9994, 0.9998, and 0.9993. The fourth table presents the outcomes of the mineral level analysis conducted on *Phyllanthus acidus* fruit.

TABLE 4 Mineral content in Phyllanthus acidus fruit samples and the percentage of differences

Mineral	Sample Rate (mg/100g)		Percentage of Level Difference
	Fresh *Phyllanthus acidus* fruit	*Phyllanthus acidus* fruit boiled in water	
Calcium	117.72	58.84	53.20%
Potassium	343.78	210.11	64.90%
Sodium	15.98	6.46	41.27%
Magnesium	7.43	4.20	60.96%
Zinc	1.61	0.482	35.40%

The boiling process can decrease the number of minerals, according to Table 4 above, which shows a decrease of up to 50% in *Phyllanthus acidus* fruit. The present study employed atomic absorption spectrophotometry to determine the mineral content of *Phyllanthus acidus* fruit. There was a reduction of 53.2% calcium content, starting from 117.72 mg/100g in fresh *Phyllanthus acidus* fruit to 58.84 mg/100 g after boiled in water. A reduction of 64.9% potassium content, starting from 343.78 mg/100 g in fresh fruit to 210.11 mg/100 g after boiled in water. A reduction of 41.27% sodium content, starting from 15.98 mg/100g in fresh fruit to 6.46 mg/100 g after boiled in water. A reduction of 60.96% magnesium content, starting from 7.43 mg/100 g in fresh fruit to 4.20 mg/100 g after boiled in water. And a reduction of 35.4% zinc content, starting from 1.61 mg/100g in fresh fruit to 0.482 mg/100 g after boiled in water.

Results of determination of total phenol levels

The maximum absorption wavelength of gallic acid, having a concentration of 400 g/mL, was determined to be 765 nm. Figure 1 displays the absorption spectrum. The calibration curve for gallic acid was established by measuring its absorbance at various concentrations, specifically 200 g/mL, 300 g/mL, 400 g/mL, 500 g/mL, and 600 g/mL, using a wavelength of 765 nm.

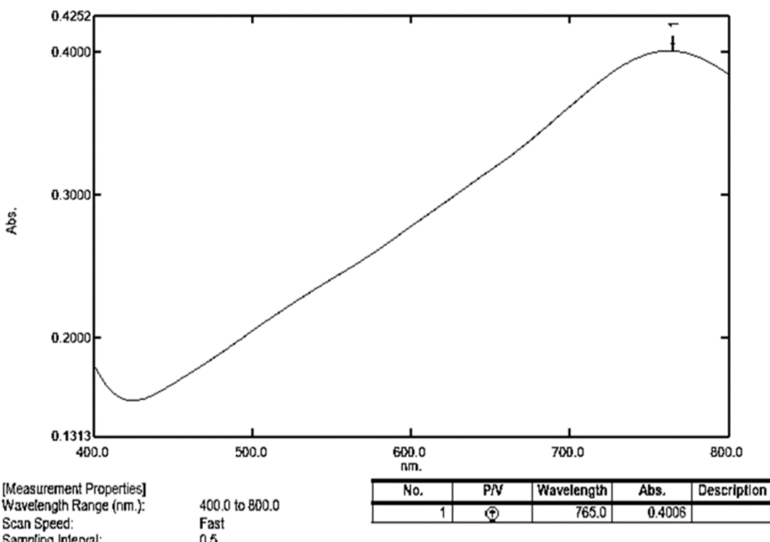

FIGURE 1 The maximum absorption of gallic acid

When gallic acid was reacted with distilled water and Folin-Ciocalteu reagent, a greenish-yellow solution was produced after being added to 20% Na_2CO_3 solution and a blue complex solution was produced. The intensity of the blue color is directly proportional to the concentration of gallic acid employed. This finding aligns with the stipulations that an aromatic nucleus in phenolic compounds can diminish phosphomolybdate phosphotungstate to blue molybdenum [16].

