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Quantification of Tannin in *Chromolaena Odorat* (Kirinyu) Leaf Extract

Purnama Ningsih*, Nurul Pratiwi, Supriadi, Dewi Staria Ahmar, Vanny Maria Tiwow, Sri Hastuti Virgianti Pulukadang, Sitti Rahmawati

Department of Mathematics and Natural Sciences Education, Faculty of Teacher Training and Education, Tadulako University, Palu 94118, Indonesia

Corresponding Author Email: purnama_ningsih@untad.ac.id

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ABSTRACT

Kirinyuh, also known as *Chromolaena odorata*, is one of the plants that can be utilized as a traditional remedy for the purpose of wound recovery. Tannins, specifically polyphenolic compounds obtained from plants and present in the stems, leaves, and epidermis, are present in these foliage parts. Young leaves are traditionally applied to wounds and crushed before being used to treat them. In fact, the medicinal properties of tannins are very broad including astringent, antimicrobial, antibacterial, antidiarrheal, anti-inflammatory, antioxidant effects, and tissue growth stimulation. Therefore, determining the tannin content in *Chromolaena odorata* is crucial in scientifically validating its traditional medicinal uses, ensuring quality control, and exploring new therapeutic applications. The objective of this study is to ascertain the tannin content in extracts of young and old leaves of the kirinyuh plant. In this reserach, kirinyuh leaves were extracted, using the soxhletation method and 96% ethanol as the solvent. The resulting extract was subsequently evaporated at 50°C using a rotary evaporator. Tannin content was ascertained at a wavelength of 765 nm utilizing a standard gallic acid solution as a comparison in conjunction with a UV-Vis spectrophotometer. The findings of the study indicated that the mean tannin content of leaf extract from young kirinyuh plants was 0.036%, while that of leaf extract from old kirinyuh plants was 0.062%.

1. INTRODUCTION

Indonesia is a mega biodiversity nation endowed with an abundance of natural resources, most notably the variety of plant species that generate primary and secondary metabolites. An abundant plant species in Indonesia that thrives in diverse habitats including drylands, swamps, and other wetland areas is *Chromolaena odorata*, also known as kirinyuh [1-6].

Kirinyuh is an edible herb that is classified as a member of the Astreaceae family. Wild in nature, this plant is not well-liked in Indonesia; consequently, its utilization within the community is suboptimal [7, 8]. Leaf parts of the kirinyuh plant are the most utilized. In comparison to other organs, kirinyuh leaves generate greater quantities of metabolite compounds [1, 9, 10]. This phenomenon is due to the preponderance of metabolic processes, particularly anabolism, in the leaf organs of plants, resulting in a greater production of metabolite compounds [11-13].

According to the findings of a study of some researchers [9, 14, 15], kirinyuh leaves contain tannin as one of the secondary metabolite compounds. Tannin, as stated by Noer et al. [16], is an exceptionally intricate organic compound composed of phenolic compounds that are present in a wide variety of plant species. Tannins are polyphenolic compounds characterized by their exceptionally high molecular weight, exceeding 1000g/mol, which enables them to interact in complex formations with proteins. Tannins are found throughout the plant, including in its fruit, stems, leaves, and bark. Due to the presence of the phenol group, tannin possesses the identical antiseptic properties as alcohol, enabling its application as an antimicrobial. Additionally, tannin has several medicinal uses, such as the ability to staunch bleeding and heal wounds [17]. In general, the leaves of the kirinyuh plant are employed medicinally. As a result, the objective of this investigation was to ascertain the tannin content of kirinyuh leaves. Tannins have unique health benefits that make them useful in both conventional and alternative medicine. Precise measurement of tannins is necessary to guarantee the effectiveness, security, and calibers of goods containing these substances.

