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Effect of Solution Concentration On Flavonoid Level Ethanol Extract Of Cassava Leaves (*Manihot esculenta Crantz*)

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Abstract: Cassava (*Manihot esculenta* Crantz) is a family of euphorbiaceae which is known that leaves contain secondary metabolites, one of which is flavonoids. The purpose of this experiment was to determine the effect of ethanol solvent concentration as a solvent on levels of flavonoids in cassava leaves. The ethanol extract was made by maceration method using variations in the ethanol concentration of 70%, 80%, and 90%, to determine the flavonoid content in cassava leaves, a quantitative test was carried out with the UV- Vis Spectrophotometic method and the standard used for comparison was quersetin. The effectof concentration variations was analyzed by SPSS. The result showed that yield of the extrac at the respective concentrations of 70%, 80%, 90% was 13.458±2.099%, 14.714±0.636%, 11.056±0.840%, and the levels of flavonoids at the respective concentrations of 70%, 80%, and 90% were 0.025±0.008 mg/g EQ, 0.031±0.01 mg/g EQ, and 0.045±0.002 mg/g EQ. The results of SPSS test with Oneway Annova test obtained a significance value of o,ooo<0,05, which means that there are differences in the levels of flavonoid in the ethanol extract of cassava leaves at concentration of 70%, 80%, and 90%.
Keywords: Cassava, Flavonoid, Solvent, Spectrophotometric UV-Vis

I. INTRODUCTOION

Flavonoids are secondary metabolites that are widely distributed in various plants, one of which is cassava leaves. Cassava leaves are known to contain phenolic compounds, especially flavonoids that can be used as antioxidants [1]. In dissolving flavonoid compounds, an appropriate solvent is needed, the solubility of a substance in a solvent is largely determined by the compatibility of the nature or chemical structure between the solvent and the compound to be dissolved. Generally, flavonoid compounds are found to bind to sugars to form glycosides which cause these compounds to be easily soluble in polar compounds [2]. So that flavonoids can be dissolved using ethanol as a solvent because ethanol is a polar solvent that has universal properties. In addition to the type of solvent, the concentration of the solvent also affects the flavonoid content to be obtained, the difference in solvent concentration causes a change in the polarity of the solvent so that it affects the flavonoid content that will be produced [3].

According to a previous study regarding differences in flavonoid levels in bay leaves with variations in solvent concentration of 70% and 96% ethanol with a comparison solution of quercetin, using a UV-Vis spectrophotometer was found that the total flavonoid content obtained in 70% ethanol extract was higher than 96% ethanol. that is equal to 350±1.76 ppm while at 96% ethanol it is 270±5.30 ppm [4].

Therefore, the aim of this study was to determine the effect of ethanol solvent concentration on the flavonoid content of cassava leaves extract and to determine the flavonoid content of cassava leaves ethanol extract obtained through the maceration extraction method with variations in ethanol concentration.

II. MATERIALS AND METHODS

2.1 Plant material Collection and Authentication

The plants studied came from the village of Weru, Sine, Ngawi, Indonesia in January, 2021, which were picked in the morning. Plant species were determined at the Laboratory of the Research Center for Traditional Medicinal Plants (BPPTOOT) Tawangmangu, Indonesia.

2.2 Chemicals

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

2.3 Preparation and Sample extraction

The extraction method used is maceration method using ethanol solvent with different concentrations of 70%, 80%, and 90% v/v. The dry cassava leaves used for each solvent was 50 g which was put into a maceration vessel and soaked with 750 mL of ethanol for 5 days with stirring 2 times a day. The results from the maceration were then filtered and evaporated with a rotary evaporator at a temperature of 58°C to obtain a liquid extract. That extract was concentrated again by being put into a porcelain dish and evaporated with a water bath at a temperature of 60°C until a thick extract was obtained.

2.4 Extract Evaluation

Extracts were evaluated organoleptically and their water content, organoleptic tests were carried out using the five senses, including shape, color, and odor of the extract [5]. While the water content test was carried out with a moisture analyzer by taking 2 g of thick cassava leaves extract which was inserted into an aluminum foil plate and then inserted into the device to measure the water content.

2.5 Qualitative Analysis

Analysis of the chemical content of flavonoids in cassava leaves using Mg and HCl with positive results indicated by the formation of yellow, orange, and red colors [6].

