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# Original Research Article

## Optimization of *Ficus deltoidea* Using Ultrasound-Assisted Extraction by Box-Behnken Statistical Design

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# Optimization of *Ficus deltoidea* Using Ultrasound-Assisted Extraction by Box-Behnken Statistical Design

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**Abstract**
In this study, the effect of extraction parameters (ethanol concentration, sonication time, and solvent-to-sample ratio) on *Ficus deltoidea* leaves was investigated using ultrasound-assisted extraction by response surface methodology (RSM). Total phenolic content (TPC) of *F. deltoidea* extracts was identified using Folin-Ciocalteu method and expressed in gallic acid equivalent (GAE) per g. Box-Behnken statistical design (BBD) was the tool used to find the optimal conditions for maximum TPC. Besides, the extraction yield was measured and stated in percentage. The optimized TPC attained was 455.78 mg GAE/g at 64% ethanol concentration, 10 minutes sonication time, and 20 mL/g solvent-to-sample ratio whereas the greatest extraction yield was 33% with ethanol concentration of 70%, sonication time of 40 minutes, and solvent-to-material ratio at 40 mL/g. The determination coefficient, $R^2$, for TPC indicates that 99.5% capriciousness in the response could be clarified by the ANOVA model and the value of 0.9681 of predicted $R^2$ is in equitable agreement with 0.9890 of adjusted $R^2$. The present study shows that ethanol-water as solvent, a short time of 10 minutes, and adequate solvent-to-sample ratio (20 mL/g) are the best conditions for extraction.

**Keywords:** Ultrasound-assisted extraction; Total phenolic content; Folin-Ciocalteu reagent; Box-Behnken statistical design.

## 1. INTRODUCTION

*Ficus deltoidea* (family: Moraceae), also named as "Mas Cotek" in Malaysia, is a capable plant in therapeutics. It is reported that 50-80% of vast majority of inhabitants in advanced and progressing nations utilize some type of the imperative traditional treatments under Traditional, Complementary, and Alternative Medicine (TCAM) for counteractive action and improvement of well-being, depending on natural plants. For example, reduction of blood glucose level, antinociceptive, ulcer healing, antioxidant, anti-inflammatory, and antimelanogenic properties can be aided by *F. deltoidea* leaves [1]. Hence, conventional prescriptions from plants have pulled in real consideration overall as a result of their potential pharmaceutical significance [2].

Extraction is the first critical stride in readiness of plant formulations. The improvement of advanced extraction procedures with noteworthy focal points over customary routines for the extraction and investigation of medicinal plants is prone to assume a vital part in the general exertion of guaranteeing accessibility of great herbal products to consumers around the world [3].

Response surface methodology (RSM) is an accumulation of scientific and measurable strategies in experimental modeling [4]. Box-Behnken design is a potent model for optimization application. This design allows factors assessment of the quadratic model, sequential plan structure, identification of fit conditions for design, and blocks utilization [5]. It has similar properties as Central Composite designs but better in the way where it could overcome experimental limitations and evade extreme conditions.

The main objective of the present study is to optimize the extraction of *F. deltoidea* leaves using ultrasound-assisted extraction method by Box-Behnken statistical design. Ultrasound-assisted method is selected for extraction due to the reasons of environment-friendly, low cost, and quickness. By referring to the result of this study, the potential for the development of *F. deltoidea* as an antioxidant product for application in pharmaceuticals in the future can be analyzed. Total phenolic content (TPC) of *F. deltoidea* extracts was identified using Folin-Ciocalteu method. To carry out the present work, the extraction parameters of ethanol concentration, sonication time, and solvent-to-sample ratio were chosen as the study aspects. Besides, some of the previous findings on optimum conditions of the factors were implemented in the present work in order to achieve the goal.

