Determination of Total Phenol Compound Content in Mango Mistletoe Leaves (*Dendrophthoe pentandra* (L.) Miq) Using the UV-Vis Spectrophotometry Method

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Abstract. Mango mistletoe leaves (*Dendrophthoe pentandra* (L.) Miq) are parasitic plants traditionally used as medicine due to their secondary metabolites and strong antioxidant activity. This study aimed to determine the total phenolic content and assess the effect of concentration on absorbance values using UV-Vis spectrophotometry, as a specific parameter for the development of herbal products. The research included sample preparation, extraction, preparation of gallic acid standards (10, 15, 20, and 25 ppm), and measurement of absorbance at 764 nm. Ethanol extracts of mango mistletoe leaves were evaluated at concentrations of 100, 200, 300, and 1000 ppm. The gallic acid standard curve showed a regression coefficient of $R^2 = 0.994$, confirming its reliability. The total phenolic content obtained was 77.19 ± 0.44 mg/mL (100 ppm), 125.05 ± 0.45 mg/mL (200 ppm), 159.58 ± 2.00 mg/mL (300 ppm), and 228.73 ± 33.06 mg/mL (1000 ppm). The results demonstrated that phenolic content increased proportionally with concentration. In conclusion, mango mistletoe leaves contain significant levels of phenolic compounds, supporting their potential as a raw material for herbal products with vigorous antioxidant activity and fulfilling the criteria as a parameter for herbal product development.
Keywords: Mango mistletoe leaves, phenol, spectrophotometry uv-vis

1 Introduction

Indonesia boasts remarkable biodiversity, with approximately 25.000–30.000 plant species distributed across its territory. It has been acknowledged as one of the seven countries with the highest biodiversity. Among these are medicinal plants, commonly referred to as herbs, which contain bioactive compounds beneficial for the natural treatment and healing of diseases [1]. Each region in Indonesia has unique practices in utilizing plants for medicinal purposes, and medicinal plants are commonly cultivated in home gardens. Herbal plants can be processed in various ways, including boiling, grinding, pounding, soaking, application, burning, kneading, chewing, or direct consumption [2]. The types of medicinal plants used

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vary depending on local availability or cultural traditions, with commonly utilized plant parts including roots, stems, leaves, and fruits.

Mango mistletoe leaves (*Dendrophthoe pentandra* (L.) Miq) are a parasitic plant classified as a higher plant belonging to the Loranthaceae family and have long been used in traditional medicine [3][4]. Mistletoe is a plant that attaches to other plants and is classified as hemiparasitic because it contains chlorophyll for photosynthesis while extracting water and nutrients from its host. Mistletoe extracts nutrients and water directly from its host plant, thereby causing harm. Consequently, mistletoe leaves are often discarded because, over time, they can weaken the host and inhibit its growth [2].

Nevertheless, mistletoe leaves contain secondary metabolites such as flavonoids and phenolic compounds. According to previous research conducted by [2], mango mistletoe leaves have been reported to contain several bioactive compounds, including flavonoids, alkaloids, steroids, tannins, saponins, phenolics, and glycosides. These compounds exhibit strong antioxidant activity and have demonstrated anti-inflammatory, antiviral, and anticancer properties, as well as the ability to lower blood pressure. They also function as antihypertensive agents [3][6]. Therefore, in this research, the total phenolic content of mango mistletoe leaves was evaluated using the UV-Vis spectrophotometry method. The objectives were to determine and quantify the total phenolic content, to assess the effect of total phenol concentrations on absorbance values, and to establish one of the specific parameters for the development of mango mistletoe-based herbal products.

2 Materials and Methods

2.1 Materials and Location

The materials used included mango mistletoe leaves, 96% ethanol, gallic acid, and distilled water. The equipment included a UV-Vis spectrophotometer (Thermo Scientific Genesys 180), an analytical balance (Dura Scale), test tubes (Iwaki), micropipettes (Dragonlab), spatulas, and beakers (Iwaki). This research was conducted at the Integrated Laboratory of Universitas Islam Malang, from August to September 2024.

2.2 Procedure

2.2.1 Sample Preparation

Mango mistletoe leaves were used as the samples. The collected leaves were initially sorted and thoroughly washed under running water to remove any adhering dirt or dust. They were then oven-dried at 65°C for 24 hours. After drying, the leaves were ground into a fine powder using a blender and subsequently sieved to ensure uniform particle size. The moisture content of the powdered sample was then determined using a moisture analyzer and confirmed to be below 10%. After this, the powder was ready for extraction [7].

