Extraction optimization for antioxidant phenolic compounds in black grass jelly (Mesona palustris BL) using response surface methodology

H Hendratama, K Harismah and A M Fuadi
Master of Chemical Engineering, Graduate School, Universitas Muhammadiyah
Surakarta, Surakarta, Indonesia

E-mail: U200166001@student.ums.ac.id, kun.harismah@ums.ac.id, Ahmad.fuadi@ums.ac.id

Abstract. Black grass jelly is a traditional plant that is more widely used as medicine to prevent carcinogenesis, diabetes, and hypertension, to overcome diarrhea, because it is antimutagenetics, stabilizers, and immunomudulators, besides black grass jelly also contains antioxidants. This study examines changes of temperature and solvent ratio toward the composition of phenols, tannins, and flavonoids which are antioxidant constituents. Analysis of phenols and tannins uses folin quality reagents, while analysis of flavonoids uses the spectrophotometer method. The change of temperature and solvent ratio gives effect to change of phenol, tannin, and flavonoids amount. Optimal extraction of phenol, tannin and flavonoids using the response surface methodology (RSM) occur at a temperature range of 94 -100°C with a solven ratio of 23-24 ml/g.

1. Introduction
Black grass jelly (Mesona palustris BL) in the Java is known as janggelan, a traditional food that is used as folk medicine. In China and Taiwan, this plant is known as hasian-tsao (Mesona procumbens Hemsl), which is used to prevent heat illness, hypertension and diabetes [1,2]. As a local plant, black grass-jelly can be used as a functional food.

Black grass jelly is a plant of Lamisaceae family that has a height of 30-60 cm and grows at an altitude of 150-1800 above sea level. It has been owned by this plant containing bioactives which are beneficial to the body [3]. Besides having bioactive ingredients, the black grass jelly plant also has benefits to prevent carcinogenesis, antiosidative, antimutagenetic, hepatoprotector, antioxidants, immunomodulators, antidiarrhea, antidiabetic and antihypertensive [3-8]. Black grass jelly plan contains polyphenol compounds which function as anti-cancer substances and are easily digested gels [9,10].
Black grass jelly has a phenolic content [11]. Phenol is an antioxidant that can protect cells from oxidative stress, namely the imbalance between relative oxygen species and the antioxidant ability of the body's antioxidants. Oxidative stress can cause tissue damage in diabetics. Antioxidant treatment can have the effect of hyperglycemia [12]. Antioxidants are compounds as having one or more hydroxyl groups on the aromatic ring, where derivatives of these antioxidants contain more flavonoids, alkaloids, tannins, and other phenolic mixtures [13].

Besides containing bioactive compounds, black grass jelly leaf also contains energy, carbohydrates, protein, fat, calcium, phosphorus, iron, vitamins, and water [14]. The main present content in black grass jelly is pectin, which is a low methoxy [15]. Pectin is a food fiber that is soluble in water, which can reduce blood fat and glycemic response [16].

Extract of black grass jelly uses 3 types of solvents, namely ethanol, ethyl acetate and water to determine the effect of the cytotoxic effects. The black grass jelly extraction was carried out for 2 hours with a solvent ratio of 20 mL/g. Then the extract is concentrated using an evaporator at a pressure of 34-36 kPa. The results of the study, black grass jelly extract has anticancer potential [17].

Based on several previous studies, black grass jelly has a bioactive composition and antioxidants. Specifically, the types of materials, bioactives, and antioxidants found in the extract, black grass jelly needs to be studied further. Therefore this study aims to study the effect of solvent and temperature ratio on the content of phenol, tannin and flavonoid compounds in black grass jelly extract, besides testing using RSM has not been done to study the effect of two types of independent variables on changes in phenol, tannin and flavonoid amount.

2. Material and Methods
2.1. Ingredients
The ingredients used are dried black grass jelly leaves obtained by black grass jelly farmers in Pakis Baru village, Nawangan District, Pacitan Regency, East Java, while the analysis material used are folin-ciocalteu (Merek), quercetin (Merek), gallic acid reagents . (Merek), aluminum chloride (Merek), Na₂CO₃, oxalic acid, sulfuric acid, sodium nitrite, and sodium hydroxide.

