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Research Article

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Qualitative and Quantitative Analysis of Hexane, Acetone, Ethanol and Water Extract from Bay Leaves (*Syzygium polyanthum* (Wight) Walp.)

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Abstract Research on the qualitative and quantitative analysis of bay leaves (*Syzygium polyanthum* (WIGHT) Walp) has been carried out from hexane, acetone, ethanol and water extracts. This study aims to analyze the chemical compounds contained in each extract of hexane, acetone, ethanol, and water from bay leaves, and determine the secondary metabolic content contained in each extract of the bay leaf. The extraction method used was maceration for hexane, acetone and ethanol solvents, infusions for water solvents. The results obtained from phytochemical screening and assay showed that bay leaf acetone extract contained phenol 0.1202% and tannin 0.1452%. Ethanol extract of bay leaves contains 0.34% alkaloids, 0.59% flavonoids, 0.1258% phenols, and 0.1688% tannins. Bay leaf water extract contains 0.486% flavonoids, phenol 0.2248% and tannin 0.1622%. The hexane extract of bay leaves was not detected to contain secondary metabolites.

Keywords Bay leaf, *Syzygium polyanthum (*WIGHT) Walp, Extraction, Qualitative Analysis, Quantitative Analysis

Introduction

Bay leaf (Syzygium polyanthum (WIGHT) Walp) (Figure 1) is used in herbal form as a medicine for diabetes mellitus. The dose is 2 x 1 sachet (5 g powder)/day, boil with 2 cups of water until it becomes one glass [1]. Ethanol extract of bay leaves can reduce blood glucose levels in mice [2]. Methanol extract from bay leaves is effective in reducing blood glucose levels given to diabetic patients [3].

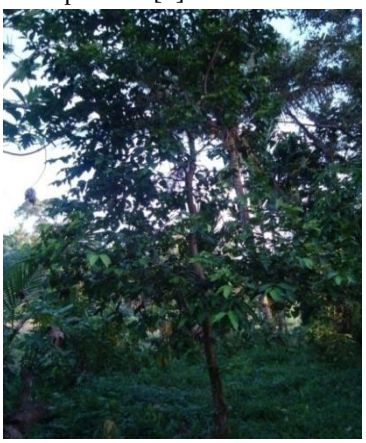

Figure 1: Bay leaf plants (Syzygium polyanthum (WIGHT) Walp.)

In phytochemical screening studies, medicinal plants of bay leaves contain flavonoids, alkaloids, steroids, and tannins [4]. Research using 95% ethanol solvent showed that bay leaves contain tannins, alkaloids, steroids, triterpenoids, and flavonoids [5]. In the research of herbal and traditional medicine, it was found that bay leaves contain tannins, essential oils, sesquiterpenes, triterpenoids, steroids, citral, saponins, and carbohydrates [6]. Other research shows that bay leaf extract with methanol solvent contains active compounds in the form of alkaloids, flavonoids, saponins, tannins, and steroids [7].

Determination of tannin in the infusion of young bay leaves and old bay leaves by visible spectrophotometry was measured at a maximum wavelength of 745 nm with reagent Folin Denis and saturated sodium carbonate. The results showed that the levels of young bay leaves and old bay leaves were 2.38 ± 0.036% and 2.45 ± 0.007% respectively [8]. The bay leaf flavonoid extract which has the best bioactivity was produced by sonication in 96% methanol in the extraction time for 15 minutes. Flavonoid levels in these conditions were obtained at 0.0116 mg / mL [9].

Based on the description above, it turns out that no research has been conducted on qualitative and quantitative analysis of extracts from hexane, acetone, ethanol, and water from bay leaves. Therefore researchers are interested in researching qualitative and quantitative analysis of extracts from hexane, acetone, ethanol, and water from bay leaves. This study aims to determine the types and levels of chemical compounds contained in extracts from hexane, acetone, ethanol, and water from bay leaves.

Materials and Methods

Tool

The tools used include: UV-Vis spectrophotometer (Shimadzu UV-1800), rotary evaporator (IKA®), analytic scales (Precisa), UV lamp (Camag), sonicator (Branson 1800), separating funnel (Iwaki), Erlenmeyer (Iwaki), measuring flask (Iwaki), measuring cup (Iwaki), beaker glass (Iwaki), measuring pipette (Iwaki), stirring rod (Iwaki), silica gel 60 F254, parchment paper, spatula, suction ball, funnel (Iwaki) , aluminum foil, maceration container (dark bottle), drop pipette, knife, blender (Philips), test tube (Iwaki), filter paper Whatmann No 42, crucible porcelain, vaporizer cup.