Drawing inspiration from the aforementioned description, scholars are intrigued by the application of UV-Vis spectrophotometry to analyze the tannin content of kirinyuh leaf extract. The objective is to ascertain the tannin levels in kirinyuh leaves, which may serve as a viable raw material for traditional medicine and facilitate the development of more effective strategies for the prevention and treatment of diverse ailments. Apart from that, determining the tannin content in young and old leaves is also important to educate the public about the use of this plant in medicine. Which type of leaf is better according to the type of treatment, whether young leaves or old leaves. Furthermore, the information on the tannin contents of young and old leaves of the kirinyuh plant, which

is native to Central Sulawesi, Indonesia, is what makes this study novel.

2. METHODS

2.1 Chemical and tools

The apparatus utilized in this study comprised a rubber suction cup, dropper pipette, Soxhlet tool, 10mL and 50mL measuring flasks, an Erlenmeyer 500mL measuring flask, a 100mL beaker, a digital balance, blender, scissors, spectrophotometer UV-Vis, cuvette, tea sieve, rotary evaporator, stir bar, spatula, and label paper. Young and old kirinyuh leaves, 96% ethanol, distilled water, aluminum foil, filter paper, gallic acid, 15% Na_2CO_3 solution, and Folin-Ciocalteu reagent were the components utilized in this study.

2.2 Powdering kirinyuh leaf

After collecting samples of young and old kirinyuh (*Chromolaena odorata*) leaves, they were washed until clean under running water, cut into small pieces, and allowed to air dry for five days without direct sunlight exposure. After the sample has dried, it is pulverized into a powder using a processor. Following this, a fine powder of kirinyuh leaves was obtained by sieving the sample through a tea sieve [18].

2.3 Extracting kirinyuh leaf

Because tannins may be recognised, described, and included into herbal product formulations that are safer and more effective, the extraction approach was chosen for this investigation. After enclosing 25g of young kirinyuh leaf powder in filter paper and placing it in the series sample holder Soxhlet device, 250 mL of 96% ethanol was added. The process of filtration continues until the particles have undergone six cycles or have lost their color. The following day, 25 grammes of immature kirinyuh leaves are extracted in the identical manner. The resulting liquid extract is subsequently concentrated at a temperature of 5.0℃ using a rotary evaporator until a viscous extract is obtained. Subsequently, employ old kirinyuh leaves in the identical manner [19].

2.4 Establishment of a standard solution of gallic acid

Tannin content can be determined accurately, quickly, and consistently by using the UV-spectrophotometric method using a standard gallic acid solution. The use of gallic acid as a standard ensures a reliable calibration foundation and excellent precision in tannin assays from *Chromolaena odorata* leaves.

A parent standard solution containing 100 ppm gallic acid is obtained by combining 10 mg of gallic acid, which has been weighed, with distilled water in a volume of 100 mL to produce a solution with that concentration [20].

2.5 Curving gallic acid with a standard solution

A standard solution of 100 parts per million (ppm) gallic acid is prepared in the following concentrations: 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm. The prepared stock solutions of lactic acid are subsequently pipetted into the following volumes: 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, and 0.5 mL. Following this, one millilitre of Folin-Ciocalteu reagent was added to each gallic acid standard solution in a 10 mL volumetric vial, which was agitated until the solutions became non-homogeneous. Shake 2 mL of 15% Na_2CO_3 solution into this solution until it becomes homogeneous. Following this, 10 mL of distilled water is added, or until the limit mark is reached, and the mixture is shaken until homogeneous before being left for a sustained period. The absorption was subsequently determined using a UV-Vis spectrophotometer at a wavelength of 765 nm [18].

2.6 Analysis of tannin concentration

In a volume of 50mL of distilled water, a 50mg extract of young kirinyuh leaves (*Cromolaena Odorata*) was dissolved. Following the transfer of 5mL of the obtained extract solution via pipette into a 10mL measuring flask, 1mL of the reagent Folin-Ciocalteu was added, followed by 2 mL of 15% Na_2CO_3 solution, which was shaken until homogeneous and left for an additional 5 minutes. After adding 10 mL or more of distilled water to reach the mark, allow the mixture to be too steep for 90 minutes. Following this, the solution is diluted to a volume of 1:10. Following this, the absorbance of the extract solution at a wavelength of 765 nm was measured. Proceed in a similar manner using the extract of old kirinyuh leaves [21].

