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**Original Research Article**

# Recovery of phenolic-rich extracts from *Handeuleum* leaves using mixture design modeling

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## Abstract

***Purpose:*** *To determine the best solvent for extracting phenolic compounds from Handeuleum leaves, which are rich in phenolic compounds.*
***Methods:*** *In optimizing the extraction solvent, the simplex centroid mixture design was used together with the extractor components, namely water, acetone, methanol, and ethanol, which were designed to produce 15 formulas. Total phenolic analysis was carried out based on the Follin-Ciocalteu method, and the results were expressed in milligrams of gallic acid equivalent per gram of dry weight of the sample.*
***Results:*** *The quadratic model with an $R^2$ value of 0.8407 is relied upon for total phenolic recovery. The best-mixed formula yielded a desirability value of 0.913 with a quarternary mixture of water (0.6910), acetone (0.3054), methanol (0.0002), and ethanol (0.0031) as solvents, obtaining a total phenolic content of 2.9613 mg GAE/g dry weight. The optimal condition validation results are also stated to be very accurate, with a residual standard error (RSE) of 0.905 %.*
***Conclusion:*** *The optimized solvent combination for extracting high amounts of phenolic compounds from Handeuleum leaves has been reported which may be upscaled for commercial purposes.*

***Keywords:*** *Solvent, Handeuleum, Phenolic, Mixture Design, Graptophyllum pictum*



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## INTRODUCTION

Bioactive compounds derived from plants are believed to have health benefits. One of them is phenolics which are mostly commonly found in every plant, especially the leaves. Phenolics play an important role in providing health-enhancing effects due to their antioxidant properties [1], anti-diabetic effect, antibacterial, cytotoxic [2], anticancer [3], anti-inflammatory [4], and diseases associated with oxidative stress [5]. A large number of phenolic compounds have different structures, and this determines their solubility in solvents with different polarities, so the extraction process has a very significant effect on obtaining the desired phenolic compounds [6]. Polar and non-polar solvents offer many choices because of their varying phenolic characteristics. Previous studies have used ethanol, methanol, hexane, acetone, and water as solvents for extracting phytochemicals.

There are several reports relating to optimizing the extraction of phenolic content. Previous study on J*uniperus procera* leaves used methanol as the best solvent [7]. However, acetone water was used on the fruit of the dates obtained. This shows that each plant matrix will exhibit different

optimal results [8]. *Graptophyllum pictum*, also known as *Handeuleum*, is part of the Acanthaceae family. This herbal plant is used as a traditional medicine, especially its leaves. *Handeuleum* empirically has antioxidant, antibacterial, anti-inflammatory, and anti-diabetic properties. Besides these activities, *Handeuleum* is a rich source of phenolic compounds [9], steroids, tannins, saponins, alkaloids, flavonoids [10], essential oils in the form of hexahydro farnesyl acetone, phytol, n-nonaosane [11], vomifoliol, and triterpenoids [12]. The diversity of these compounds holds potential for the development of drugs for health. This study is the first attempt to identify the best solvent for the extraction of phenolic compounds using mixture design modeling.

## EXPERIMENTAL

### Sample preparation

*Handeuleum* leaves or *Graptophyllum pictum* were grown in June 2022 at the LPPM IPB's Tropical Biopharmaceutical Cultivation Conservation Unit in Dramaga (6º3'49" S and 106º42'57"t). The leaves were collected, rinsed with running water, and dried for three days in an oven at 50 °C. The dried leaves were crushed with a disk mill and sieved through an 80-mesh sieve.

### Mixture design optimization

The optimization of solvent extraction was done using a simplex centroid method from Design Expert 13.0 software (Stat-Ease Inc, Minneapolis, USA). Independent variables include single solvents (ethanol, methanol, acetone, and water), binary mixtures, ternary mixtures, and quaternary mixtures (Figure 1). The dependent variable is the concentration of total phenolic content. Because each component of the mixture was in the proportion range of 0 to 1, the appropriate model was picked for each response. Figure 1 below shows a graph that includes the symbols from the design results consisting of A (water); B (acetone); C (methanol); and D (ethanol) for a single solvent, AB; BC; CD; AD; AC for binary mixture, and ABCD for a quaternary mixture.

### Sample extraction

Dry *Handeuleum* leaves (4 g) were mixed with the solvent following the simplex centroid design formula (Table 2) with a total volume of 40 mL (1:10 b/v). The sample was sonicated to mix for 30 mins and then macerated using a water bath shaker (DAIHAN Wise bath) for 3 h at 30 °C. The mixture was concentrated with a rotary evaporator (Hanvapor HS-2005V, Hanshin Scientific, Korea). The resulting filtrate was obtained and prepared at a concentration of 0.2 g/mL.

