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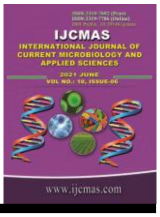

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Optimization of Dielectric Constant and Ratio Material to Solvent using Response Surface Methodology on Antioxidant Activity Teter Leaves Extract (*Solanum erianthum*)

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ABSTRACT

Keywords

Optimization, Teter leaf, Extraction, Antioxidant and RSM

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This research aimed at getting the optimum solvent dielectric constant and ratio teter leaves to solvents on the highest antioxidant activity of teter leaves extract. Response Surface Methodology (RSM) was used for the optimization of extraction conditions with the experimental design was a Central Composite Design (CCD) in two factors, namely solvent dielectric constant and ratio of teter leaves to solvent. The results showed that the optimum treatment was solvent dielectric constant value 40.74 and the ratio of teter leaves to solvent 1:10.19 to produced teter leaves extract which had the highest antioxidant activity was 84.34% with IC50 was 161.988 ppm, extract yield was 17.41%, total phenolic content was 175.151 mg GAE/g extract, total flavonoid content was 82.60 mg QE/g extract, total tannin was 12.30 mg TAE/g extract and vitamin C was 82.30 mg AAE/g extract.

Introduction

Solanum erianthum or Teter is a plant that is classified into the Solanaceae family. This plant grows up in dry or damp areas thus they are easy to be cultivated. Teter plant has a lot of potentials which are useful for the health sector. Its leaf is one of the plant's parts that can be utilized. Modise and Mogotsi (2008) state that teter leaves' stew usually is used as diuretic medicine, to heal malaria, leprosy, venereal disease, and also used to stimulate liver function. Additionaly, Essien *et al.,* (2012) also report that teter leaves are useful for traditional medicine especially to treat various skin and gastric problems because it contains essential oil. Priyadharsini and Sujatha (2013) add another fact that teter leaves can act as antioxidants because of their bioactive components, such as flavonoids, phenol, tannins, and vitamin C. The bioactive component such as flavonoids in teter leaves has a powerful ability as electron donors, can react with free radicals to be converted into more stable compounds ending the radical chain reactions and as a chemopreventative agent (Asolu *et al.*, 2010).

The uptake of the bioactive component from teter leaves can be done by extraction. Maceration is used as one of the extraction methods. The advantage of the maceration method is its low cost. The method is also simple in which it is done without a heating process thus it will not damage the bioactive components (Mukhriani, 2014). The extraction process is influenced by many factors such as dielectric constant, the ratio of materials and their solvent, type of solvent, time, temperature, and particle size (Chew *et al.*, 2011). One important factor in the extraction process is the dielectric constant of its solvent. The solvent in the dielectric constant is very closely related to the polarity of the solvent. Each material needs different solvent polarity thus it also needs different solvent dielectric. Solvent polarity can be seen by one of which the values of solvent dielectric constant are used. Moreover, the materials and the solvent ratio are some of the factors that can influence the extraction process. This is because the greater the volume of solvent used, the higher the ability to dissolve the material (Handayani *et al.*, 2016). However, each material needs material comparison with a different solvent.

The optimization process in this research used Response Surface Methodology (RSM). RSM is a collection of mathematical and statistical techniques used for modeling and analysis of problems in response which is influenced by several variables and aims to obtain response optimization (Montgomery, 2001). Central Composite Design (CCD) second-order fit is widely used. Generally, CCD has 2^k factorials with a lot of data (n_f), axis (2^k), and center (n_c). CCD is significantly efficient to the second-order fit. The two parameters in the specific design are the axis distance of α run from the design center and the number of center points n_c (Montgomery, 2001). The research about the teter leaves extraction process to get the highest antioxidant activities has not been widely carried out. Thus, another research about the optimization of solvent dielectric constant and the ratio between the materials and solvent using RSM to get the highest level of teter leaves to extract with antioxidant is urgently needed.

Materials and Methods

The present research was conducted in the Food Processing Laboratory and Food Analysis Laboratory, Faculty of Agricultural Technology of Udayana University, and Agricultural Analysis Laboratory, Faculty of Agriculture of Warmadewa University. The research was carried out from September 2020 until February 2021.

The materials used in this research were teter leaves with such criteria: dark green leaves on the third to the ninth leave from the tip of the leave obtained from Banjar Taro Kelod, Desa Taro, Gianyar, Bali. The sample was taken in the morning to avoid over respiration. The chemical used in this research were technical ethanol solvent 96% (Merck, Germany), DPPH pro analysis (Sigma-Aldrich, USA), reagen folin-ciocalteu (Merck, Germany), sodium carbonate (Merck, Germany), Concentrated HCl (Merck, Germany), $NaNO_2$ 5% (Merck, Germany), AlCl3 10% (Phyfo Technology Laboratories, USA), gallic acid (Sigma-Aldrich, USA), quercetin (Sigma-Aldrich, USA), Na_2CO_3 (Merck, Germany), folinedenis reagent (Merck, Germany), and ammonium molybdate (Merck, Germany).

The tools used in this research were sieve 60 mesh (ABM, Indonesia), oven (Blue M, USA), shaker (H-M-SR, Swiss), analytical scales (Shimadzu, Jepang), micropipette (Dragon Lab, Indonesia), spectrophotometer (Biochromsn 133467, UK), test tube (Pyrex¸

USA), rotary vacuum evaporator (Butchi Rotavapor R-300, Switzerland), and fabric.

The obtained teter leaves then were washed with clean water and wiped using a clean fabric. The leaves then were dried using the oven at a temperature of 40 ° C ± 5 ° C for 24 hours. After that, the dried teter leaves were smoothed using a blender, then sieved using sieve 60 mesh thus modified teter leaf powder was obtained (Kemit *et al.,* 2019). After the leaf powder was obtained, the process then was continued by the extraction process.