2.2.2 Sample Extraction

The extraction process of mango mistletoe leaves was performed using the maceration method. A total of 100 g of powdered simplicia was macerated with 1000 mL of ethanol solution. The maceration was carried out in two stages: in the first stage, 100 g of powdered simplicia were mixed with 700 mL of 96% ethanol, homogenized using a shaker for 2 hours, and stored at room temperature in the dark for 24 hours. After 24 hours, the mixture was shaken again for 2 hours and then filtered. In the second stage (remaceration), the residue from the first maceration was mixed with 300 mL of 96% ethanol, homogenized for 2 hours,

filtered, and allowed to stand for an additional 24 hours. The purpose of shaking and standing was to break down the cell walls of the simplicia, thereby facilitating the release of active compounds into the ethanol solvent. After 24 hours, the macerated mixture formed two layers: the upper layer (supernatant), containing the active compounds, and the lower layer (residue). The supernatant was collected and concentrated using a rotary evaporator at 70°C. The resulting thick extract was stored in vials and kept refrigerated [8].

2.2.3 Preparation of Gallic Acid Stock Solutions

A gallic acid stock solution (1000 ppm) was prepared by dissolving 50 mg of gallic acid in 50 mL of distilled water in a beaker. From this stock, working solutions of 100 ppm, 200 ppm, and 300 ppm were prepared using a micropipette and transferred into appropriately labelled containers [9].

2.2.4 Determination of Maximum Wavelength

A 0,1 mL aliquot of gallic acid solution (100 ppm) was mixed with 1 mL of Folin–Ciocalteu reagent and incubated for 5 minutes. Subsequently, 1 mL of Na_2CO_3 solution was added, and the mixture was incubated for 90 minutes. After incubation, the maximum wavelength was determined using a UV-Vis spectrophotometer (Thermo Scientific Genesys 180). The identified maximum wavelength, 764 nm, was then used as the reference for measuring the absorbance of the samples [10].

2.2.5 Total Phenolic Content Determination

The first step involved pipetting 0,1 mL of the sample solution at each concentration (100, 200, 300, and 1000 ppm) into a microplate. Subsequently, 1 mL of Folin–Ciocalteu reagent was added and incubated for 5 minutes. After incubation, 1 mL of Na_2CO_3 solution was added, and the mixture was further incubated for 90 minutes [10]. The total phenolic content was then calculated by measuring the absorbance of the samples at a wavelength of 764 nm using a UV-Vis spectrophotometer [11].

2.3 Data Analysis

The absorbance values obtained were then processed using Microsoft Excel and ANOVA using the following formula:

$$\frac{Concentration\ \frac{(Y-b)}{a} \times V \times FP}{sample\ height} \quad (1)$$

In this formula, **V** refers to the volume of extract utilized, and **FP** indicates the influencing factor incorporated in the calculation process. Upon completing the calculation according to the specified equation, the total phenolic content was determined.

After performing these calculations, the total phenol content was obtained [12].

3 Results and Discussion

3.1 Gallic Acid Standard Curve

Determination of total phenolic content was performed using the Folin–Ciocalteu reagent with gallic acid as the reference standard. Gallic acid was selected as the reference standard due to its effectiveness in forming complexes with the Folin–Ciocalteu reagent, its high reactivity, and its stability as a natural phenolic compound [13]. Gallic acid possesses hydroxyl groups and conjugates double bonds on each benzene ring. The phenolic hydroxyl groups reduce the heteropoly acids (phosphomolybdate–phosphotungstate) in the Folin reagent to form a blue molybdenum–tungsten complex, which can be detected using a UV-Vis spectrophotometer. The addition of Na_2CO_3 serves to alkalinize the medium, facilitating the dissociation of protons from phenolic compounds into phenolate ions [10]. Consequently, the reaction between gallic acid, the Folin–Ciocalteu reagent, and Na_2CO_3 produces a blue-colored complex.
The results of the gallic acid standard solution test are presented in Figure 1.

