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Phytochemical Screening and Determination of Phenolic and Flavonoid Contents in Ethanol Extract of *Phyllanthus emblica L.* Fruit

Hafid Syahputra[1] Masfria[1], Muhammad Fauzan Lubis[2], Mita Oktria Ginting[3], Nawang Pramesti[3], Putri Aisyah Nasution[3]
[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia*
[2]*Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia*
[3]*Undergraduate Program, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia*
*Corresponding Author: hafid@usu.ac.id

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ABSTRAK
Buah *Phyllanthus emblica* L. dikenal memiliki aktivitas antioksidan yang kuat, yang erat kaitannya dengan kandungan fenol dan flavonoidnya. Penelitian ini bertujuan untuk menganalisis kandungan metabolit sekunder dan menentukan kadar total fenol serta flavonoid dalam buah Phyllanthus emblica. Proses ekstraksi dilakukan menggunakan metode maserasi dengan pelarut etanol 96%, kemudian dilanjutkan dengan penguapan menggunakan rotary evaporator. Ekstrak etanol yang diperoleh selanjutnya diuji skrining fitokimia menggunakan pereaksi warna, dan kadar total fenol serta flavonoid diukur menggunakan metode kolorimetri. Pereaksi Folin–Ciocalteu diaplikasikan untuk mengomplekskan senyawa fenolik dengan asam galat sebagai standar pembanding. Penentuan kadar total flavonoid dilakukan dengan metode aluminium klorida menggunakan kuersetin sebagai standar pembanding. Hasil penelitian menunjukkan bahwa ekstrak etanol buah Phyllanthus emblica mengandung alkaloid, saponin, flavonoid, tanin, triterpenoid, dan glikosida. Kadar total fenol yang diperoleh adalah 61,1271±3,86 mg GAE/g ekstrak, sementara kadar total flavonoid mencapai 3,3742±0,06 mg QE/g ekstrak.
Keyword: *Phyllanthus emblica L.*, Fenol, Flavonoid, Maserasi

ABSTRACT
Phyllanthus emblica L. fruit is known for its potent antioxidant activity, closely associated with its phenolic and flavonoid content. This study aims to analyse the secondary metabolite composition and determine the total phenol and flavonoid levels in *Phyllanthus emblica* fruit. The extraction process was carried out using the maceration method with 96% ethanol as the solvent, followed by a rotary evaporator evaporation. The ethanol extract was subjected to phytochemical screening with colour reagents, and the total phenol and flavonoid contents were assessed using a colourimetric method. TheFolin–Ciocalteu reagent was employed for complex phenolic compounds, with gallic acid as the standard reference. The total flavonoid content was determined using the aluminium chloride method, with quercetin as the standard. The results indicated that the ethanol extract of *Phyllanthus emblica* fruit contained alkaloids, saponins, flavonoids, tannins, triterpenoids, and glycosides. The study found that the total phenol content was 61.1271±3.86 mg GAE/g extract, while the total flavonoid content was 3.3742±0.06 mg QE/g extract.
Keywords: *Phyllanthus emblica L.* Fruit, Phenol, Flavonoids, Maceration

1. Introduction

Phyllanthus emblica L., commonly known as Kemloko, is a plant widely found in Indonesia, particularly on the island of Sumatra. As a member of the Phyllanthaceae family, it is distributed across subtropical and tropical regions and has been traditionally utilised for various therapeutic purposes, such as

promoting hair growth, alleviating constipation, and relieving fever and pain. The fruit of *Phyllanthus emblica* L. is rich in vitamin C and phenolic compounds, which contribute to its antibacterial, antioxidant, anticancer, antidiabetic, analgesic, and antipyretic properties [1]. *Phyllanthus emblica* plant contains various secondary metabolites, including saponins, alkaloids, flavonoids, steroids, triterpenoids, tannins, and glycosides [2]. In particular, flavonoids exhibit multiple pharmacological effects, such as antioxidant, anti-inflammatory, antidiabetic, anticancer, antiviral, anti-ageing, and antibacterial activities, as well as protecting against cardiovascular diseases [3].