The extraction process was carried out after obtaining the teter leaves powder. The making process of teter leaves extract was conducted by using the maceration method. The treatment carried out in this process was solvent dielectric constant and the ratio between materials and solvent. The teter leaves powder was taken; each 10 g for every sample, dissolved using ethanol dielectricum constant solvent and the ratio with materials and solvent-based on the treatment condition obtained through model CCD using RSM supported by Minitab 19 software according to Table 1.

The solution was put into erlenmeyer (all sides of erlemeyer were wrapped in aluminum foil), then was shaken for 48 hours with the help of a shaker with 100 rpm speed on the room temperature. The solution was filtered using paper Whatman No.1 supported by a vacuum pump. The obtained filtrate was evaporated using a rotary vacuum evaporator at the temperature of ± 45^{o}C, 90 rpm, 95 mbar with the vapor temperature 23°C. The evaporation process was considered finished when the whole solvent was evaporated thus the modified ethanol teter leaves were gained (Kemit *et al.,* 2019). The extract was then analyzed yield extract, total phenolics contents, total flavonoids contents, total tannins contents, vitamin C, antioxidant leaves, and IC$_{50}$. The observation variables done in the research were extracted yield (AOAC, 1990), total phenolics contents by the Folin – Ciocalteau method (Sakanaka *et al.,* 2005), total flavonoidscontents by method AlCl3 (Singh *et al.,* 2012), total tanninscontents using the Folin-Denis method (Suhardi, 1997), vitamin C using a spectrophotometer (Vuong *et al.,* 2014) and antioxidant activities with DPPH method (Shah dan Modi, 2015).

The data analysis was carried out using Minitab 19 software. The research was carried out with the extraction optimization process calculation of the teter leaves towards the influence of solvent dielectric constant with the ratio materialsto solvent using RSM with the coherence model of CCD using a two-order equation:

$$Y = \beta o + \sum_{i=1}^{k} \beta i X i + \sum_{i=1}^{k} \beta ii X i^{2}$$

$$+ \cdots + \sum\sum_{i<j} \beta ij X i X j + \varepsilon$$

Where Y was a respond (parameter), βo is constanta, βi, βii, and βij are coeficent from independent variable (X). X isindependent variable without code ($X_1$is solvent dielectric constants, 35 with level 40, 45 and X_2 is the ratio materials to solvent with level 1: 7, 1: 10 and 1: 13.

Results and Discussion

Yields Extract

The graphic of surface plot and contour plot formed a maximum response with was marked with the surface plot graphic resembles an inverted parabola. The analysis result of the yield extract of teter leaves shows that teter leaves extract has yield extract amounted of 7,99-11,67%. The statistic analysis using RSM with the model CCD compatibility was gained an equation: $Y = -39{,}2 + 1{,}662\ X_1 + 3{,}134\ X_2 - 0{,}02082\ X_{12} - 0{,}1501\ X_{22} + 0{,}0027\ X_1X_2$. The incompatibility test of the model (lack of fit) from the teter leaves yield extract model obtaining P>0,05 as many as 0,113. This shows that the incompatibility of quadratic equation models was strongly rejected, thus the quadratic equation model shown above was valid and coherent so that it could be used to predict extract yield on the optimum condition. This is mentioned to know the validity of one equation of quadratic RSM was determined by the values of regression test and the lack of fit from the presented data.

The same result was also reported by Montgomery (2001) that the incoherency model of quadratic equation test can be seen from the p-value on the lack of fit, where the incoherency is significantly rejected when the p-value is bigger than the significance level of 5%. The increase in the yield value of the teter leaf extract to the dielectric constant value of the solvent and the optimum ratio of the material with ethanol solvent is due to the similarity in polarity between the solvent and the extracted compound to produce the optimum yield of teter leaf extract. The dielectric solvent constant is strongly related to the solvent polarity. The bigger value of the constant dielectric of a solvent is, the smaller the polar solvent itself and the smaller the solvent dielectric constant then the more non-polar the solvent is. For getting the highest extract yield on the teter leaves, it is predicted that it needs an ethanol solvent dielectric constant of 40,64 with the material and solvent ratio of 1: 10,81. The prediction test using a respon optimizer thus it is gained graphic D-optimally which can be observed in Figure 2.

The value prediction of the dielectric constant itself had more polar value than ethanol (24, 30) and more non-polar than aquades solvent (80,40). This means that the solluted component on the yield extract had the same polarity with the solvent dielectric constant 40,64. The increasing value of extract yield to the solvent dielectric constant was caused by the increasing point of polarity similarity between the solvent and extracted compound. When the optimum point has reached the solvent which has the same polarity as the extracted compound so that it was then able to attract many bioactive components contained in the material so that the yield value of teter leaf extract was the highest at its optimum condition. However, there was a decrease in the yield value of teter leaves extract after the optimum point was reached, this was because the similarity of the polarity between the solvent and the extracted compound had decreased so that the yield value produced also decreased. This result is also strongly supported by Lestari *et al.*, (2014) who report that the solvent which has the same polarity with the extracted compound will give the more maximum result. According to Priyadharsini and Sujatha (2013), teter leaves contain several compounds which are polar, such as flavonoids, phenolics, tannins, and vitamin C.

The ratio of materials and solvent also influenced the value of teter leaves produced. The higher the ratio between materials and solvent would make a bigger and wider contact between the materials and solvent. However, when the optimum point had been reached, there would be a decrease in the yield value of the teter leaves extract. This is supported in the statement of Benedicta *et al.*, (2016) who state that the higher the ratio of the material and the solvent, the greater the distribution between the solvent and the material which will increase the yield produced. Moreover, Alara *et al.*, (2020) also report that there was a decrease in the yield extract value after reaching the optimum concentration of ethanol solvent and the ratio of the material to the optimum ethanol solvent. The argument is strengthened by the report of Teresa *et al.*, (2016) who add that the more the amount of solvent addition used will cause the yield to decrease because the equilibrium state between the solid and liquid has been reached.