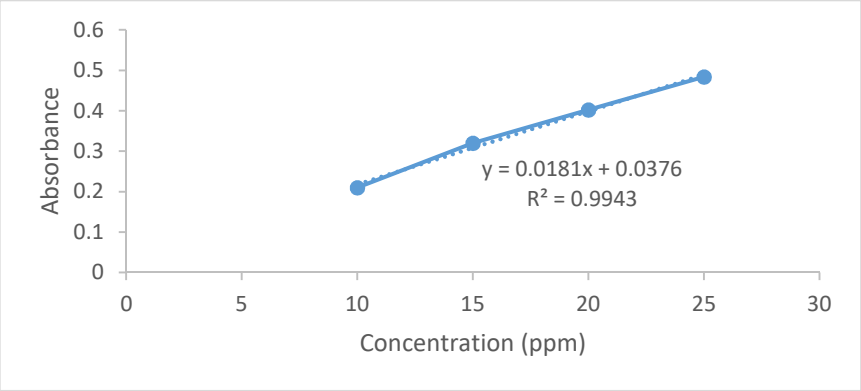

Fig. 1. Gallic acid solution test graph (nm)

Based on the results in Figure 1, gallic acid concentrations were measured in the range of 10-25 ppm, with three replicates, resulting in a curve coefficient of $R^2 = 0.9943$. According to research conducted by [14], when the R2 value is close to 1, the total phenol content can be calculated. This indicates that the coefficient value of y and y on the curve are nearly identical, indicating a linear relationship between concentration and absorbance of 99,43%. Therefore, the test can be continued with the total phenol test on the sample.

3.2 Total Phenolic Content

Table 1. Total phenol test results

Concentration (ppm)	Repetition	Absorbance (Y)	Total phenol content (GAE mg/mL sample)	Average total phenol content ± SD
100	1	1,430	76,92818	77,19 ± 0,44504
	2	1,428	76,81768	
	3	1,446	77,81215	
200	1	2,301	125,0497	125,05 ± 0,4511
	2	2,291	124,4972	
	3	2,311	125,6022	
300	1	2,971	162,0663	159,58 ± 2,00779
	2	2,882	157,1492	
	3	2,925	159,5249	
1000	1	3,593	196,4309	228,73 ± 33,06547
	2	5	274,1658	
	3	3,840	215,6022	

In the total phenol test, the maximum wavelength obtained from the gallic acid reference solution was 764 nm, which was subsequently used for measuring the absorbance of the samples. Determining the maximum wavelength was essential to evaluate absorbance changes at each concentration, thereby achieving maximum analytical sensitivity and optimal absorbance during measurements [13]. Based on the results presented in Table 1, at a concentration of 100 ppm, the mean absorbance was 1.434, with a total phenolic content of 77.19 mg/mL. At 200 ppm, the mean absorbance was 2.301, corresponding to a total phenolic content of 125.05 mg/mL. At 300 ppm, the mean absorbance was 2.926 with a total phenolic content of 159.58 mg/mL, and at 1000 ppm, the mean absorbance was 4.44 with a total phenolic content of 228.73 mg/mL.

These findings suggest that increasing the extract concentration leads to a higher total phenolic content. This suggests that concentration has a significant impact on the phenolic content of the analyzed samples. As the concentration of phenolic compounds in the solution increases, more phenolate ions are formed, leading to a greater amount of blue complex formation and, consequently, an increase in absorbance and the measured total phenolic content [15]. The formation of the blue complex highly influences absorbance measured by spectrophotometry, the stability of phenolic compounds, optimal alkaline conditions, and the absence of interfering compounds. The data obtained showed a positive correlation between concentration and phenolic content, indicating that the reaction mechanism proceeded as expected within the tested concentration range. However, at very high concentrations, reaction efficiency may decrease, as observed by [5], where high concentrations yielded lower absorbance values than lower concentrations. This may be attributed to reagent saturation at high concentrations and excessive sample concentrations, which can inhibit and interfere with color development in the Folin–Ciocalteu reaction. The presence of other interfering compounds in the sample, such as ascorbic acid, sugars, or other substances, may also influence this process.

4 Conclusion

Based on the results and discussion, it can be confirmed that mango mistletoe leaves (*Dendropthoe pentandra* (L) Miq) contain phenolic compounds. The findings demonstrate that concentration has a significant effect on absorbance values, with higher concentrations yielding higher total phenolic content. The presence of substantial phenolic compounds supports the potential of mango mistletoe leaves as a raw material for herbal products with strong antioxidant activity. Therefore, mango mistletoe leaves fulfil the criteria as a specific parameter for the development of herbal products with significant phenolic content.

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