Phenolic and flavonoid compounds in *Phyllanthus emblica* L. are the primary contributors to its antioxidant activity. Phenolic compounds function as free radical acceptors and chain-breaking agents, rapidly inhibiting lipid oxidation and other molecules by donating hydrogen atoms to radical species. Besides their antioxidant role, phenolic compounds may also exert preventive effects against various diseases through different mechanisms, including cellular signalling, gene expression, and modulation of enzymatic activity. Structurally, phenolic compounds possess one or more aromatic rings with one or more hydroxyl groups [4].

Flavonoids act as primary antioxidants capable of neutralising free radicals at low concentrations and may function as pro-oxidants when free radical concentrations are excessively high. As antioxidants, flavonoids undergo oxidation by radicals, forming more stable or neutral radicals [5]. Their pharmacological activities, including antioxidant properties, are closely related to their ring structure, allowing them to function as reducing agents, hydrogen donors, superoxide radical scavengers, and metal chelators [6].

The extraction process of phenolic and flavonoid compounds from plants is crucial in determining the total yield of active compounds extracted by the solvent. This study analyses the total phenolic and flavonoid content of *Phyllanthus emblica L.* fruit sourced from Padang Lawas Utara, North Sumatra. The choice of extraction method is vital, as it depends on the characteristics of the target compounds and the solvent's properties. In this research, the maceration method was employed, a simple and effective extraction technique involving soaking plant material in a single or mixed solvent at room temperature, protected from light, for a defined period [7]. Ethanol, utilised as the solvent in this study, is widely recognised as one of the most effective solvents for extracting polyphenolic compounds and has demonstrated superior efficiency in extracting a greater quantity of chemical constituents than water or methanol [8]. This approach ensures the optimal recovery of phenolic and flavonoid compounds from *Phyllanthus emblica L.* fruit.

2. Methods

2.1 Materials and Sample Preparation

The primary instruments employed in this study included a UV-Vis spectrophotometer, water bath, rotary evaporator, and analytical balance. *Phyllanthus emblica* L. fruit, sourced from Padang Lawas Utara, North Sumatra, was the primary raw material. For the extraction process, 96% ethanol was utilised as the solvent. The reagents employed for phytochemical screening included Mayer's, Bouchardat's, and Dragendorff's reagents, along with concentrated hydrochloric acid, magnesium powder, amyl alcohol, iron (III) chloride, Lieberman-Burchard reagent, Molisch reagent, and lead (II) acetate. The study utilised gallic acid, Folin-Ciocalteu reagent, sodium carbonate, quercetin, sodium acetate, aluminum chloride, analytical-grade methanol, and distilled water to assess the phenolic and flavonoid content. The *Phyllanthus emblica* L. fruit samples were meticulously cleaned, sliced, and deseeded, then dried in a controlled cabinet until a brittle texture was achieved. The dried fruit was subsequently ground into a fine powder using a blender and stored in an airtight container to preserve its integrity for further analysis [9].

2.2 Extraction of Phyllanthus emblica Fruit

Phyllanthus emblica fruit was extracted using the maceration method with 96% ethanol as the solvent. The process involved placing one part of the dried powdered plant material (simplisia) into a glass container and adding 10 parts of ethanol. The mixture was soaked for 6 hours with intermittent stirring to ensure thorough contact between the solvent and plant material. It was then left to stand for an additional 18 hours without agitation. The resulting macerate was separated through filtration, and the extraction process was repeated at least twice to ensure maximum active compound yield. All collected macerates were combined and concentrated by evaporation using a rotary evaporator to obtain the final ethanol extract [10].

2.3 Phytochemical Screening

The alkaloid identification process was initiated by accurately weighing 0.5 g of the dried powdered plant material (simplisia), adding 1 mL of 2 N hydrochloric acid (HCl) and 9 mL of distilled water. The mixture was heated in a water bath for 2 minutes, then allowed to cool and filtered. The resulting filtrate was divided into three portions for further analysis using Mayer's, Bouchardat's, and Dragendorff's reagents. The presence of alkaloids was confirmed by forming a precipitate in at least two of these tests, indicating a positive result [11].

