

Optimization of Microwave-Assisted Extraction of Flavonoids from Binahong (*Anredera cordifolia*) Leaves Using Response Surface Methodology

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Article info	Abstract
<p>History Submission: 08-01-2020 Review: 30-05-2020 Accepted: 26-08-2020</p> <p>*Email: zaldy.rusli@unpak.ac.id</p> <p>DOI: 10.33096/jffi.v7i3.596</p> <p>Keywords: Binahong leaf; Response Surface Method; Flavonoids; Antioxidants</p>	<p><i>Binahong (Anredera scandens (L.) Moq.) leaves are one of the plants that empirically can be used for wound healing and contain flavonoids which have antibacterial and antioxidant activities. Determination of the optimum conditions of the Binahong leaf extraction process needs to be done to ensure the extraction quality of the Binahong leaves associated with the given activity. Microwave Assisted Extraction (MAE) is an extraction that utilizes microwave radiation to heat the solvent quickly and efficiently. This study aims to determine the most effective binahong leaf extraction conditions that produce optimal levels of flavonoids and antioxidant activity. The extraction process was carried out using the Microwave assisted extraction (MAE) method. Determination of optimum conditions is done based on Response Surface Method (RSM) with variable ethanol concentration (70%, 80% and 90%), extraction time (4, 12 and 20 minutes) and power (450, 600, and 800 watts) using Box- Behnken Design (BBD) with Design Expert 7.0 software. The extract quality parameters measured were total flavonoid levels and antioxidant activity. The results showed the optimum conditions with the BBD method were obtained at an ethanol concentration of 81.49%, extraction time of 13.84 minutes, and power of 626.19 watts with flavonoid levels of 3.8561% and antioxidant activity (IC₅₀) of 95.51834 ppm with active categories.</i></p>

I. Introduction

Binahong leaves have several benefits such as reducing blood glucose levels (Sukandar et al., 2011), reducing high blood pressure (Siswantari, 2015), improving kidney function (Sukandar et al., 2010) and reducing uric acid levels (Lidinilla, 2014). The content of active compounds contained in binahong leaves are phenolic compounds, triterpenoids, alkaloids and flavonoids (Anasta et al., 2013). These compounds have antioxidant properties. Based on the results of research Parwati et al., (2014) showed that the ethanol extract of binahong leaves has a very active antioxidant activity with an IC₅₀ value of 40.27 ppm.

In order to provide the desired activity, a process is needed to separate or isolate the compounds. Extraction is the process of separating compounds from a matrix or a simplisia using an appropriate solvent. The extraction method used depends on the type, physical properties, and chemical properties of the compound to be extracted (Hanani, 2015). One of the extraction methods that can be used is the Microwave Assisted Extraction (MAE) method. MAE is an extraction technique using microwave energy. The advantages of using the MAE method include helping to increase the

amount of crude extract yield in a short extraction time and the amount of solvent that is lower than conventional extraction methods (Nawrot et al., 2012). The results of the study of Dewi et al., (2018) stated that the value of *Pleurotus ostreatus* extract flavonoids obtained with MAE was twice as high as in maceration and waterbath heating.

Some factors that influence extraction with MAE are the type of solvent, time and microwave power. Based on optimization research Lu et al., (2013) on *Cryptotaenia japonica* Hassk using ethanol concentrations (70%, 80%, 90%) obtained the optimum concentration at a concentration of 70% with the obtained flavonoid levels of 27.01 mg / g or 2,701%. Research on barley leaves using extraction time before the optimization experiment that is 4 minutes, 12 minutes and 20 minutes using MAE obtained optimum time at 11.12 minutes, the results of this flavonoid extraction increased by 5.47% (Gao et al., 2016). Based on research Handayani et al., (2014) using 90-900 watts of power shows that the optimal power obtained at 450 watts with a flavonoid content of 1.04 %. The three variables need to be optimized so that the extraction process can run efficiently and produce extracts that have maximum flavonoid levels and antioxidant



activity. One way to determine optimal data is Response Surface Methodology (RSM) This study uses the Box-Behnken Design (BBD) design method of RSM by using 3 variables including ethanol concentration, extraction time, and power.

Based on the description above, an optimization of the MAE binahong leaf method has been carried out with a total flavonoid response using Response Surface Methodology (RSM) data with parameters namely ethanol solvent concentration (70%, 80%, 90%), extraction time (4, 12, 20 minutes) and power (450 watts, 600 watts and 800 watts). The best optimization results of total flavonoid levels of binahong leaves were continued for the determination of antioxidant activity.

