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Flavonoid Level Determination in Jamu Pegel Linu in Magelang Regency Using Uv-Visible Spectrophotometry

Selma Septi Pratiwi, Perdana Priya Haresmita*, Missya Putri Kurnia Pradani, Arief Kusuma Wardani

Department of Pharmacy, Faculty of Health Sciences, Universitas Muhammadiyah Magelang, Indonesia
*Corresponding author: perdanapriyaharesmita@unimma.ac.id

Abstract

Jamu is made from the toga plant, which is still used medicinally in Indonesia. Rutin, a type of flavonoid component, is frequently present in herbal medicine pegel linu. Rutin serves as an antioxidant and anti-inflammatory by neutralizing free radicals, which helps to prevent tissue damage and relieve soreness. This research's objective was to evaluate the amounts of flavonoids in samples using UV-Visible spectrophotometry, employing three samples of herbal medicine (A, B and C) as well as the standard solution, reagents $AlCl_3$ and sodium acetate. In this investigation, the protocol utilized to determine the maximum wavelength was 400–800 nm, and the result was 413.5 nm. The linear regression equation is y = 0.0471x + 0.0624 with a correlation coefficient of R^2 of 0.9934 and a LOD value of 3.9694 mg / L and a LOQ value of 13.231 mg / L. The determination of total flavonoid levels considered as rutin uses concentrations of 10, 12.14, 16 and 18 ppm with operating time of 30 minutes. The findings of rutin content analysis for samples A, B, and C were 1.6683%, 2.8763%, and 3.0923%, respectively.

Keywords: jamu, flavonoid, rutin, spectrophotometry UV-Visible

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1 Introduction

One of the common traditional treatments used by Indonesians, known as jamu, is made from family medicinal plants (TOGA), which are typically used as part of traditional medicine to preserve local culture or custom [1]. To make their products more effective and to have a quicker effect, certain manufacturers of herbal medicines frequently add BKO to their products. Since BKO has analgesic properties and can alleviate pain, it is frequently included to herbal medicines. As a result, the impact of BKO is comparable to the effectiveness of linu pegel herbal medication in reducing bodily aches and linu's effects [2]. More than 50% of Indonesians were said to have used herbal medicine in 2010, according to a research. Up to 95.60 % of Indonesians who take herbal medicine report feeling better physically. The three most commonly utilized herbs are ginger 50.36%, kencur 48.77% and temulawak 39.65%, which are all used in finished potion liquid 48.0% [3]. Flavonoid groups like quercetin, catechin, rutin, epicathecin, naringenin and kaempferol are among the high antioxidant substances commonly found in materials used to make herbal medicines [4].

Flavonoids, which are secondary metabolites of polyphenols and are abundantly present in both plants and foods, have a variety of bioactive properties, such as antiviral and anti-inflammatory actions [5]. anti-cancer, diabetic-friendly and cardioprotective [6]. Flavonoids have anti-aging, antioxidant properties [7], and they are also effective as inhibitors of hydrolysis, oxidative enzymes and anti-inflammatories [8]. Antioxidants work to counteract free radicals, preventing bodily harm and the onset of degenerative diseases [9]. To address this need, the search for natural antioxidant compounds is focused on natural resources. New natural antioxidant compounds must be continuously sought for or at least updated in order to become a safer antidote to free radicals for the human body [10]. Flavonoids devided into the following subclasses: flavanols, flavanones, flavones, isoflavones, anthocyanidins and flavonols. The flavonoid subclass is divided based on structural characteristics [11]. Figure 1 illustrates derivatives of flavonoid substances.