2.7 Data analysis

After enclosing 25g of young kirinyuh leaf powder in filter paper and placing it in the series sample. The concentrations of tannin in the kirinyuh leaf extract were determined quantitatively in this study using the UV-Vis spectrophotometric method and a standard curve that was acquired to ascertain the tannin concentration in the leaf extract. The data analysis pertaining to tannin levels is as follows [20]. In the Lambert-Beer law, the absorbance is linearly related to the concentration of the analyte solution and inversely proportional to the transmittance. The Lambert-Beer law equation can be written as:

$$A = abC \tag{1}$$

This means that the absorbance (A) is directly proportional to the concentration of the solution (C) and the thickness of the substance through which the light passes (b). The Lambert-Beer law is a reliable method for quantifying sample concentration in data analysis. The concentration of the sample can be determined by applying a linear regression equation, where the obtained absorbance of the sample is used as the y value to compute the corresponding x value, which represents the concentration of the analyzed sample. In addition, the determination of sample concentration can be achieved using a calibration curve, which is based on the Lambert-Beer law. This rule states that the absorbance of a sample solution is directly proportional to its concentration. Absorbance data was utilized in research to determine the levels of tannin compounds in kirinyuh leaf extract (*Chromolaena odorata*). Information gathered utilizing a UV-Vis spectrophotometer. Using the Eq. (2), tannin concentration in gr/L is computed [21].

$$X = \frac{(Y - b)}{a} \tag{2}$$

where,

Y = Absorbance value

a = Constant (constant)

b = Slope of line

X = Concentration of the sample solution

Afterwards, the tannin content in % was determined by employing the Eq. (3).

$$Tannin\ content(\%) = \frac{x\left(\frac{mg}{L}\right) \times VolumeSample(L) \times FP}{Bobot\ sample\ (mg)} \times 100\% \quad (3)$$

where, x = Tannin Concentration (mg/L); FP = Dilution Factor.

3. RESULT AND DISCUSSION

The objective of this study was to ascertain the concentrations of tannin compounds present in the extract derived from the leaves of Kirinyuh (*Chromolaena odorata*). The utilized samples originated from Palasa Village, located in the Palasa District of the Parigi Regency of Moutong, Sulawesi Tengah (Figure 1).

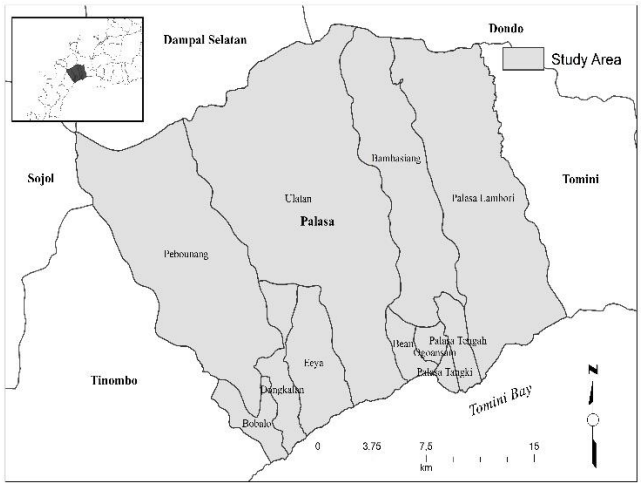

Figure 1. Map of location the kirinyuh plants

Quantifying tannins in *Chromolaena odorata* is crucial for several reasons. Accurate quantification ensures the efficacy of medicinal products by confirming that sufficient levels of tannins are present to provide therapeutic benefits. It also allows for the standardization of herbal preparations, ensuring consistent potency and quality across different batches. Furthermore, understanding tannin levels is essential for safety, as excessive intake can lead to adverse effects like gastrointestinal discomfort and nutrient absorption issues. Reliable quantification supports research and development, enabling the study of tannins' pharmacological effects and the potential creation of new treatments. Lastly, quantification is necessary for regulatory compliance, ensuring that medicinal products meet required safety and efficacy standards.