II. Research Method

II.1 Materials

Binahong leaves used are all leaf parts obtained from the Ciapus region, Bogor, aluminum chloride, aquadest, Iron (III) chloride, Bouchardat LP, Dragendrof LP, ethanol 70%, ethanol 80%, ethanol 90%, ethanol 90% (Sigma®), Mayer LP, Na-acetate (Emsure®), DPPH powder (Sigma®), Zn powder, Mg powder, vitamin C (Emsure®).

II.2 Instruments

The tools used in this study include glassware, mesh 40 sieves, grinders (Philips®), Microwave (Samsung®), Oven (Mettler®), UV-Visible Spectrophotometry (V-730®), Analytical balance (AND®).

II.3 Plant Determination

Binahong leaves were determined at Herbarium Bogoriense, Biology Research Center of the Indonesian Institute of Natural Sciences (LIPI), Cibinong, Bogor.

II.4 Simplisia

A total of 15 kg of binahong leaves were sorted wet and the best leaves were selected, then washed using running water and drained. Leaves that have been drained, dried by roasting at 40°C, then sorted again for parts that cannot be cleaned during the previous sorting, grinded and sieved with a mesh 40 sieve to obtain a uniform size. Simplisia binahong leaves powder is stored in a tightly closed container. The yield of simplisia is calculated using the formula:

$$\text{Yield (\%)} = \frac{\text{Final Weight}}{\text{Initial Weight}} \times 100\% \quad (1)$$

II.5 Simplisia Characterization

1. Determination of Water Content

Determination of water content is done using the gravimetric method by entering a sample of 2 g into a cup that has been tapped. Dried at 105°C for 5 hours then weighed. Continue drying and weigh every 1 hour until the difference between 2 consecutive weighing is not more than 0.25% (DepKes RI, 2015).

Water Content (%)

$$= \frac{W_1 - W_2}{\text{Weight simplisia}} \times 100\% \quad (2)$$

W_1 = Cup Weight before heating; W_2 = Cup Weight + contents after heating.

2. Determination of Ash Content

Determination of ash content is done using the gravimetric method by entering a sample of 2 g into a cup. Dried at 105 °C for 5 hours then weighed. Continue drying and weigh every 1 hour until the difference between 2 consecutive weighing is not more than 0.25% (DepKes RI, 2015).

Ash Content (%)

$$= \frac{W_{\text{Crucible+ash}} - W_{\text{Crucible empty}}}{\text{Weight of simplisia}} \times 100\% \quad (3)$$

3. Phytochemical Screening

Alkaloids

0.5 g of sample was added 1 mL of HCl 2 N and 9 mL of distilled water, heated on a water bath for 2 minutes, cooled and filtered. Then tested with 3 alcohol reagents namely bouchardat reagents, dragendrof and major reagents. Positive test results are obtained when red brown colour precipitation are formed in bouchardate reagents, red to orange colour precipitation in dragendrof reagents and yellowish white colour precipitation in major reagents (Hanani, 2015).

Flavonoids

0.5 g of sample was dissolved in 5 mL 96% ethanol. The sample solution was taken 2 mL, added a little 0.1 g of Mg powder and added 10 drops of concentrated HCl from the side of the tube and shaken slowly. The red or orange color formed indicates the presence of flavonoids, if there is an orange-yellow color indicating the presence of flavones, chalcon and auron (Hanani, 2015).

Saponin

A total of 0.5 g of sample is put into a test tube, 10 mL of hot water is added, cooled, and shaken vigorously for 10 seconds. Positive results are characterized by the formation of froth to stabilize for less than 1 minute (Hanani, 2015).

Tannin

0.2 g of sample was dissolved in 2 mL of distilled water in a test tube. Two or three drops of 1% FeCl₃ solution are added to the solution. If it is formed a positive blue-green color containing tannin (Catechin tannin). Whereas if positive blue-black formed contains tannins (Galic tannins) (Hanani, 2015).

II.6 Analysis of Total Flavonoids

1. Reagents

Na Acetate Solution 1 M

Carefully weighed 8.2 grams of sodium acetate, then dissolved with distilled water to mark the boundary in a 100 mL volumetric flask, then stir until homogeneous.

