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Optimization of the Ultrasonic Extraction Process of Kasumba Turate (*Carthamus tinctorius Linn*) Using the Response Surface Metodology (RSM) Technique

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ABSTRACT

Kasumba turate (Carthamus tinctorius Linn) or safflower is a traditional medicine used by South Sulawesians. Polyphenol compounds and antioxidant activity are active components of kasumbu turate. This component can be obtained through extraction. The goal of this study was to find the best conditions for extracting kasumba turate with high total phenol levels and strong antioxidant activity, as well as encapsulating the resulting extract. The ultrasonic assisted extraction (UAE) method was used to extract Kasumba turate with methanol, and the Responses surface Methodology method was used to optimize the extraction. Behnken Box Variations in composition solutions of 50, 60, 70, 80, and 90%, as well as time variations of 20, 30, 40, 50, and 60 minutes. A solvent ratio of was used for sample extraction 1: 1 using Methanol solvent and water, determined which is the best result of this variable, and continued by analyzing is a test analysis with Spectrophotometry, DPPH, and the best results of the variable are analyzed using Spectrophotometry to see the content of flavonoid compounds in Kasumba Turate Flowers.

Introduction

Kasumba Turate (*Carthamus tinctorius L*) could be a conventional therapeutic plant utilized observationally by the individuals of South Sulawesi to treat measles and is brewed in bubbling water to fortify the body's resistance or perseverance of the persistent. (Hamshidi et al., 2019). Kasumba turate (*Carthamus tinctorius L*) may be a plant utilized in medication to treat dysmenorrhea, menopause, chest torment, and stomach torment due to its solid bioactive properties. cancer, cardiovascular malady, cerebrovascular malady, Alzheimer's malady, and ischemia/reperfusion-induced intense kidney damage. In this manner, Cancer prevention agents are broadly utilized within the field of pharmacology. Cancer prevention agents are another vital bioactive fixing separated from safflower. Cancer prevention agents contain numerous hydroxyl bunches and Kasumba turate has the potential to be created into utilitarian nourishment, since it contains polyphenols and cancer prevention agents that are advantageous to ward off free radicals. Kasumba turate/Kasumba ogi (Carthamus tinctorius L) is known by the Bugis-Makasssar community. The request for Kasumba turate as a characteristic antioxidant compound utilized within the economy of the pharmaceutical and utilitarian nourishment businesses is expanding as buyer mindfulness develops. In any case, financial contemplations constrain producers to utilize certain proportionate fixings at a cheaper cost. Subsequently, kasumba turate (Carthanmus tinctorius L.), a cheap elective to crocus saffron (Crocus sativus L.) and wealthy in bioactive substances is utilized in different financial segments. Kasumba turate is known for its wealthy chemical composition. It contains 444 monosaccharides and complex sugars, amino acids, proteins, lipids, cellulose, minerals and vitamins (counting thiamin and riboflavin), little sums of

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alpha and beta carotene, and more tin and lycopene (Adamska, 2021).

Botanical Characteristics and Development Prerequisites of kasumba turate

The quality of the raw material obtained from safflower is determined by the conditions of the growing area, such as temperature and humidity, soil moisture, solar radiation, and soil fertility. The intensity of sunlight affects the content of flavonoids. Flavonoid synthesis is higher under sunlight-limited conditions in plants. The characteristics of the plants being grown are also important. Research is currently underway to obtain safflower varieties that contain as much pigment as possible in their petals. Safflower (Carthamus tinctorius) belongs to the Asteraceae family. Its natural range includes Asia (Territory of India) and the Middle East. The plant has a bushy habit and reaches a height of 100-130 cm. It produces large lance-shaped leaves with jagged edges. Safflower flowers grow in a radial tube shape, forming large inflorescences (flower heads). Groups of cultivars were distinguished according to flower color before and after drying. (1) yellow flowers and red dried flowers, (2) yellow flowers and dried flowers, (3) orange flowers and dark colored flowers, red dried flowers and (4) white flowers and dried flowers.

Methods

Material

Kasumba turat is dried in the sun for 12 hours and pureed into a smooth paste. Stored in a closed container and stored at room temperature and protected from sunlight.

Experimental Design

In this study, UAE conditions were optimized using RSM and central composite design in terms of total phenolic content, antioxydant activity, extraction yield dan DPPH. (X_1) ratio solution and (X_2) time extraction were selected as the independent variabels

Ultrasound assisted extraction (UAE) process

Kasumba turate samples were prepared as follows: 10 g of kasumba turate was added to 50, 60, 70, 80, 90% (v/v) methanol. And the 20, 30, 40, 50 60 min. a group without sample addition was defined as a control group and was also prepared at the same time.