Total Phenolics Contents

The graphic of surface plot and contour plot formed maximum response which is marked by the form of surface plot graphic resembling an inverted parabola. The analysis result of total phenolics content of teter leaves extract shows that the extract of teter leaves has total phenolics content between 93,90-184,38 mg GAE / g extract. Statistic analysis using RSM with the model coherence CCD was gained through an equation of: $Y = -1792 + 85{,}2\ X_1 + 38{,}7\ X_2 – 1{,}023\ X_{12} - 1{,}791\ X_{22} + 0{,}034\ X_1X_2$. The lack of fit test model from the data of a phenolics content of teter leaves extract gained the value of $P>0{,}05$ amounted 0,445. The result means that the incoherence of the quadratic equation was strongly rejected, which can be concluded that the quadratic equation model showed was valid and can be used to predict the phenolics content extract at optimum conditions. One way to find out whether an RSM quadratic equation is valid or not was by testing the lack of fit of the data. Quadratic equation model mismatch is highly rejected if the p-value is greater than the degree of significance 5% (Montgomery, 2001).

The total phenolics content increase is more influenced by the dielectric constant of the solvent and the ratio of the material to the solvent used. The dielectric constant of the solvent was 41.49 and the ratio of the substance to the solvent 1:10,42 was an optimum condition which was predicted that would produce total phenolics content amounted of 174, 226 mg GAE / g extract. Testing the prediction of the optimum conditions used the response optimizer so that the D-optimally graph was obtained which could be seen in Figure 4. The dielectric constant value amounted to 41,49 had more polar characteristics than aquades (80,40). To get the highest totalphenolics content value on the teter leaves extraction, the 41,49-ethanol solvent dielectric constant was needed. According to the polarization principle, a compound would be dissolved on the solvent which had the same polarity. The statement was supported by Turkmen *et al.*, (2006) who report that the change of polarity of the solvent can change the ability of the solvent to solve the phenolic compound. This is because the ability and properties of the solvent in dissolving phenolic compounds are different, depending on the degree of polarity of the solvent and the extracted compound (Suryani *et al.*, 2016).

The ratio of the material with the solvent also affects the total phenolics content value of the resulting teter leaf extract. The ratio of the material with the optimum solvent will produce the optimal total phenolics content. This is because the phenol will continue to dissolve in the solvent used until it reaches the saturation point, when the saturation point has been reached, there will be a decrease in the total phenol value of the resulting teter leaf extract. The more solvent is used; the concentration of the compounds contained in the material will decrease so that it also causes the total phenol obtained will also decrease. This is according to the report from Wati *et al.,* (2015) who state that that the greater the volume of solvent used in the extraction process will cause more and more compounds to dissolve in it until the saturation point is reached.

Total Flavonoid Contents

Surface plot and contour plot graphic formed maximum response which is marked with the surface plot graphic resembling reverse parabola. The analysis of total teter leaves extract shows that the leave extract has total flavonoid between 61,16-85,93 mg QE / g extract. The statistic analysis using RSM with the coherence model CCD gains equation: $Y = -583{,}5 + 29{,}90\ X_1 + 11{,}18\ X_2 – 0{,}3701\ X_{12}- 0{,}564\ X_{22} + 0{,}015\ X_1X_2$. The incoherence model test (lack of fit) from the data of total flavonoids of the teter leaves extract, it is gained $P>0{,}05$ amounted 0,841. This means that the mismatch of the quadratic equation model is strongly rejected, which means that the quadratic equation model shown is valid and can be used to predict the total flavonoids of teter leaf extract at optimum conditions. According to Montgomery (2001), one way to find out whether a quadratic equation RSM is valid or not is from testing the lack of fit of the data where the mismatch of the quadratic equation model is strongly rejected if the p-value is greater than the degree of significance 5%.

The increase in total flavonoids was due to the similarity in polarity between the solvent and the extracted compound and the ratio of the material to the solvent used. The polarity of a solvent can be seen from the dielectric constant value of the solvent. The dielectric constant of ethanol solvent which is suitable for obtaining the highest total flavonoids in teter leaf extract as predicted is 40.64 with a solvent ratio of 1: 10.42. Testing the prediction of the optimum conditions uses the response optimizer so that the D-optimally graph is obtained which can be seen in Figure 6.

The dielectric constant is more polar than the dielectric constant value of ethanol (24.30) and is more non-polar than distilled water (80.40). Flavonoid compounds are divided into several types and each type of flavonoid has a different polarity depending on the number and position of the hydroxyl groups of each type of flavonoid so that this will affect the solubility of flavonoids in solvents (Harborne, 1987). This statement is reinforced by research by Lestari *et al.,* (2014) which reports that a solvent that has the same polarity as the extracted compound will provide maximum results.

The ratio of the material with the solvent also affects the total flavonoid value of the resulting teter leaves extract. The ratio of material with solvent 1: 10.42 is the optimum condition which is predicted to produce the highest total flavonoid value of teter leaves extract. According to Delazar *et al.,* (2012), the increase in total flavonoids along with the increase in the ratio of the material to the solvent is caused by the more solvent used, so the capture of the target compound into the solvent can run more optimally. After reaching the optimum point, there was a decrease in total flavonoids due to the decrease in the polarity similarity between the solvent and the extracted compound and the extraction process had reached its saturation point. The more solvent is used; the concentration of the compounds contained in the material will decrease so that the total value of flavonoids obtained will also decrease. This is confirmed by the statement reported by Radojkovic *et al.,* (2012) that there was a decrease in total flavonoids in mulberry (*Morus alba* L.) leaf extract after reaching the optimum concentration of ethanol solvent and the ratio of the material to the optimum ethanol solvent.

