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THE EFFECT OF MACERATION TIME ON TOTAL ALKALOID LEVELS IN BROCOLI (Brassica oleracea var. italica) BY USING UV-Vis SPECTROPHOTOMETRY METHOD

Pengaruh Waktu Maserasi terhadap Kadar Alkaloid Total pada Brokoli (Brassica oleracea var. italica) dengan Metode Spektrofotometri UV-Vis

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ABSTRACT

Broccoli is known to be very rich in secondary metabolites, such as alkaloids, flavonoids, tannins, polyphenols, essential oils, and polypeptides. Alkaloids are one of the secondary metabolites that are commonly found in nature and have physiological activities. The extraction time factor is the thing that can affect the quality of the extraction results which in this study is the alkaloid content. This study aims to determine the optimal maceration time so that it can produce optimum total alkaloid content. The stages in this study include sample preparation and then sample extraction by maceration using 96% ethanol with differences in maceration time of 1 day, 2 days and 3 days. Furthermore, identification using TLC and analysis of total alkaloid content using UV-Vis spectrophotometer. Kruskal Wallis test was used to determine whether there was an effect of maceration time on total alkaloid content. The results showed that the highest average total alkaloid content in the second sample was maceration for 2 days with a concentration of 17.5%. The Kruskal Wallis test showed that there was no effect between maceration time on total alkaloid content.

Keywords: Total alkaloid concentration, broccoli flower extract and effect of maceration time

BACKGROUND

Indonesia has natural wealth with various types of plants that are useful as traditional medicine. One type of plant that contains active substances and can be useful as traditional medicine is broccoli. Broccoli is known as the Crown Jewel of Nutrition because it contains various important nutrients such as vitamins, fiber, minerals, and secondary metabolites (Fatharanni and Anggraini, 2017).

Alkaloids are one of the secondary metabolites found in broccoli plants. Alkaloids have the ability to act as antibacterials by interfering with the peptidoglycan constituent components in bacterial cells, so that the cell wall layer is not fully formed which then causes the death of the cell (Chairani and Harfiani, 2018). In addition, in the world of medicine, alkaloid compounds have analgesic effects (morphine and codeine), antitussive (codeine), antimalarial (quinine), spasmolytic (Papaverin), antiamoebic, and antiemetic (Emetin) (Sumardjo 2008 in Salamah *et al*., 2017).

Alkaloid levels in a plant can be influenced by several factors, one of which is the extraction time. Extraction time can affect the quality of the extraction results. Extraction time that is too short can result in the bioactive components extracted from the material not being maximized so that the bioactive components obtained are low (Yuliantari *et al*., 2017). In addition, the longer the extraction time, the greater the chance of contact between the simplicia and the solvent so that the yield will increase to the saturation point of the solution, however, after reaching the optimal time the amount of extract obtained will decrease (Kristian *et al*., 2016).

But so far, research on the optimal maceration time in order to produce optimum total alkaloid content has not been studied much, so research is carried out on the effect of maceration time on total alkaloid content in broccoli (*Brassica oleracea* var. *italica*) using UV-Vis spectrophotometry method. Identification of alkaloids using UV spectrophotometric method is based on the reaction of alkaloids with bromocresol green (BCG), forming a yellow product (Ajanal *et al*., 2012).

METHOD

This research is included in an experimental quantitative type that emphasizes the determination of total alkaloid content in broccoli (*Brassica oleracea var. italica*). Quantitative analysis using UV-Vis spectrophotometer was carried out by entering the absorbance data of the analyzed sample into the regression equation obtained from the calibration curve, in order to obtain the total alkaloid content. One Way ANOVA test was used to determine the effect of maceration time on total alkaloid content. If the data cannot be tested using the One Way Anova test, then the data is tested using the Kruskal-Wallis test.

Data collection techniques using non-participant observation, namely the observation of an object under study. The observations were made, namely thin layer chromatography and determination of total alkaloid content using a UV-Vis Spectrophotometer. The time of research and data collection was carried out in April 2021-June 2021. The location of the research was the Chemical and Microbiology Laboratory of STIKes AKBIDYO. The samples in this study were broccoli flowers taken in the Genikan area, Ngablak District, Magelang, Central Java.