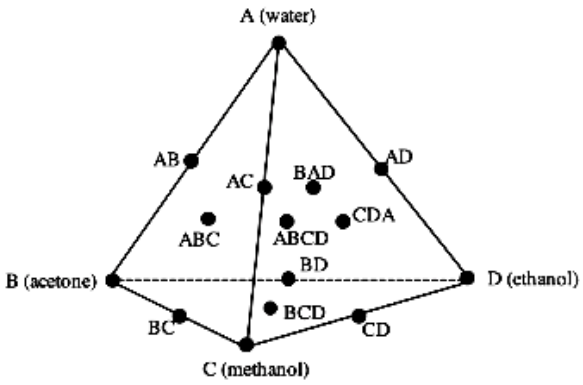


**Figure 1:** Simplex centroid design in optimizing solvent extraction of phenolic content through the design expert program

### Evaluation of parameter/indices

#### *Total phenolic content*

The extracted sample (20 µL) was transferred into 96-well microplate, and 120 µL of 10 % Follin-Ciocalteu solution was added and incubated for 5 min. Thereafter, 80 µL $Na_2CO_3$ was added and incubated for 30 min. Absorbance was measured using microplate reader (Spectrostar Nano, BMG Labtech, Ortenberg, Germany) at 759 nm. Gallic acid values ranging from 20 to 300 ppm were used in the calibration curve. Total phenolic content concentration was represented in milligrams dry weight of *Handeuleum* leaves (DW) as comparable to gallic acid (GAE).

### Data analysis

Statistical data testing based on one-way analysis variance with Duncan's further test (confidence level 95 %) was carried out. Optimization was done using Design Expert program (Stat-Ease Inc, Minneapolis, MN).

## RESULTS

### Model fitting and ANOVA

For the simplex centroid design modeling, 15 trials (runs) were obtained resulting from the Design Expert program (Table 2). The best-suggested model was the quadratic model with the fit statistic shown in Table 1($p$ > 0.05, $F$-value of 2.93). The $R^2$ coefficient value was 0.8407 or 84.07 %, the $R^2$ (an indicator of the extent to

which the experimental factors and their interactions may account for the observed response value's variability), CV. The model is said to be getting better when $R^2$ is close to 1.00 and when the $R^2$ is smaller it indicates an irrelevant model. Coefficient of variation (CV) is a comparison between the level of precision and the experiment, the higher the CV, the lower the reliability of the experiment. In this study the CV obtained was 13 %. Furthermore, the Adeq precision of 5.5691 indicates an adequate signal if the value exceeds 4.00, where this parameter is used as an evaluation of the model in navigating the design space.

**Table 1:** ANOVA summary of quadratic model for total phenolic content

| Fit Statistics | Value |
|---|---|
| P | 0.1243 |
| F | 2.93 |
| $R^2$ | 0.8407 |
| Adjusted R2 | 0.5541 |
| Mean | 2.38 |
| CV % | 13.00 |
| Adeq Precision | 5.5691 |
| Standard Deviation | 0.3095 |

Significant at $p < 0.05$

## Effect of the solvent system

Each treatment resulted in a response value of total phenolic concentration that was significantly different ($p < 0.05$). Total phenolic content of *Handeuleum* leaf extract ranged from 1.608 to 3.089 mg GAE/g dry weight. The solvent used was polar, and the results obtained depended on the chemical characteristics of the phenolic structure found in *Handeuleum* leaves.

## Contour and surface response plots

The contour plot (Figure 2) shows that phenolic recovery is optimal with the formulation of a quaternary mixture or a mixture of water, acetone, methanol, and ethanol with a total phenolic value of 3.089 mg GAE/g dry weight. This result was also not significant with the ternary mixture of water-methanol-acetone with a value of 2.970 mg GAE/g dry weight. The polynomial equation represents the synergy of the solvent effect in Eq 1.

Total phenolic content = 2.43A + 1.52B + 1.87C + 2.21D + 3.83AB + 2.42AC + 1.24AD + 0.5379BC + 0.2899BD + 2.13CD ……. (1)

The polynomial equation above represents the quantitative effect of factor interactions on total phenolic response by comparing the factor coefficients. A positive sign implies that there is a synergistic interaction impact, whereas a negative sign suggests that there is antagonistic interaction effect. It was observed from the equation that the factors involved gave a synergistic effect, namely the binary mixture of water and acetone was the highest, followed by water, then ethanol. While the smallest synergistic effect is a mixture of binary acetone and methanol

## Optimal conditions for improving phenolic extraction

Results of the total phenolic response were optimized to obtain desirability values. The final optimal conditions obtained showed a desirability of 0.913 with a total phenolic of 2.9613 mg GAE/g dry weight.