The regression equation Y = 0.0010X + 0.0072 was derived from the Gallic acid calibration curve, which yielded a correlation coefficient of r = 0.9994. The absorption curve is generated through the representation of the absorbance value along the Y-axis and the concentration along the X-axis. The utilization of the correlation coefficient denoted as r is employed in the context of linear regression models, where the dependent variable Y is expressed as a linear function of the independent variable X, represented as Y = axe + b. A linear relationship is considered ideal when the coefficient of correlation (r) is either +1 or -1, depending on the direction of the line, and the intercept (b) is equal to 0. Table 5 summarizes the outcomes of the assessment of the overall phenol content in the fruit fraction and ethanol extract of *Phyllanthus acidus*.

TABLE 5 Phyllanthus acidus (L) Skeels fruit phenol content in extracts and fractions

Sample	Content of Total Phenol (mg GAE/g sample)
Ethanol extract	39.27
n-hexane fraction	5.99
Ethyl acetate fraction	48.01
Remaining fraction	25.1

The analysis outcomes revealed that the extract and test fraction exhibited distinct total phenol content. The ethyl acetate fraction exhibited the highest concentration of 48.01 mg GAE/g sample, followed by the thick ethanol extract with 39.27 mg GAE/g sample. The remaining fraction demonstrated a total phenol content of 25.1 mg GAE/g sample, while the n-hexane fraction exhibited the lowest concentration of 5.99 mg GAE/g sample. The ethyl acetate fraction of *Phyllanthus acidus* fruit exhibited the highest

total phenol content, owing to a greater number of polyphenolic compounds compared to the ethanol extract, n-hexane fraction, and residual fraction. In the ethanol extract, there are still primary and secondary metabolites that have hydroxyl groups in aromatic groups such as polyphenolic can affect the reaction results after the addition of reagents. The polarity level of the solvent plays a crucial role in determining the structure and kind of phenolic compounds that are extracted. This implies that different solvents can selectively extract certain compounds based on their polarity. The ethyl acetate fraction exhibits a significantly elevated concentration of total phenol, suggesting the presence of a polyphenol group with similar polarity to that of the ethyl acetate solvent [16].

Results of determination of total flavonoids level

The investigation aimed to determine the highest level of absorption of quercetin. This was achieved by introducing 1 M $AlCl_3$, CH_3COONa reagent, and distilled water with a concentration of 14 g/mL to the sample and measuring the absorption using a UV-Vis spectrophotometer. The maximum wavelength observed was 427 nm, and the measurement was taken 30 minutes after adding the reagents. Figure 2 depicts the wavelength of absorption and calibration curve for quercetin.

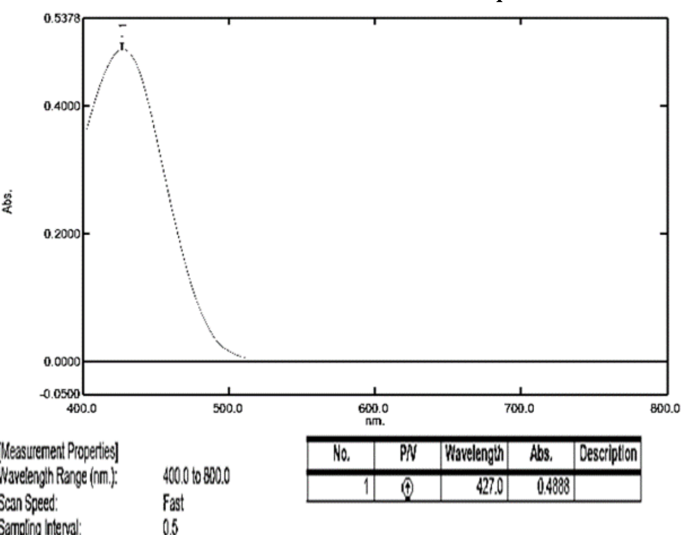

FIGURE 2 The maximum uptake of quercetin

Determination of the calibration curve using a standard solution of quercetin at a concentration of 6 - 22 g/mL and measured at a wavelength of 427 nm. The addition of $AlCl_3$ solution to the measurement of flavonoids forms a more yellow color complex so that there will be a shift in wavelength towards the visible (visible). The addition of sodium acetate maintains the wavelength in the visible region.