The tools used in this research are: beaker glass (Iwaki), volumetric flask (Iwaki), test tube (Iwaki), Erlenmeyer tube (Iwaki), Blender, Sieve, Filter paper, Analytical balance (Ohaus), Vaccum Pump (Rocker 300), Water bath (HH-6), Magnetic stirrer (Heidolph), UV lamp 254 nm and 366 nm, UV-Vis Spectrophotometer (Genesys 150), Chamber, and other laboratory glassware. Materials needed are: broccoli flower, ethanol 96%, TLC plate silica gel GF254, ethyl acetate, chloroform, aquades, Dragendrof reagent, caffeine, BCG (bromocresol green) and Phosphate buffer pH 4.7.

Sample Preparation

Dry sample preparation was carried out by means of 3 kg of broccoli flowers cut into small pieces and washed and then air-dried. Then it was dried in an oven at a temperature of around 38°C for 48 hours, then mashed using a blender until a powder was formed, sieved using a sieve. 100 g of broccoli flower simplicia powder was macerated using 1 L of 96% ethanol as a solvent. Simplicia powder was soaked with variations in maceration time of 1, 2, and 3 days. Then the macerate was filtered using a vacuum pump. The maceration results were then concentrated using a water bath to obtain a thick extract of broccoli flowers.

Thin Layer Chromatography (TLC)

The silica gel GF_{254} TLC plate was activated by oven at 100°C for 1 hour to remove the water present on the TLC plate (Sastrohamidjojo, 2007). The stationary phase used silica gel

60 GF_{254} and the mobile phase used ethyl acetate:chloroform eluent with a ratio of 8:2 (Jannah, 2014). Then to check the presence of alkaloids, sprayed Dragendorf reagent. The spots or spots formed were marked and observed at UV light 254 and 366 nm. A positive result will show an orange color in Dragendof's reagent.

Analysis of Total Alkaloid Levels with UV-Vis Spectrophotometer

Preparation of caffeine standard solution

A total of 250 mg of caffeine was dissolved in hot distilled water and put into a 250 mL volumetric flask to obtain a concentration of 1000 ppm. Then 2.5 mL pipette was added and distilled water was added to a 25 mL volumetric flask to obtain a concentration of 100 ppm (Wahyuni and Marpaung, 2020).

Determination of the maximum wavelength (λmax) of caffeine

The maximum wavelength of the caffeine solution was determined using a UV-Vis spectrophotometer at λ: 250-290 nm (Wahyuni and Marpaung, 2020).

Caffeine standard curve determination

Take 0.1; 0.3; 0.6; 0.9; 1,2; and 1.5 mL of 100 ppm caffeine standard solution and diluted to 10 mL so that the concentration of the standard solution is 1; 3; 6; 9; 12; and 15 ppm. Then measured the absorbance at the maximum wavelength using a UV-Vis spectrophotometer (Wahyuni and Marpaung, 2020).

Preparation of 100 ppm broccoli flower extract mother liquor

Weighed 10 mg of broccoli flower extract and dissolved to 10 mL, then shaken until homogeneous to obtain a concentration of 1000 ppm. Then pipette 1 mL of the sample and added ethanol up to 10 mL, then shaken until homogeneous to obtain a concentration of 100 ppm (Wahyuni and Marpaung, 2020).

Preparation of broccoli flower extract solution

Pipette 0.1 mL of the mother liquor extract and then put it into a 10 mL volumetric flask. Then 96% ethanol was added to the mark to make each extract concentration of 1 ppm (Wahyuni and Marpaung, 2020).

Determination of total alkaloid content of broccoli flower extract

Take 2 mL of broccoli flower extract and then add phosphate buffer and BCG solution. Then added with chloroform and stirred using a magnetic stirrer for 10 minutes and let stand 10 minutes. Then the chloroform phase was taken and put into a 10 mL volumetric flask and then chloroform was added to the limit mark. Then the absorbance was measured at the experimental maximum wavelength (Wahyuni and Marpaung, 2020).

RESULTS AND DISCUSSION

Material collection and plant determination

The sample in this study was broccoli flower obtained from the Genikan Village area, Ngablak District, Magelang Regency, Central Java. The results of plant determinations carried out at the Plant Systematics Laboratory, Faculty of Biology, Gadjah Mada University stated that the plant sample used was actually Broccoli (*Brassica oleracea* var. *italica*).

Sample preparation

Sample preparation aims to increase the surface area of the sample, so that the maceration process will produce a maximum yield. Preparation is done by washing, drying and making sample powder.