Total Tannin Contents

The graphic of surface plot and contour plot forms a maximum response which is marked by the graphic form of surface plot resembling reverse parabola. The results of the total tannin analysis showed that the teter leaf extract had a total tannin extract ranging from 7,30-13,08 mg TAE/g ekstrak. Statistic analysis using RSM with the CCD model coherence gained an equation: $Y = -72{,}7 + 3{,}556\ X_1 + 2{,}280\ X_2 – 0{,}04361\ X_{12}- 0{,}1005\ X_{22} + 0{,}0050\ X_1X_2$. The incoherence test model (lack of fit) from the total tannins data gained a value of P>0,05 as much as 0,906. This result means that the incoherence of the quadratic equation is strongly rejected, which resulted in the conclusion that the model of the quadratic equation is valid and can be used as a prediction of the teter leaves extract of total tannins on the optimum condition. One way to know the validity of the RSM quadratic equation is from the lack of fit test from the data where the incoherency of the quadratic equation model will be highly rejected when the p-value is bigger than the significant level of 5% (Montgomery, 2001).

The solvent dielectric constant and the ratio of materials and solvent influence the teter leaves extract total tannins produced. The higher the dielectric constant of the solvent and the ratio of the material to the solvent causes an increase in the total tannin produced until the optimum point is reached, but after the optimum point is reached, the total tannin value of teter leaves extract has decreased. The prediction of the optimum condition to produce the highest total tannins is on the ethanol solvent constant 40,21 and the ratio materials with the solvent is 1:10,30. The test of optimum prediction condition was carried out using respon optimizer thus the graphic of D-optimally can be seen in Figure 8.

The ethanol solvent dielectric constant 40,21 is polar more than the pure ethanol solvent (24,30) and is more non-polar than the aquades solvent (80,40) thus in the solvent polarization with the dielectric constant 40,21 is predicted able to produce the highest amount of teter leaves extract total tannins. The thing is caused by the similarity of polarity between the solvent and extracted compound. The argument is strengthened by the research from Lestari *et al.*, (2014) who report that the solvent which has the same polarity with the extracted compound will give more maximum result. After reaching the optimum point, the decreasing level of total tannins happened because the decreasing similarity of polarity between the solvent and extracted compound and the number of extracted compounds had lessened, thus the total tannin produced was decreasing. The result was gained by Rodrigues *et al.*, (2016) who report that the decreasing amount of total tannins in the extract Eugenia uniflora (*Myrcia amazonica* DC) after reaching the optimum ethanol solvent concentration.

The ratio of materials and solvent also influences the produced total tannins. The higher the ratio of the material to the solvent, the higher the total tannin from teter leaves extract until the optimum conditions have been reached. This is because the greater the area of contact between the solvent and the extracted material so that the penetration of the solvent into the cell has a greater chance until the optimum point is reached. The decrease in the total value of tannins after achieving the optimum conditions is because the more solvent is used, the concentration of the compounds contained in the material will decrease so that the total tannin value obtained will also decrease. The statement is strengthened by Jayanudin *et al.*, (2014) who report that l the contact area between the solvent and the material affects the yield of the extract produced. Moreover, Tan *et al.*, (2017) also report that There was a decrease in the total tannins from the leave extract of *Pouzolzia zeylanica* L. after reaching the optimum ratio of materials and ethanol solvent.

Vitamin C

The graphic of surface plot and contour plot formed a maximum response which was marked by the form of the graphic resembling reverse parabola. The analysis result of the teter leaves extract of vitamin C shows that the extract teter leaves have a vitamin C range between 56,86-98,80 mg AAE/g extract. The statistic analysis used RSM with the coherence CCD model obtained equation: Y = -714 + 34,76 X1 + 21,17 X2– 0,3976 X12- 0,449 X22 + 0,315 X1X2. The test of incoherence model (lack of fit) from the data of teter leaves vitamin C gained value of P>0,05 which was 0,401. The result shows that the incoherency of the quadratic equation was strongly rejected and the quadratic equation model was valid and could be used to predict the optimum condition of vitamin C of teter leaves extract. One way to determine the validity of the RSM quadratic equation is the lack of fit test from the data where the incoherency of the quadratic equation model is highly rejected if the p-value is greater than the significant level of 5% (Montgomery, 2001).

The solvent dielectric constant and the ratio of materials and solvent influence the total vitamin C that the teter leaves extract produced. The higher solvent dielectric constant and the ratio of materials and solvent create the increased total of vitamin C that the teter leaves extract produced thus the optimum point was reached. Yet, when the optimum point was reached, the total value of vitamin C from the teter leaves extract to experience its decrease. The prediction of the optimum condition to produce the highest total of vitamin C is put in the ethanol solvent dielectric constant 39,92 and the ratio of materials and solvent is 1:9,57. The prediction test of the optimum condition used response optimizer thus the graphic D-optimally obtained can be seen in Figure 10.

The thing that can increase the vitamin C of teter leaves extract is the same polarity with the solvent. According to Zumdahl (2007), vitamin C has a lot of polar O-H and C-O bonding thus can make vitamin C is significantly polar and can be extracted well with the solvent which has the same polarity. The result is supported by the report of Najoan *et al.*, (2016) who state that a polar compound will be more effective if it is extracted with the polar solvent. The solvent dielectric constant which was needed to extract vitamin C (39,92) is lower than the value of the solvent dielectric constant which was needed to extract a total of phenol (41,49), the total of tannins (40,21), and the total of flavonoids (40,64). The result indicated that to extract vitamin C, a lower polarized solvent is needed compared to the solvent needed to extract tannin, flavonoids, and phenol compounds. This is supported by the report from Verdiana *et al.*, (2018) who state that the solvent with the lower polar is indicated able to extract more effective vitamin C.

The ratio of materials and solvent also influenced the teter leaves to extract that was produced. The optimum condition to produce the highest vitamin C of teter leaves extract is in the materials and solvent ratio 1: 9,57. The result shows that the more the solvent used, the more extracted compounds will be produced thus the optimum point will be reached.

This is also in line with the other parameters such as flavonoids, a total of phenol, a total of tannins and teter leaves extract, and the extract yield of teter leaves, when the optimum condition from the ratio of materials and solvent has reached, then the decreased value of vitamin C from the teter leaves extract possibly happens.