Materials

The materials used in this study were bay leaf simplicia (*Syzygium polyanthum* (Wight) Walp.), Quercetin (Sigma), catechins (Sigma), gallic acid (Sigma) and all solvents and reagents purchased from Merck, hexane, acetone, ethanol and distilled water. Other chemicals needed to carry out qualitative and quantitative analyses were purchased from Merck.

Procedures

Making and standardizing bay leaf simplicia

Making bay leaf simplicia was carried out following the Department of Health of the Republic of Indonesia 1985 [10].

Standardization of bay leaf simplicia was carried out following the Ministry of Health of the Republic of Indonesia in 2011 [11].

Extract Making

A total of 50 grams of simplicia powder is added to 500 mL hexane, macerated for 24 hours. Maserate is separated by filtration. This extraction process is repeated twice, using the same type and amount of solvents. All macerates were collected, then evaporated with a rotary evaporator at a temperature below ± 50 ^{o}C so that the liquid extract was obtained. Do the same for acetone and ethanol solvents until a liquid extract is obtained.

Furthermore, making bay leaf infusion: As much as 50 grams of bay leaf simplicia are inserted into the infusion pan with 500 mL of water solvent added, then put in a water bath for 15-20 minutes at 98 ^{o}C, strain using a flannel cloth to obtain bay leaf water extract.

Qualitative analysis of bay leaf extract

Qualitative analysis was carried out by Materia Medika Indonesia VI [12] and Hanani [13] towards Carbohydrate, Alkaloids, Flavonoids, Tannin, Terpenoid, Essential oil, Saponin, Phenol, Fatty acid, and Steroids.

Quantitative analysis of bay leaf extract

Quantitative analysis of alkaloid was carried out following Indonesian Herbal Pharmacopoeia Edition I [14].
Quantitative analysis of flavonoids was carried out following Indonesian Herbal Pharmacopoeia Supplement I [15].
Quantitative analysis of phenol was carried out following Pharmacopoeia Hermal Indonesia Supplement II [11]
Quantitative analysis of tannins was carried out by Indonesian Herbal Pharmacopoeia Supplement I [15].

Results and Discussion

Standardization of bay leaf simplicia

The results of macroscopic standardization of bay leaf simplicia are shown in Figure 2.

Figure 2: Macroscopic of bay leaf simplicia

The results of microscopic standardization of bay leaf simplicia are shown in Figure 3.

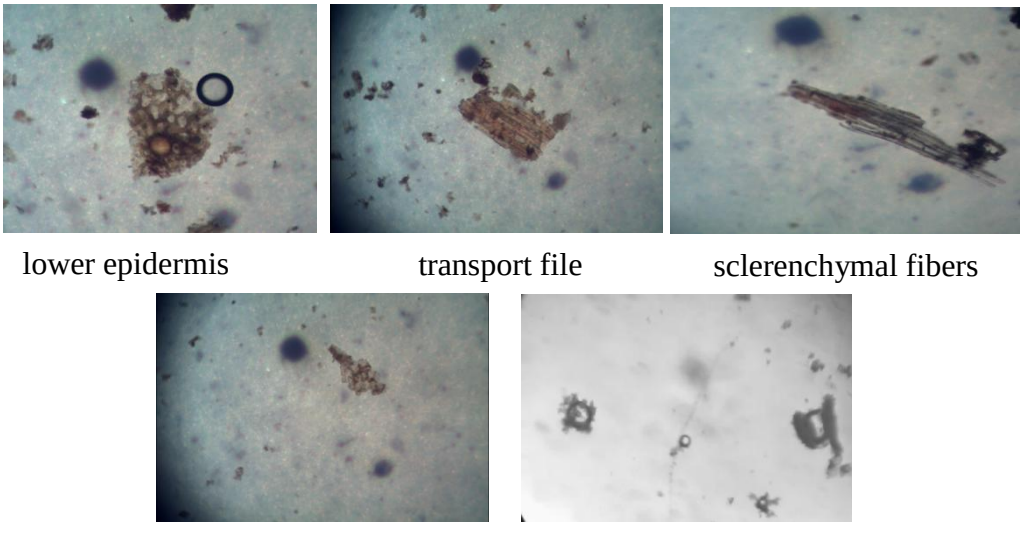

Figure 3: Microscopic of bay leaf simplicia

The thin layer chromatographic pattern of bay leaf simplicia is shown in Figure 4.