## 2. MATERIALS AND METHODS

### 2.1. Sample Preparation

The *F. deltoidea* leaves were procured from the Institute of Sustainable Agrotechnology Universiti Malaysia Perlis (UniMAP), Uniciti Alam, Perlis. Then, the *F. deltoidea* leaves were washed in tap water and dried using conventional-type laboratory-oven (Memmert, United States) for 48 hours at 40°C [6]. Next, the leaves were grinded using an electronic blender (Electrolux Sdn Bhd, PRC) into powder form. After that, the powder was sieved with AS 200 sieve shaker (Retsch GmBH, Haan, Germany) for constant particle size, 250 μm. Before conducting the experiment, the grinded and sieved powder was kept in a closely covered container.

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### 2.2. Extraction Solvent Preparation

The extraction solvent used, ethanol, was prepared at different concentrations 10, 30, 50, 70, and 90%. The total amount of extraction solvent prepared was 5 L. All the extraction solvents were prepared in volumetric flask of 1L.

### 2.3. Preliminary Experiment

In order to determine a particular range for optimization of the parameters, a preliminary experiment process was conducted. For each test, one parameter was manipulated to identify the significant effect of the specific factor on the result. The ranges for the selected parameters are as follows: ethanol concentration (10-90%), sonication time (10-50 minutes), and solvent-to-sample ratio (10-50 mL/g). Triplicate was carried out for a more precise result using the digital ultrasonic cleaner model PS-40A at constant temperature (60°C) and ultrasonic power (40 kHz). After the extraction process, the extracts underwent filtration and drying. Then, the average percentage of yield was calculated using the Equation (1):

$$\%\,\text{Yield} = \frac{W_{\text{extracts}}}{W_{\text{samples}}} \times 100\% \tag{1}$$

### 2.4. Standard Curve Preparation

The standard curve using gallic acid with concentration at 1, 0.2, 0.04, 0.008, and 0.0016 mg/mL was plotted. A sample absorbance measurement was conducted at 750 nm by Genesys 20 spectrophotometer (Thermo Electron Corporation, Madison, USA).

### 2.5. TPC Determination

The TPC determination was made by modified Dzolin *et al*. [7] method. Folin-Ciocalteau's reagent was used as the assay for determination of TPC. Firstly, 0.1 g of the extracts was weighed and dissolved in 100 mL of distilled water. Then, 0.1 mL of aqueous extracts was mixed with 0.15 mL of Folin-Ciocalteau's reagent and vortex for a minute, followed by 0.35 mL of 20% (w/v) saturated sodium carbonate ($Na_2CO_3$). The mixture was then incubated at 40°C for 30 minutes after being vortex for another 1 minute. A sample absorbance measurement was conducted at 750 nm by Genesys 20 spectrophotometer (Thermo Electron Corporation, Madison, USA). The absorbance obtained was proportional to the polyphenol concentration. The TPC was then calculated in mg gallic acid equivalent (GAE) per g using Equation (2) [7, 8]:

$$\text{TPC} = \frac{(\text{Absorbance of sample} - \text{Intercept})}{\text{Slope}} \div \text{Concentration of extracts} \tag{2}$$

### 2.6. Response Surface Methodology (RSM)

Box-Behnken statistical design (BBD) was the tool used in optimizing the *F. deltoidea* extraction conditions for each parameter. After running the software, the analysis recommended the number of experiments to determine the optimum conditions for the TPC. Every experiment was repeated twice for precise result. Then, the correlation relationship between the three parameters was determined from ANOVA and graphical analysis.

## 3. RESULTS AND DISCUSSION

### 3.1. Preliminary Result

*3.1.1. Effect of Ethanol Concentration*

Both the yield and TPC rise with the increase of ethanol concentration as shown in Figure 1. The extractable yield increases 3% between 10 and 50% ethanol concentration but drops 1% after attaining 31% at 70%. Meanwhile, TPC rises from 376.75 mg GAE/g till 430.17 mg GAE/g. However, it plunges after 70% ethanol concentration due to low water content. Accumulation of water content creates greater interaction of solvent with the internal wall of plant tissues that significantly increases the amount of extracts [9]. The highest extractable yield and TPC achieved are 31% and 430.17 mg GAE/g respectively at 70% ethanol concentration. Therefore, the suitable range for optimization is 50 to 90%.