Aluminum Chloride 10%

Weighed carefully 10 grams of aluminum chloride, then dissolved with sodium acetate until dissolved, then added distilled water quantitatively to the 100 mL volumetric flask.

Blank Solution

In a 25 mL volumetric flask, pipette 2.5 mL 10% aluminum chloride, then added 2.5 mL of sodium acetate 1 M and added 15 mL of ethanol p.a, then distilled water was added quantitatively.

Standard solution of Quersetin (100 ppm)

Put in a 100 mL measure of 100 mg quersetin which has been weighed carefully, then dissolved with ethanol p.a quantitatively, then homogenized (1000 ppm). To get a 100 ppm quersetin standard solution, it is done by using a 10 mL pipette of 100 ppm standard solution, put in a 100 mL volumetric flask and then dissolved with ethanol quantitatively (100 ppm).

2. Determination of Maximum Wavelength

As much as 5 mL of standard solution of quersetin in a concentration of 100 ppm was put into a 50 mL volumetric flask, 15 mL of ethanol p.a were added, then 1 mL of 10% aluminum chloride was added, 1 mL of sodium acetate 1 M and distilled water to the mark. Homogeneously shaken and then allowed to stand for 30 minutes, measured absorbance at a wavelength of 380-780 nm using a UV-Vis spectrophotometer.

3. Determination of Optimum Incubation Time

A total of 5 mL of 100 ppm quersetin standard solution was put into a 50 mL volumetric

flask, 15 mL of ethanol p.a were added, then 1 mL of 10% aluminum chloride was added, 1 mL of sodium acetate 1 M and distilled water to the mark. Shaken homogeneously and incubated in room rates. Absorption is measured at maximum wavelengths at 5, 10, 15, 20, 25, 30, 40 and 45 minutes so that a stable optimum time is obtained.

4. Calibration Curve

The standard series quersetin 2, 4, 6, 8, and 10 ppm are made from 100 ppm solution of 1, 2, 3, 4, 5 mL of 100 ppm standard solution pipetted into a 50 mL volumetric flask. Then added 15 mL of p.a ethanol, then added 1 mL of 10% aluminum chloride, 1 mL of 1 M sodium acetate and diluted with distilled water quantitatively. Shaken homogeneously then left for 30 minutes, absorbance measured at maximum wavelength. A standard series calibration curve is created by plotting between the concentration (x-axis) and absorbance (y-axis), then looking for a standard series regression equation.

5. Determination of Total Flavonoids

A total of 50 mg of ethanol extract of binahong leaves was weighed, then dissolved with ethanol up to 50 mL. Pipette as much as 10 mL of the extract solution into a 50 mL volumetric flask and then add 15 mL of ethanol p.a, add 1 mL of 10% aluminum chloride, 1 mL of sodium acetate 1 M and distilled water to the mark mark. Shaken homogeneously then allowed to stand for optimum time, then absorption is measured by UV-Vis spectrophotometry at maximum wavelength. The resulting absorbance is entered into the quersetin standard series regression equation. Then flavonoid levels are calculated using the formula:

$$\begin{aligned} & \% \text{ Total Flavonoids (w/w)} \\ &= \frac{c \text{ (ppm)} \times \text{volume (mL)} \times \text{fp} \times 10^{-6}}{\text{weight of simplisia} - (\text{weight of simplisia} \times \text{water content})} \times 100\% \end{aligned} \quad (4)$$

II.7 Optimization of Binahong Leaves Extraction

1. Extraction Process

Binahong leaf extraction is carried out by microwave extraction by weighing 30 g of simplicia powder then put into an erlenmeyer and adding 300 mL of solvent (1:10). The parameters to be used are ethanol solvent concentration (60%, 70%, 80%), extraction time (4, 12, 20 minutes) and power (300, 450, 600 Watt). This parameter is first inputted into the RSM data using Expert 7.0 Design software with BBD design so that 17 data will be obtained (Table 1). Then the sample is extracted following the parameter data obtained. Samples are irradiated in a microwave oven at regular intervals (radiation 1 minute and 2 minutes turned off). The extraction product is left at room temperature then filtered, the filtrate is evaporated using a rotary evaporator and then thickened. Total flavonoid levels from the extract were measured.

2. Data Analysis

Results (total flavonoid levels) were analyzed using Design Expert 7.0 software. After obtaining the optimum data, an antioxidant activity test is performed.