The sample used was 3 kg of broccoli flowers. Broccoli flowers are washed with water to remove impurities that can interfere with the extraction process. The sample is then dried to remove moisture in the sample and to minimize damage due to degradation of microorganisms and prevent the growth of fungi so that it can be stored for a long time and

does not damage the chemical composition in it (Anam, 2015). The sample powder was made by blending the dry sample and then sifting it. The results obtained from this process are 352 g which are green with a yield of 11.73%.

Extraction

Sample extraction of broccoli flower was carried out using the maceration method. Maceration is done by immersing the sample powder in a solvent. The solvent will penetrate the cell wall and then enter the cell cavity containing the active substance in the cell, this results in a concentrated solution being pushed out (Anam, 2015). The selection of this maceration method was carried out on the grounds that the compounds contained in the sample were not damaged by heating. In addition, the maceration method uses simple, easy and inexpensive procedures and equipment.

In general, the solvent that is often used in extraction is ethanol. The advantages of ethanol solvent are that it is non-toxic, selective, has good absorption power, and is able to extract various active compounds. According to Harbone (1996) in Wahyuni and Marpaung (2020), ethanol is able to attract active compounds with different levels of polarity, namely polar, semipolar, and nonpolar such as flavonoids, alkaloids, tannins, anthraquinones, terpenoids, and saponins.

As a solvent ethanol has the ability to extract the active compound in the extract based on the principle like dissolves like. This principle means that a compound can be dissolved in a solvent when it has the same polarity. In addition, Wahyuni and Marpaung (2020) research explained that the highest total alkaloid content was found in 96% ethanol extract. Therefore, in this study, 96% ethanol solvent was used for maceration.

The samples were macerated as much as 100 g and soaked in 1 L of 96% ethanol. Immersion was carried out at various time variations, namely 1 day, 2 days and 3 days. The macerate was filtered using a vacuum pump rocker to separate the filtrate and residue. The filtrate that has been obtained is concentrated using a water bath at 50°C. The yield of broccoli flower ethanol extract can be seen in Table 1.

Table 1. Yield of extract ethanolic broccoli flower

Sample	Simplicia weight	Extract weight	Yield
Sample 1 (1 day maceration)	100 g	7,55 g	7,55%
Sample 2 (2 days maceration)	100 g	12,74 g	12,74%
Sample 3 (3 days maceration)	100 g	13,73 g	13,73%

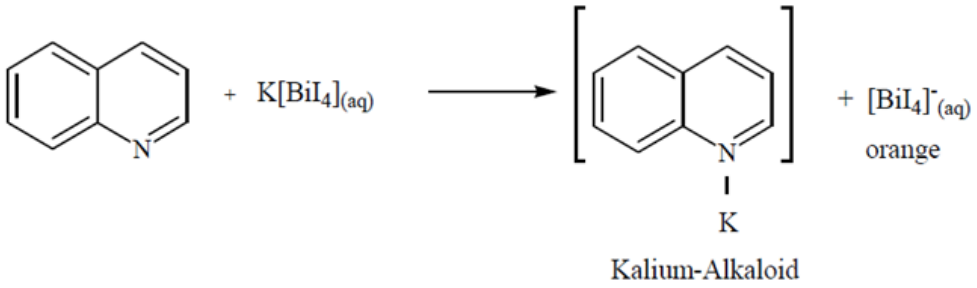
The yield results in Table 1 inform that the highest yield is in sample 3 with a maceration time of 3 days, which is 13.73%. This is in line with the research by Handrianto and Wardani (2019), that soaking for 3 days resulted in the highest extract weight when compared to soaking for 1 day or 2 days. Extracts with high yields are expected to have a high content of compounds as well. According to (Dewatisari *et al*., 2018), the yield value has a correlation with the amount of bioactive content present in the plant. In addition, according to Budiyanto (2015) states that the higher the yield of the extract, the higher the content of the substance components that are attracted to a raw material.

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is a qualitative analysis method by separating the sample components based on differences in polarity. There are two phases used in thin layer chromatography, namely the stationary phase and the mobile phase. The mobile phase will dissolve the components of a mixture of substances and the stationary phase will hold the

components of the mixture. Components that have a strong interaction with the stationary phase will be left behind, while components that are easily soluble in the mobile phase will move faster (Misfadhila *et al*., 2017). The stationary phase used in this research is silica gel 60 GF_{254} plate which is polar, so that silica gel can be used to separate substances containing polar alkaloid compounds. The mobile phase used was ethyl acetate: chloroform in a ratio of 8:2. Subsequently, the sample was spotlighted on the TLC plate and then placed in a chamber containing the mobile phase (ethyl acetate: chloroform).