2.6 Quantitative Analysis

Quantitative analysis was carried out using the UV-Vis spectrophotometry method using a quercetin comparison solution based on the modified method [4], which used 1000 ppm mother liquor, then continued with the manufacture of standard series solutions with concentrations of 20, 40, 60, 80, 100, and 120 ppm. Before measuring the standard curve, the maximum wavelength was determined by making a 50ppm quercetin solution then 0.5 mL pipetted and put into a 5 mL volumetric flask, added with 1.5 mL ethanol pa, 0.1% aluminum chloride 10% mL, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water, shaken until homogeneous. The absorbance was measured at a wavelength of 350-500 nm. Then determine the operating time by making a 50ppm quercetin solution, then 0.5 mL pipetted and put into a 5 mL volumetric flask, added with 1.5 mL of ethanol pa, 0.1 mL of 10% aluminum chloride, 1 M of potassium acetate 0.1 mL and 2.8 mL distilled water, shaken until homogeneous. The absorbance of the solution was measured at the obtained wavelength with an interval of 5 minutes until a stable absorbance was obtained. After obtaining the maximum wavelength and operating time, measure the standard curve using a standard series solution in which 0.5 mL of each solution was pipetted into a 5 mL volumetric flask, added with 1.5 mL of ethanol p.a. 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water, were shaken until homogeneous. The solution was incubated for the time obtained at the operating time and then measured with a UV-Vis spectrophotometer with the maximum wavelength obtained.

The next step is making a sample solution of cassava leaves, with a sample concentration of 2000 ppm, 0.5 mL of the solution is pipetted into a 5 mL volumetric flask, added with 1.5 mL of ethanol p.a. 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water, were shaken until homogeneous. The solution was incubated for the time obtained at the operating time and then measured with a UV-Vis spectrophotometer with the maximum wavelength obtained.

2.7 Statistic Analysis

The method used in statistical testing is SPSS (Statistical Package for the Social Sciences) using Oneway Annova test method, where the decision is made if the significance value is less than 0.05 then there is a difference in each concentration, but before carrying out the Oneway Annova test, first conduct a normality test where the decision is if the value is If the significance value is more than 0.05, the data is normally distributed, in addition to the normality test to perform the Oneway Annova test, it is a homogeneity test where the significance value is more than 0.05, so it can be said that the variance of two or more groups is the same [7].

III. RESULTS AND DISCUSSION

This study used cassava leaves as plant samples, because cassava leaves are known to contain flavonoid compounds [1]. Cassava leaves extract was obtained by the maceration method, because the maceration method used the principle of immersion at room temperature, so as not to reduce the flavonoid content of the plant. Flavonoid compounds are known to be reduced if extracted by heating [8]. Meanwhile, to dissolve cassava leaves flavonoid compounds, ethanol solvent is used, because ethanol is a polar and universal solvent which can dissolve all types of secondary metabolites, both polar and non-polar. Maceration with ethanol was carried out for 5 days with stirring 2 times a day, the purpose of this stirring was so that all surfaces of the powder could be in contact with the solvent, so that the flavonoid compounds could be dissolved completely [9].

3.1 Extract evaluation

The results of the maceration are concentrated with a rotary evaporator so that the solvent in the macerate can evaporate to produce a thicker extract, while the use of a water bath is to concentrate the extract in order to obtain a thick extract. The yield value can be seen in **Table 1**, where the highest yield value was obtained at 80% ethanol concentration. According to the research, it was stated that the highest extract yield of weed rhizome extract was obtained at a concentration of 70% and the extract yield would be lower at 80% and 90% ethanol concentrations [3].

Table 1:Cassava Leaves Extract Yield

Solvent (v/v)	Extract Yield
Ethanol 70%	13.453±2.095%
Ethanol 80%	14.714±0.636%
Ehtanol 90%	11.056±0.840%

The viscous extract obtained was then tested organoleptically, it was found that the ethanol extract of cassava leaves had a thick extract form, a characteristic odor of the extract, and a greenish-brown color. The extract became viscous because the content of ethanol and volatile substances at a temperature of 60°C had been exhausted and what remained were secondary metabolites that did not evaporate at that temperature. The greenish-brown color is produced from the simplicia color of the cassava leaves which are slightly green in color. The next evaluation is the water content test, which aims to determine the water content contained in the extract, determining the water content is very important to determine the freshness and durability of the extract. The value of the water content of the ethanol extract of cassava leaves produced was in accordance with the literature, which was <10% [5]. The value can be seen in **Table 2**.