The detection of saponins involved the preparation of 0.5 g of the dried powdered material, which was transferred into a test tube with 10 mL of hot water. After allowing the solution to cool, it was vigorously shaken for 10 seconds. The presence of saponins was indicated by forming a stable foam layer, measuring between 1-10 cm in height, which remained intact for a minimum of 10 minutes, even after adding a drop of 2 N hydrochloric acid [11].

To identify flavonoids, 10 g of the dried powder was accurately weighed and mixed with 100 mL of hot water. The mixture was boiled for 5 minutes and filtered while still hot. A 5 mL aliquot of the filtrate was then combined with 0.1 g of magnesium powder, 1 mL of concentrated hydrochloric acid, and 2 mL of amyl alcohol. Following thorough mixing and separation, the presence of flavonoids was confirmed by a colour change to red, yellow, or orange in the amyl alcohol layer, indicating a positive reaction [11].

Tannins were identified by weighing 0.5 g of the dried powdered material, which was subsequently extracted with 10 mL of distilled water and filtered. The filtrate was diluted with distilled water until a colourless solution was obtained. A 2 mL aliquot of this solution was then treated with 1-2 drops of a 1% iron (III) chloride solution. The emergence of a blue or greenish-black colouration confirmed the presence of tannins within the sample [7].

To detect steroids and triterpenoids, 1 g of the dried powder was macerated with 20 mL of n-hexane for 2 hours, followed by filtration. The resulting filtrate was evaporated using an evaporating dish, and the residue was treated with 2 drops of acetic anhydride and 1 drop of concentrated sulfuric acid. The formation of a red, pink, or purple colouration indicated the presence of triterpenoids, whereas a greenish-blue colouration signified the presence of steroids [7].

Glycoside identification was performed by weighing 3 g of the dried powdered material, which was then extracted with a mixture comprising 30 mL of 95% ethanol and water (7:3) and 10 mL of 2 N sulfuric acid, followed by refluxing for 10 minutes. After cooling, the solution was filtered, and 20 mL of the filtrate was treated with 25 mL of 0.4 N lead (II) acetate solution, thoroughly shaken, and allowed to stand for 5 minutes before being filtered again. The filtrate was then extracted three times with 20 mL of a chloroform-isopropanol mixture (3:2). The chloroform layer was treated with anhydrous sodium sulfate, filtered, and evaporated at a temperature not exceeding 50°C. The residue was dissolved in 2 mL of methanol, and a 0.1 mL portion of this solution was placed in a test tube and evaporated using a water bath. The remaining residue was combined with 2 mL of water and 5 drops of Molisch reagent, then carefully adding 2 mL of concentrated sulfuric acid. The formation of a violet ring at the interface of the two layers confirmed the presence of glycosides [7].

2.4 Determination of Total Phenolic Content

The total phenolic content of the *Phyllanthus emblica* fruit was determined using gallic acid as the standard reference. Initially, 50 mg of gallic acid was accurately weighed and transferred into a 50 mL volumetric flask, dissolved, and made up to volume with methanol and distilled water to obtain a final 1 mg/mL concentration. Subsequently, a 3 mL aliquot of this stock solution was pipetted into a 10 mL volumetric flask further diluted with methanol and distilled water to reach a final volume of 10 mL, resulting in a concentration of 300 µg/mL. From this, 0.3 mL of the 300 µg/mL gallic acid solution was pipetted into a 10 mL volumetric flask, followed by adding 7.2 mL of distilled water and 1 mL of 10% Folin-Ciocalteu reagent. The solution was vortexed and allowed to stand for 3 minutes. Subsequently, 1.5 mL of 20% Na_2CO_3 solution was added, homogenised, and left to stand at room temperature. The absorbance was measured at a wavelength range of 400-800 nm. The operating time for quercetin was recorded every minute for 60 minutes at the maximum wavelength. A calibration curve was then constructed using a series of gallic acid concentrations (15, 20, 30, 40, and 45 µg/mL) to establish the relationship between gallic acid concentration (µg/mL) and absorbance.