Responses Surface Methodology

In this study were used CCD was run 25 times to determine the optimal values of important extraction factors and their effects on Antioxydant activity, total phenolic, and yield. Each variabel was examined factor point (-1, +1) and the central point (0).

Table 1. Compositions Variabels Kasumba Turate

Factor	Name	Level	Low Level	High Level
a	X1 Pelarut Metanol	70.00	50.00	90.00
B	X2 Waktu	40.00	20.00	60.00

Extraction Yield

The extracts prepared duirng the experiment was transferred to bottom flask, Evaporate the remaining solvent in the round-bottomed flask to obtain the desired yield, then pour it into a glass bottle and close it tightly.

Antioxydant activity

Sampling was performed by pipetting 1 ml of sample solution and adding 3 ml of 50 ppm DPPH. Then homogenize and incubate for 30 minutes at room temperature in the dark. Absorbance was measured at a wavelength of 520 nm. The same treatment was applied to the blank and ascorbic acid comparison tests. The result of this analysis is the amount of ascorbic acid as a DPPH inhibitor in the sample.

$$\%DPPH = \left|\frac{(Abssample - Abscontol)}{(Abscontrol - Absblank)}\right| \times 100\%$$

Total Phenolic Content

0.2 ml of sample was added to 0. 5 ml Folin-Ceocalteu 10% (v/v) and left for 3 minutes. Then add 1 ml of 10% (w/v) Na2CO3, homogenize, and leave at room temperature for 30 min. Then add 1 ml of 10% (b/v) Na2CO3, homogenize and leave at room temperature for 30 min. The absorbance is then measured with a spectrophotometer. Gallate compounds were used as a standard curve. The result of this analysis is the total phenol concentration in the kasumbaturic acid extract. Absorbance was measured with a spectrophotometer. Gallic acid compound was used as a standard curve. The result of this analysis is the concentration of total phenols in the Kasumba Turate extract.

$$TPC\left(\frac{mg}{g}\right) = \frac{ncv}{m \times 1000}$$

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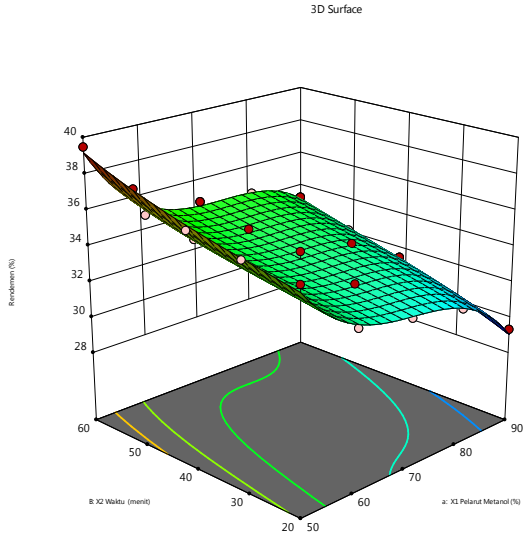
Results and Discussions

Screening of important variables with a focused composite design

Central composite design (CCD) was used to describe the effect of solvent concentration, time extraction on the TPC, antioxidant activity, and yield.



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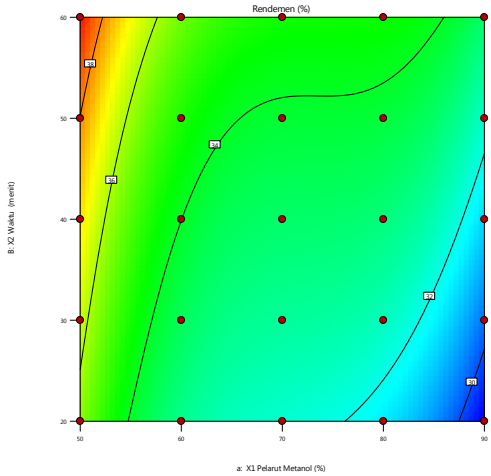


(b)

Figure 1. Contour Yield kasumba Turate : (a) 3-D and (b) 2-D

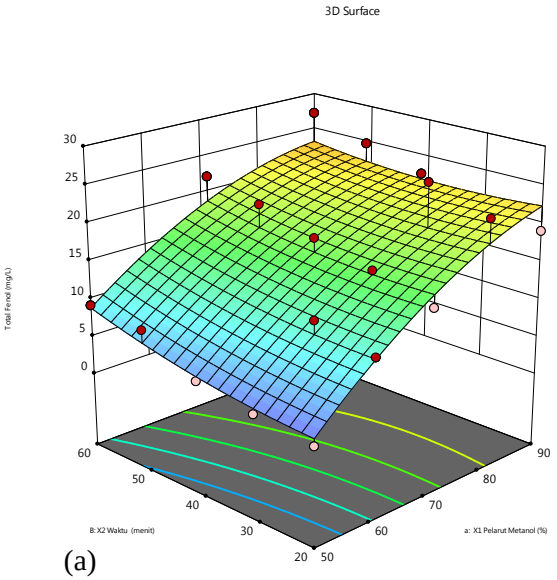
Effect of extraction conditions on yield