2.2. Extraction
The research was conducted in two stages, namely extraction and analysis of results. The ingredients of black grass jelly are dry, then dried and mashed to 40 mesh. Then, the material is extracted by using water for 120 minutes and temperatures 80, 90, 100°C. The remaining solids are squeezed for 3 minutes to increase the amount of comparison coming out of the material [18, 19]. Determination of optimal temperature is carried out at a solvent ratio of 24 mL/g. Then, the extraction is carried out to analyze the optimal solvent ratio, at optimal temperature.

2.3. Analysis
- Determination of total phenolic
The total phenolic from the extract was estimated using the folin-ciocalteu method with gallic acid standard [20]. The reaction mixture consists of 1 ml of extract diluted with 9 ml distilled water. 1 ml of folin-ciocalteu was treated to the mixture and 7% of sodium carbonate solutions was treated to the mixture, after 5 minutes. Standard solution of gallic acid (10, 20, 30, 40 and 50 μg/ml) were prepared,
then incubated for 90 minutes at room temperature [21]. Absorbance for test and standard solutions were determined against the reagent blank at 550 nm with a visible spectrophotometer.

- **Determination of tannin**
  The tannins were determined by the Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of tannic acid (10, 20, 30, 40, 50 μg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/Visible spectrophotometer [21].

- **Determination of flavonoid**
  Total flavonoid content was measured by the aluminium chloride colorimetric. The reaction mixture consists of 1 ml of extract and 4 ml of water. 0.3 ml of 5% sodium nitrite was treated and after 5 minutes, 0.3 ml of 10% aluminium chloride was mixed. After 5 minutes, 2 ml of 1 M Sodium hydroxide was treated and diluted to 10 ml with water. A set of reference standard solutions of quercetin (10, 20, 30, 40 and 50 μg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer [22].

### 3. Result and Discussion

Extraction efficiency is determined by the five parameters issued are temperature, time, solvent ratio, size of material and type of solvent. These parameters can be either independent or interactive effects [23]. This study uses an independent variable solvent ratio (x) and temperature (z). While the interactive variables are analyzed are phenol (y₁), tannin (y₂), and flavonoids (y₃). Based on the equations from standard graph, the concentration of phenol, tannins and flavonoids obtained in the Table 1.

**Table 1. Amount of phenol, tannin, and flavonoid toward Changes of temperature and solvent ratio**

| Solvent Ratio (mL/g) | Temperature (°C) | Phenol (mg GAE/mL) | Tannin (mg GAE/mL) | Flavonoid (mg Qc/mL) |
|---------------------|------------------|--------------------|--------------------|----------------------|
| 18                  | 100              | 0.06586            | 0.03957            | 0.14347             |
| 20                  | 100              | 0.06390            | 0.03817            | 0.12109             |
| 22                  | 100              | 0.06915            | 0.04113            | 0.11254             |
| 24                  | 100              | 0.07502            | 0.05008            | 0.13479             |
| 24                  | 90               | 0.05910            | 0.04442            | 0.12902             |
| 24                  | 80               | 0.06203            | 0.03678            | 0.10193             |

3.1. **Phenol**

Based on Table 1, it can be seen that the phenol content increases at a ratio of 20-24 mL/g, while the decrease in phenol amount occurs at the solvent ratio of 18-20 mL/g. This increases the nature of phenol which is easily dissolved in water [24, 25], while the decrease in other effects of black grass jelly
extraction. Increased temperature causes changes in the questionnaire to changes in phenol [25, 26]. This happened because the temperature on phenol causes two optimum extraction peaks. At temperatures more than 100°C there is an optimum global phenol extraction.