For the sample measurement, 10 mg of the ethanol extract of *Phyllanthus emblica* fruit was accurately weighed and dissolved in analytical-grade methanol in a 10 mL volumetric flask to achieve a 1000 µg/mL concentration. A 0.3 mL aliquot of this sample solution was then transferred into a 10 mL volumetric flask, and 7.5 mL of distilled water, followed by 1 mL of 10% Folin-Ciocalteu reagent, was added. The solution was vortexed and left to stand for 3 minutes. Subsequently, 1.5 mL of 20% Na_2CO_3 solution was added, homogenised, and allowed to stand at room temperature. The absorbance was measured at the wavelength range of 400-800 nm. The total phenolic content obtained was expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/g extract) ± standard deviation.

The total phenolic content (TPC) was calculated using the linear regression equation obtained from the calibration curve, with the formula:

$$\mathrm{TPC} = \frac{\mathrm{C.V.FP}}{\mathrm{g}}$$

Note:
TPC = Total Phenolic Content (mg GAE/g extract)
C = concentration of the compound in the sample solution (µg/mL)
V = volume of the sample solution (mL)
W = weight of the sample (g)
FP = Dilution factor.

2.5 Determination of Total Flavonoid Content

The total flavonoid content of the *Phyllanthus emblica* fruit was determined using quercetin as the reference standard. Initially, 10 mg of quercetin was accurately weighed and dissolved in a 100 mL volumetric flask using analytical-grade methanol to reach the mark, resulting in a stock solution with a 100 µg/mL concentration. From this stock solution, 6 mL was pipetted into a 10 mL volumetric flask and further diluted to the mark with analytical-grade methanol to obtain a secondary solution with a 60 µg/mL concentration. Subsequently, 0.5 mL of this solution was transferred into a 5 mL volumetric flask, followed by adding 1.5 mL of analytical-grade methanol, 0.1 mL of 10% $AlCl_3$ solutions, 0.1 mL of 1M sodium acetate, and 2.8 mL of distilled water. The mixture was homogenised and allowed to stand for 30 minutes. The absorbance was then measured at the maximum wavelength within the 400-800 nm range. The operating time for quercetin was recorded every minute for 60 minutes at the maximum wavelength. A calibration curve was established by preparing a series of quercetin concentrations (30, 40, 50, 60, 70, and 80 µg/mL) to create a correlation between quercetin concentration (µg/mL) and absorbance.

For the sample analysis, 10 mg of the ethanol extract of *Phyllanthus emblica* fruit was accurately weighed and dissolved in analytical-grade methanol in a 10 mL volumetric flask to obtain a final concentration of 1000 µg/mL. A 0.5 mL aliquot of this sample solution was pipetted into a 5 mL volumetric flask, followed by the addition of 1.5 mL of analytical-grade methanol, 0.1 mL of 10% $AlCl_3$ solution, 0.1 mL of 1M sodium acetate, and 2.8 mL of distilled water. The mixture was homogenised and allowed to stand under ambient conditions for the optimal time. The absorbance was measured at the maximum wavelength, and the procedure was repeated three times to ensure accuracy and minimise experimental errors.

The total flavonoid content was calculated using the linear regression equation obtained from the calibration curve and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract) ± standard deviation. The calculation of total flavonoid content (TFC) was performed using the following formula:

$$\mathrm{TFC} = \frac{\mathrm{C\ x\ V\ x\ FP}}{\mathrm{W}}$$

Note:
TFC = Total flavonoid content (mg QE/g extract)
C = concentration of the compound in the sample solution (µg/mL)
V = volume of the sample solution (mL)
W = weight of the sample (g)
FP = Dilution factor.