The percent yield is then entered into Design Expert 13 to determine the influence of the independent variables on the yield of the sample extract. Response data are analyzed using Design Expert and analysis of variance (ANOVA). The significance of the coefficients is evaluated using probability values (p-values). If the probability value is less than 0. 05 ($p < 0.05$), reject the first hypothesis (H0) and accept the alternative hypothesis (H1). Increasing solvent concentration and time. For the model fitted, the coefficient of determination (R^2) was 0.9885. P-value for the lack of was siginificant ($P<0.05$) thereby confriming the validity of model. The value of adjusted determination coeficient (adjusted R^2= 0.9788) also confirmed that the model was highly significant. At the samre time value, very low 0.95 of CV clearly indicated a very high degree of precision and good deal of reliability of experimental values. Anova for the selected cubic. Final equation in terms of coded factors.

Extraction yield (Y) = 51.4956 + -0.451412 * a + 0.035647 * B + 0.00042969 * aB + 0.00217396 * a^2 + 0.000113629 * B^2

Effect of extraction conditions on Total Phenolic Content

The main part of polyphnols in Kasumba Turate are likely flavonoids, which may describe the results as flavonoids are associated with many biological activities such as antioxidant and antiviral. In this study, RSM was performed to define the appropriate solvent concentration and time extraction for optimization of flavonoid extraction conditions from Kasumba turate. Based on the table above.



(a)

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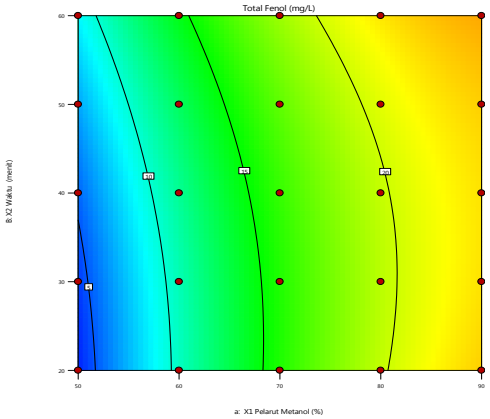


(b)

Figure 2. Contour Total Phenolic Kasumba Trate: (a) 3-D and (b) 2-D

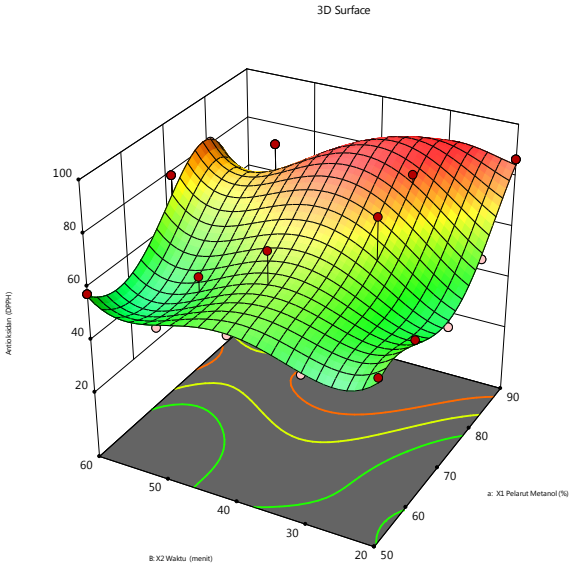
This model is a significant methanol solvent model with p-values between 0. 0003 and 0. 05. The above significant values > 0. 05 indicate non-significance, so the model used between extraction time and total phenols is not appropriate and there is no linear relationship. The ANOVA results show that extraction time and extraction power (BD) have a significant ($p < 0.05$) negative impact on the overall evaluation value. The coefficient of variation (CV) isdefined as the quotient between the standard deviation and the mean value. CV is used for statistical data analysis. The CV value is related to the reproducibility of the experiment. A lower CV value indicates less variation in the mean value and better reproducibility of the experiment. CV value is 21.76

Total Fenol= 51.4807 + 1.45974 * a + 0.033621 * B + -0.0022765 * aB + -0.00682329 * a^2 + 0.002575 * B^2

Effect of extraction conditions Antioxidant

The highest antioxidant activity of Kasumba Turat crude extract is 27,250 samples A90B20 (methanol 90%, time 20 min). The antioxidant activity produced is the number of compounds extracted by different solvents with different solubility (Syahbirini et al., 2005). Solvents with higher solubility produce more potent antioxidant activity compared to solvents with lower solubility. The antioxidant activity responses were then entered into a statistical application to determine the influence of the independent variables on the amount of phenols in the sample extracts. Response data are analyzed using analysis of variance (ANOVA).