*3.1.2. Effect of Sonication Time*

Figure 2 shows the effect of sonication time on the yield and TPC. The yield surges 5% within 20 minutes of extraction, and then it constantly falls till it reaches 20% of yield at 50 minutes. The peak of TPC for this parameter is 444.53 mg GAE/g at 20 minutes. TPC stops at 364.82 mg GAE/g, which is the lowest TPC obtained when the extraction time is extended to 50 minutes. It reveals a difference of 79.71 mg GAE/g from the peak of TPC. From the result collected, it is clear that the damage of plant cell is caused by the effect of acoustic cavitation by ultrasound as time is prolonged. According to Rajaei *et al.* [10], 20 minutes is sufficient for ultrasound-assisted extraction. Therefore, range of sonication time for optimization selected is between 10 and 30 minutes to avoid plant cell from damage and lead to less yield and TPC [10].

**Figure 1: Effect of ethanol concentration on yield and TPC.**

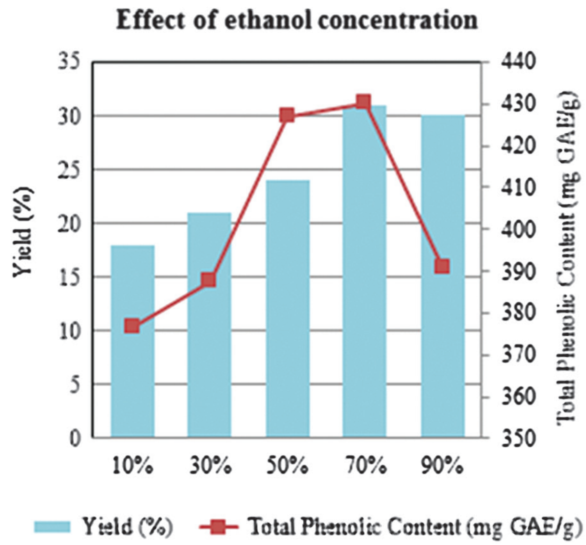



**Figure 2: Effect of sonication time on yield and TPC.**

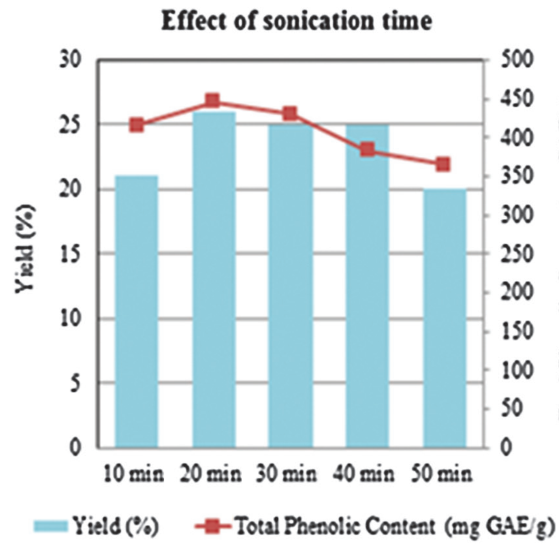
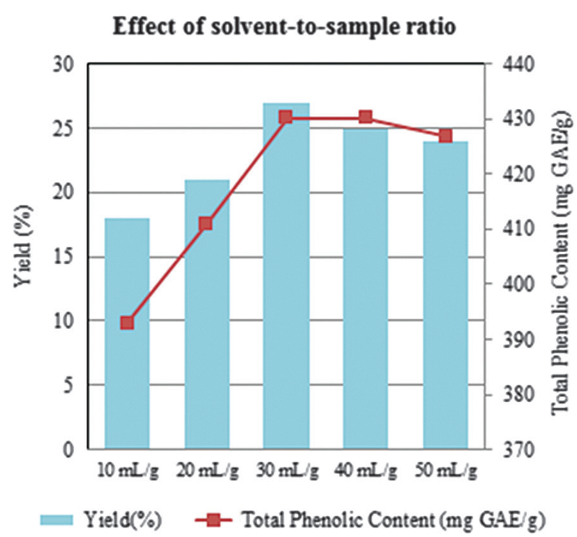