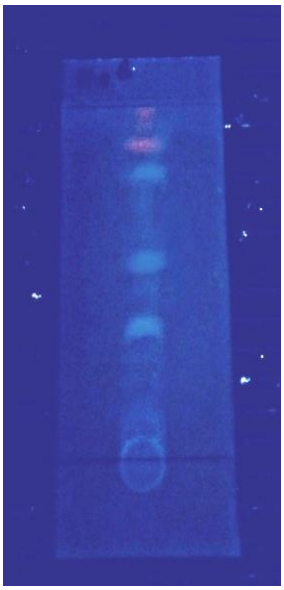

Figure 4: The thin layer chromatographic pattern of bay leaf simplicia

Description:

Stationary phase: Silica gel 60 F254

Mobile phase: Ethyl acetate P-formic acid P-acetic acid P-water (10: 0.5: 0.5: 1)

Detection: Sitroborate LP and UV (λ 366 nm)

The loss on drying was 8.1909% ± 0.2377%.

The total ash content was 5.0905% ± 0.0134%.

The level of insoluble acid ash was 0.7394% ± 0.0842%.

The level of extract dissolved in water was 8.5886% ± 1.0044%.

The level of soluble extract in ethanol was 11.2617% ± 0.5816%.

The total flavonoid content of the simplicia of bay leaves was 3.2321%.

Qualitative analysis results from hexane extract, acetone extract, ethanol extract, and water extract from bay leaves

Bay leaves which have been extracted using four different solvents, namely hexane, acetone, ethanol and water then carried out a qualitative test of the chemical content. The results are shown in Table 1.

Table 1: Qualitative test results of extracts of hexane, acetone, ethanol, and water from bay leaves

Chemical content	Reagent	Results			
		Hexane	Acetone	Ethanol	Water
Alkaloids	Wagner	(-)	(-)	(+)	(-)
	Mayer	(-)	(-)	(-)	(-)
Flavonoids	Mg Powder + 1 mL hydrochloric acid (concentrated)	(-)	(-)	(-)	(-)
	Lead acetate	(-)	(+)	(+)	(-)
Steroids	0.5 mL Chloroform + 0.5 mL acetic acid + sulfuric acid (concentrated)	(-)	(-)	(-)	(-)
Saponin	Foam test (2 mL water)	(-)	(-)	(-)	(-)
Essential oil	Potassium Permanganate	(-)	(-)	(-)	(-)
	Acetate Anhydride	(-)	(-)	(-)	(-)
Terpenoid	Acetate Anhydride	(-)	(-)	(-)	(-)
Tanin	$FeCl_3$	(-)	(+)	(+)	(+)
Phenol	$FeCl_3$	(-)	(+)	(+)	(+)
Fatty acid	Sulfuric acid	(-)	(-)	(-)	(-)
Carbohydrate	Benedict	(-)	(-)	(-)	(-)
	Fehling (A dan B)	(-)	(-)	(-)	(-)
	Molish	(-)	(-)	(-)	(-)

Table 1 shows the negative test results on steroid test, saponin test, essential oil test, terpenoid test, fatty acid test and carbohydrate test in hexane extract. Positive results were shown in alkaloid testing, flavonoid test, tannin test, and phenol test. In hexane extract, there is no secondary metabolite. The result is because hexane has non-polar characteristics, is volatile, has a distinctive odor and generally hexane is used to extract vegetable oils [16]. Besides the age of plants, the type and environment where they grow can also influence the results of the analysis of the chemical content of plants which results in differences in the content of chemical compounds and the content of chemical compounds [10]. Other factors that also influence are the nature of chemical compounds, solvents used and available tools [13].

The results of the determination of the chemical components of an extract of hexane, acetone, ethanol, and water from bay leaves

The total alkaloid content in the ethanol extract from bay leaves was 0.34% (Table 2).Determination of total alkaloid levels was carried out by the gravimetric method. The gravimetric method is one method of determining quantitatively by measuring the weight of components in a pure state after going through the separation process [13]. The addition of ammonia is intended for ammonia to react with hydrochloric acid which forms salts that are soluble in water while the alkaloid returns in basic form and is not dissolved in water but is easily soluble in chloroform. Free alkaloids can be extracted with chloroform solvents, resulting in chloroform extract which is a total alkaloid. Based on the results of the experiment and the calculation of the total alkaloid content of the ethanol extract obtained levels of 0.34% (Table 2). The results obtained are by existing standards in Indonesian Herbal Pharmacopoeia Edition I [14], which is not less than 0.30%.