Table 1. Design experiment of optimization

No. Experiment	Ethanol Concentration (%)	Time (minutes)	Power (watts)
1	70	12	450
2	90	12	450
3	80	4	450
4	80	20	450
5	70	4	600
6	90	4	600
7	70	20	600
8	90	20	600
9	80	12	600
10	80	12	600
11	80	12	600
12	80	12	600
13	80	12	600
14	70	12	800
15	90	12	800
16	80	4	800
17	80	20	800

II.8 Antioxidant Activity Test

1. Making reagent solutions

Preparation of reagent solutions, including:

DPPH 1 mM Solution

DPPH powder weighed as much as 39,432 mg, then put into a 100 mL volumetric flask, then dissolved with methanol p.a to the boundary markers (previously the volumetric flask was covered with aluminum foil).

Blank Solution.

1 mL of DPPH 1 mM solution is taken, methanol p.a to 10 mL is added, then homogenized. The blank solution was incubated at around 25-30°C (room temperature) for 30 minutes (measuring flask wrapped in aluminum foil).

Preparation of a 1000 ppm Vitamin C Standard Solution

A total of 100 mg of ascorbic acid was weighed and then put into a 100 mL volumetric flask, then dissolved with methanol p.a to the quantitatively (1000 ppm).

2. Determination of Maximum Wavelength

1 mL of DPPH 1 mM latency was then diluted to the limit with methanol p.a in a 10 mL volumetric flask and incubated at room temperature for 30 minutes. After that the absorption is measured at a wavelength of 500-530 nm (previously the measuring flask was coated with aluminum foil).

3. Determination of Optimum Incubation Time

A total of 1 mL of 1000 ppm vitamin C standard solution is then diluted with methanol p.a to a 10 mL volumetric flask, then homogenized. Added 1 mL of 1 mM DPPH solution and then allowed to stand for optimum time at room temperature. Absorption is measured at the maximum wavelength and measured at 10, 20, 30,

40, 50, and 60 minutes so that the optimum absorption time is stable (previously the measuring flask was coated with aluminum foil).

4. Preparation of test solutions series

Binahong leaf extract obtained was weighed 50 mg and then put into a 50 mL volumetric flask, which had been coated with aluminum foil and dissolved in methanol p.a until the boundary markers were then homogenized (1000 ppm). Test solution series 20, 40, 60, 80, and 100 ppm was made by transfer 0.2, 0.4, 0.6, 0.8 and 1.0 of 1000 ppm vitamin C standar solutin into a 10 mL volumetric flask then added 1 mL of 1 mM DPPH 1mM solution in which all parts of the measuring flask were covered with aluminum foil and diluted with methanol pa to the mark limit, then homogenized. Then it is left in the dark at room temperature for the optimum incubation time.

5. Determination of Antioxidants by DPPH Method

The test solution series, the standar solution series (Vitamin C) and the blank solution are measured at the maximum wavelength determined by a spectrophotometer. Percentage value of resistance to DPPH is calculated by the following formula:

% Inhibition Concentration (IC)

$$= \frac{\text{blank absorption} - \text{sample absorption}}{\text{blank absorption}} \times 100\% \quad (5)$$

A standard series calibration curve is created by plotting between the concentration (x-axis) and IC (y), then the standard series regression equation and test series are searched. IC₅₀ value with the concentration of inhibition center (50%) with the

equation $y = bx + a$, where $y = 50$ and x is the concentration of the test solution that is able to inhibit 50% of free radical solution 1,1-diphenyl-2-picrylhydrazil.

III. Result and Discussion

III.1 Plant Determination

The leaves of binahong (*Anredera cordifolia*) used in this study were all leaf parts obtained from the Ciapus area, Bogor. Determination was carried out at the Herbarium Bogoriense Research Center for Biological Sciences Natural Sciences Indonesia (LIPI), Cibinong, Bogor. The results of the determination state that the sample used in this study is *Anredera cordifolia* (Ten.) Steenis of the *Basellaceae*.