The regression test of order 2 polynomial was used to determine the changes in temperature and ratio of changes to phenol amounts. The temperature and solvent ratio have a significant effect on the probability of 0.009. While the temperature and solvent ratio did not give a significant effect, the square of the temperature and the ratio of solvents had a significant effect on changes in phenol amounts. The use of this regression model shows that changes in temperature and ratio have a very strong influence on changes in phenol levels, this is a multiple correlation value (R) which approaches 1. The value of R this regression model is 0.9932, with the correlation between temperature and the number of seafarers on the phenol level is mathematically written as follows:

\[ y_1 = -0.0121z + 0.005x^2 + 0.0001z^2 - 0.0002xz + 0.4947 \]  

(1)

Based on the mathematical equation, the correlation between phenol amount, temperature and solvent ratio was connected in 3 dimensions. The results of analyzed variables indicate that phenol amount increase with increasing amounts of solvents. This is in accordance with the properties of phenol compounds dissolved in water [26].

At 80°C, the increase of phenol amount from the changes of solvent ratio showed a less significant increase. While the temperature of 100°C the solvent ratio showed a significant change in changes in phenol amounts. This happens because the temperature and ratio of solvents have a strong influence on changes in phenol levels. This result shows that the maximum condition of phenol occurs at 100°C and the solvent ratio is 24 mL/g. While the minimum content occurs at 85°C and the solvent ratio is 19 mL/g. Unlike the maximum conditions that exist in maximum operating conditions, the minimum requirements at a point in Figure 1.

![Figure 1. Correlation temperature and solvent ratio toward phenol amount](image-url)
3.2. Tannin

Tannins is a group of constituents of antioxidants that dissolves in the water, but it dissolves in the water due to a bitter taste. Tannin is compound that decomposed at 2100°C. Based on the data obtained from Table 1 obtained from tanning levels increasing with temperature and solvent ratio [25, 27]. This happened because tannins dissolve easily in water and decomposes at high temperatures.

The regression method of order 2 polynomial is used to determine the correlation between temperature, and the ratio of solvents to tannin levels. This regression method is very significant because it provides a probability value of 0.00002, when compared with a linear regression model that gives a probability value of 0.075. Based on the regression method of order 2 polynomial, it was found that the temperature and ratio of the solvent simultaneously had a significant effect toward changes of tannin amount. While in parallel only the temperature and the solvent ratio have a significant effect. The temperature and ratio have a very strong influence toward changes of tannin because the R value of this regression model is 0.9994 with a constant correlation (R²) of 0.9988. While the mathematical correlation of the regression model is written as follows.

Based on the equation of Figure 2 which represents a 3-dimensional correlation between temperature and the ratio of solvents toward tan amount. At 80°C, the effect of increasing the solvent ratio is not significantly increased on tannin amount, while at 100°C, there is a significant increase in the amount of the solvent ratio of 22-24 mL/g. This result shows that the maximum extraction conditions are 100°C and a solvent ratio of 24 mL/g.

\[ y_2 = 0.0086z + 0.00065x^2 - 1.03E^{-0.5}z^2 - 0.00025xz - 0.471 \]  

\[ (2) \]

**Figure 2.** Correlation temperature and solvent ratio toward tannin amount

3.3. Flavonoid

Table 1 shows an increase in temperature which causes an increase in flavonoid amount [24, 25, 28]. The increase of solvent ratio causes changes toward changes of flavonoid amount [24]. These results prove
that not all antioxidants combinations are dissolved in water. From these results it can be seen that the increase in temperature causes an increase in flavonoid amount, but increases in the solvent ratio does not always increase flavonoid amount.

The regression method of order 2 polynomial is used to determine the correlation between temperature, and the ratio of solvent toward flavonoid amount. The temperature and the solvent ratio simultaneously have a very strong influence on flavonoids, this causes the R value = 0.9937. While the regression model shows a significant difference and the solvent ratio has a significant effect on the probability of 0.007. While the temperature and solvent ratio squared have a significant effect toward changes of flavonoid amount. From this regression model is obtained mathemetic correlation between temperature and solvent ratio toward flavonoids amount as follows:

\[ y_3 = 0.05z + 0.0028x^2 - 0.0001z^2 - 0.0012xz - 2.51984 \]  

(3)

From this mathematical equation, it can be known that realistic between temperature and solvent ratio toward flavonoids the amount in a 3-dimensional plot The result, the maximum extraction conditions occur at 100°C and the solvent ratio is 18 mL/g. This result indicates that an increase of temperature causes an increase of amount, while the increase of solvents ratio does not cause an increase in flavonoid amount. Minimum flavonoid amount occurs at 80°C with a solvent ratio of 20-22 mL/g (Figure 3).