**Figure 3: Effect of solvent-to-sample ratio on yield and TPC.**




*3.1.3. Effect of Solvent-to-Sample Ratio*

Figure 3 shows the effect of solvent-to-sample ratio on yield and TPC. The amount of yield and TPC increases at the same time from 18% (392.90 mg GAE/g) at 10 mg/L till 27% (430.17 mg GAE/g) at 30 mg/L. However, the percentage of yield declines after 40 mg/L. This does not show a significant effect on TPC. This may be due to the fact that greater solvent-to-sample ratio will facilitate mass transfer by providing greater concentration gradient [11]. The adequate extraction solvent-to-sample ratio is 30 mL/g because the continuous addition of solvent will become saturated, resulting in the decline of TPC. The trend in present study is almost the same with the extraction of *Euryale ferox* seed shells using ultrasound [12]. Thus, the optimum range for selected percentage of yield is 20-40 mL/g.

**3.2. Optimization**

For the optimization study, Design Expert software package (version 7.1.5, State-Ease Inc., Minneapolis, MN, USA) was employed where response surface methodology was based on a three-variable, three-level BBD. A total of 17 experimental runs are required for this optimization study. The ethanol concentration, sonication time and solvent-to-sample ratio are independent variables selected to be optimized for the extraction of *F. deltoidea*. Each variable was set at three levels. TPC was taken as the response of the designed experiments and the extraction yield of each condition was measured. The ranges for each variable

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selected from preliminary experiments were used in the 17 experiments. The 17 experimental runs and the observed responses are showed in Table 1.

*3.2.1. Analysis of Variance (ANOVA)*

Statistical analysis is made by analysis of variance (ANOVA) on the regression coefficients for the intercept, linear, quadratic and interaction terms of the model. The results of ANOVA shown in Table 2 signify the importance of the quadratic model.

The F value of 160.93 shows this model is significant with 0.01% chance of noise occurrence. The significance of each coefficient distinguished from the F-test and p-value. The corresponding variables would be more significant if the absolute F value becomes greater and the p-value becomes smaller. The probability > F values that are less than 0.05 and greater than 0.1 respectively, reveal the significance and insignificance of this model. In this case, A, B, C, AB, AC, BC, $A^2$, $B^2$, and $C^2$ are significantly displayed with p-values less than 0.05. Lack of fit is used for checking quality of the fitted models in order to have the

**Table 1: Data to obtain amount of TPC for every experimental runs in BBD and percentage of yield.**

| Ethanol concentration (%) | Sonication time (min) | Solvent-to-sample ratio | Total Phenolic Content (mg GAE/g) | Yield (%) |
|---|---|---|---|---|
| 50 | 30 | 30 | 420.39 | 30 |
| 50 | 10 | 30 | 442.54 | 26 |
| 70 | 20 | 30 | 435.93 | 31 |
| 70 | 20 | 30 | 442.54 | 31 |
| 50 | 20 | 40 | 379.35 | 25 |
| 70 | 20 | 30 | 435.93 | 31 |
| 90 | 20 | 20 | 372.87 | 26 |
| 70 | 30 | 20 | 424.71 | 31 |
| 70 | 10 | 40 | 465.22 | 31 |
| 90 | 20 | 40 | 402.03 | 29 |
| 90 | 30 | 30 | 364.77 | 30 |
| 70 | 30 | 40 | 382.59 | 33 |
| 70 | 20 | 30 | 439.3 | 31 |
| 50 | 20 | 20 | 435.52 | 24 |
| 70 | 20 | 30 | 443.08 | 31 |
| 70 | 10 | 20 | 454.96 | 28 |
| 90 | 10 | 30 | 447.4 | 26 |