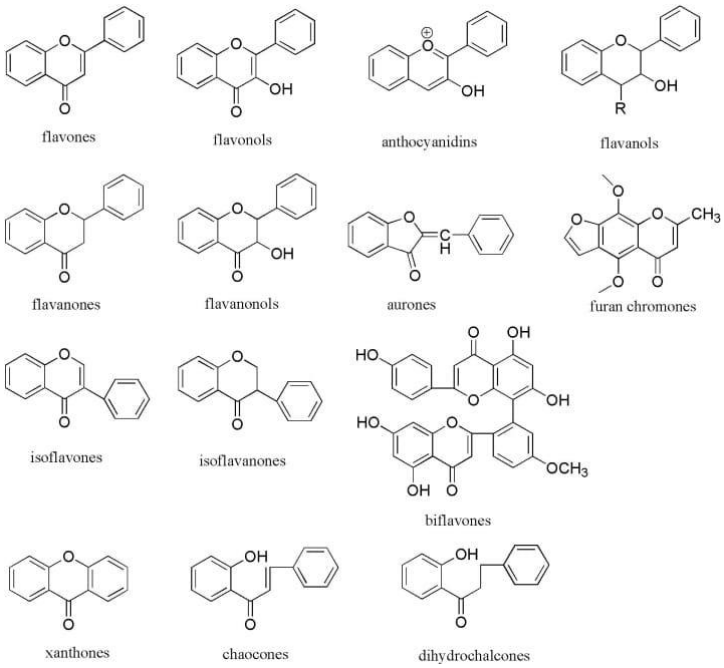

Figure 1 Structure and derivatives of flavonoid compounds [12].

Rutin is produced when quercetin and glicon rutinosa condensing together (rhamnose and glucose). The most popular variety of glycoside quercetin is rutin. The chemical compound rutin is known as 3′,4′,5,7-tetrahydroxyflavon-3-D-rutinosida by IUPAC. In the presence of a hydroxyl group, this molecule has anti-radical properties. Rutin has antioxidant, neuroprotective, cardioprotective and anticarcinogenic properties in addition to its antioxidant activity [8]. Figure 2 illustrates the rutin's chemical structure. The findings of this study are anticipated to offer details on

flavonoid concentrations and a number of validation parameter values in samples of the herbal remedy pegel linu that were assessed using the UV-Visible spectrophotometry method.

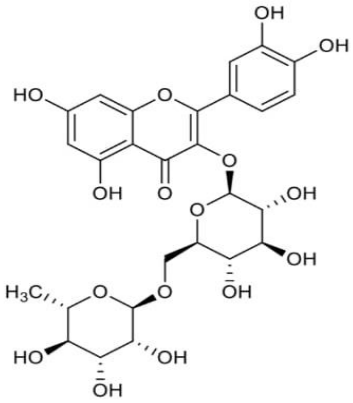

Figure 2 Rutin structure [13].

2 Materials and Methods

2.1 Tools and Materials

Digital scales (SHS), micro pipettes, measuring flasks measuring 10 ml, 25 ml and 100 ml, petri plates, stretcher spoons, stirring rods, test tubes, test tube racks, blue tips, yellow tips, micro pipettes, waterbath (DFS), cuvettes and UV-visible spectrophotometry were the tools utilized in this work. Jamu samples A, B, and C, ethanol 80% (Bratachem), ethanol 96% (Merck), ethanol P, $AlCl_3$, sodium acetate, aquadest and rutin standard.

2.2 Methods

The procedures used in this study included sampling, sample preparation using an extraction method, preparation of reagents, standard solutions, preparation of blanks, determination of the maximum wavelength, determination of the standard curve, analysis of the flavonoid content of the sample using the UV-Visible spetrophotometry method, and validation of the method used.

2.3 Sampling

the samples requirements of jamu pegel linu, the samples were bought from a number of shops in Magelang Regency.

2.4 Preparation of Samples

A measuring flask with a capacity of 10 ml is used to hold the sample, which can weigh up to 500 mg. The sample was then extracted with a magnetic stirrer at 50°C for 60 minutes after receiving up to ethanol 80% 25 ml. In order to attain a level of 1000 ppm [14], the sample was filtered using filter paper and then deposited in volume to the limit mark [15].

2.5 Reagent Production

2.5.1 Aluminium Chloride Solution 10% ($AlCl_3$)

Aquades were used to dissolve 2.5 grams of aluminum chloride ($AlCl_3$) in a 25 ml measuring flask. Aquades were then used to reach the limit and homogenized the mixture.

2.5.2 Solution of Sodium Acetate 1 M

Sodium acetate 2.05 gram were dissolved in 25 ml of aquades in a measuring flask to the limit mark, then homogenized [16].

2.6 Production of Standard 1000 ppm Standard Solutions

Ten mg of rutin are added to a measuring flask 10 ml, which is then homogenized after being thoroughly dissolved in ethanol 80%.