Prior to the separation process, the eluent was first saturated. Saturation is done by inserting the mobile phase into the chamber and then inserting filter paper as high as the chamber. The saturation process aims to saturate the eluent in the chamber with solvent vapor, so that the elution is able to produce good and regular propagation (Nurmalasari *et al*, 2019). When the solvent starts to wet the plate, the solvent will dissolve the compounds in the sample. Then the compound will move on the plate like the movement of the solvent, then after it will form several tone spots because the sample will interact with the silica on the plate (Misfadhila, *et al*, 2017). Then to check for the presence of alkaloids, it was done by spraying Dragendorph's reagent which was marked with an orange color (Figure 1).

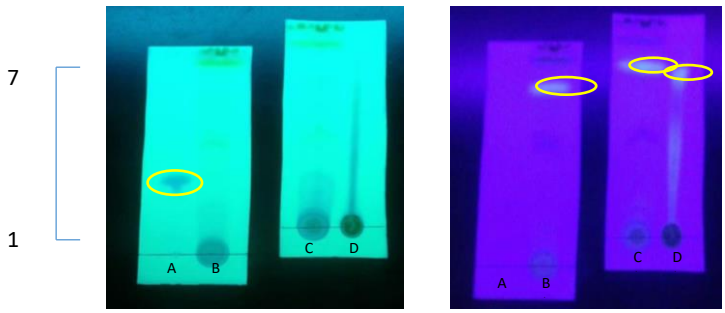


Picture 1. Reaction of Dragendrof test (Fadlila RN., 2011)

The result of spraying Dragendrof reagent, caffeine does not look orange stain. While samples 1, 2, and 3 have orange stains. These results can be seen in Figure 2.

Figure 2. Results of Spotting and Spraying with Dragendorff's Reagent.
A= Caffeine, B=Sample 1, C=Sample 2, D=Sample 3

The spot on the TLC plate were detected under UV light at 254 nm and 366 nm. Caffeine spot are visible under UV light of 254 nm while under UV light of 366 nm caffeine stains are not visible. The spots of the three samples were clearly visible under 366 nm UV light and faintly visible under 254 nm UV light as shown in Figure 3.



(A) (B)

Figure 3. Spots at UV 254 (a) and UV 366 (b)
A= caffeine, B=sample 1, C=sample 2, D= sample 3

The Rf value of caffeine and the three samples is shown in Table 2. The Rf value of caffeine produced from this study is not much different from the results of Misfadhila *et al.* (2017), which is 0.30, while in this study it is 0.34. The results of the Rf values of the three samples did not differ much, namely 0.77 in the first sample, 074 in the second sample and 0.71 in the third sample. According to (Herlianawati, 2007), the Rf value of scopolamine (tropane alkaloid) is 0.52.

Table 2. Rf value

Sample	**Rf**	
	UV 254	UV 366
Caffeine	0,34	
Sample 1		0,77
Sample 2		0,74
Sample 3		0,71

Analysis of Total Alkaloid Levels

Determination of maximum wavelength

The absorbance of the standard solution of caffeine was measured in the wavelength range of 250-290 nm using a UV-Vis Spectrophotometry instrument. Based on the measurement results, the maximum wavelength value of caffeine standard solution is 272.873 nm with an absorbance value of 0.771. This is in line with research conducted by Sari and Kuntari (2019) and Wahyuni and Marpaung (2020) that the maximum wavelength of caffeine is 273 nm.

Determination of caffeine standard curve

The standard curves is intended to produce a curve of the relationship between the absorbance and the standard concentration of caffeine. According to the Lambert-Beer law, the intensity transmitted by a solution of an adsorbent is directly proportional to the concentration of the solution. The standard concentration series of caffeine used were 1, 3, 6, 9, 12, and 15 ppm. Measurements were made using a UV-Vis spectrophotometer at the maximum wavelength of caffeine, which is 273 nm. The standard curve for caffeine is shown in Figure 4.

Figure 4 shows that the greater the concentration of the caffeine standard solution, the greater the absorbance value. Based on the standard curve, the regression equation obtained is y= 0.0505x + 0.009 where y is the absorbance and x is the standard concentration of caffeine.

The squared value of the correlation coefficient (R2) is 0.9997, which shows the relationship between extract concentration and absorbance is very strong. The value (r) close to 1 has a very strong relationship between the two variables by forming a linear curve (Winahyu *et al*., 2019).