**Table 2: ANOVA for response surface of quadratic model.**

| Source | Sum of squares | Degree of freedom | Mean squares | F value | p-value prob > F | |
|---|---|---|---|---|---|---|
| Model | 15141.103 | 9 | 1682.344767 | 160.927 | <0.0001 | Significant |
| A = Ethanol concentration | 1028.9916 | 1 | 1028.991613 | 98.4295 | <0.0001 | |
| B = Sonication time | 5921.9845 | 1 | 5921.98445 | 566.475 | <0.0001 | |
| C = Solvent-to-sample ratio | 433.20961 | 1 | 433.2096125 | 41.4392 | 0.0004 | |
| AB | 914.4576 | 1 | 914.4576 | 87.4736 | <0.0001 | |
| AC | 1820.3022 | 1 | 1820.302225 | 174.123 | <0.0001 | |
| BC | 685.9161 | 1 | 685.9161 | 65.6122 | <0.0001 | |
| $A^2$ | 3185.1948 | 1 | 3185.194813 | 304.684 | <0.0001 | |
| $B^2$ | 201.81638 | 1 | 201.8163813 | 19.305 | 0.0032 | |
| $C^2$ | 874.21678 | 1 | 874.2167813 | 83.6243 | <0.0001 | |
| Residual | 73.178695 | 7 | 10.45409929 | | | |
| Lack of fit | 25.694575 | 3 | 8.564858333 | 0.72149 | 0.5894 | Not-significant |
| Pure error | 47.48412 | 4 | 11.87103 | | | |

suitable model, and lack of fit must not be significant. The F value 0.72, representing comparative lack of fit to the pure error, is insignificant. Moreover, 58.94% possibility of F value is not fit.

$R^2$ is the coefficient of determination or the coefficient of multiple determination for multiple regression for statistical measurement on the closeness of data to the fitted regression line. The $R^2$ value of 0.9952 indicates that 99.5% capriciousness in the response could be clarified by this model. The 0.9681 of predicted $R^2$ is in equitable agreement with the 0.9890 of adjusted $R^2$. Singh *et al.* [13] adjusted with greater values resulting in a more prominent model. In present study, the standard deviation and CV with low values, which are 3.23 and 0.76% respectively, proved that the experiment is well performed. Hence, it means that the model has excellent accuracy and less dispersion. Adequate precision functioning as noise ratio indicator to errors that may occur on the range of predicted response. The satisfied signal favorable is at ratio greater than 4. The adequate precision resulted in the study is 40.208. So, this model can be utilized as navigator on design space [13].

*3.2.2. Quadratic Equation for TPC*

The results obtained from the BBD were fitted to a second-order model equation to explain the reliance of TPC on the effect of ethanol concentration, sonication time, and solvent-to-sample ratio in terms of coded values A, B, and C respectively.

$$TPC = 439.36 - 11.34\,A - (-27.21\,B) - 7.36\,C - 15.12\,AB + 21.33\,AC - 13.10\,BC - 27.50\,A^2 + 6.92\,B^2 - 14.41\,C^2 \quad (3)$$