The decreasing value of vitamin C after the optimum condition reached out was caused by the more solvent used, the compound concentration will be decreased thus the obtained vitamin C will be decreased as well. The result is in line with Wati *et al.*, (2015) who state that the bigger volume of solvent is in the extraction process, will cause more compound that is dissolved inside until the saturation point is reached.

Antioxidant Activity

The surface plot and contour plot graphic formed in Figure 11 shows a maximum response marked by the form of the graphic resembling a reverse parabole. The analysis result of antioxidant activities of the teter leaves extract shows that the extract of teter leaves has antioxidant activities range between 74,32-81,42 %. The statistic analysis using RSM with the coherency of CCD model gained an equation: $Y = -173,7 + 10,99\ X_1 + 5,72\ X_2 - 0,1358\ X_{12} - 0,354\ X_{22} + 0,0313\ X_1X_2$. The testing process of the model (lack of fit) from the antioxidant activities teter leaves extract gained from the values of $P>0,05$ which is 0,153. The result shows that the incoherency of the quadratic equation model is significantly rejected and the quadratic equation model shown valid and can be used to predict the extract of teter leaves antioxidant activities on the optimum condition. One of the ways that can be used to know a valid RSM quadratic equation is from the lack of fit testing from the data where the incoherency of quadratic equation is highly rejected when the p-value is bigger than the significant level of 5% (Montgomery, 2001).

The solvent dielectric constant and the ratio of materials and the solvent highly influences the values of antioxidant activities of the teter leaves extract which is produced. The optimum condition to produce the highest antioxidant activities is predicted on the ethanol dielectric solvent constant 41,64 and the ratio of materials and the solvent is 1:9,95.

The optimum condition prediction testing used a response optimizer thus the graphic D-optimally obtained which can be seen in Figure 12.

Ethanol solvent dielectric constant 41,64 is more polar than pure ethanol (24,30) and has a character of non-polar than the aquades solvent (80,40). According to Priyadharsini dan Sujatha (2013), teter leaves contain several polar compounds such as flavonoids, phenolics, tannins, and vitamin C which can function as antioxidants. The increase of antioxidant activities is caused by the bioactive components functioned as antioxidants such as phenol, flavonoids, tannins, and vitamin C. The increase of antioxidant activities based on the increase of total phenol, flavonoid, tannins, and vitamin C. But, after the optimum point is reached, antioxidant activities is decreasing based on the decreasing process of total phenol, flavonoids, tannins, and vitamin C, thus, it can be concluded that there is a positive correlation between the number of bioactive components such as total flavonoids, tannins and vitamin C which are contained in the teter leaves extract with the values of antioxidant leaves. The statement is strengthened by the research of Pujimulyani *et al.*, (2010) who report that there is a correlation between bioactive component which functioned as an antioxidant with the value of antioxidant activities of white turmeric (*Curcuma mangga* Val.).

IC_{50}

The graphic resembles a parabole that forms minimum response which shows that the values of IC_{50} are influenced by the solvent dielectric constant and the ratio of materials and solvent. The analysis result of IC_{50} of the teter leaves extract shows that the teter leaves extract has IC_{50} range extract between 173,748- 201,878 ppm. The statistic analysis using RSM with the coherency of CCD model gained an equation: $Y = 1055 - 39,3\ X_1 - 15,3\ X_2 + 0,489\ X_{12} + 1,086\ X_{22} - 0,135\ X_1X_2$. The test of incoherency model (lack of fit) from the data IC_{50}teter leaves extract gained values of $P>0,05$ is as much as 0,053.

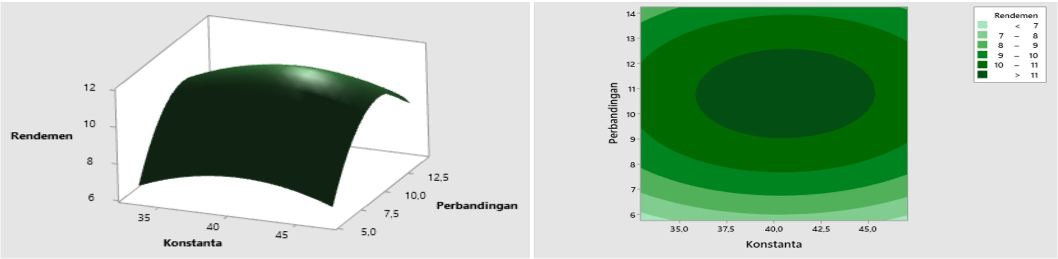
Table.1 The treatments combination

No.	Code Variation		Treatment	
	X_1	X_2	Dielectric Constant	Ratio Material To Solvent
1	-1	-1	35	1:7
2	+1	-1	45	1:7
3	-1	+1	35	1:13
4	+1	+1	45	1:13
5	-1,414	0	32,92	1:10
6	+1,414	0	47,07	1:10
7	0	-1,414	40	1:5,75
8	0	+1,414	40	1:14,24
9	0	0	40	1:10
10	0	0	40	1:10
11	0	0	40	1:10
12	0	0	40	1:10
13	0	0	40	1:10

Table.2 The response values and the response actually in optimum conditions

Response	Prediction Value	Average the true value	Standard deviation	Coefficien Of Variation (%)
Yield Extract	11,41 %	17,41%	1,12	6,43%
Total phenolic Contents	173,62mg GAE/g	175,151mg GAE/g	2,12	1,21%
Total Flavonoid Contents	81,82mg QE/g	82,60mg QE/g	2,85	3,45%
Total Tannin Contents	10,51mg TAE/g	12,30mg TAE/g	0,86	6,99 %
Vitamin C	80,84mg AAE/g	82,30mg AAE/g	1,21	1,47 %
Antioxidant Activity	82,97%	84,34%	1,49	1,77%
IC_{50}	166,30 ppm	161,988ppm	1,88	1,16 %

Fig.1 Chart of surface plot and contour plot teter leaves yield extract



(a). Chart of Surface Plot

(b). Chart of Contour Plot

Fig.2 Charts of d-optimally teter leaves yield extract.