From the quercetin absorption curve, the value of r = 0.9999 with the regression equation Y = 0.0353X + 0.0008. The attainment of an optimal linear association is contingent upon the condition that the coefficient b equals zero, and the correlation coefficient r assumes a value of either positive or negative, the direction of which is determined by the orientation of the line. Table 6 presents the outcomes of the assessment of the complete phenolic content in the fraction and ethanol extract of *Phyllanthus acidus* fruit.

TABLE 6 Total flavonoids content of Phyllanthus acidus (L.) Skeels fruit extracts and fractions

Sample	Average Total Flavonoids Content (mg QE/g sample)
Ethanol Extract 96%	7.72
n-hexane Fraction	0.57
Ethyl Acetate Fraction	8.11
Remaining Fraction	3.38

The analysis revealed that the extracts and fractions tested exhibited varying levels of total flavonoids content. Notably, the ethyl acetate fraction demonstrated the highest concentration of flavonoids, measuring up to 8.11 mg QE/g sample. The thick ethanol extract followed closely with the 7.72 mg QE/g sample, while the remaining fraction exhibited a lower concentration of 3.38 mg QE/g sample. The n-hexane fraction of *Phyllanthus acidus* fruit exhibited the lowest concentration of flavonoids, measuring only 0.57 mg QE/g sample. The ethyl acetate fraction of *Phyllanthus acidus* fruit exhibited the greatest aggregate flavonoids content, owing to the comparatively higher concentration of flavonoids compounds present in this fraction compared to the ethanol extract, n-hexane fraction, and residual fraction. The presence of primary and secondary metabolites in the ethanol extract can influence the outcome of reactions after the introduction of reagents [17].

The study investigated the total phenol and flavonoids content and the antimicrobial activity of ethanol extract and fractions (n-hexane, ethylacetate, and residue) obtained from *Phyllanthus acidus* fruit. The results revealed that the ethyl acetate fraction exhibited the highest total phenol content of 48.01 mg GAE/g sample and total flavonoids content of 8.11 mg QE/g sample. Furthermore, the ethylacetate fraction demonstrated the most potent antimicrobial activity against *E. coli*, *S. aureus*, *S. mutans*, *P. aeruginosa*, *C. albicans*, and *M. canis*. In contrast, the n-hexane fraction exhibited the weakest activity.

Antimicrobial activity test

Antibacterial testing using Nutrient Agar media with Staphylococcus aureus, Escherichia coli, Streptococcus mutans, and Pseudomonas aeruginosa bacteria while the antifungal testing using potato dextrose agar (PDA) with the fungus Candida albicans, Microsporum canis (Table 7).

TABLE 7 Results of bacterial growth inhibitory area test diameter measurement

Bacterial		Concentration (mg/mL)													
		500	400	300	200	100	80	60	40	35	30	25	20	15	10
Staphylococcus aureus	A	17.53	16.63	15.13	13.93	10.43	10.0	8.87	8.07	7.23	-	-	-	-	-
	B	-	7.20	6.93	6.60	6.30	-	-	-	-	-	-	-	-	-
	C	21.90	20.80	18.80	17.97	17.73	17.27	16.70	15.50	12.83	12.47	11.43	12.47	9.43	8.17
	D	15.23	13.23	11.53	11.07	9.53	8.77	8.33	8.07	7.90	7.67	7.23	6.87	6.47	-
Escherichia coli	A	16.33	15.07	13.87	12.47	9.37	8.57	8.07	7.33	-	-	-	-	-	-
	B	-	7.00	6.63	6.30	6.17	-	-	-	-	-	-	-	-	-
	C	19.77	18.97	17.93	15.97	15.27	14.97	14.63	14.17	11.97	11.50	10.20	9.87	9.20	7.77
	D	14.13	12.53	11.00	9.70	9.17	8.63	8.27	7.97	7.63	7.20	6.50	6.27	-	-
Streptococcus	A	19.26	17.53	16.46	14.63	11.80	10.98	9.80	9.40	9.06	8.33	7.73	-	-	-