*3.2.3. Graphical Analysis*

Figure 4 demonstrates the correlation of ethanol concentration and sonication time. The significant of interactive effect between the variables is supported by ANOVA in Table 2. The TPC rises with the increasing of ethanol concentration when the time is prolonged. This trend is similar with the study on *Cosmos caudatus* by Zulkiply [14], with the reason that correlate to the effectiveness of sonication bath [14]. However, at 90% concentration, low TPC is exhibited. This is the impact of solvent polarity. The greatest TPC (455.531 mg GAE/g) is obtained at 70% ethanol concentration at 15 minutes. Figure 5 reveals the significant interactive effect of ethanol concentration and solvent-to-sample ratio on TPC. It is also supported by ANOVA, as shown in Table 2. The more concentrated the ethanol solution, the more lower the usage of the quantity of solvent. The primary effect of the solvent-to-sample ratio was to adjust the solubility and equilibrium constant, consequently speeding up the sample diffusion and achieving high TPC [15]. Water content causes the swelling of cell and facilitates better mass transfer [16]. Hence, 30 mL of 70% ethanol concentration reveals the maximal TPC (431.98 mg GAE/g) from 0.1 g of the extracts. In the economical point of view, less consumption of solvent in extraction is practical and reasonable. Figure 6 determines the impact of collaboration of sonication time with solvent-to-sample ratio on TPC. ANOVA, in Table 2, acts as supportive data on the significance for these factors. The TPC rises with the increasing of sonication time and solvent-to-sample ratio. However, it drops after the achieved peak of 30 mL/g. Prolonged time of heating effect to ultrasound extraction may have resulted in the deprivation of TPC in extracts. Furthermore, in state of economical, high usage of time is not applicable. This trend is

**Table 3: $R^2$ Analysis.**

| Standard deviation | CV (%) | $R^2$ | Adjusted $R^2$ | Predicted $R^2$ | Adequate precision |
|---|---|---|---|---|---|
| 3.23 | 0.76 | 0.9952 | 0.9890 | 0.9681 | 40.208 |

**Figure 4: 3D plot of interaction between ethanol concentration and sonication time on TPC.**

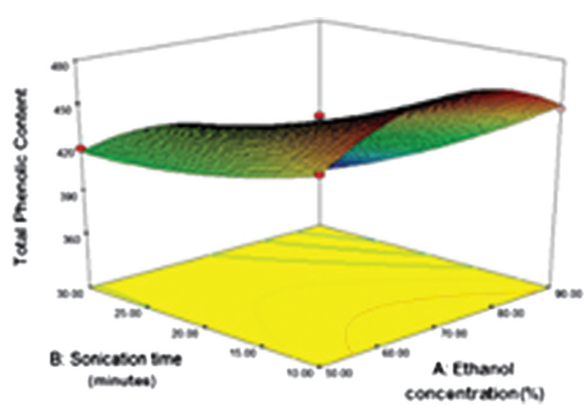


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**Figure 5: 3D plot of interaction between ethanol concentration and solvent-to-sample ratio on TPC.**