**Table 2:** Observed response of the total phenolic content with mixture design simplex centroid

| Run | Solvents | | | | Total phenolic content (mg GAE/g dry weight) |
|---|---|---|---|---|---|
| | *A* | *B* | *C* | *D* | |
| 1 | 0 | 0 | 0.5 | 0.5 | 2.589±0.12$^{b}$ |
| 2 | 0.5 | 0 | 0.5 | 0 | 2.644±0.13$^{b}$ |
| 3 | 0 | 0 | 0 | 1 | 2.235±0.09$^{c}$ |
| 4 | 0 | 0.5 | 0 | 0.5 | 1.826±0.13$^{de}$ |
| 5 | 0.5 | 0.5 | 0 | 0 | 2.692±0.29$^{b}$ |
| 6 | 0 | 0 | 1 | 0 | 1.926±0.14$^{d}$ |
| 7 | 0.5 | 0 | 0 | 0.5 | 2.629±0.07$^{b}$ |
| 8 | 0 | 0.33 | 0.33 | 0.33 | 2.188±0.03$^{c}$ |
| 9 | 0 | 0.5 | 0.5 | 0 | 1.608±0.13$^{e}$ |
| 10 | 0.25 | 0.25 | 0.25 | 0.25 | 3.089±0.02$^{a}$ |
| 11 | 1 | 0 | 0 | 0 | 2.483±0.01$^{b}$ |
| 12 | 0.33 | 0.33 | 0.33 | 0 | 2.970±0.08$^{a}$ |
| 13 | 0.33 | 0.33 | 0 | 0.033 | 2.673±0.18$^{b}$ |
| 14 | 0 | 1 | 0 | 0 | 1.610±0.22$^{e}$ |
| 15 | 0.33 | 0 | 0.33 | 0.33 | 2.546±0.11$^{b}$ |

***Note:*** A: water; B: acetone; C: methanol; D: ethanol; mg: milligram; GAE: gallic acid equivalent. Mean values followed by different superscripts (a, b, c, d, e) indicate significant differences at a 95% confidence level based on Duncan's further test

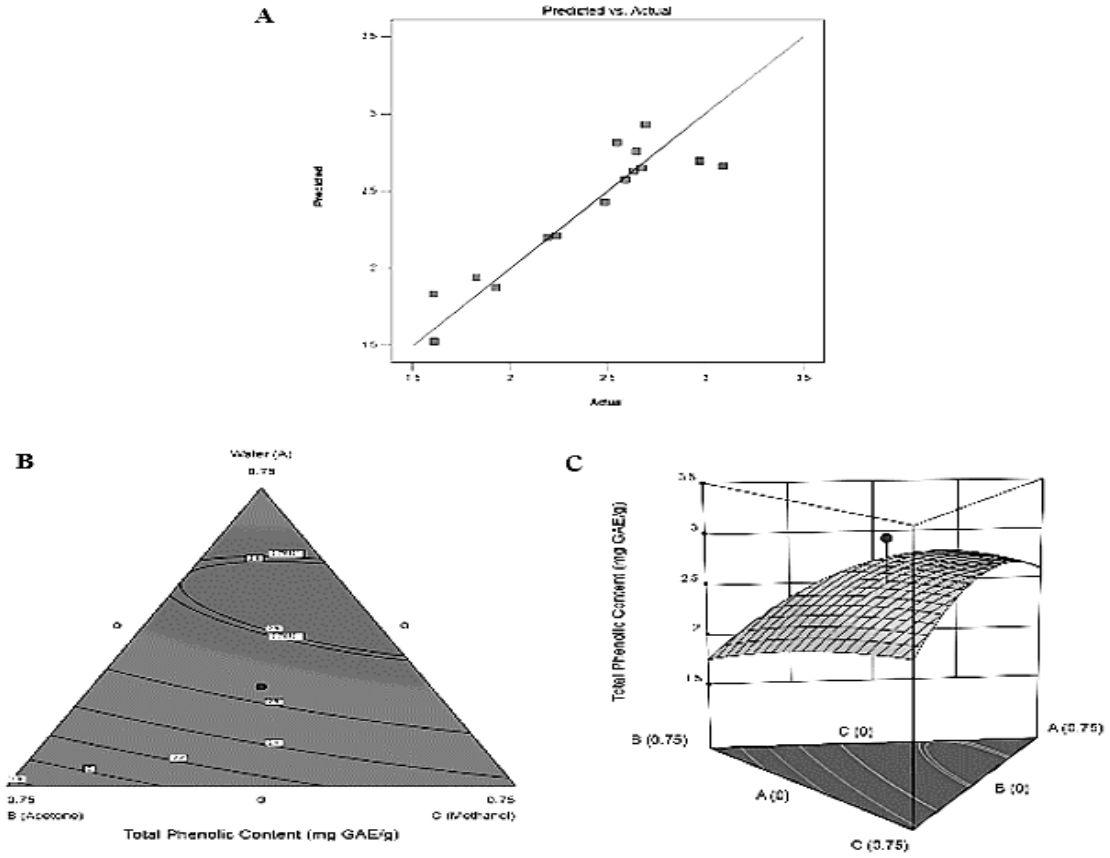


**Figure 2:** (A): Predicted versus actual plot, (B): contour plot, and (C): 3-D surface plot of the effect of four components solvents on total phenolic contents

