Analysis of Alkaloid Compound The Ethanol Extract Of Akar Bulu (Merremia Vitifolia) Leaves Using UV-VIS Spectrofotmetry Methode

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Abstract. This study aims to determine the levels of alkaloids compound in the extract of *Akar Bulu(Merremia vitifolia)* leaves. The leaves of *M.vitofilia* are widely used by the people of Luwu (South Sulawesi) to treat wounds in diabetics. Stages in this work include sample preparation (collection, drying and sieving); ethanol maceration, evaporation and UV-Vis Spectrofotmetry analyzing. Extraction of the chemical content of the leaves of *M.vitifolia* was carried out by maceration method using 96% ethanol 1600 mL. Determination of alkaloid levels in the ethanol extract of *M.vitifolia* leaves using the UV-Vis spectrophotometer method at a maximum wavelength of 345 nm. The results showed that the alkaloid content of *M.vitifolia* leaves was 9.51 mg/g.

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1.Introduction

Indonesia's tropical forest area has the second highest biodiversity in the world after Brazil. Indonesia have more than 20,000 types of medicinal plants, but only 1,000 have been recorded and only 300 types have been used for traditional medicine. With the development of the times, various types of diseases cause some people to be bored with consuming synthetic drugs, so that the enthusiasm to explore natural medicines, known as herbal remedies, arose again. At this time, the tendency of people to return to nature makes plants have high economic value, however, the problem that must be of concern to the current government is how to ensure that herbal-based drugs have measurable quality and are able to support health [1].

One of the plants that often used by Indonesian people, especially in the area of South Sulawesi, Luwu district, is commonly called Bilajang Bulu (Merremia vitifolia). Bilajang extract is believed by the local community to reduce blood sugar levels and is used as a medicine to speed up the recovery of a diabetic patient's injury. The people of Mamuju (West Sulawesi) also believe that bilajang Bulu (Merremia vitifolia) can cure malaria, therefore a phytochemical test has been carried out on the bilajang Bulu plant (Merremia vitifolia). Based on research by Sukarti [2], it shows that the stems and leaves of bilajang Bulu contain secondary metabolites, one of which is alkaloids.

Alkaloids are compounds that can be used as anti-diabetes, anti-diarrhea, and anti-malaria. The usefulness of alkaloid compounds in the field of pharmacology and medical science, namely, has a role to stimulate the nervous system, raise or lower blood pressure, and fight against microbial infections so that the number of alkaloid compounds in a plant affects the benefits of these plants [3]. Until now there has been no research discussing alkaloid levels in feather bilajang, therefore this study aims to analyze the alkaloid levels in the ethanol extract of Bilajang Bulu (Merremia vitifolia) leaves using a UV-Vis spectrophotometer.

Based on the results of phytochemical tests for secondary metabolites on the stems of M. vitifolia, it is known that they contain steroid and alkaloid compounds, while the leaves have a positive test on phenolic compounds, flavonoids, saponins, steroids, alkaloids, and caratenoids [2]. The flavonoid content of M. vitifolia was 163.4 mg [4]. It has been Reported that one of the plants related to M. vitifolia, namely, M. peltata. Phytochemical test results of M. peltata ethanol extract showed the presence of flavonoids, steroids, alkaloids and tannins which have pharmacological functions, namely, as antioxidants, free radical scavengers, and active antimicrobials [5]. M. Peltata (L) Merr or known by the regional name Aka lambuang is a traditional medicinal plant belonging to the Convolvulaceae family, which is used by the community to treat various diseases, especially diarrhea and skin wounds [6].

2. Experimental Method

2.1 Materials

The materials used in this study were BCG (Bromocressol green) ethanol 96% aquades, phosphate buffer Ph 7, berberine chloride HCl 0.02 N, NaOH, and M. vitifolia leaves collected from Binturu village, Wara Selatan subdistrict, city. Palopo.

2.2 Instrumentation

The instruments used in this study were a rotary evaporator, UV-Visible spectrophotometer and a blender.

2.3 Procedure

2.3.1. Sample Preparation

The leaves of M. vitifolia which have been collected as much as 5 kg are cleaned and then dried without exposure to sunlight. Furthermore, the leaves of M. vitifolia were mashed using a blender and then sieved into powder (simplicia) and obtained 500 grams of simplicia, then stored in a container and ready to be extracted

2.3.2. Extraction Stage

500 grams of Bilajang Bulu (Merremia vitifolia) leaf powder, macerated with 96% ethanol. During the maceration process, it stirred for 3 hours. Maceration carried out once until the alkaloid was completely extracted.