By exploring the specific health benefits of tannins and emphasizing the importance of their quantification, this study aims to deepen our understanding of *Chromolaena odorata*'s medicinal properties and its potential applications in both traditional and modern medicine.

In this work, after sanitizing and dehydrating the samples according to the prescribed procedure for five days, a 50-gram sample of both young and old kirinyuh leaves was obtained by crushing the specimens. A four-day Soxhlet extraction process was conducted, during which 50 grammes of young and old kirinyuh leaves were solvent twice each. Consequently, 25 grammes of young and old kirinyuh leaves were solvented per day for a duration of six hours, as the equipment utilised was insufficient to accommodate the 50 gramme sample. The use of 96% ethanol as a solvent in the extraction of polar tannin compounds is primarily due to its effectiveness in dissolving these compounds. Ethanol is a polar solvent, which means it can dissolve other polar compounds, such as tannins, due to its ability to form hydrogen bonds with them. This property makes ethanol an ideal choice for extracting polar tannin compounds from plant materials. The high concentration of 96% ethanol was chosen to maximize the solubility of the tannins, thereby improving the efficiency of the extraction process. Moreover, the choice of ethanol as a solvent can also be influenced by its relatively low toxicity levels and the short extraction times it allows, which are important considerations in many extraction processes. However, it's important to note that the specific choice of solvent can depend on various factors including the type of tannin compounds being extracted, the extraction method used, and the desired purity of the extracted tannins [21-25].

The ethanol extracts of 500mL of juvenile kirinyuh leaves and old kirinyuh leaves were subsequently concentrated at a temperature of 50°C in a rotary evacuator until a thick extract of kirinyuh leaves was obtained. With this, the solvent utilized in the extraction process is eliminated. Rotary evaporation facilitates the solvent evaporation process by decreasing the pressure within the rotary evaporator in comparison to the ambient pressure. This adjustment allows for easier evaporation of substances at temperatures below the boiling point [26].

Prior to determining the tannin content of leaf extract from Kirinyuh, it is necessary to prepare a standard solution of gallic acid. A total of five different concentrations of gallic acid solution were utilized in this investigation, with the absorbance of utilizing a UV-Vis spectrophotometer, these concentrations were acquired. The preparation of a standard solution of gallic acid is crucial for the accurate quantification of tannin content in plant extracts using the spectrophotometric method. It ensures the precision, accuracy, and reliability of the results by providing a consistent and known reference for the titration process [27-29]. Table 1 presents the absorbance outcomes of the standard gallic acid solution.

Table 1. Absorbance of gallic acid solution

Gallic Acid Concentration (ppm)	Absorbance
1	0.340
2	0.798
3	1.333
4	2.183
5	2.508

A standard solution curve was constructed using the absorbance data of the gallic acid solution presented previously. Utilizing the curve, the tannin content of the extract of kirinyuh leaves is ascertained. Gallic acid is a phenolic compound, which can react with other phenolic compounds, including tannins, under certain conditions. This

reaction is utilized in the Folin-Ciocalteu method, commonly used to determine the total phenolic This equation can be used to determine the concentration of an unknown solution based on its absorbance. The reaction between gallic acid and phenolic compounds results in a colour change that can be measured spectrophotometrically [30]. The solution curve for gallic acid is illustrated in Figure 2.

