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Total Flavonoids of Patikan Kebo Extract (*Euphorbia hirta L.*) in Various Drying Methods

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Abstract: Drying is the most important process to maintain the stability of chemical compounds from plants. Chemical compounds from plants that have medicinal properties, one of them is flavonoids which are secondary metabolites. To get high levels of flavonoids, we must keep to how to drain any plants. The aim of this study was to investigate the best drying methods wich obtained higher levels of flavonoids. The study was such an experimental analytical research consisting of two groups. The herbs was dried by two different methods aerated and sun heating. Extraction was conducted by maceration with 70% ethanol solvent. Flavonoid levels were calculated using UV-Vis spectrofometry. The average flavonoid content in the aerated group and the sun heating group 2 mg/gram and 1.89 mg/gram respectively.

Keywords: drying methods, flavonoids, patikankebo

I. INTRODUCTOION

People sometimes do not realize that plants that grow wild around them can be used as medicine to cure disease and maintain health. Secondary metabolite compounds found in plants are bioactive substances related to the chemical content in plants [1]. One of them is patikankebo (*Euphorbia hirta L.*). This plant is a wild weed that is commonly found in the tropics, growing at an altitude of 1-1400 meters above sea level (masl). The use of patikankebo is to help treat sore throat, bronchitis, asthma, dysentery, stomach ulcers, diarrhea, blood urine, eczema [2]. Patikankebo (*Euphorbia hirta L.*) contains the terpenoid compounds eufol, betaeufol, and eufosterol. Other chemical constituents are alkaloids, tannins, waxes, tannins, polyphenolic compounds (such as gallic acid), the flavonoid quercetin, and xanthoramnin[3].

Quality, safety and efficacy of plants as natural medicines must be improved.It is necessary to standardize their raw materials, either in the form of simplicial, extracts or galenic preparations. One of the influencing factors is the condition of the drying process of medicinal plants, especially those derived from plants[4]. Previous research had proven that the choice of drying method affects the flavonoid, phenolic and antioxidant levels of an extract[5][6]. Previous research about different drying methods affected the total phenol, alginate and proximate content in *Sargassum polycystum*[7].

Proper drying method will produce good quality of simplicia and will not damage the content of active substances in the plant which will be used for further processing, either for analysis or for making a preparation. Therefore, this research was conducted on the effect of drying plants in order to obtain high levels of flavonoids in patikankebo. The plant drying process was carried out with two variations of drying methods, namely by air drying and air-sundrying.The extraction method used maceration with 70% ethanol. The results of this study were expected to obtain an appropriate drying method for patikankebo herbs in order to obtain high levels of flavonoids using UV-Vis spectrophotometry method.

II. MATERIALS AND METHODS

2.1 Plant material Collection and Authentication

The materials used in this study were patikankeboherbs. Patikankebo was taken from Tempel Village, Jembungan, Banyudono, Boyolali Regency, Indonesia. Make sure the plantwas taken all parts of the plant (leaves, stems, and flowers) except the roots. The plants were picked in the morning before sunrise.

2.2 Chemicals

Aqua, quercetin, ethanol p.a., HCl, Mg metal, $AlCl_3$ and potassium acetate.

2.3 Plant Drying

Patikan kebo herb washed and chopped about 1 cm to speed up the drying process. Weighed 8 kg, then divided into 2 portions of 4 kg each. The first part was aerated without being exposed to the sun (air method) and the second part is dried in direct sunlight (air-sun method). The parameter used to stop the drying process waswhen the patikan kebo can be crumbled.

2.4 Preparation and Sample extraction

The dried patikan kebo plants were weighed 200 grams for each drying method, put in a glass bottle and 2000 ml of solvent was added, allowed to stand for 4 x 24 hours (4 days), shaking twice a day. The maceration process was carried out in a tightly closed container at room temperature. After 4 x 24 hours the macerate was filtered using flannel, the filtrate obtained was collected. The obtained filtrate was evaporated with a distillation apparatus at a temperature of 78^{o}C until half the solvent evaporated, then continued evaporation in a porcelain cup on a water bath at a temperature of 78^{o}C.

2.5 Extract Moisture Test

The viscous extract obtained was weighed, the yield was calculated for further qualitative analysis of flavonoids and the calculation of flavonoid levels.The extract was weighed as much as 2 grams in a silicate crucible that had been previously sized, then dried in an oven at 105^{o}C for 3 hours, cooled in a desicator for 15 minutes. Then weighed, the same treatment was repeated until the weight remained[8].