2.3.3. The alkaloid level analysis stage

The alkaloid level analysis stage was carried out using the UV-Vis spectrophotometer method. The following are the stages of analyzing alkaloid levels in Bilajang Bulu [7].

a. Determination of wavelength

The standard solution of berberine coride with a concentration of 100 ppm is pipette 1 mL and put in a beaker glass then added with 0.5 mL of phosphate buffer pH 7 and 5.0 mL of BCG solution, then the absorbance is determined at 200-800 nm

b. Standard curve determination

The standard solution of berberine chloride with a concentration of 100 ppm each is taken 0.5; 1; 1.5; 2; and 2.5 mL then put into a test tube added with 0.5 mL of phosphate buffer pH 7 and 5.0 mL of BCG solution, then observed the absorbance at maximum wavelength and stability time.

c. Alkaloid level determination

A total of 10 mg of ethanol extract of Bilajang Bulu leaves were dissolved in 50 mL of 0.2 N HCl. Stirring was carried out with a magnetic stirrer then 2 mL of 0.1 N NaOH was added. The bilajang Bulu leaf extract solution was put into a test tube as much as 3 mL then added with 5 mL of phosphate buffer pH 7 and 5 mL of BCG solution, then observed the absorbance results at the maximum wavelength resulting from the maximum wavelength treatment.

2.3.4. Data Analysis

The analysis in this study were the result of the UV-Vis spectrophotometer analysis by entered the absorbance of the sample into the regression equation obtained from the calibration curve to obtain alkaloid levels.

$$y = ax + b$$

Note: y: Absorbance of the sample
A: Slope
x: Concentration of the sample (from the instrument)

3. Result and Discussion

3.1 Results of sample preparation

The sample used in this study was 5 kg of fresh leaves of bilajang Bulu (Merremia vitifolia) taken from the Binturu Village, Wara Selatan District, Palopo City. The sample washed with clean water to carried out and remove impurities in the Akar Bulu leaves. After that the leaves of the feather bilajang dried to remove the moisture content. The dried and brown leaves were mashed to obtain a powder sample. Pollination carried out to reduce the sample size so that when extracting the sample and the solvent can react quickly. If the sample size was large, the surface area will be smaller, on the other hand, if the sample size was small, the surface area will be bigger [8]. The results of sample preparation obtained a fine powder sample of 500 grams.

3.2 Extraction Results with Ethanol

The results of the extraction of alkaloid compounds in Bilajang Bulu leaves were carried out by weighing the sample powder as much as 500 grams, then macerated using 96% ethanol for 2x 24 hours which was then macerated again for 1x 24 hours so that the ethanol extract was obtained as much as 1600 mL. The results of the Bilajang Bulu leaf sample extraction are shown in Table 1.

Table 1.The results of maceration of leaves of Bilajang Bulu (Merremia vitifolia) simplicia

	Sampel (gram)	Repeat	Maserat	Filtrate
	500	I	2400	1200
		II	800	400
Total	500		3200	1600

The alkaloid compound extraction method used maceration by immersing the fine simplicia powder in a solvent. The principle of maceration is that the solvent will penetrate the cell wall so that the active substance will be dissolved as a result of the high concentration difference that will be pushed out of the cell [7]. Simplicia placed in a jar with the solution of the predefined sprays, then occasionally stirred and closed tightly so that the solvent enters the entire surface of the simplicia. The yield stored and protected from direct light to prevent reactions catalyzed by light or discoloration. The maceration time is generally 3-5 days, after which the balance between the extracted material on the inside of the cell and the outside of the cell has been achieved, with stirring it guaranteed that the balance of the extracted substance concentration were faster in the liquid because the idle state during maceration causes a decrease in the transfer of the active ingredient. 8]

3.3 Analysis of alkaloid levels using Uv Vis

3.3.1 Maximum Wavelength

The maximum wavelength of alkaloids obtained using a UV-Vis spectrophotometer was carried out by measuring 100 ppm berberine chloride at a wavelength of 200-400 nm. Based on the research,

it was found that the maximum wavelength of berberine chloride is 345 nm. The results of the maximum wavelength can be seen in Figure 1.

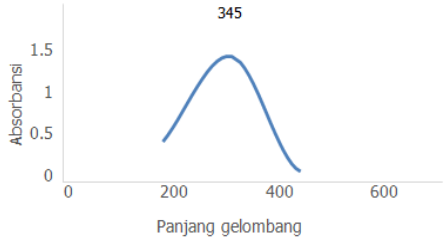

Figure 1. The maximum wavelength of berberin chloride

3.3.2 Standard Curve Creation

The standard curve in this study aims to connect the concentration of berberine chloride with the absorbance of each concentration variation. Several variations of the concentration of berberine chloride used include, 0 ppm 5 ppm 10 ppm 15 ppm 20 ppm and 25 ppm. The absorbance of each berberine chloride concentration was measured using Uv-Vis spectrophotometry at a wavelength of 345 nm. The results of absorbance measurements for the Berberine Chloride Standard solution are shown in Table 2. In accordance with Lambert Beer's law, the concentration of the solution is directly proportional to the continued intensity for the absorbent solution. The determination of berberine chloride can be varied, namely 0 ppm 5 ppm 10 ppm, 15 ppm, 20 ppm, and 25 ppm [9]

Table 2. The results of the absorbance measurement of the standard solution of berberine chloride.