![Figure 3. Correlation temperature and solvent ratio toward flavonoid amount](image)

3.4. Analysis Regresion

Regression analysis was conducted to determine the accuracy of the use of regression model of order 2 polynomial toward the results of the analysis of the effect of temperature and solvent ratio of phenol, tannin, and flavonoid amount. The use of this regression model gives a value of R and R² above 0.98. Then, the standard error value of the regression model also has a relatively small value of less than 0.005.
Based on R value of square found in Table 2 shows that the magnitude of the independent variables and influences changes in interactive variables. The accuracy of the regression model is very accurate used to determine unknown data. Based on the adjusted square values, there are several other factors that influence the extraction of phenols, tannins, and flavonoids from black grass jelly.

**Table 2. Regression statistic**

| Phenol | Multiple R | 0.9931612 |
|--------|------------|-----------|
|        | R Square   | 0.9863691 |
|        | Adjusted R Square | 0.9318459 |
|        | Standard Error | 0.0014726 |
|        | Observation  | 6         |
| Tannin | Multiple R | 0.999434  |
|        | R Square   | 0.998868  |
|        | Adjusted R Square | 0.994340 |
|        | Standard Error | 0.000267 |
|        | Observation  | 6         |
| Flavonoid | Multiple R | 0.993714  |
|        | R Square   | 0.987468  |
|        | Adjusted R Square | 0.937341 |
|        | Standard Error | 0.003792 |
|        | Observation  | 6         |

The test model is shown in Table 3. The diversity of statistical models occurs significantly in the calculated less than 0.01 with the magnitude (F) shown in Table 3. From these results it can be used for the use of second-order polynomial regression models, which are used for comparison with temperature and ratio of solvents with phenols, tannins, and flavonoids.

**Table 3. Analysis of variance (ANOVA)**

| Phenol | df | SS   | MS    | F     | Significance F |
|--------|----|------|-------|-------|----------------|
|        | 4  | 0.000156 | 3.92E-05 | 18.09 | 0.174331 |
|        | 1  | 2.17E-06 | 2.17E-06 |       |                |
|        | 5  | 0.000159 |       |       |                |
| Tannin |

The diversity of statistical models occurs significantly in the calculated less than 0.01 with the magnitude (F) shown in Table 3. From these results it can be used for the use of second-order polynomial regression models, which are used for comparison with temperature and ratio of solvents with phenols, tannins, and flavonoids.
The optimization model used is the selection of the maximum area conditions with the contour model. The optimal condition is determined based on the conditions of the amount of phenol, tannin, and flavonoids at high concentrations, namely the conditions shown in red on the contour. Figure 4 shows the correlation between temperature and solvents ratio toward amount of phenol, tannins, and flavonoids. Based on Figure 4, the optimum conditions for extracting phenol, tannin, and flavonoids from black grass jelly occur in the temperature range of 94-100°C and the amount of solvent is 23-24 mL/g.

Based on the results of Figure 4, it is known that the distribution of maximal extraction conditions occurs in several variations of the solvent ratio. But in general all maximum conditions occur at 100°C. Thus, it can be seen that the increase in temperature causes an increase in the amount of phenol, tannins, and flavonoids. But the increase in the solvent ratio does not cause an increase in flavonoid levels. Nevertheless the determination of optimal extraction conditions by using this contour model does not show optimal conditions that produce an optimal condition. The optimal condition is chosen according to the maximal conditions which gave the condition of phenol amount more than 0.07 mg GAE/mL, tannins more than 0.05 mg GAE/mL, and flavonoid levels more than 0.123 mg Qc/mL.
4. Conclusion

Based on the Response Surface Methodology (RSM) analysis, it is known that the maximum amount of phenol and tannin occurs in the same conditions, but the maximum amount of flavonoids occurs at the solvent ratio of 18 mL/g. Overall, the increase in temperature causes increases in the amount of phenol, tannins, and flavonoids. With the contour model, it can be seen that the optimal extraction conditions occur in the temperature range of 94-100°C and the ratio of solvent ratios is 23-24 mL/g, while the minimum extraction conditions occur in the temperature range 80 - 84°C with solvent ratio 19-21 mL/g.