**Table 3:** The estimated optimal conditions for extraction with desirability value

| Solvent | Formulation | Total phenolic content (mg GAE/g DW) | Desirability value |
|---|---|---|---|
| Water | 0.6910 | 2.9613 | 0.913 |
| Acetone | 0.3054 | | |
| Methanol | 0.0002 | | |
| Ethanol | 0.0031 | | |

**Table 4:** Predicted and actual response values for the optimized extraction

| Solvent | Formulation | Predicted value of TPC (mg GAE/g DW) | Actual value of TPC (mg GAE/g DW) | RSE (%) |
|---|---|---|---|---|
| Water | 0.6910 | 2.9613 | 2.9881 | 0.905 |
| Acetone | 0.3054 | | | |
| Methanol | 0.0002 | | | |
| Ethanol | 0.0031 | | | |

### Validation of the optimal condition

The validation stage uses the percentage of residual standard error (RSE) to assess the suitability of ideal conditions at the end of phenolic extraction. The validation results obtained an actual value of 2.9881 mg GAE/g dry weight, and RSE was 0.905 %. These results explain that the model applied in this study is very adequate (Table 4).

## DISCUSSION

This study investigated the effect of extraction solvent in obtaining the best levels of phenolic compounds through a mixed design approach, namely the simplex centroid design. Selectivity of the extraction of phenolic compounds is achieved with different solvents or mixed solvents by modeling the simplex centroid mixture design. This design involves water, ethanol, methanol,

and acetone as solvents. The four types of solvents were designed to produce 15 treatments which include pure solvents, binary mixtures, ternary mixtures, and quaternary mixtures. This technique is based on statistics which are usually used to improve performance and produce optimal processes [13]. Measurement of total phenolic levels according to the Folin-Ciocalteu method forms a blue complex (phosphotungstate-phosphomolybdenum), measured using spectrophotometer at 759 nm. This method is well-known and widely used because of its accuracy, specificity and sensitivity [14]. Recovery of phenolic compounds depends on the solvent used in the extraction and its polarity [15]. Water, methanol, ethanol, and acetone have different polarity indices. However, water is the most polar solvent, followed by methanol and ethanol, while acetone is the least polar solvent. Phenolics extracted using a less polar solvent gave a smaller total phenolic value compared to a more polar solvent with varying proportions of water content. This means that the phenolic compounds extracted would be mostly polar.

The results of the total phenolic response are optimized to obtain desirability values. This value is an evaluation of the level of confidence from the optimal conditions of the extraction process based on the mixture design modeling, a value closer to 1.00 indicates the highest level of confidence from the optimization results. In the final optimal, various solutions provided by the Design Expert program were produced, then the solution that showed the highest desirability value was selected and then validation was carried out on the formulation obtained. An RSE value of less than 5 shows that there is no significant difference between the actual and projected values, indicating that the model developed in this study is quite acceptable [14].

It is known that water gives a better effect in pure solvent conditions and with other solvent mixtures as evidenced by a higher total phenolic value. Water has the highest polarity index, while acetone has the lowest among the test solvents used [16]. This is supported by findings from previous studies [13].

High phenolic extraction in water-miscible solvents is possible because water helps the diffusion of extractable compounds through plant tissues [17]. The use of mixed solvents is better than a single solvent for extracting phenolic compounds in plant samples [18]. However, using a different plant matrix, a previous study revealed that the highest recovery of phenolic compounds was produced by a mixture of several organic solvents compared to a single solvent [19]. Furthermore, a ternary mixture of water, ethanol, and methanol gave simultaneous results in total phenolic extraction from *Pimpinella anisum* [20]. This shows that the optimizing technique using a mixture design is the right thing in extracting bioactive compounds, this technique is considered to shorten the time in the optimizing process.

## CONCLUSION

This study describes the variance of the data better through a quadratic model, and a quaternary mixture of water, acetone, methanol, and ethanol as the optimal condition for increasing phenolic extraction with a high desirability. Validation of optimal conditions shows a high level of accuracy with a lower percentage residual standard error. This study provides commercially valuable information for the future development of phenolic compounds from Handeuleum leaves.

## DECLARATIONS

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***Ethical approval***

None provided.

***Availability of data and materials***

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

***Conflict of interest***

No conflict of interest is associated with this work.

***Contribution of authors***

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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