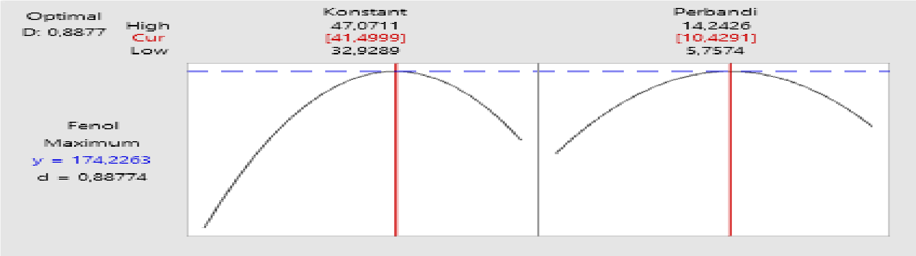


Fig.3 Chart of surface plot and contour plot total phenolics contents of teter leaves extract

(a). Chart of Surface Plot (b). Chart of Contour Plot

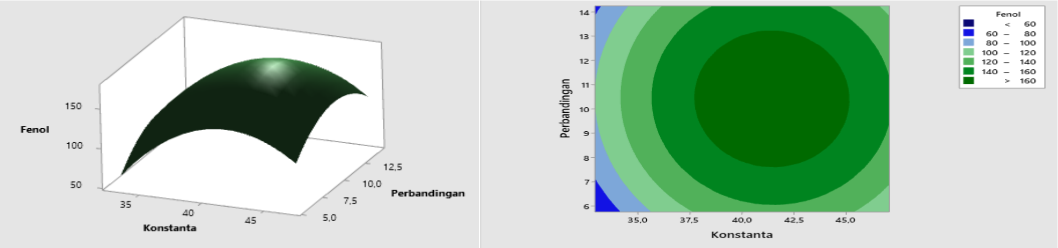


Fig.4 Chart of d-optimally total phenolic contents of teter leaves extract.

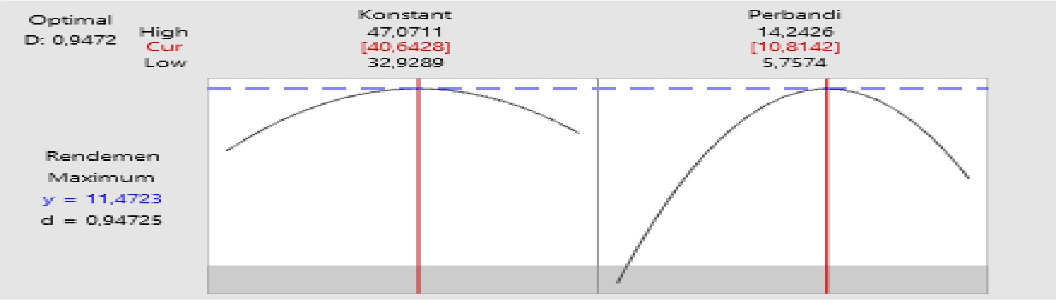


Fig.5 Chart of surface plot and contour plot total flavonoid contents of teter leaves extract

(a). Chart of Surface Plot (b). Chart of Contour Plot

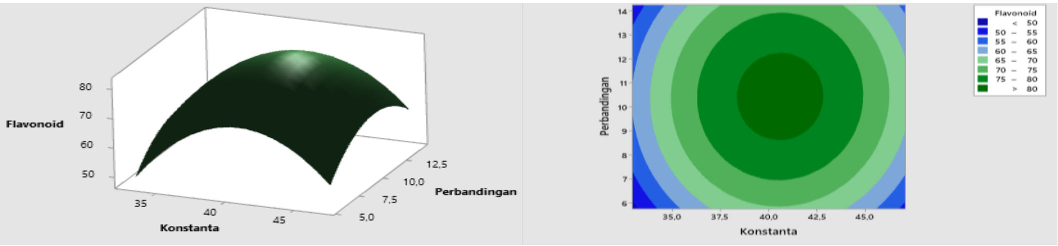


Fig.6 Chart of d-optimally total flavonoid contents of teter leaves extract

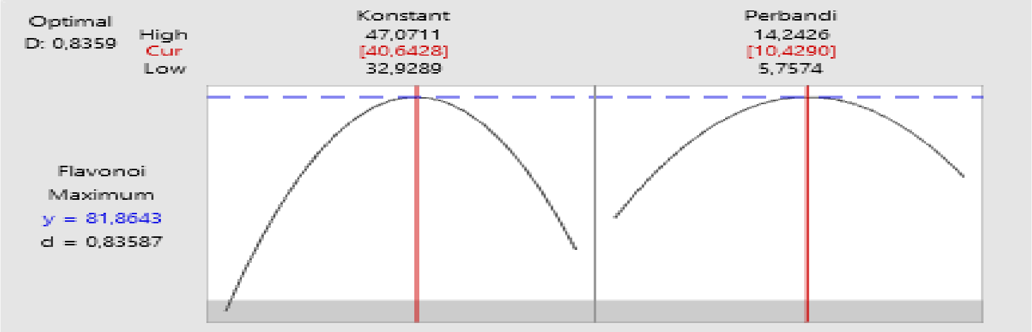
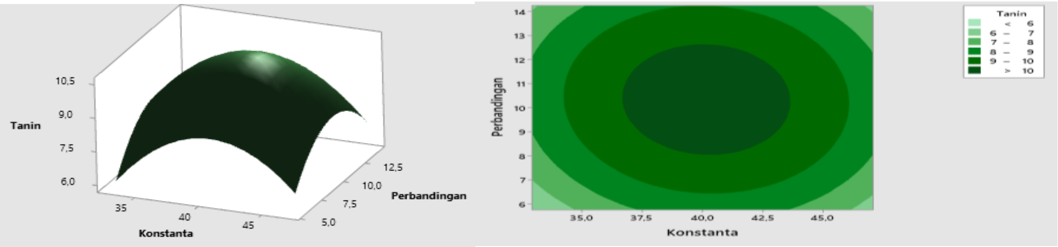