Figure 1. Simplisia Binahong Leaf Powder

The result of the determination of the water content of Binahong leaf simplicia was 8.3604%, according to the Depkes RI (2008) the condition for the water content of simplicia was $\leq 10\%$. These results indicate that binahong leaf simplicia has met the requirements. The ethanol extract of binahong leaves obtained water content in the range of 4% - 6%. The results of the determination of ashah simplicia ash content of the leaves are 15.3042 %, according to (Kemenkes RI, 2011) the condition of the binahong leaf simplicia ash content is no more than 16.3 %, these results indicate that binahong simplicia leaves have met the requirements. The phytochemical screening is carried out to determine the content of secondary metabolites contained in plants. Phytochemical test results can be seen in Table 2.

Table 2. Phytochemical test results

Phytochemical Screening	Results
Alkaloid	+
Flavonoid	+
Tanin	+
Saponin	+

Based on phytochemical tests that have been done, alkaloid compounds were detected in

III.2 Simplisia Characterization

Simplisia powder (Figure 1) was obtained as much as 1,100 g of 15 kg of binahong leaves, so that a rendement of 7.33% was obtained. These results have met the requirements according to the Kemenkes RI (2011) which states that the yield of thick binahong leaf extract is not less than 11.91%. Sudirman (2011) said that the higher the rendement value indicates that the raw material has greater opportunity to be utilized.

powder simplicia and ethanol extract of binahong leaves. Analysis of the alkaloid group used three reagents namely Boucharlat LP, Dragendorff LP and Mayer LP, with positive results each of which formed brown colour precipitation, white potassium-alkaloid deposits and orange colour precipitation, this occurs because of the content of bismuth nitrate added to HCl to prevent hydrolysis with alkaloids (Harborne et al., 2006). In the research of Hanani et al., (2005) showed that alkaloids have antioxidant properties. Alkaloid compounds, especially indols, have the ability to stop free radical chain reactions efficiently (Yuhernita & Juniarti, 2011).

Flavonoid testing showed positive results on powder simplicia and extract of binahong leaves with the formation of red color. This is due to the reduction of flavonoids with Mg and Zn producing red or orange complexes (Depkes RI, 2008). Flavonoid compounds are often known for their benefits as antioxidants The ability of antioxidants in the flavonod itself is in the hydroxyl group of phenols that are able to capture free radicals directly through the contribution of hydrogen atoms (B. Arifin & Ibrahim, 2018).

In tannin testing showed positive results with the formation of blue-green deposits when

added FeCl_3 , this is because tannins will form complex compounds with Fe^{3+} ions. Tannins are active secondary metabolites which are known to have antioxidant properties (Malangngi et al., 2012). Phenolate compounds in tannins that work as antioxidants occur through terminating radical chain reactions and donating hydrogen atoms to produce more stable free radicals (Nimse & Pal, 2015).

The saponin test showed positive results which were marked by froth after shaking for 10 seconds and survived within 1 minute, this is because the saponins were colloids which were soluble in water and would foam after being shaken (Mien et al., 2015). In the research of Ahmad et al. (2012) said that saponin has potential as an antioxidant, where saponin is able to reduce superoxide through the formation of hydroperoxide intermediates so as to prevent biomolecular damage by free radicals.

III.3 Analysis of Total Flavonoid Levels

Determination of flavonoid content of binahong leaf extract using the colorimetric method with AlCl_3 reagent which has the principle of complex formation, so that the wavelength shifts towards visible (Anwar and Triyasmono, 2016). Measurement of maximum wavelength absorption is carried out in the range 380-780 nm. In this study the wavelength obtained is 430 nm. Measurement of the maximum wavelength aims to determine the value of the wavelength that has a maximum absorption value. A stable absorbance value obtained from the maximum wavelength occurs in the 30 minute. This shows that the reaction was complete at the 30th minute. Determination of the optimum incubation time is done to find out the time needed by a substance in order to react optimally so that a stable absorption value is obtained. From the results of the calibration curve, the equation $y = 0.0773x + (-0.0255)$ with a correlation coefficient (r) of 0.999. A value of r close to 1 indicates that the calibration curve obtained is linear.

III.4 Optimization of Binahong Leaf Extraction

$$Y = 3.79 + 0.22 A + 0.46 B + 0.001 C + 0.11 AB - 0.11 AC + 0.043 BC - 0.84 A^2 - 1.01 B^2 - 0.56 C^2 \quad (6)$$

Information : Y = levels of flavonoids; A = As ethanol concentration (%); B = As microwave extraction time (minutes); C = As power (watts); AB = As the effect of ethanol concentration and time; AC = As the effect of ethanol concentration and power; BC = As the effect of extraction time and power; A^2 = As the ethanol concentration is increased twice (%); B^2 = As the microwave extraction time is increased twice (minutes); C^2 = As power is increased twice (watts)

Based on equation (6) it is known that a positive effect of ethanol concentration (A). time (B). power (C), AB and BC indicates an increase of each factor increased the flavonoid levels. While the negative effect of AC, A^2 , B^2 and C^2 indicate a

The results of optimization of binahong leaf extraction (Table 3) were carried out by plotting the absorbance into the calibration curve.