2.7 Creating the Blank Solution

The following ingredients were added to a 10 ml measuring flask: ethanol P 1.0 ml, ethanol 80% 1.5 ml, $AlCl_3$ 10% 0.1 ml, sodium acetate 1M 0.1 ml and aquades 2.8 ml.

2.8 Determination Of Maximum Absorbance Wavelength

From the standard solution of 1000 ppm, a pipette full up to 1 ml is poured into a measuring flask with 10 ml and ethanol 80% is then added until the limit mark is reached to produce 100 ppm. Then, after adding up to 0.5 ml to the pipette, homogenizing it with ethanol 80% 1.5 ml, $AlCl_3$ 0.1 ml, sodium acetate 0.1 ml and aquades 2.8 ml. Utilizing a visible beam spectrophotometer, wait 30 minutes before

determining its absorbance at wavelengths between 400 until 800 nm.

2.9 Rutin Standard Curve

Typically, the standard solution is measured out using a micropipette with a volume of 1; 1,2; 1,4; 1,6 or 1,8 ml and then poured into a measuring flask 10 ml. The standard liquor is then mixed with ethanol 80% to reach the set limit. Then, 1.5 ml of ethanol 80% is added to each solution after it has been diluted by 0.5 ml using a micropipette and a 10 ml measuring flask. Next aluminum chloride 10% ($AlCl_3$) 0.1 ml, sodium acetate 1M 0.1 ml, and aquades 2.8 ml are added to achieve a rutin level of 10, 12, 14, 16, and 18 g/ml. An ordinary maximum absorbance wavelength is used to measure the absorbance of this homogenized solution using a visible beam spectrophotometer after it has been allowed to stand for 30 minutes. In order to find its linear regression equation, next develop a calibration curve.

2.10 Analysis of the Sample Solution's Total Flavonoid Levels

The sample is pipetted into a measuring flask 10 ml with the extracted material 1.0 ml. Ampelous then adds ethanol 80% 1.5 ml, aluminum chloride 10% ($AlCl_3$) 0.1 ml, sodium acetate 1M 0.1 ml and aquades 2.8 ml. Following a 30-minute standing period, this solution is agitated until homogeneous. Using a visible beam spectrophotometer, the absorbance was assessed after 30 minutes, and the procedure was then repeated three times. The linear regression equation of the calibration curve uses the total flavonoid levels present in the sample to calculate the total flavonoid compound content.

2.11 Validation of Method

2.11.1 Linearity

The results for absorbance are used to compute the correlation coefficient (r), slope, and intercept values. The response of measurement findings with concentrations close to straight lines demonstrates the linearity of a procedure [17]. The correlation coefficient (r) of the linear regression analysis (y = bx + a) of the calibration curve is used to determine the linearity of the standard curve. When r 0.99 and Vxo, the coefficient of the regression function, are both less than 5%, the coefficient of cleration is said to be good.

2.11.2 Limits of Detection (LOD) and Quantification (LOQ)

The calibration curve's standard deviation and slope data were used to calculate LOD and LOQ. Yb 3 for the detection limit and 10 for the quantity limit in the calculation of the value of Y [18]. The detection limit and quantity limit can be determined using the equations 1, 2, and 3.

$$SD = \sqrt{\frac{(y-y')^2}{n-2}} \quad \text{(Equation 1)}$$

$$LOD = \frac{3 \times SD}{slope} \quad \text{(Equation 2)}$$

$$LOQ = \frac{10 \times SD}{slope} \quad \text{(Equation 3)}$$

Information:
SD : standard deviation.
Slope : slope of the calibration curve.

2.11.3 Calculation of Flavonoid Levels

The final flavonoid value is calculated using the formula equation 4, which was derived in earlier studies.

$$\text{Identifying Flavonoid} = \frac{m}{g} = \frac{Y \times N \times V}{W} x\ 100\% \quad \text{(Equation 3)}$$

Information :
Y : flavonoid concentration (mg g^{-1})
N : dilution value
V : volume of extraction yield (mL)
W : sample of herbal medicine mass (g) [19].