Fig.7 Chart of surface the plot and contour plot total tannin contents of teter leaves extract



(a). Chart of Surface Plot

(b). Chart of Contour Plot

Fig.8 Chart of d-optimally total tannin contents of teter leaves extract

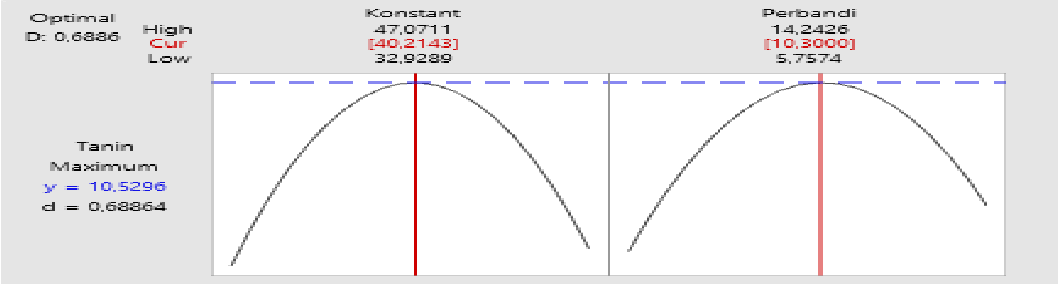
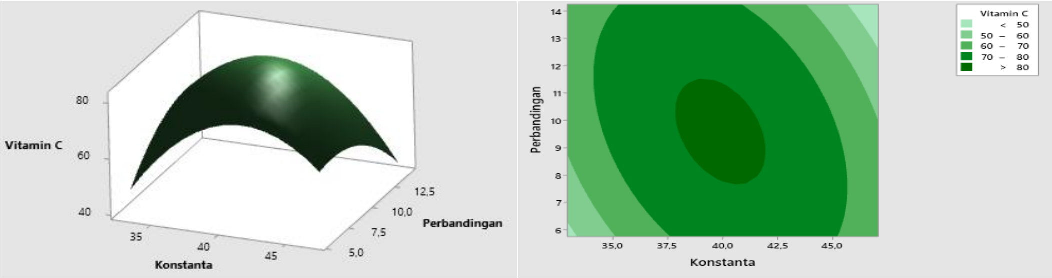


Fig.9 Chart of surface plot and contour plot vitamin C of teter leaves extract



(a). Chart of Surface Plot

(b). Chart of Contour Plot

Fig.10 Chart of d-optimally vitamin c of teter leaves extract

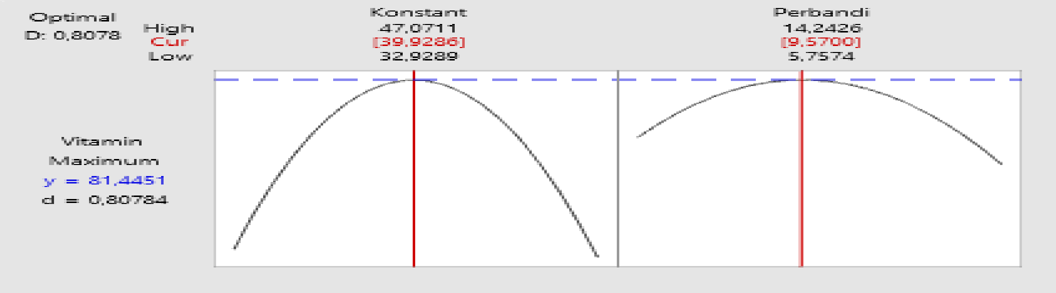


Fig.11 Charts of surface the plot and contour plot antioxidant activity of teter leaves extract

(a). Chart of Surface Plot (b). Chart of Contour Plot

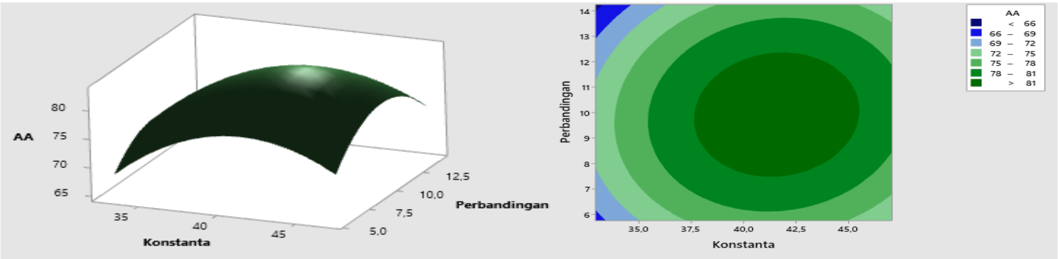


Fig.12 Charts of d-optimally antioxidant activity of teter leaves extract

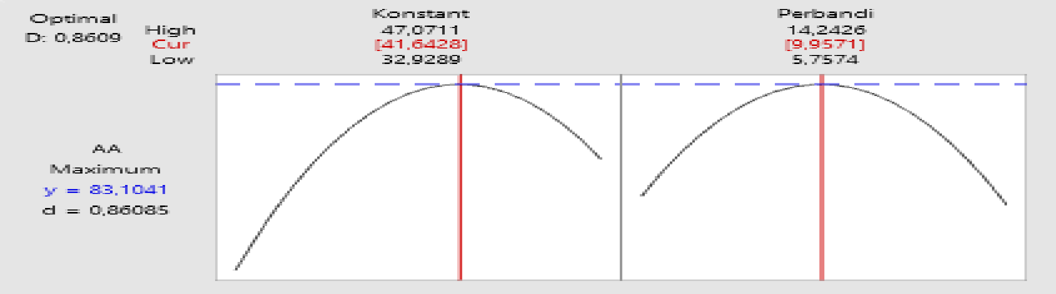


Fig.13 Chart of surface the plot and contour plot IC_{50} of teter leaves extract