Total flavonoid levels in ethanol and bay leaf water extracts were 0.512% and 0.486% respectively as quercetin (Table 2).Determination of the maximum wavelength of the standard quercetin solution is 403.5 nm at a concentration of 50 µg / mL (Figure 5).

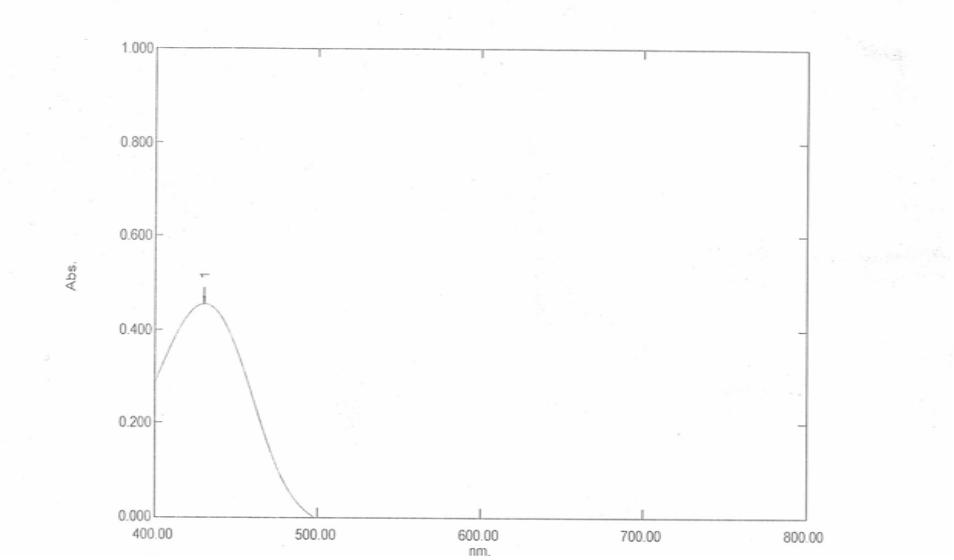

Figure 5: Visible light spectrum for quercetin at a concentration of 50 ppm with aluminum chloride reagent (Maximum at 430.5 nm)

Based on the calibration curve in Figure 6, the regression equation obtained y = 0.0101x - 0.0579. The correlation coefficient r = 0.9996, this number is close to 1, which means that there is a very high correlation between absorptions and levels of compounds and shows a linear relationship between the two. Table 2 shows the total flavonoid levels in each water extract and ethanol extract by 0.486% and 0.512%. These results meet the requirements contained in Indonesian Herbal Pharmacopoeia Edition I [14], which is not less than 0.40%.

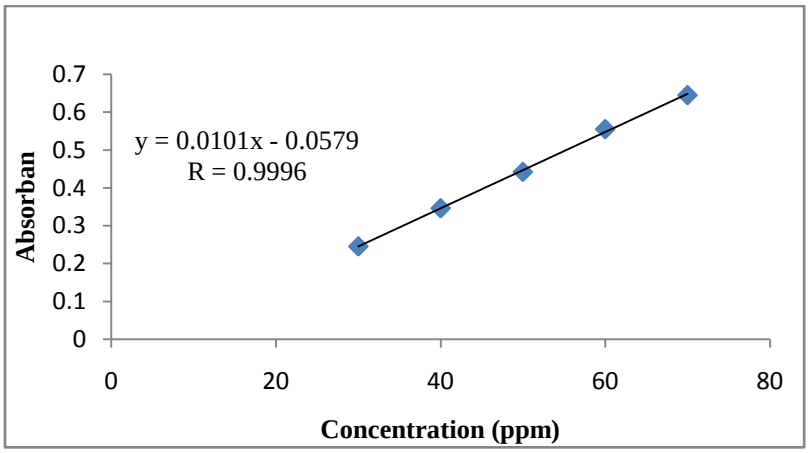

Figure 6: Quercetin calibration curve

Total phenol levels in acetone, ethanol, and water from bay leaves were respectively 0.1202%, 0.1258% and 0.2248% calculated as gallic acid (Table 2).In determining total phenolic content, the maximum absorption of gallic acid was obtained at 765 nm at a concentration of 15 µg / mL (Figure 7). Based on the calibration curve in Figure 8 the regression equation obtained y = 0.0066x + 0.1979. The correlation coefficient r = 0.9997, this number is close to 1, which means that there is a very high correlation between absorptions and levels of compounds and shows the relationship between the two. Based on this equation, the total phenolics content of water extract, ethanol extract, and acetone extract were 0.2248%, 0.12586%, and 0.1202% respectively.