2.6 Qualitative Analysis

Extract as much as 10 mg dissolved in 10 ml of ethanol, heated. The solution was taken 2 mL in a test tube, added 1 cm of magnesium (Mg) band and added 5 drops of concentrated HCl. The formation of a pale yellow, greenish yellow to brick red solution indicates the presence of flavonoids [9].

2.7 Quantitative Analysis

The standard solution was prepared by weighing 10 mg of quercetin, dissolved with ethanol pa to 10 mL (1000 ppm) in a 10 ml volumetric flask. Take 1 ml of 1000 ppm mother liquor diluted to 10 ml in a 10 ml volumetric flask (100 ppm concentration). The standard solution of quercetin was made in various concentrations of 2, 4, 6, 8, and 10 ppm diluted from a solution of 100 ppm.

Maximum Wavelength measured by prepared 2 ml of quercetin solution with a concentration of 10 ppm was put in a test tube and reacted with 0.1 ml of 10% $AlCl_3$, added 0.1 ml of 1M sodium acetate and 2.8 ml of distilled water, the readings were taken at a wavelength range of 300-500 nm.

Operating timemeasured by prepared 2 mL of 10 ppm quercetin solution was put in a test tube and reacted with 0.1 mL of 10% $AlCl_3$, added 0.1 mL of 1M sodium acetate, and 2.8 mL of distilled water. The absorbance of the solution was measured at the maximum wavelength that had been obtained with an interval of 2 minutes until a stable absorbance was obtained.

The Quercetin standard curvewas made by prepared quercetin concentrations of 2, 4, 6, 8 and 10 ppm. Each solution was taken 2 mL each into a test tube, each solution was reacted with 0.1 mL of 10% $AlCl_3$, added 0.1 mL of 1M sodium acetate, and 2, 8 mL of distilled water, shaken well and allowed to operate according to the optimized operating time at room temperature, the absorbance was measured using a UV-Vis spectrophotometer at the previously optimized maximum wavelength. The standard quercetin curve was made by connecting the concentration of the quercetin standard solution with the absorbance results obtained in measurements using a UV-Vis spectrophotometer.

Flavonoid levelof extract was determained by weighed 10 mg on a watch glass, dissolved with 10 mL of ethanol pa in a 10 mL volumetric flask to obtain an extract solution with a concentration of 1000 ppm. Took 2 mL, react with 0.1 mL of 10% $AlCl_3$, add 0.1 mL of 1M sodium acetate and 2.8 mL of distilled water,silenced according to the optimized operating time. Absorbance readings were carried out at the maximum wavelength. If the absorbance of the sample did not meet the standard, namely the range between 0.2-0.8, dilution was carried out until it reached that range.

2.8 Statistical Analysis

Data analysis used in this study is Mann Whitney. Mann Whitney is used to test the difference in the mean of two independent samples, whether the two independent samples are two populations with the same mean.

III. RESULTS AND DISCUSSION

3.1 Extract evaluation

Table 1. Extract Yield

Drying Method	Simplicia Weight (grams)	Extract WeightThick(grams)	Yield (%)	AverageYield(%)
Air drying	200	35.02	17.51	17.26
	200	34.80	17.40	
	200	33.72	16.86	
Air-sun drying	200	45.02	22.51	22.60
	200	45.27	22.64	
	200	45.27	22.60	

The yield obtained in Table 1. shows that drying in air-sun method is higher (22.60%) than air drying method (17.26%). The higher drying temperature causes the plant cells to be damaged, so that the cell organelles are more easily removed. Meanwhile, during drying, the simplicia cells were not badly damaged so that only a small part of the cell organelles came out. Extraction was carried out by the maceration method in accordance with research conducted by IkkeSafitri, et al (2018) regarding the extraction method that produces the highest to lowest flavonoid and phenolic levels, respectively, percolation, maceration, soxhletation, then reflux.

The purpose of determining the water content of the extract is to provide a minimum limit or range of the amount of water content in the extract. The results of the water content test of the patikankebo herb extract obtained are as follows:

Table 2. Moisture Content of Patikan Kebo Herba Condensed Extract

Method Drying	Replication	Sample Weight Extract (grams)	Water content (%)	Average Water content (%)
Air drying	1	2.00	10.00	10.33
	2	2.00	10.00	
	3	2.00	11.00	
Air-sun drying	1	2.00	11.00	10.00
	2	2.00	9.00	
	3	2.00	10.00	

The requirements for a good thick extract of the patikankebo herb are that the water content is not more than 14.6%[10].The results obtained (Table 1) all extracts had met these requirements. The water content obtained was different, this was because at the time of evaporation to become a thick extract it takes time, place and different conditions. All the water content test took 4 hours to constant weight.