3 Results and Discussion

Contains flavonoids almost every component of a plant, including fruits, roots, leave and the outer bark of stems. Because flavonoids have a conjugated aromatic structure that exhibits substantial absorption bands in the

ultraviolet and visible light spectrums, study of flavonoids was carried out using UV-visible spectrophotometry [20]. Due to the fact that flavonoids are polar molecules, they will disintegrate easily in polar solvents including acetone, ethanol, methanol, butanol and others. Figure 3 illustrates the chemical structure of flavonoid molecules.

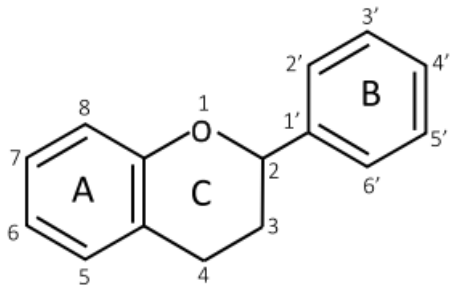

Figure 3 Flavonoids' Chemical Structure [21].

Process of filtering effective components or active substances from plant parts is called an extraction method, and it is utilized to acquire the required chemical compound. By choosing the right solvents, it is possible to maximize the removal of phenolic and flavonoid chemicals from the source. Because ethanol is generally less hazardous and has the ability to dissolve practically all compounds, including polar, semipolar, and non-polar ones, it was chosen as the solvent for this investigation. If the variables of concentration, temperature, duration and method of extraction are chosen properly, the use of ethanol solvents can be most effective. Due to the unique features of each plant component, these four elements cannot be balanced in any extraction procedure [22].

3.1 Maximum Wavelength Determination

Using a UV-Visible spectrophotometry technique with a wavelength range of 400–800 nm, one middle concentration in a standard series was measured in this work to determine the maximum wavelength [23]. A maximum wavelength of 413.5 nm was discovered after numerous experiments. The aim of figuring out the maximum wavelength is to figure out the absorption region that can be formed in the form of the absorbance value of the typical standard solution that has been measured using a UV-Visible spectrophotometer. To determine the duration of the ideal reaction and the stability of the reaction to ensure that the absorbance value does not decline and reduce the possibility of measurement errors, the maximum wavelength must be operating time at room temperature for 30 minutes [24].

3.2 Calibration Curve Determination

Linearitas is the calibration curve links absorbance (y) with concentration is known as linearity (x). Through a single measurement at several concentrations, linearity can be determined. The production of the standard curve starts with the production with a concentration of 1,000 ppm, followed by dilution to a concentration of 100 ppm, and then further dilution to a concentration of 10, 12, 14, 16 and 18 ppm with a combination of reagents, namely $AlCl_3$ 10% and sodium acetate 1 M, and analysis of the data using UV-Visible spectrophotometry such that the equation y = bx + a can be created and the correlation coefficient (r) can be obtained and the absorbance value of the rutin solution can be obtained. Rutin, a flavonoid of the flavonol group with a keto group in C-4 and a hydroxy group on the C-3 or C-5 atom so that it can form a color complex with $AlCl_3$, is frequently used as a comparison [10]. $AlCl_3$ 10% is added to produce a batochromic effect, which involves shifting to a higher wavelength. There is also an increase in the intensity of the common standard solution, resulting in a yellower color, allowing the color reaction to be seen with the naked eye and measured using visible spectrophotometry. Sodium acetate is also added as a reaction stabilizer so that the batochromic effect that occurs can be maintained [25]. Then, aquadest is added and let to stand for 30 minutes in order to ensure that the reaction between the regular standard solution and the added reagents can proceed flawlessly. Table 1 displays the findings from the concentration versus absorbance data.

Table 1 Concentration versus Absorbance

Concentration (ppm)	Absorbance
10	0,406
12	0,501
14	0,595
16	0,711
18	0,772

Blank solution was employed as a control in quantitative analysis testing using UV-visible spectrophotometry. Blank solution serves as a blank (multiplies zero) chemicals that do not need to be evaluated. The standard curve line equation derived from the data is y = 0.0471x + 0.0624 with a correlation coefficient (r) of 0.9934. Linearity is carried out by determining the straight-line equation linking concentrations to absorption variations using a rutine standard solution. There is a correlation between the concentration of the common solution and the absorption value and a r value that is close to 1 denotes a linear standard curve. From these observations, it can be inferred that the absorbance observed increases with increasing concentration (ppm). The rutin acquired quercetin values are displayed between their absorbance and levels. Figure 4 depicts the calibration curve of rutin in detail.