Optimization results indicate that there are differences in the concentration of ethanol solvent, extraction time and power to flavonoid levels. The highest levels of flavonoids obtained at 3.8543% at 80% concentration, this is due to the nature of polarity to attract a compound, as well as polar flavonoids. While at a concentration of 90% the flavonoid levels have decreased, this occurs because at that concentration the polarity has decreased so that the flavonoids attracted to the solvent are smaller. Luginda et al., (2018) said that variations in ethanol concentration affect the level of flavonoids, the higher the concentration of ethanol used in extracts of beluntas leaves, the smaller the levels of flavonoids produced.

At the time of extraction, the highest flavonoid levels are at 12 minutes, this is because at 4-12 minutes the flavonoid levels have increased and at more than 20 minutes a decrease in flavonoid levels due to the influence of heat on the microwave associated with the power used, resulting in the loss of compounds contained. In the study of Gao et al., (2016) showed that flavonoid extraction appeared to be reduced when it exceeded 12 minutes or under 4 minutes. Budiyanto and Yulianingsih (2008) states that the right extraction time will produce optimal compounds, extraction time that exceeds the optimal time will cause hydrolyzed extracts, so that it will reduce the levels of extracted compounds.

At 450 watts of power, 600 watts and 800 watts is obtained optimal power at 600 watts with the highest levels of flavonoids, and has decreased at 800 watts of power. According to the research of Chen et al., (2008) said that the higher the power will produce higher heat resulting in the compound that is drawn can be damaged, causing the levels of flavonoids obtained will decrease. The results of data analysis of the ethanol concentration. extraction time and power to flavonoid levels using Design Expert 7.0 software obtained the following equation:

decrease of the factors lowered the flavonoid levels. Based on ANOVA data in Table 4. it can be seen that the value of desirability obtained is 0.996 or 99.6% which explains that 99.6% of the influential factors come from the independent variables while the rest are other factors. for the value of the coefficient of determination (R^2) obtained at 0.9565 which is almost close to 1 which shows that the model of flavonoid levels can be accepted. while the value of p-value lack of fit obtained by $0.0014 < 0.05$ which indicates that the second order equation cannot explain the phenomenon of increased levels of flavonoids this can be caused by the predicted value of flavonoid levels obtained is not much different only increased by 0.0018% of the results of

the study . Data analysis in this study uses RSM which is an effective way to find optimum conditions by looking at the response system when the level of these factors is involved (Harvey, 2000). The results of the optimum conditions obtained at RSM (Figure 2) show that the higher the ethanol concentration, the lower the flavonoid levels. this is due to differences in polarity to attract polar flavonoid compounds such as the principle of like dissolve like that polar compounds will dissolve easily in polar solvents. whereas nonpolar compounds will dissolve easily in nonpolar solvents. Similarly, the results of the extraction time and the longer the power level of flavonoids decreases due to the heat generated by microwaves at the time of extraction associated with the length of extraction time. so that it can cause damage to compounds

contained in plants and result in decreased levels of flavonoids.

RSM predict the optimization conditions can be obtained at a concentration of 81.49%, extraction time of 13.84 minutes and 626.19 watts of power with the results of optimal flavonoid levels of 3.8561%. While the results of research conducted the highest levels of flavonoids obtained by 3.8543% at 80% concentration. 12 minutes and 600 watts of power. These results indicate that the value of flavonoid levels in the study results is not much different from the results of the analysis at RSM.