3.2Qualitative Analysis Result

Qualitative flavonoid tests were carried out to ensure that the patikan kebo herbal extract produced contained flavonoids or not. The results of the qualitative test with the addition of concentrated Mg and HCl bands can be seen in table 3.

Table 3. Flavonoid Qualitative Test

Drying method	Replication	Results	Information
Air drying	1	Yellow	positive containsflavonoids
	2	Orange	positive containsflavonoids
	3	Brick red	positive containsflavonoids
	1	Orange	positive containsflavonoids
	2	Yellow	positive containsflavonoids

Air-sun drying	3	Yellow	positive containsflavonoids

The color of the qualitative test results from yellow to brick red indicated that the patikankebo extract contains flavonoids. The color produced in qualitative testing of flavonoids was different because in one sample there are different types of flavonoids. The reaction of the patikankebo extract added with concentrated Mg and HCl serves to reduce the benzopiron core contained in the flavonoid structure to form a red flavilium salt [11].

3.3 QuantativeAnalysis Result

The maximum wavelength was used to measure the absorbance of the calibration curve and samples of the patikankebo herb extract. The maximum wavelength was the wavelength with the highest absorbance. The maximum wavelength of the complex between quercetin (10 ppm) and $AlCl_3$ obtained the highest absorbance of 0.613 at a wavelength of 441 nm.

Determination of the operating time was carried out to determine that the reaction between quercetin and $AlCl_3$ had been completed, which is indicated by the absorbance being stable or constant. The operating time showed that the time required for quercetin and $AlCl_3$ to react to form complex compounds was at least 14 minutes (the absorbance was constant) before being read on a UV-Vis spectrophotometer. From the determination of the standard curve of quercetin obtained the following data:

Table 4. Standard Curve Data

X (Concentration)	Y (Absorbance)
2 ppm	0.226
4 ppm	0.359
6 ppm	0.466
8 ppm	0.566
10 ppm	0.642

Based on the standard curve above, the linear regression formula y = 0.05195X + 0.1401 is obtained with a correlation coefficient (R^2) of 0.9902. R^2 value close to 1 indicates that the calibration was linear and there was a relationship between concentration and absorbance[12].

Based on UV-Vis spectrophotometric measurements, the results showed that the quercetin flavonoid content contained in the patikankebo herb extract with the dried herbs aerated (2 mg/gram) compared to sun heating drying (1.89 mg/gram), such as in Table 4.6, this was due to sunlight emitting UV radiation which causes the formation of free radicals so that they can damage the compounds contained in the patikankebo herb. The high extract yield did not affect the content of the extract, although the high extract yield did not necessarily mean that the flavonoid content contained in the extract was also high.

Table 4. Flavonoid Content of Patikan Kebo Herba Extract

Drying Method	Replication	Absorbance	Level (mg/gram)	Average (mgEQ/gram)	Average Content (mgEQ/gram)
Air Drying	1	0.736	2.01	2.00±0.39	2.00±0,24
		0.617	1.61		
		0.847	2.39		
	2	0.800	2.21	1.99±0.21	
		0.725	1.96		
		0.675	1.79		
	3	0.725	1.90	2.01±0.20	
		0.721	1.89		
		0.830	2.24		
Air-Sun Drying	1	0.615	2.06	2.02±0.08	1.89±0,13
		0.585	1.93		
		0.620	2.08		
	2	0.545	1.77	1.84±0.10	
		0.590	1.96		

		0.551	1.79		
	3	0.578	1, 92	1.80±0.10	
		0.545	1, 77		
		0.536	1.73		

3.4 Statistical analysis result

*Mann Whitney*used to test the difference in the mean of two independent samples, whether the two independent samples are two populations with the same mean. The results of the Mann Whitney analysis have a significance value of 0.340, thus the probability of 0.340 > 0.05 which means that the two variants are the same, this means that there was no significant difference between the air drying method and air-sun drying method.

IV. CONCLUSION

The flavonoid content produced in the dried patikankebo herbs with the air dryingand air-sun drying respectively 2QEmg/gram and 1.89 mgQE/gram. There was no significant difference between the air drying method and air-sun drying method.

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