3. Results and Discussion

3.1 Extraction of Phyllanthus emblica Fruit

The extraction process used the maceration technique with 96% ethanol as the solvent. 1200 g of *Phyllanthus emblica* fruit powder was immersed in ethanol at a ratio of 1:10 (sample to solvent) by weight. During the maceration process, intermittent stirring was carried out to enhance the extraction efficiency. After the maceration process, the solvent was evaporated to obtain a concentrated extract, resulting in 580 g of concentrated extract, equivalent to a yield of 48%, as seen in Figure 1.

Maceration is a cold extraction method that relies on dissolving active constituents from the plant material into the solvent, allowing equilibrium between the intracellular and extracellular concentrations. 96% ethanol in this study was intended to extract polar metabolites effectively, as phenolic and flavonoid compounds possess multiple hydroxyl groups, rendering them highly polar. This method was chosen to maximise the extraction of phenolic and flavonoid compounds from *Phyllanthus emblica* fruit, ensuring an efficient recovery of these bioactive constituents.

a. b.

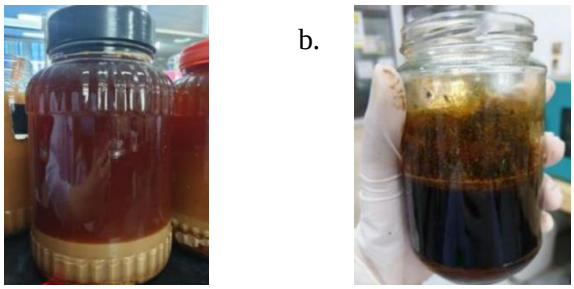

Figure 1. (a) Maceration Process; (b) Ethanol Extract Results of Phyllanthus emblica Fruit

3.2 Phytochemical Screening

The obtained ethanol extract was subjected to phytochemical screening to identify the classes of compounds present within the plant material. Phytochemical screening uses colourimetric reactions with specific reagents to detect chemical groups [12]. The results of the phytochemical analysis revealed that the *Phyllanthus emblica* fruit extract contained several classes of bioactive compounds, including alkaloids, flavonoids, tannins, saponins, triterpenoids, and glycosides. These findings indicate that the ethanol extract is rich in secondary metabolites known for their potential pharmacological activities.

3.3 Determination of Total Phenolic Content

The total phenolic content of the *Phyllanthus emblica* ethanol extract was determined using a colourimetric method with the Folin-Ciocalteu reagent. Phenolic compounds in the extract react with the Folin-Ciocalteu reagent to form a blue-coloured complex, which absorbs light at a specific wavelength, quantifying phenolic content. The absorbance measurement was conducted at a maximum wavelength of 744 nm, with the optimal operating time recorded between 57-60 minutes.

The calibration curve was established using gallic acid as a standard reference to ensure accurate phenolic content determination. The calibration curve displayed a linear relationship, with a correlation coefficient (R) of 0.99920 and the regression equation $Y = 0.01497x + 0.00626$. The high R-value, approaching 1, indicates a strong linearity, confirming that the regression equation reliably represents the relationship between phenolic concentration and absorbance [13]. This linearity is consistent with the Lambert-Beer law, which states that absorbance is directly proportional to the concentration of the analyte, provided the system behaves ideally. The maximum wavelength and calibration curve can be seen in Figure 2.

The calculated total phenolic content of the *Phyllanthus emblica* ethanol extract, expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract), is presented in Table 1. These results highlight the presence of phenolic compounds in the extract, suggesting its potential antioxidant capacity and reinforcing the value of *Phyllanthus emblica* as a source of phenolic-rich bioactive compounds.