Table 2: Water Content of Cassava Leaves Extract

Solvent (v/v)	Water Content
Ethanol 70%	2.793±0.371%
Ethanol 80%	3.236±0.796%
Ehtanol 90%	2.530±0.371%

3.2 Qualitative Analysis Result

Before carrying out a quantitative test on the ethanol extract of cassava leaves, a qualitative test was conducted to determine the chemical components of the plant. Flavonoids showed positive results if there was a change in color to red, yellow, or orange in the sample after the addition of Mg and HCl metals. Mg and HCl serve to reduce the binzopyron core contained in the flavonoid structure and form red or orange flavilium salts [10].

3.3 Quantative Analysis Result

After conducting a qualitative test, to determine the levels of flavonoids contained in the ethanol extract of cassava leaves, a quantitative test was carried out using a UV-Vis spectrophotometer and a comparison solution of quercetin. In testing the levels of flavonoids with a UV-Vis spectrophotometer, it is important to determine the wavelength first, to determine the wavelength value so as to produce the maximum absorption value. 10% aluminum chloride is used for the color reaction which can form a complex with quercetin to facilitate reading on a UV-Vis spectrophotometer. Meanwhile, 0.1 M potassium acetate was used to determine the wavelength in the visible region [11]. The result of determining the maximum wavelength obtained is 440 nm. Next is the determination of operating time which aims to find out how long it takes between aluminum chloride and quercetin to finish reacting so that the reaction can run perfectly. The operating time obtained was 95 minutes, this time was used for incubation of the sample solution before measurements were made on a UV-Vis spectrophotometer.

After determining the maximum wavelength and determining the operating time, the next step is to make a standard curve of quercetin with concentrations of 20, 40, 60, 80, 100, 120 ppm. The graph of the curve can be seen in **Figure 2**. The value of r which is close to 1 indicates that the calibration curve is linear and there is a relationship between the concentration of the quercetin solution and the absorbance value.

Quantitative analysis of cassava leaf flavonoids with different solvent concentrations using a UV-Vis spectrophotometer. The average results of 3 measurements of absorbance and levels of flavonoids in the extract can be seen in **Table 3**.

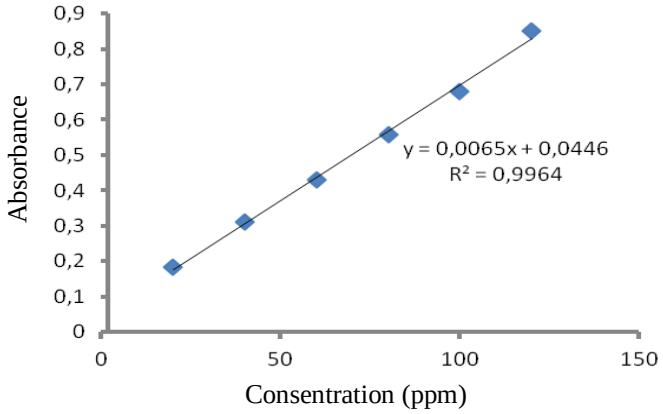

Fig 1: Quercetin Standard Curve

Table 2: Cassava Leaves Extract Flavonoid Content

Solvent (v/v)	Flavonoid Content
Ethanol 70%	0.025 mg/g EQ
Ethanol 80%	0.031 mg/g EQ
Ehtanol 90%	0.045 mg/g EQ

From the Table 2, it can be seen that the highest flavonoid content of cassava leaves was obtained from cassava leaves macerated with 90% ethanol. The results obtained were influenced by the solvent concentration of 70% ethanol and 80% containing more water than 90% ethanol. Previous research by Yunita & Khodijah (2020) stated that quercetin is a class of polar compounds that have low solubility in water and are more soluble in organic solvents and ethanol. The study stated that 90% ethanol concentration resulted in higher flavonoid levels because 90% ethanol contained less water. According to research conducted by Yunita & Khodijah (2020) stated that 96% ethanol in tamarind leaves produces more flavonoid levels than 70% ethanol. This statement shows that a higher concentration of ethanol solvent at the time of extraction can produce higher flavonoid levels.