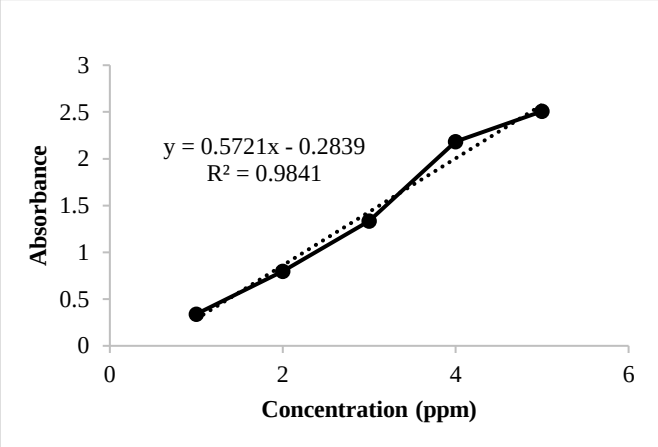

Figure 2. Curve gallic acid solution

The outcomes derived from the analysis of the standard curve indicate that as the concentration increases, so does the absorbance value. This is due to the direct relationship between the concentration of a solute in a solution and the amount of light it absorbs. To ascertain the tannin content of a sample via linear regression from the gallic acid calibration curve, it is necessary to generate a curve. By plotting absorbance against known concentrations of a solute in a series of solutions, a standard curve is generated. Typically, linearity is defined as the extent to which values deviate from the regression line's direction, as determined by mathematical equations; thus, the standard curve equation derived from the concentration of gallic acid is y=0.572x-0.284, with a R^2 value of 0.984. The correlation coefficient (R^2) is 0.984, representing absorption, concentration, and kirinyuh leaf extract. Its value is approximately one, indicating that the regression equation is linear and applicable for calculating the tannin content of the extract [31]. This equation can be used to determine the concentration of an unknown solution based on its absorbance, in this study is tannin.

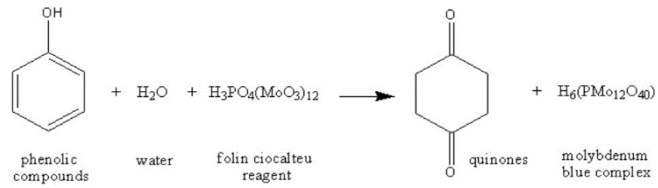

Figure 3. Phenolic compound reaction with Folin-Ciocalteu

The quantification of tannin compounds was performed at a wavelength of 765nm using a UV-Vis spectrophotometer. The quantification of tannin levels was accomplished by employing a tannin standard curve. Gallic acid is the tannin standard that is employed. Gallic acid is utilized as a tannin standard because it is a hydrolyzed tannin group, which enables it to function as a comparison when quantifying tannin levels. Before analyzing samples that contain tannin compounds, it is necessary to incorporate the Folin-Ciocalteu reagent, the absorption of which will be measured in the visible ultraviolet range. The hydroxy group present in the tannin compound initiates a reaction with Folin-Ciocalteu under alkaline conditions and with the addition of Sodium Carbonate (Na_2CO_3). This results in the formation of a phenolic ion, which subsequently undergoes a phospholybdate-phosphotungstate reduction reaction and contributes to the formation of a blue molybdenum-tungsten complex. Sodium carbonate's purpose is to establish an alkaline environment to facilitate the ciocalteu reduction reaction. through the hydroxyl groups of polyphenols in the sample and produce molybdenum-tungsten in the colour blue (Figure 3) [26].