Table 3. Results of determination of flavonoid levels

Concentration ethanol (%)	Time (minutes)	Power (watt)	Flavonoid levels (%)	Prediction (%)	Difference
70	12	450	1.9716	2.0512	-0.0796
90	12	450	2.8047	2.7142	0.0905
80	4	450	1.5193	1.8004	-0.2811
80	20	450	2.896	2.6256	0.2704
70	4	600	1.6652	1.3459	0.3193
90	4	600	1.7602	1.5933	0.1669
70	20	600	1.8557	2.0225	-0.1668
90	20	600	2.4019	2.7175	-0.3156
80	12	600	3.7299	3.7276	0.0023
80	12	600	3.7099	3.6741	0.0358
80	12	600	3.8543	3.8391	0.0152
80	12	600	3.7655	3.7401	0.0254
80	12	600	3.8195	3.7758	0.0437
70	12	800	2.21	2.6363	-0.4263
90	12	800	2.5491	2.9537	-0.4046
80	4	800	1.5157	1.8031	-0.2874
80	20	800	2.9257	3.0331	-0.1074

Table 4. results of determination of flavonoid levels

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	11,67	9	1,30	17,12	0,0006	Significant
A-kons	0,38	1	0,38	5,00	0,0604	
B-waktu	1,64	1	1,64	21,69	0,0023	
C-daya	$9,901 \times 10^{-6}$	1	$9,901 \times 10^{-6}$	$1,308 \times 10^{-4}$	0,9912	
AB	0,051	1	0,051	0,67	0,4393	
AC	0,051	1	0,051	0,68	0,4368	
BC	$7,399 \times 10^{-3}$	1	$7,399 \times 10^{-3}$	0,098	0,7637	
A ²	2,99	1	2,99	39,49	0,0004	
B ²	4,32	1	4,32	56,99	0,0001	
C ²	1,26	1	1,26	16,61	0,0047	
Residual	0,53	7	0,076			
Lack of Fit	0,52	3	0,17	46,98	0,0014	Significant
Pure error	0,015	4	$3,657 \times 10^{-3}$			
Cor total	12,20	16				