Concentration (ppm)	Absorbance (A)
0	0.000
5	0.315
10	0.662
15	1.111
20	1.355
25	2.146

Based on the absorbance measurement of the standard solution of berberine chloride, the form of a calibration curve between the concentration of berberine chloride and the absorbance was obtained as shown in Figure 2.

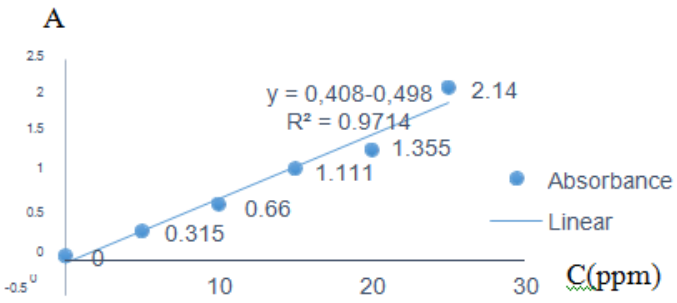

Figure 2. The standard curve between the concentration and the absorbance of berberine chloride

Figure 2 shows that the greater the concentration of the standard solution of berberine chloride, the greater the absorbance value. Based on the standard curve, the regression equation is obtained, namely y = 0.408x - 0.498 with a value of R2 = 0.9714 where x is the standard concentration of

berberine chloride and y is the standard absorbance of berberine chloride. The regression equation with berberine chloride can be used a regression equation which can then be used to calculate the alkaloid levels in M. Vitophilia leaves [10].

3.3.4 Determination of Alkaloid Levels in Bilajang Bulu Leaves

Determination of the amount of alkaloid content of M. Vitofilia leaf extract using the UV-Vis spectrophotometer method begins by extracting the alkaloid compounds from bilajang Bulu leaves used BCG reagent. BCG (Bromocresol green) as a yellow-forming reagent and will bind with alkaloids to form complex alkaloids. BCG can react with certain classes of alkaloids (alkaloids having nitrogen in their structure) [10]. Alkaloid detection preparation was by adding 5 phosphate buffer pH 7 into 5 mL of the sample extract to provide optimum results. The reaction of complex formation between alkaloids is shown in Figure 3. The concentrated extract of Bilajang Bulu leaves with the addition of 0.02 N HCl is used to form alkaloid salts. Strong acids react with alkaloids to form alkaloid salts. The purpose of adding 0.1 N NaOH is used so that alkaloid liberation occurs from the salt to form free alkaloids. Free alkaloids are insoluble in water but soluble in organic solvents. The release of amines from their salts can be done by adding bases [10].

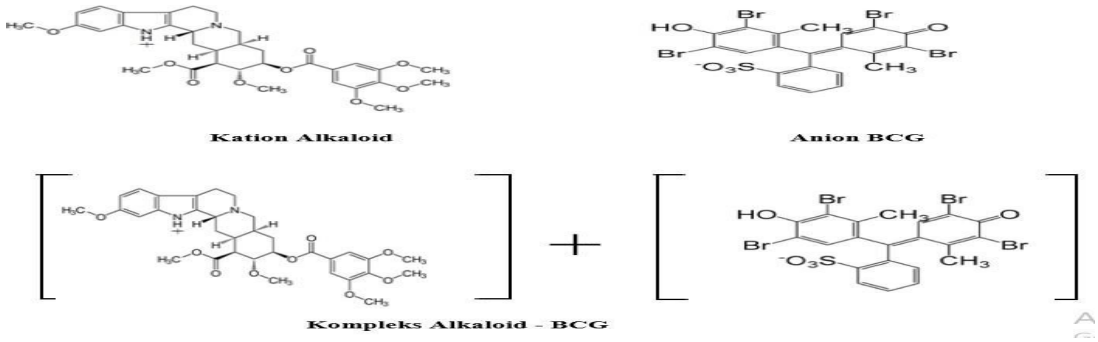

Figure 3.The reaction for complex formation between alkaloids and BCG (Source: Patel et al, 2015)

4. Conclution

The choice of method for calculating alkaloid levels in a plant can use a UV-Vis Spectrophotometer and a standard solution was required. The standard solution used in this study was a solution of berberine chloride which serves to identify the number of alkaloids in M. Vitofilia leaves. Determination of the alkaloid levels of bilajang Bulu leaves was carried out by the UV-Vis spectrophotometer method so that the alkaloid content of M. Vitofilia extract was obtained, namely 9.51mg / g or 0.0951%. The amount of alkaloid levels found in M. Vitofilia leaves were quite high compared to several studies that have been conducted on a number of other plants. The research report on alkaloid levels in earring flowers was only 0.286 mg / g [10] and the results of research on alkaloid levels in papaya flower extract were only 0.02981 mg [11]. Based on these conditions, this research can be the basis for further research, namely isolating alkaloid compounds and their derivatives so that they can be developed into drugs.

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6.References

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