5. Acknowledgment

Thanks for the Institute for Research and Community Service (LPPM) of the Muhammadiyah University of Surakarta, which has funded the research process.

References

[1] Harjadi D P, dan Bangun P Nuswantoro. 2002. Purification og gel forming component extracted from janggelan (Mesona palustris BL) and characterization of the resulted gel. Seminar PATPRI

[2] Ruhayat A. 2002. Cincau hitam tanaman obat penyembuh. Penebar Swadaya Indonesia

[3] Tri Dewanti Widyaningsih, dan B T F Sari. 2017. International Journal of Chem Tech Reserc 10 45
[4] L S Lai, S T Chou, and W W Chao. 2001. *J. Agric. Food Chem*., 49: 96

[5] C G Yen, P D Duh, and Y L Hung. 2001. *J. Agric. Food Chem*., 49: 5000

[6] Tri Dewanti Widyaningisih, A P Sukardiman, Djoko and D Win. *J. Technol. Food Ind.*, 23: 29

[7] C T Yeh, W H Huang, and G C Yen. 2008. *J. Nutr. Biochem*

[8] Tri Dewanti Widyaningisih, E Martanti, and D M Lukitasari. 2017. *Asian Journal of Pharmaceutical and Clinincal Research*, 10: 326

[9] S U Nudin, R K L Liu, G P Young, J C R Stangouliis, C T Christophersen, and C A Abbott. 2017. *Nutrients*, 9: 355

[10] J Lim, S Adisakwattana, and C J Henry, 2018. *Asia Pac J. Clin Nutr*. 27: 336

[11] T D Widiyaningsih, J Mahar, N Wijayanti, Rahmani, and M Najmuuddin. 2013. *Food and Public health*, 3: 142

[12] R Amalia, and T D Widyansih. 2014. *Jurnal pangan dan agroindustri*, 2: 28

[13] M Sayuti, dan R Yenrina. 2018. *Antioksidan alami dan sintetik*. Andalas University Press. Indonesia

[14] T D Widyaningisih, S D Wijayanti, and N Tisnaningrum. 2018. *International Journal of Phytomedicines and Related Industries*, 10: 185

[15] T D Widyaningisih, and P Adilaras. 2008. *Journal of food since and technology*, 10: 1390

[16] S A Moharib, and S A El-Batran. 2008. *Reserch Journal of Agricultural and Biological Science*, 4: 455

[17] T D Widyaningisih. 2012. *ACPBEE Procedia*, 2: 110

[18] T D Widyaningisih, and R M Safitri. 2014. *J. Agroteknologi*. 8: 74

[19] B P Nuswantoro, and Haryadi. 2003. *Agritech*, 23: 28

[20] S Tabasum, S Khare, and K Jain. 2016. *Asian J. Pharm Clin. Res.*, 9: 371

[21] C K Chandran, and G Indira. 2016. *Journal of Medicinal Plants Studies*, 4: 282

[22] V D Tambe, and R S B Gambar. 2014. *Journal Of Pharmacognosy And Phytochemistry*, 9

[23] Montgomery. 2001. *Design and analysis of experiments*. John Wiley & Sons. New york

[24] M Radojkovic, Z Zekovic, S Jokic, and S Vidovic. 2012. *Romanian Biotechnological Letters*, 17: 7295

[25] E N Fombang, and W R Saa. 2016. *Journal of Nutrition & Food Sciences*, 6: 1

[26] A Rajaeni, M Barzegar, Z Hamidi, and M A Sahari. 2010. *J. Agr. Sci. Teach*, 12: 605

[27] R Tabraki, A Safari, and A Y Faal. 2013. *Journal of Applied Chemical Research*, 7: 67

[28] H N Rajha, N El Darra, Z Hobaika, N Baussetta, E Vorobiev, R G Maroun, and N Louk. 2014. *Food and nutrition sciences*, 5: 397