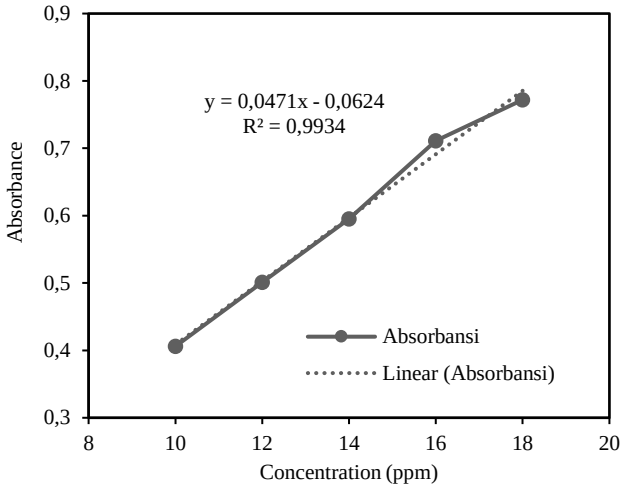

Figure 4 Calibration Curve of Rutin

3.3 Detection Limit (LoD) and Quantification Limit (LoQ)

The detection limit is the smallest amount of analytes in the sample that can still be detected by the instrument and give a meaningful response when compared to blanks (LoD). The quantification limit (LoQ), however, is the lowest concentration of analytes in a sample that still satisfies the cautious and cautious criteria [23]. The analysis of LoD required three trials, and the determination of LoQ required as many as ten measurements of the absorbance of a blank solution devoid of analytes. By diluting analyte concentrations in the sample on multiple levels, detection limit validation (LoD) parameters for non-instrumental methods are assessed [26].

From the aforementioned findings, it can be concluded that the UV-visible spectrophotometry equipment has a detection limit of 3.9694 mg / L for the lowest common solution. The analyte has a limit for the least amount of 13.231 mg / L, meaning that measurements can still be accurate at that concentration [27]. The slope values from the calibration curve are used to generate linear regression lines, which are then used to determine the LoD and LoQ values statistically.

The standard deviation (SD) of a series of measurements is required to compare the accuracy of a result; the smaller the SD number, the more appropriate the approach was utilized [25]. With a slope of 0.0471, the SD in this study was 0.0624.

3.4 Determination of Flavonoid Levels in Samples

The final part of this study, the estimation of flavonoid levels, was done three samples A, B and C. Samples that have been extracted using the maceration process are then examined using UV-visible spectrophotometry with ethanol 80%. An absorbance value that is the percentage of the flavonoid concentration in the samples. The samples A, B and C were duplicated three times and all had average flavonoid levels considered a rutin of 1.6683%, 2.8763% and 3.0923% respectively.

The most frequently found glycoside quercetin type is rutin. Quercetin is one of the best flavonols. Falvonoids are present in a wide variety of fruits and vegetables, but they are also present in red apples, onions, red wine, tea, cranberries, kale, peppers, and broccoli. Using a 70% ethanol solvent, the total flavonoids present in beluntas leaves are 33.80% [28]. The soursop leaf extract's 7.3% total flavonoids were found to be flavonoids when it was extracted with 70% ethanol [29]. Using ethanol solvent, the raru bark's *(Cotylelobium melanoxylon* Pierre*)* flavonoid levels were measured, and the average value was 4.3939% p.a [30].

4 Conclusions

The method of analysis by UV-visible spectrophotometry produces results that are pretty good in terms of linearity, LOD and LOQ. Based on the study of the maximum absorbance wavelength of 413.5 nm, the linear regression equation y = 0.0471x + 0.0624 was found, with a correlation coefficient (r) value of 0.9934. Sample C had the highest flavonoid content, at 3.0923%, while Sample A had the lowest, at 1.6683%.

5 Declarations

5.1 Acknowledgments

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5.2 Author Contributions

The names of the authors listed in this journal contributed to this research.

5.3 Funding Statement

This research was not supported by any funding sources.

5.4 Conflicts of Interest

The authors declare there is no conflict of interest.

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