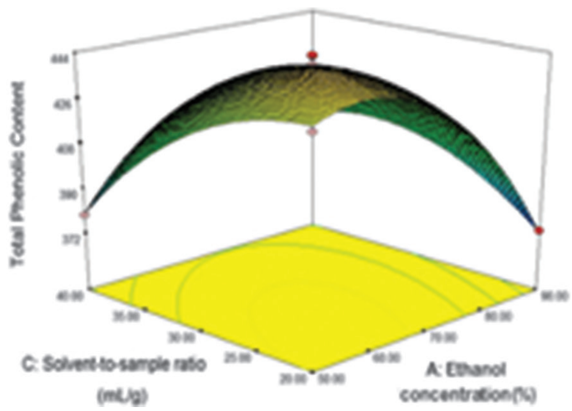


**Figure 6: 3D plot of interaction between ethanol concentration and solvent-to-sample ratio on TPC.**

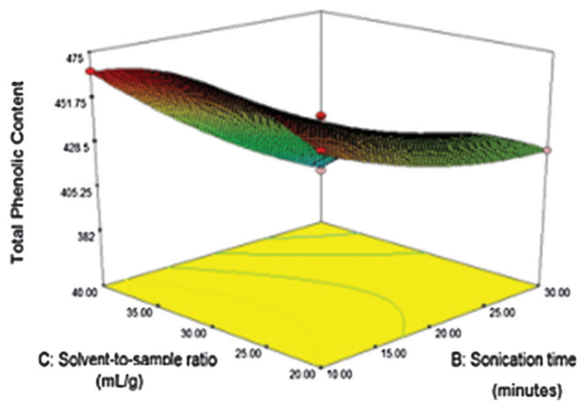


**Table 4: Validation data for optimum conditions of TPC for *F. deltoidea*.**

| Run | Ethanol concentration (%) | Sonication time (minutes) | Solvent-to-sample ratio (mL/g) | TPC (mg GAE/g) | Actual TPC (mg GAE/g) | Error (%) |
|---|---|---|---|---|---|---|
| 1 | 64 | 10 | 20 | 456.142 | 455.98 | −0.036 |
| 2 | 64 | 10 | 20 | 456.142 | 456.09 | −0.011 |
| 3 | 64 | 10 | 20 | 456.142 | 455.28 | −0.189 |
| Average | | | | | 455.78 | −0.079 |

similar with the optimized solvent-to-sample ratio condition for ultrasound-assisted extraction of flavonoids from *Cryptotaenia japonica Hassk* [17].

### 3.3. Validation of Model

Validation is done to signify the capability of RSM through the predicted TPC generated by the Design Expert software package (version 7.1.5, State-Ease Inc., Minneapolis, MN, USA). Table 4 demonstrates the validation data of triplicate runs. The predicted TPC was 456.142 mg GAE/g whereas the average of actual TPC was 455.78 mg GAE/g, which reveals a gently diverse with −0.079 of percentage error as shown in Table 4. The small percentage error proves that RSM is a reliable tool for optimization. Equation (4) is used to calculate the percentage error.

$$\%\,\text{Error} = \frac{\%\,\text{experimental} - \%\,\text{predicted}}{\%\,\text{predicted}} \tag{4}$$

## 4. CONCLUSION

Optimization was carried out for TPC with the suggested value on each factor by Design Expert software. ANOVA and graphical analysis demonstrated the significance of the model and interactive effects between factors. Ethanol concentration and sonication have a significant effect, which is implied by the three-dimensional graphical analysis. However, solvent-to-sample ratio showed gently effect with the two parameters. The optimum point predicted by Design Expert was 64% of ethanol concentration, 10 minutes of sonication time, and 20 mL/g of solvent-to-sample ratio. The predicted TPC was 456.142 mg GAE/g. The present study shows the precision of this software to make assumption on the TPC by evaluating the percentage error, which is only −0.079%. It is verified that Design Expert is a noteworthy and productive software for optimization study. In conclusion, all the objectives of this study were successfully attained.

## 5. RECOMMENDATION

There are some recommendations to improve the quality of future studies. In the present study, only female leaves of *F. deltoidea* were used as sample, although this plant has two types of leaf structure. Extraction of male leaves could be examined in selection of samples. Meanwhile, qualitative analysis, such as high performance liquid chromatography (HPLC), can be done to observe the efficiency of extracts. This would properly indicate the phenolic content of extracts. In the present study, the parameters that were used for screening and optimization were ethanol concentration, sonication time, and solvent-to-sample ratio. There are other factors that can be applied in future studies, including ultrasound power and temperature.

## 6. COMMERCIALIZATION POTENTIAL

*F. deltoidea* has been reported to be valuable in pharmaceuticals, as an antidiabetic, anti-inflammatory, antioxidant, and antibacterial agent. It is also widely planted in many countries. The present work revealed that the cost of ultrasound-assisted extraction is low, but it can produce more yield with good quality of TPC. Ethanol water as extraction solvent is less hazardous to the environment, and subsequently, disposal cost could be saved. Besides that, minimizing the usage of solvent can save the usage of energy as well. Therefore, *F. deltoidea* has been proved to have a market value.

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**Author Contributions**
Conceived and designed the experiments: L.J. Ong and Muhammad Shahzad Aslam. Implemented the experiments: L.J. Ong. Analyzed the data: L.J. Ong. Contributed reagents/materials/analysis tools: Awang Soh Mamat, Muhammad Shahzad Aslam and Muhammad Syarhabil Ahmad. Wrote the paper: L.J. Ong.

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**Conflict of Interest**
None.

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