mutans	B	-	9.90	9.63	8.26	8.03	7.86	7.50	7.33	7.00	-	-	-	-	-
	C	28.93	27.33	26.13	24.43	19.16	18.73	16.70	15.60	15.33	14.56	13.63	12.23	10.13	9.20
	D	20.50	19.63	15.53	14.60	12.23	10.96	9.33	8.76	8.06	7.86	7.70	7.46	7.20	6.93
Pseudomonas aeruginosa	A	18.63	17.90	16.03	15.00	12.30	11.10	10.50	9.03	8.80	7.63	-	-	-	-
	B	-	10.03	9.40	8.90	8.93	8.70	8.13	7.86	7.40	-	-	-	-	-
	C	28.83	28.50	26.00	24.03	20.40	19.70	18.06	16.46	15.60	14.30	13.26	12.20	11.13	10.10
	D	24.50	20.46	19.46	16.40	13.06	12.06	10.26	9.83	9.40	8.63	8.43	8.13	7.83	7.50

A: Ethanol extract C: Ethylacetate fraction
B: n-hexane fraction D: residual fraction

The findings of the antibacterial activity measurement conducted on four bacterial strains indicate that the ethylacetate fraction exhibited the most potent inhibition in comparison to the ethanol extract, n-hexane fraction, and residual fraction. This is attributed to the extraction of numerous secondary metabolites with the ethyl acetate solvent. The antibacterial activity of the n-hexane fraction is comparatively weak due to its exclusive composition of steroid/triterpenoid compounds (Table 8).

TABLE 8 Measurement of test fungus growth inhibitory area diameter

No.	X	*Candida albicans*				*Microsporum canis*			
		A	B	C	D	A	B	C	D
1	500	25.50	-	44.97	22.4	25.37	-	33.13	21.8
2	400	22.10	11.93	42.70	21.0	23.53	11.60	30.40	20.4
3	300	20.83	10.73	38.27	19.4	22.07	10.53	27.77	18.9
4	200	17.63	9.90	33.47	17.3	18.57	9.37	25.80	15.7
5	100	13.33	-	29.63	13.3	12.93	-	14.70	13.1
6	80	10.53	-	26.70	10.2	11.57	-	13.3	11.9
7	60	8.83	-	19.97	9.4	9.70	-	12.4	10.9
8	40	6.77	-	18.37	8.8	7.73	-	10.8	9.8
9	35	-	-	14.67	8.4	-	-	10.3	9.3
10	30	-	-	11.20	8.0	-	-	9.13	8.5
11	25	-	-	8.87	7.6	-	-	8.1	7.9
12	20	-	-	7.93	7.1	-	-	7.5	7.4
13	15	-	-	6.80	6.5	-	-	6.6	6.4
14	blank	-	-	-	-	-	-	-	-

X: Concentration (mg/mL)
A: Ethanol extract C: Ethylacetate fraction
B: n-hexane fraction D: residual fraction

According to conventional wisdom, the inhibition zone's sensitivity degree is determined by its diameter. Specifically, if the diameter of the inhibition zone exceeds 20 mm, it is considered strongly sensitive. It is deemed moderately sensitive if the diameter falls between 16-20 mm. Inhibition zones with diameters ranging from 10-15 mm are classified as intermediate, while those below this range are considered resistant 0 (zero). As per the guidelines provided by the Clinical Laboratory and Standard Institute (CLSI), the antifungal inhibition area is considered strong if the inhibition area's diameter exceeds 18 mm. In case the diameter of the inhibition area falls within the range of 13-17 mm, it is classified as moderate. In contrast, if the diameter of the inhibition area is less than 12 mm, it is deemed resistant [18].