The Folin-Ciocalteu reaction is a colorimetric assay used to measure the total phenolic content in a sample, which includes phenolic compounds such as tannins found in *Chromolaena odorata* leaf extract. This method is essential for determining the total phenolic content because it provides a quantitative measure of the phenolic hydroxyl groups present, regardless of their specific identity. The Folin-Ciocalteu reagent consists of phosphomolybdenum and phosphotungstate. It is prepared by mixing sodium tungstate and phosphomolybdic acid in phosphoric acid. A modification of this reagent, the Folin-Ciocalteu reagent, includes the addition of lithium sulfate and bromine, which helps prevent turbidity and allows for the reaction to proceed without the precipitation of solids. When phenolic compounds react with the Folin-Ciocalteu reagent, they form a blue complex that can be quantified by visible-light spectrophotometry. This reaction forms a phosphotungstic-phosphomolybdenum complex, where the maximum absorption of the chromophores depends on the alkaline solution and the concentration of phenolic compounds. The reagent rapidly decomposes in alkaline solutions, necessitating the use of an excess of reagent to ensure complete reaction. This excess can lead to precipitates and high turbidity, which is why the addition of lithium salts is crucial [31]. The method measures the total reducing capacity of a sample, not just phenolic compounds. This means it quantifies the total phenolic content, including tannins, by reacting with any reducing substance present in the sample. The Folin-Ciocalteu method is particularly useful for analyzing plant extracts due to its ability to measure the total phenolic content, which is critical for understanding the antioxidant and other phytochemical properties of the extract. The Folin-Ciocalteu method has been successfully validated for determining the total phenolic content of various plant extracts, including those from *Chromolaena odorata*. It complies with the requirements for analytical use and ensures the reliability of the results. This method is easy to perform, rapid, applicable in routine laboratory use, and low-cost, making it a valuable tool for researchers studying the phytochemical properties of plant extracts. Based on the explanation above, the Folin-Ciocalteu reaction is necessary for determining the total phenolic content in *Chromolaena odorata* leaf extract because it provides a quantitative measure of phenolic compounds, including tannins, by reacting with a reagent that forms a blue complex that can be quantified spectrophotometrically. This method is reliable, accurate, and widely used in the field of phytochemistry [31-34].

The tannin content of kirinyuh leaf extract was determined by performing two repeated measurements using a UV-Vis spectrophotometer (Duplo). The present study involved the dual measurement of each sample. Specifically, absorbance

data were obtained twice for young kirinyuh leaves, while old kirinyuh leaves exhibited marginally different absorbance patterns. To calculate the average tannin content of the two samples of Kirinyuh leaves the sum of the total tannin values from both measurements was calculated, and the result was subsequently divided by two. The mean tannin content of juvenile kirinyuh leaf extract was determined to be 0.036%, while that of old kirinyuh leaf extract was 0.062% (Table 2). The variation in tannin concentrations between young and mature leaves of *Chromolaena odorata* is principally influenced by the plant's adaptation mechanisms aimed at maximising survival and growth. Young leaves, because to their increased vulnerability to herbivory and environmental stresses, have elevated tannin levels as a defensive mechanism. As leaves get more developed and less susceptible to damage, the amount of tannins in them drops. This change indicates a shift in the plant's distribution of resources and metabolic focus.

Table 2. The findings of the tannin content determination of kirinyuh leaf extract

Sample	Absorbance	Tannin Concentration (gr/L)	Tannin Content (%)
Young Kirinyuh Leaf Extract 1	0.387	0.00018	0.036
Young Kirinyuh Leaf Extract 2	0.387	0.00018	0.036
	Average		0.036±0
Old Kirinyuh Leaf Extract 1	0.464	0.000314	0.063
Old Kirinyuh Leaf Extract 2	0.463	0.000312	0.062
	Average		0.062±0.001

4. CONCLUSIONS

Based on research determining the tannin content in *Chromolaena odorata* leaf extract or kirinyuh leaves, it can be concluded that the tannin content in young kirinyuh leaves using the UV-Vis spectrophotometric method is 0.036% and the tannin content in old kirinyuh leaves using the UV-Vis spectrophotometric method is 0.062%. This conclusion demonstrates a discernible disparity in tannin levels between young and old kirinyuh leaves. This disparity suggests that ancient kirinyuh leaves may hold promise for utilisation in diverse applications, such as herbal medicine or pharmacological research.

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