Table 1. Determination of Total Phenolic Contents

No	Sample Weight (g)	Absorbance	GAE Content (µg/ml)	Content (mgGAE/g extract)	Total Phenolic Content (mgGAE/g extract)
1	0.01	0.8605	57.0612	57.0612	61.1271±3.86
2	0.01	0.9523	63.1936	63.1936	
3	0.01	0.9513	63.1267	63.1267	

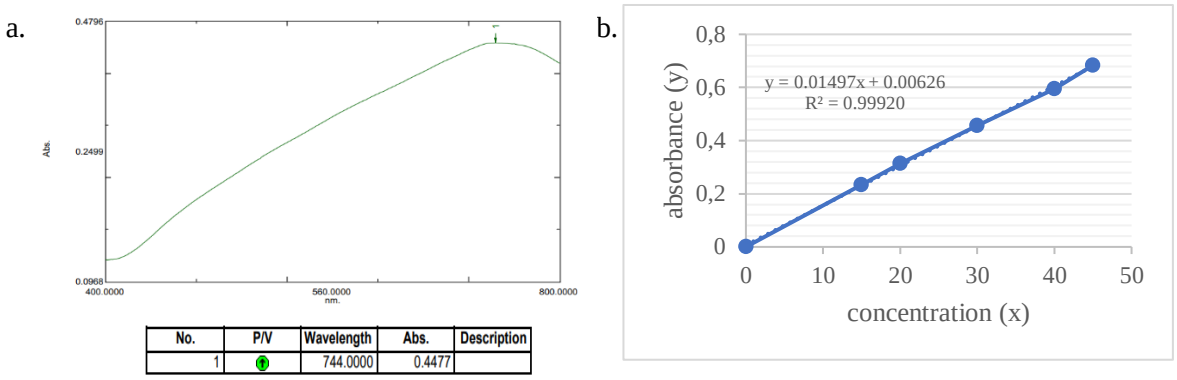

Figure 2. (a) Maximum Wavelength Curve of Gallic Acid; (b) Standard Curve of Gallic Acid

3.4 Determination of Total Flavonoid Content

The total flavonoid content of the *Phyllanthus emblica* ethanol extract was analysed using a colourimetric method involving 10% aluminium chloride ($AlCl_3$) and 1M sodium acetate. The use of $AlCl_3$ facilitates the formation of a complex with flavonoid compounds, resulting in a bathochromic shift (redshift) in the absorption spectrum, which is characterised by the development of yellowish colour in the solution. Quercetin was employed as the standard reference for quantifying flavonoid content, as it belongs to the flavonol group and contains a keto group at the C-4 position and hydroxyl groups at the C-3 and C-5 positions, characteristic of flavonol and flavone structures. The addition of sodium acetate stabilises the complex and maintains the wavelength in the visible region [14].

The absorbance of the ethanol extract was measured at a maximum wavelength of 437 nm, with consistent readings observed between the 47th and 49th minutes, indicating optimal stability. The flavonoid content was determined by establishing a calibration curve using quercetin as the standard, and the resulting curve exhibited a linear relationship with a correlation coefficient (R) of 0.9994 and a regression equation of Y = 0.0076x – 0.0038. The high R-value, close to 1, confirms the linearity of the regression equation, indicating an accurate relationship between quercetin concentration and absorbance. This follows the Lambert-Beer law, which postulates that absorbance is directly proportional to the concentration of the analyte under ideal conditions [13]. The maximum wavelength and calibration curve can be seen in Figure 1.

The calculated total flavonoid content of the *Phyllanthus emblica* ethanol extract is expressed in milligrams of quercetin equivalent per gram of extract (mg QE/g extract) and is presented in Table 2. The high flavonoid content observed in the extract underscores the potential pharmacological properties of *Phyllanthus emblica*, particularly its antioxidant activity, which is attributed to the presence of flavonoid compounds. These findings suggest that the ethanol extract of *Phyllanthus emblica* could serve as a valuable source of natural flavonoids with potential therapeutic applications.