The antimicrobial activity provided by the ethanol extract and ethyl acetate fraction was thought to be due to the secondary metabolites identified; namely, flavonoids and saponins. Flavonoids the largest group of compounds in nature that have antibacterial and antifungal effects because they contain phenol groups. Flavonoids identified phenol groups can also coagulate proteins and lower the surface tension of microbial cells [19,20].

The researcher has hypothesized that the residual fraction's antimicrobial properties are attributed to the presence of secondary metabolites, specifically saponins. The antifungal properties of saponins are attributed to their ability to interact with membrane sterols, thereby affecting their mechanism of action. Saponin compounds are known to exhibit antifungal properties by reducing the surface tension of sterol membranes present in microbial cell walls, thereby enhancing their permeability. The heightened permeability of the cell membrane results in the extraction of a denser intracellular fluid, leading to the release of cellular components such as nutrients, metabolic substances, enzymes, and proteins, ultimately resulting in microbial death [19,20].

The ethanol extract of *Phyllanthus acidus* fruit identified more secondary metabolites than the ethylacetate fraction but gives a smaller diameter of inhibition. This can be caused by differences in the quantity of antimicrobial secondary metabolites extracted by each solvent.

The n-hexane fraction showed weak antimicrobial activity against the test fungi because it only had steroid/terpenoid compounds. The presence of oils and fats identified in the n-hexane fraction can interfere with antimicrobial activity. Oils and fats interfere with the diffusion process and protect microbes from antimicrobial compounds so that they are unable to inhibit microbial growth [19,20]

Conclusion

The present study employed atomic absorption spectrophotometry to determine the mineral content of *Phyllanthus acidus* fruit. The results indicated that calcium starting from 117.72 mg/100g in fresh *Phyllanthus acidus* fruit to 58.84 mg/100 g in *Phyllanthus acidus* fruit boiled in water representing a reduction of 53.20%, potassium starting from 343.78 mg/100 g in fresh *Phyllanthus acidus* fruit to 210.11 mg/100 g in *Phyllanthus acidus* fruit boiled in water representing a reduction of 64.90%, sodium starting from 15.98 mg/100 g in fresh *Phyllanthus acidus* fruit to 6.46 mg/100 g in *Phyllanthus acidus* fruit boiled in water representing a reduction of 41.27%, magnesium starting from 7.43 mg/100 g in fresh *Phyllanthus acidus* fruit to 4.20 mg/100 g in *Phyllanthus acidus* fruit boiled in water representing a reduction of 60.96% and zinc starting from 1.61 mg/100g in fresh *Phyllanthus acidus* fruit to 0.482 mg/100g in *Phyllanthus acidus* fruit boiled in water representing a reduction of 35.40%. The study investigated the total phenol and flavonoids content and the antimicrobial activity of ethanol extract and fractions (n-hexane, ethylacetate, and residue) obtained from *Phyllanthus acidus* fruit. The results revealed that the ethyl acetate fraction exhibited the highest total phenol content of 48.01 mg GAE/g sample and total flavonoids content of 8.11 mg QE/g sample. Furthermore, the ethyl acetate fraction demonstrated the most potent antimicrobial activity against *E. coli*, *S. aureus*, *S. mutans*, *P. aeruginosa*, *C. albicans*, and M. canis. In contrast, the n-hexane fraction exhibited the weakest activity.

Based on this conclusion, Phyllanthus acidus (L.) fruit has potential as an alternative treatment, so it is recommended that for further research we can find out about the therapeutic activity of the extract and fraction

as an antioxidant, anti-diabetic, or anti-inflammatory.

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Conflict of Interest

The authors declare no conflict of interest.

Orcid:
Masfria Masfria:
https://orcid.org/0000-0001-6087-4349
Hafid Syahputra:*
https://orcid.org/0000-0001-6628-7727
Laili Khairani:
https://orcid.org/0009-0002-6731-9633
Pani Reflesia Nainggolan:
https://orcid.org/0009-0004-0086-2855
Gitta Yohana:
https://orcid.org/0009-0003-2884-3595
Elda Lastry Marta Yanti:
https://orcid.org/0009-0000-5557-6013

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