Table 3. Caffeine Standard Absorbance

Concentration (ppm)	Absorbance
1	0.066
3	0.159
6	0.308
9	0.458
12	0.615
15	0.772

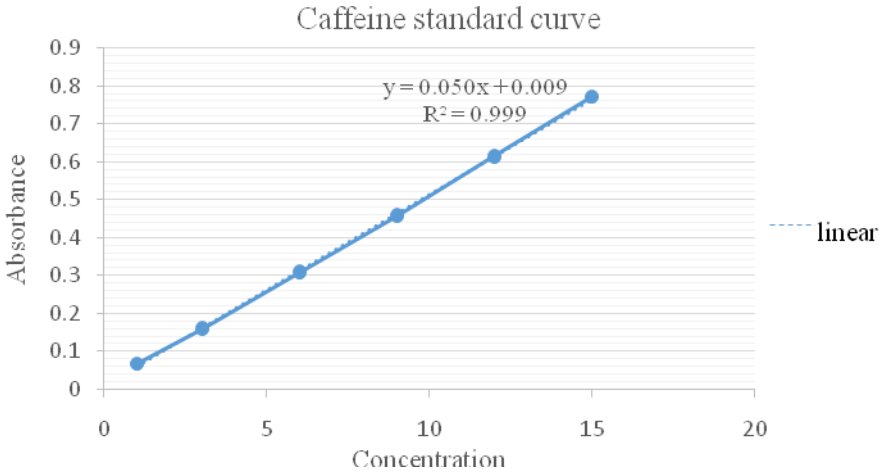


Figure 4. Caffeine standard curve

Determination of total alkaloid levels in the sample

Determination of alkaloid levels was carried out by taking a solution of broccoli flower extract and then adding a solution of phosphate buffer pH 4.7, which aims to provide optimum results when BCG reacts with alkaloids. The addition of BCG serves as a color reagent that will bind to the alkaloids to form an alkaloid-BCG ion complex.

Determination of the total alkaloid content of broccoli flowers was carried out using the UV-Vis Spectrophotometry method which resulted in the data in Table 4. Based on Table 4 it can be concluded that the highest average alkaloid content was found in sample 2 with a maceration time of 2 days, which was 17.5%.

Table 4. Total Alkaloid Content of Broccoli Flower Extract

Sample	Absorbance	Alkaloid level (ppm)	Total alkaloid level	Average level of total alkaloids
1	0.019	0,198	9,9%	9,6%
	0.019	0,198	9,9%	

	0.018	0,178	8,9%	
2	0.032	0,455	22,8%	17,5%
	0.027	0,356	17,8%	
	0.021	0,238	11,9%	
3	0.025	0,317	15,9%	16,7%
	0.026	0,337	16,9%	
	0.026	0,337	16,9%	

Statistical Data Analysis

Based on the results in Table 4, a One Way ANOVA analysis was then carried out to determine the effect of the difference in maceration time of the three samples. The conditions that must be met to perform the One Way ANOVA test are data that are normally distributed and homogeneous. If the data cannot be tested using One Way ANOVA, then the data is tested using the Kruskal-Wallis test.

The results of the Shapiro-Wilk normality test, there are data that are not normally distributed as evidenced by a significance level value less than 0.05. The data that are not normally distributed are found in sample 1 and sample 3 with a significance value of 0.000. While sample 2 is normally distributed as evidenced by a significance level value greater than 0.05. The results of the Shapiro-Wilk normality test can be seen in Table 5. Thus, the One Way ANOVA test cannot be continued and is continued with the Kruskal-Wallis test.

Table 5. Shapiro-Wilk Normality Test

Sample		Shapiro-Wilk		
		Statistic	df	Sig.
Level	Sample 1	.750	3	.000
	Sample 2	.998	3	.909
	Sample 3	.750	3	.000

The results obtained from the Kruskal Wallis test are shown in Table 6. Based on Table 6, it is known that the Asymp value. sig of 0.058 which means greater than 0.05, so that the average levels of the three samples are the same or there is no significant difference. Therefore, it can be concluded that from the three samples there was no significant difference between the maceration time and the total alkaloid content contained in the extract.

Table 6. Kruskal Wallis Test Results

	Kadar
Chi-Square	5.695
Df	2
Asymp. Sig.	.058

CONCLUSION

Based on the results of the Kruskal Wallis test, it was stated that there was no significant effect of maceration time on the total alkaloid content of broccoli flower extract. The highest average level was found in sample 2 with maceration for 2 days of 17.5%.

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