Flavonoid levels

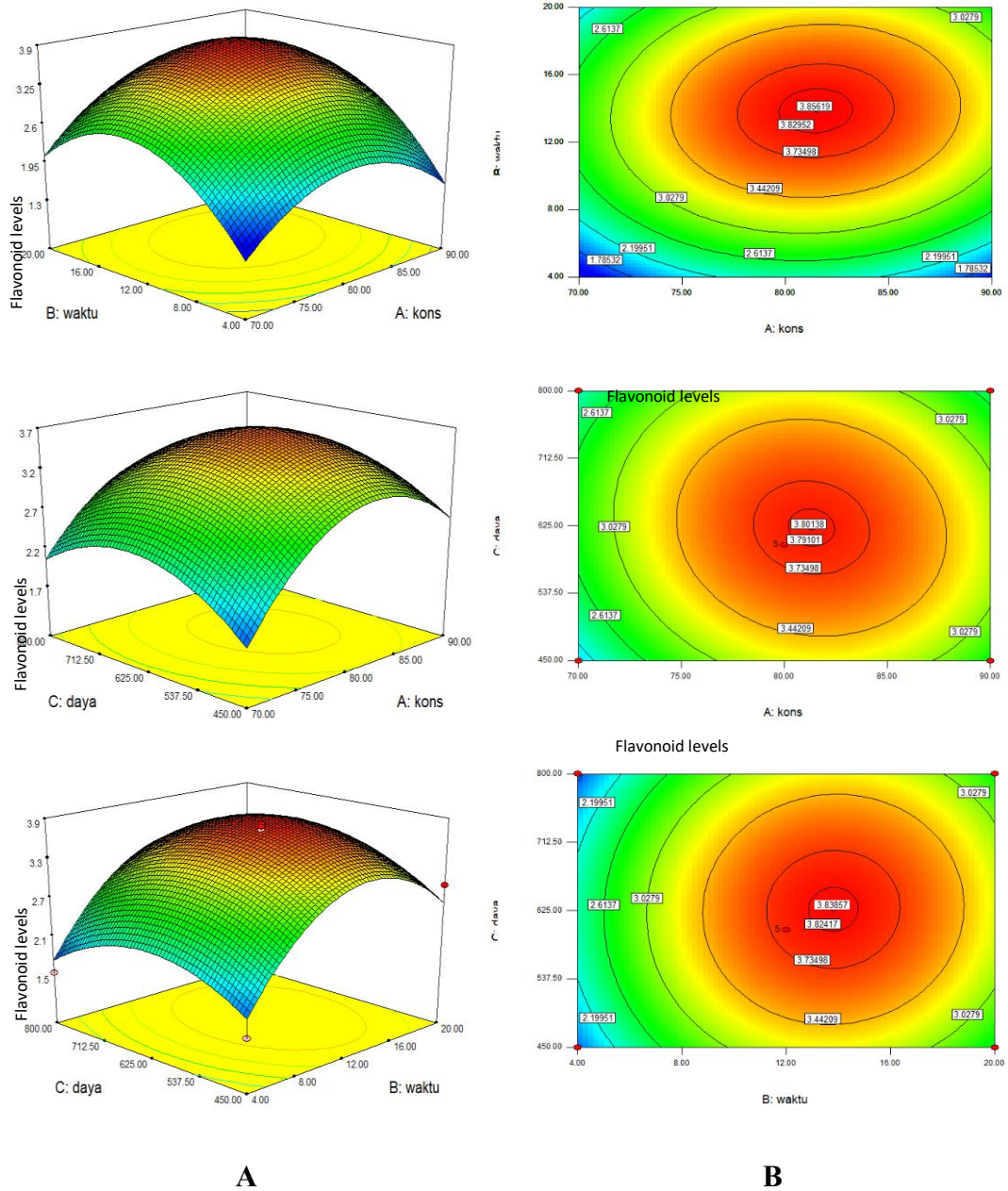


Figure 2. (A) Surface Plot and (B) Contour Plot of Flavonoid levels

III.5 Antioxidant Activity Test

The measurement of antioxidant activity in binahong leaves extract was based on the optimum conditions of flavonoid levels, namely the extract with a concentration of 80%, 12 minutes and 600 watts of power. This measurement is carried out at a wavelength of 515 nm, which is the maximum wavelength of DPPH. As a comparison, vitamin C is used in standard curve measurements and incubation time because vitamin C is a powerful natural antioxidant. The incubation time obtained is at the 30 minute. Whereas the results of calculation of%

vitamin C inhibition obtained linear regression equation $y = 9.14287x + 3.55265$ with an r value of 0.995, so an IC_{50} value of 5.0802 ppm was obtained with the very active category. The results of the antioxidant activity test for vitamin C can be seen in Table 5.

Table 5. Antioxidant activity test for vitamin c and binahong extract

Vitamin C Antioxidant Activity Test		
Concentration	% inhibition	IC ₅₀
Blanks		
2 ppm	18.6583 %	
4 ppm	44.1945 %	5.0802 ppm
6 ppm	59.4098 %	
8 ppm	75.6052 %	
10 ppm	94.5815 %	
Binahong Extract Antioxidant Activity Test		
Concentration	% inhibition	IC ₅₀
Blanks		
20 ppm	15.4900 %	
40 ppm	23.5816 %	95.5183 ppm
60 ppm	31.3024 %	
80 ppm	41.9568 %	
100 ppm	53.6267 %	

In the measurement results of the antioxidant activity of binahong leaves extract obtained a linear regression equation $y = 0.4732x + 4.7969$ with an r value of 0.995. IC₅₀ value obtained in binahong leaf extract was based on calculations of 95.51834 ppm with active categories. The presence of antioxidant activity from the sample causes a change in color in the DPPH solution in ethanol which was originally purple to yellow. The absorbance value obtained in binahong leaf extract decreases with increasing concentration. This is due to the reduction of DPPH radicals by antioxidants, where the higher the concentration of binahong leaf extract, the more particles of antioxidant compounds contained will increase the antioxidant activity and cause its absorbance to decrease (Molyneux, 2003). Binahong leaves extract activity test results can be seen in Table 5.

The ability of binahong leaf extract as an antioxidant is related to the flavonoid compounds it contains. Flavonoids act as antioxidants because they have hydroxyl groups that can donate hydrogen atoms to free radical compounds and stabilize reactive oxygen (Rezaeizadeh et al., 2011). The flavonoid content in binahong leaves plays a role in the healing of peptic ulcers, where the content of flavonoids is responsible through anti-inflammatory mechanisms (Arifin et al., 2014).

IV. Conclusion

Based on the research conducted it can be concluded that Ethanol concentration of 81.49%, extraction time of 13.84 minutes and 626.19 watts of power obtained the optimum flavonoid content of binahong leaves at 3.8561%. The antioxidant activity of binahong leaves based on the optimum conditions of flavonoid levels obtained IC₅₀ values was 95.51834 ppm with active categories.

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