The significance of the coefficients is evaluated using probability values (p-values). If the probability value is less than 0. 05 ($p < 0.05$), reject the initial hypothesis (H0) and accept the alternative hypothesis (H1).



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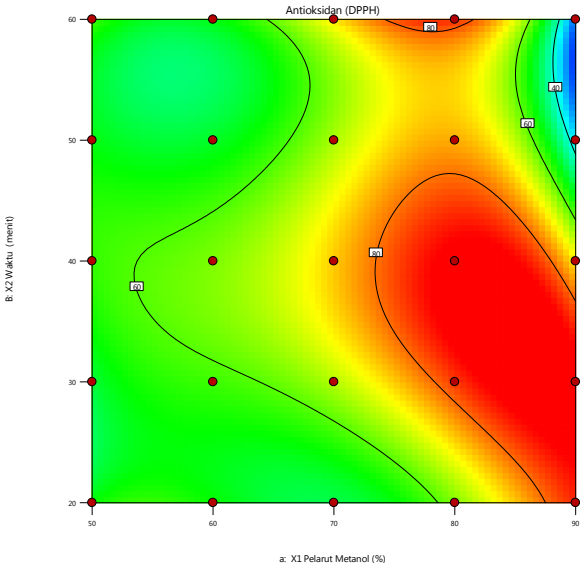

(b)

Figure 3. 3D and 2D Contour antioxidant activity

The solvent methanol is significant in the analysis of antioxidant activity, with p-value of 0. 0361 < 0. 05. Model selection based on R coefficient with value 0.8165 has a significant impact of 81.65 %. Additionally, the interaction between the independent variables, i.e., methanol and solvent values, is significant with 0.0361 > 0. 05. Significant values < 0.05, indicating non-

significance, so the model used does not have a linear relationship between methanol solvent and antioxidant activity. The extraction time model is significant at p-value < 0. 05 in the analysis of antioxidant activity. The coefficient of variation (CV) is defined as the quotient between the standard deviation and the mean value. CV is used for statistical data analysis. The CV value is related to the reproducibility of the experiment. A lower CV value indicates less variation in the mean value and better reproducibility of the experiment. A CV value of 0. 05 indicates non-significance with a significant value greater than 0.05. Therefore, the model used between extraction time and antioxidant activity is not appropriate and there is no linear relationship. The antioxidant effect of DPPH is thought to be due to its ability to supply hydrogen. Radical scavenging activity is important in preventing the harmful role of free radicals in various diseases, including cancer. Free radical scavenging using the DPPH method has been recognized as a mechanism for screening the antioxidant activity of plant extracts. In the DPPH assay, the purple DPPH solution is reduced to the yellow product diphenylpyrrolehydrazine by adding the extract in a concentration-dependent manner. The results showed that methanol extract had the highest antioxidant activity compared to other extracts. The results indicate the activity depending on the chemical content of Kasumbuturate extract. In addition, the content of polyphenols and tocopherols removes DPPH radicals that have the ability to release hydrogen. This is inversely proportional to the change in methanol solvent, with higher methanol solvent content increasing the antioxidant activity of the sample (Aktsar, Abd, Malik 2023). Antioxidant Anctivity= 74.28 + 32.13 * a -3.33 * B + 3.05 * aB + 10.55* a^2 -39.96 * B^2- 25.24 a^2B

Conclusions

The coclusion of these research are as follows:

1. From the results of the study we can conclude that by using a variety of methanol solvents, and time shows the highest yield obtained at 50% methanol concentration with an extraction time of 60 minutes with a value of 39, 466%, for total phenol obtained the highest results at a concentration of 90% at a time of 60 minutes with a value of 27,250 mg/L and for antioxidants obtained the highest results at a concentration of 90% at a time of 20 minutes with a value of 27,250 DPPH

2. The optimal operating conditions based on yield, total phenol, and antioxidants are at 90% solvent concentration and 60 minutes, this can be seen from the p-value for yield which is <0.05, namely 0.00003 while for total phenol and antioxidants it is still high above <0.05 and the results are not significant, this shows that there is still a need for more in-depth research and the need to add other factors so that optimization of application design can be done.

3. In the characterization results that have been carried out to detect the presence of total phenols and antioxidants in the sample obtained by using a sample concentration of 60%, the results for total phenols are 7.40 mGEA/g, and antioxidants are 54.58 mg/L.

4. From the experimental results also obtained Anova results for yield with C.V 0.955, total phenol, with C.V 21.26 and antioxidant C.V 27.37 where the results for yield are significant, while for phenol and antioxidant insignificant.

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