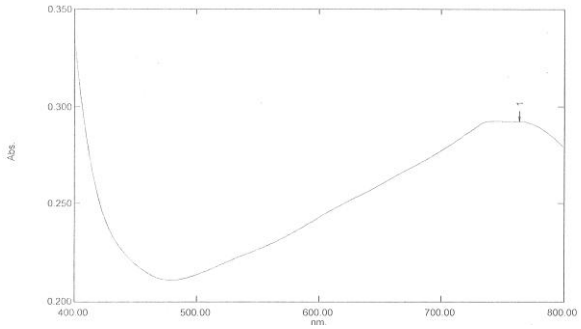

Figure 7: Visible light spectrum for gallic acid at a concentration of 15 ppm with Folin-Coucalteau reagent (Maximum at 765 nm)

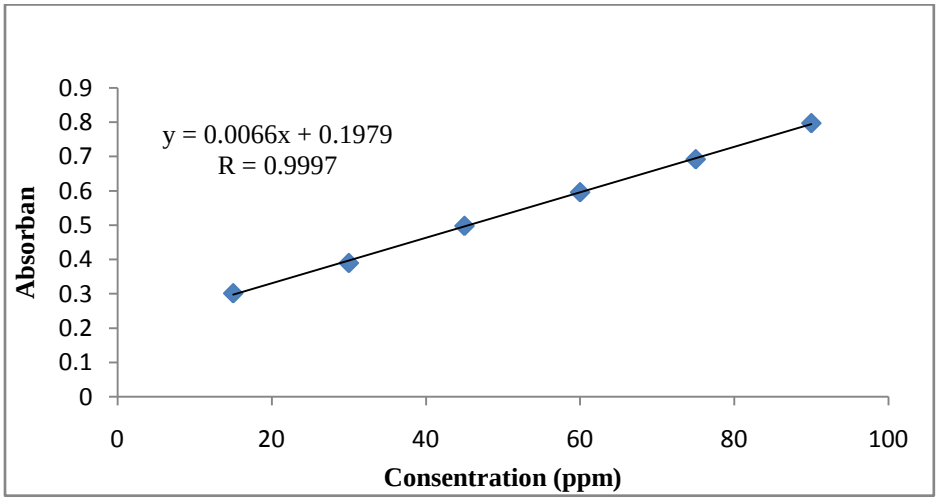

Figure 8: Gallic acid calibration curve

The total tannin levels in acetone, ethanol and water extract from bay leaves were 0.1457%, 0.1688% and 0.1622% respectively calculated as catechins (Table 2).Determination of tannin was carried out using UV-Vis spectrophotometry using standard catechin compounds. Determination of total tannin content was carried out by UV-Vis Spectrophotometry (Shimadzu UV-1800) method. The measurement results of the absorption of the comparable solution at the wavelength of 279 nm showed that the tannin levels obtained in acetone extract were 0.1457%, ethanol extract 0.1688%, and water extract 0.1622%.

Table 2: Results of quantitative analysis of the chemical content in extracts of hexane, acetone, ethanol, and water from bay leaves

Type of Extract	Total alkaloids (%)	Total flavonoids (%)	Total phenol (%)	Total tannin (%)
Hexane Extract	-	-	-	-
Acetone Extract	-	-	0.1202	0.1452
Ethanol Extract	0.34	0.512	0.1258	0.1688
Water Extract	-	0.486	0.2248	0.622

Conclusion

Bay leaf acetone extract contains phenol and tannin compounds; the ethanol extract contains alkaloids, flavonoids, phenols, and tannins; the water extract contains flavonoids, phenols, and tannins.

The total alkaloid content of the bay leaf ethanol extract is 0.34%; the total flavonoid levels in ethanol and water extracts were 0.512% and 0.486% respectively; total phenol levels in a row of acetone, ethanol and water extracts were 0.1202%, 0.12586%, and 0.2248%; total tannin levels of acetone, ethanol and water extracts were 0.1452%, 0.1688%, and 0.1622% respectively.

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