Table 2. Determination of Total Flavonoid Content

No	Sample Weight (g)	Absorbance	GAE Content (µg/ml)	Content (mgGAE/g extract)	Total Flavonoid Content (mgGAE/g extract)
1	0.01	0.0209	3.2544	3.2544	3.3742±0.06
2	0.01	0.0227	3.4868	3.4868	
3	0.01	0.0219	3.3816	3.3816	

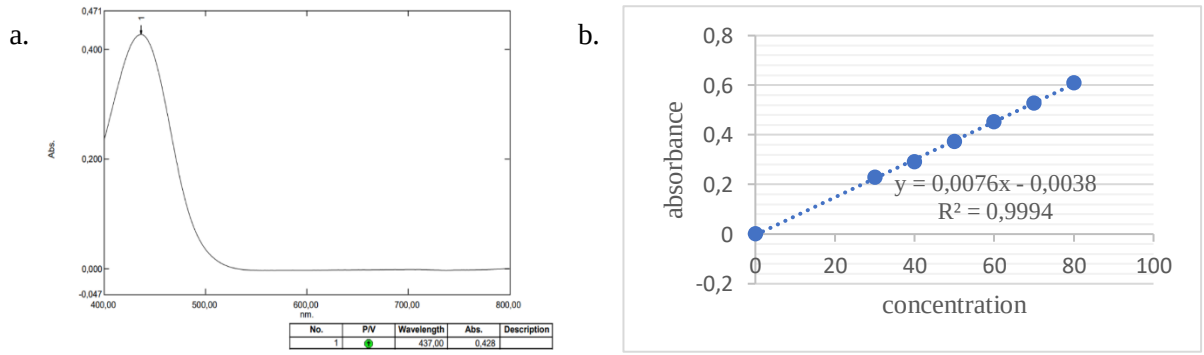

Figure 3. (a) Maximum Wavelength Curve of Quercetin; (b) Standard Curve of Quercetin

The total phenolic content of the ethanol extract of *Phyllanthus emblica* fruit was determined to be 61.1271 ± 3.86 mg GAE/g extract, while the total flavonoid content was found to be 3.3742 ± 0.06 mg QE/g extract. Phenolic compounds constitute plants' most significant group of natural antioxidants, characterised by one or more phenolic rings with hydroxyl groups attached to the aromatic ring. These structural features enable phenolic compounds to readily donate hydrogen atoms to neutralise free radicals, thereby preventing oxidative damage. The ability of phenolic compounds to form stable phenoxyl radicals during oxidation reactions makes them highly effective as natural antioxidants [4].

Numerous studies have indicated that medicinal plants contain significant amounts of phenolic compounds primarily responsible for their antioxidant activities. In particular, phenolic compounds, such as flavonoids and phenolic acids, have been recognised as the main contributors to antioxidant properties in plant extracts [4]. According to a study by Manikandan et al. (2019), *Phyllanthus emblica* L. is reported to be rich in polyphenolic compounds and is widely distributed throughout Asia, which supports the findings of the current study that highlights the high phenolic content in the extract [15].

Antioxidants are compounds capable of neutralising free radicals in the human body, preventing cellular damage caused by oxidative stress and reactive oxygen species (ROS). Due to their high reactivity, antioxidants undergo oxidation, protecting other cellular molecules from oxidative damage. The observed biological activities of *Phyllanthus emblica*, including its antioxidant, antibacterial, anticancer, antidiabetic, anti-inflammatory, cardioprotective, and anti-anaemia properties, are likely attributed to the presence of phenolic compounds, which confer these health benefits [16]. The substantial levels of phenolic and flavonoid compounds in *Phyllanthus emblica* fruit suggest its potential application as a natural antioxidant source, offering therapeutic advantages for managing oxidative stress-related health conditions.

4. Conclusion

The findings of this study indicate that *Phyllanthus emblica* fruit contains a diverse range of bioactive compounds, including phenolics, flavonoids, alkaloids, saponins, tannins, triterpenoids, and glycosides. The total phenolic and flavonoid content were quantified as 61.1271 ± 3.86 mg GAE/g extract and 3.3742 ± 0.06 mg QE/g extract, respectively. These results suggest that *Phyllanthus emblica* is a rich source of natural phenolic and flavonoid compounds with potential pharmacological properties. To further enhance the extraction yield and efficacy of these bioactive compounds, future research should consider employing different solvents or extraction methods to optimise the recovery of phenolic and flavonoid constituents from the fruit.

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