(a). Chart of Surface Plot (b). Chart of Contour Plot

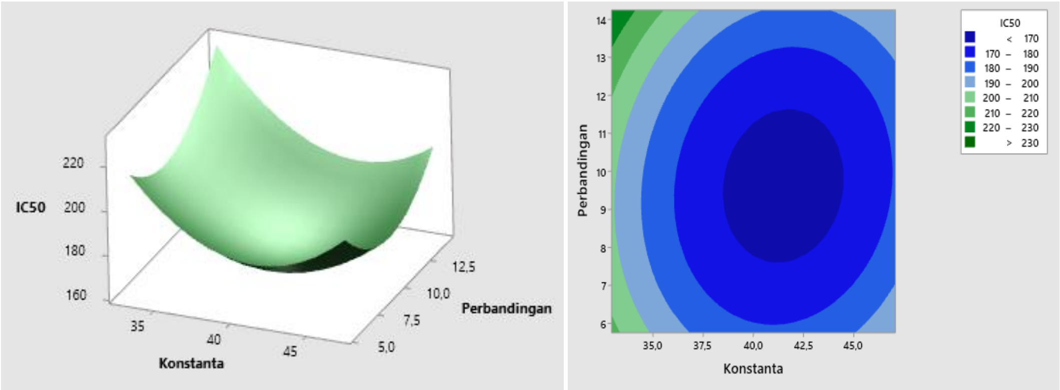
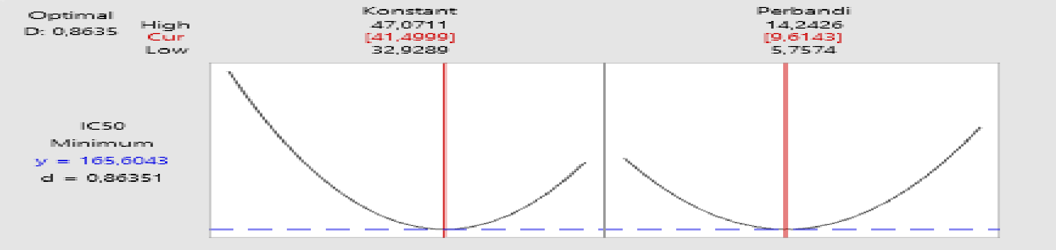


Fig.14 Charts of d-optimally IC_{50} of teter leaves extract



This shows that the incoherency of the quadratic equation model is highly rejected and the quadratic equation model shown is valid and can be used to predict IC_{50} of the teter leaves extract in the optimum condition. One way to identify a valid quadratic equation of RSM is from the testing of lack of fit from the data where the incoherency of quadratic equation model is highly rejected when the p-value is bigger than the level of significance 5% (Montgomery, 2001).

The solvent dielectric constant and the ratio of materials and solvent influence the value of IC_{50} produced of teter leaves extract. The optimum condition to produce the lowest IC_{50}teter leaves extract is predicted on the ethanol dielectric solvent constant 41,49 and the ratio of materials and solvent is 1:9,61. The optimum condition prediction test used a response optimizer thus a result of the graphic of D-optimally can be seen in Figure 14.

The decreasing values of IC_{50}teter leaves extract were caused because the value of IC_{50} is influenced by the antioxidant activities values, where the values of IC_{50} decreased based on the increase of antioxidant activities values. But, after reaching the optimum point, the values of IC_{50} increased based on the decrease of antioxidant activities. The low value of IC_{50}, shows a high antioxidant activity meanwhile, the high values of IC_{50} show low antioxidant activities. This happened because the values of IC_{50} were versus inversely with the antioxidant activities. The condition is based on the statement of Husni *et al.*, (2014) who report that the lower values of IC_{50}, the higher antioxidant activities.

Validation Condition Steady

The determination of optimum conditions on the research was carried out using a respon optimizer supported by the Minitab 19 software. This validity test is important to be conducted to prove a result from the optimum condition and to investigate the accuracy which can be acceptable to gain the optimum response (Montgomery, 2001). The response data which were obtained from each optimation were: yield extract, total flavonoids content, total phenolics content, vitamin C, total tannins content, antioxidant activities, and IC_{50} combined to optimize those five responses. The values of the responses of prediction and the value of the real respons on the optimum validity condition process can be seen in Table 2.

The optimum condition formed was on the dielectric constant 40,74 and the ratio of materials and solvent was 1:10,19 predicted will produce yield extract value amounted 11,41%, total flavonoids content were 81,82 mg QE/ g extract, total tannins content were 10,51 mg TAE/g extract, total phenolics content was173,63 mg GAE/ g extract, vitamin C was 80,84 mg AAE/g extract, antioxidant activities was 82,97% and IC_{50} was 166,30 ppm. The validity of the optimum condition was carried out by looking at the coefficient of variation (CV) from each response. The coefficient of variation is a ratio between the standard deviation and the average values of data which is presented in percentage. Based on the optimum validity result in Table 2, it is known that the values of CV yield extract, total phenolics content, total flavonoids content, total tannins content, vitamin C, antioxidant activities, and IC_{50} which is relatively small. This result proofs that the optimum condition on each response is acceptable. The statement is supported by Gomez dan Gomez (1984) who report that the optimum condition on a response can be accepted when the CV is less than 15% to each experiment.

Based on the discussion above, it can be concluded that ethanol solvent dielectric constant was 40,74 and the ratio between materials and ethanol solvent was 1:10,19 as the treatment of the optimum condition to produce teter leaves extract which has high antioxidant activities which were 84,34%, with IC50 161,988 ppm, extract yield 17,41%, total flavonoids 82,60 mg QE/g extract, total phenol 175,151 mg GAE/g extract, vitamin C 82,30 mg AAE/g extract dan total tannins 12,30 mg TAE/g extract.

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