3.4 Statistic analysis result

The results of flavonoid levels were carried out by SPSS statistical testing with the Oneway Annova test to determine whether there was a significant difference in flavonoid levels between 70%, 80%, and 90% solvents. Based on the tests carried out, a significance value of 0.000 (0.000 <0.05) was obtained, it can be concluded that there were significant differences in the three solvent concentrations on the flavonoid content of the ethanol extract of cassava leaves.

IV. CONCLUSION

According to the research that has been done, it can be concluded that there are significant differences between the levels of flavonoid extracts of 70%, 80%, and 90% v/v ethanol extracts with the levels of each concentration being 0.025 mg/g EQ, 0.031 mg/g EQ, 0.045 mg/g EQ.

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REFERENCES

[1] Hasim, S. Falah, and L. K. Dewi, "Effect of Boiled Cassava Leaves (Manihot esculenta Crantz) on Total Phenolic, Flavonoid and its Antioxidant Activity," *Curr. Biochem.*, vol. 3, no. 3, pp. 116–127, 2016.

[2] E. Hanani, *Analisis Fiokimia*. Jakarta: Buku Kedokteran EGC, 2016.

[3] C. P. Suhendra, I. W. R. Widarta, and A. A. I. S. Wiadnyani, "PENGARUH KONSENTRASI ETANOL TERHADAP AKTIVITAS ANTIOKSIDAN EKSTRAK RIMPANG ILALANG (Imperata cylindrica (L) Beauv.) PADA EKSTRAKSI MENGGUNAKAN GELOMBANG ULTRASONIK," *J. Ilmu dan Teknol. Pangan*, vol. 8, no. 1, p. 27, 2019, doi: 10.24843/itepa.2019.v08.i01.p04.

[4] E. S. Syamsul, Y. Y. Hakim, and H. Nurhasnawati, "PENETAPAN KADAR FLAVONOID EKSTRAK DAUN KELAKAI (Stenochlaena palustris (Burm. F.) Bedd.) DENGAN METODE SPEKTROFOTOMETRI UV-VIS," *J. Ris. Kefarmasian Indones.*, vol. 1, no. 1, pp. 11–20, 2019, doi:

10.33759/jrki.v1i1.46.
[5] R. Depkes, *Parameter Standar Umum Ekstrak Tumbuhan Obat*. Jakarta, 2000.
[6] J. B. Harborne, *Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan*. Bandung: Institut Teknologi Bandung, 1987.
[7] Hari Purnomo, *Statistika Farmasi*. Yogyakarta: CV. Grafika Indah, 2017.
[8] N. Tomayahu, Z. Abidin, F. Farmasi, and U. M. Indonesia, "PENETAPAN KADAR FLAVONOID TOTAL EKSTRAK ETANOL KULIT BUAH ALPUKAT (Persea americana Mill .) DENGAN METODE SPEKTROFOTOMETRI UV-VIS," vol. 4, no. 2, pp. 226–230, 2016.
[9] E. Yunita and Z. Khodijah, "Pengaruh Konsentrasi Pelarut Etanol saat Maserasi terhadap Kadar Kuersetin Ekstrak Daun Asam Jawa (Tamarindus indica L .) secara Spektrofotometri UV-Vis Effect of the Different Ethanol Concentration during Maceration on Quercetin Level of Tamarind (Tama," vol. 17, no. 02, pp. 273–280, 2020.
[10] N. N. P. Dewa Gede Eka Prayoga, Komang Ayu Nocianitri, "ANTIOKSIDAN EKSTRAK KASAR DAUN PEPE (Gymnema reticulatum Br .) PADA BERBAGAI JENIS PELARUT," vol. 8, no. 2, pp. 111–121, 2019.
[11] D. M. Sholihin, A. O. T. Dewi, and E. D. Antari, "PENETAPAN KADAR FLAVONOID PADA PELEPAH PISANG RAJA (Musa acuminata Colla. forma cerifera Back.) DENGAN VARIASI LAMA WAKTU EKSTRAKSI," *Farm. Politek. Indonusa Surakarta*, vol. 4, no. 2017, pp. 1–5, 2020.

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