

## OPTIMIZATION OF PHENOLIC COMPOUNDS EXTRACTION FROM PURPLE BASIL LEAF BY CONVENTIONAL AND MICROWAVE ASSISTED EXTRACTION METHODS

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**Abstract**— The present study aimed to determine the optimum extraction conditions of conventional solvent extraction (CSE) and microwave-assisted extraction (MAE) techniques to obtain maximum total phenolic (TPC), total flavonoid (TFC), total anthocyanin (TAC) and antioxidant capacity (AA). Response surface methodology (RSM) and central composite design (CCD) were used to determine optimum points of CSE and MAE. Both two extraction methods, all parameters significantly affected TPC, AA, TFC and TAC ( $p < 0.05$ ). MAE showed higher bioactive compounds yield than that of CSE. Optimum point for CSE and MAE was found to be 60°C and 30 min and 591.83 W and 2.98 min respectively. TPC, AA, TFC and TAC were obtained as 33.81 mg/g, 160.27 mg/g, 11.89 mg/g and 331.01 mg/kg for CSE and 62.99 mg/g, 214.62 mg/g, 21.80 mg/g and 3462.93 mg/kg for MAE respectively. This study recommended that the MAE should be used for the extraction of PBL to increase phenolic extraction yield.

**Keywords**— Extraction, response surface methodology, phenolic compounds, purple basil, *Ocimum basilicum* L.

### I. INTRODUCTION

Purple basil (*Ocimum basilicum* L.) is a member of the Lamiaceae family. Fresh leaves of purple basil are widely used in sauces, food, salads, kinds of vinegar and aromatic oils to provide for taste, smell and color (Angers *et al.*, 1996). It could be considered as the medicinal and functional plants in terms of containing essential oils and phenolic compounds such as flavonoids, anthocyanins and phenolic acids. Anthocyanin of the purple basil is cyanidin and peonidin derivatives (Phippen and Simon, 1998), the flavonoid is quercetin, luteolin, apigenin, kaempferol (Grayer *et al.*, 2002) and phenolic acid are rosmarinic acids (Javanmardi *et al.*, 2002). Therefore, purple basil has bioactive properties due to the great diversity of phenolic compounds and should be utilized in different food formulation or directly consumed as new food products. For that reason, phenolic compounds of the purple basil should be extracted from different methods.

The conventional solvent extraction method (CSE) is one of the most used methods in the extraction of phenolic from different food matrix. However, the CSE is a time-consuming method due to the low mass transfer rate and extraction yields. To increase mass transfer rate CSE

is conducted at high temperatures. Phenolic compounds have high sensitivity to temperature and can be degraded in case of prolonged exposure to high temperatures. Therefore, alternative extraction techniques should be applied to increase mass transfer rate and decrease the thermal degradation rate. Microwave extraction methods could be used to overcome low mass transfer rates and long extraction time (Proestos and Komaitis, 2008). The microwaves affect the polar molecules in the extraction medium and increase the internal pressure of the solid materials, therefore, the extraction efficiency is increased (Ince *et al.*, 2013).

In this study, two different extraction techniques namely CSE and Microwave-Assisted Extraction (MAE) were used to evaluate phenolic compounds of purple basil by extraction efficiency. These phenolic compounds are very sensitive to heat therefore, optimization of extraction parameters is necessary to increase the extraction yield of phenolic compounds of PBL. Response surface methodology (RSM) could provide information about extraction temperature, time and microwave power ranges. However, there are no studies on the optimization of phenolic compound extraction from purple basil leaf with different methods in the literature.

The present study aims to determine optimum extraction conditions to obtain the phenolic compounds from purple basil using conventional solvent, and microwave-assisted extraction techniques. In addition, the effects of different extraction methods were compared in terms of some bioactive properties namely, total phenolic (TPC), total flavonoid (TFC), Total anthocyanin (TA) and antioxidant capacity (AA).

### II. METHODS

#### A. Materials

Purple basil (*Ocimum basilicum* L.) samples were obtained from local markets in Elazığ, (Turkey). The leaves of purple basil were removed from the body and dried at 35 °C by Hot air drier at a constant air velocity of 2 m/s. The air velocity was determined by a Testo 440 vane probe anemometer (Lutron, AM-4201, Taiwan). The air flow was circulated horizontally through the surface of the purple basil samples. Dry leaves were crushed and kept at room temperature in airless boxes until analysis. The chemicals used in the study were acquired from Merck (Darmstadt, Germany).

#### B. Methods

##### Extraction Procedure

The distilled water was used as a solvent in CSE and MAE methods. The solid-to-solvent ratio was 1:20 g mL<sup>-1</sup>. CSE was carried out according to the method described by Karasu *et al.* (2015). The samples were incubated at a predetermined temperature and time period in a water bath (Daihan WB-22, Korea) for the extraction of phenolic compounds. A microwave extraction system (Samsung GE83X) was used for microwave assisted extraction. The appropriate microwave power and time were determined with preliminary studies. As a result of the experiments, the high values of microwave power was observed to negatively affect on the bioactive components of purple basil, although the processing time is short. After each extraction process, extracts were roughly filtered through a piece of paper and were centrifuged (Hettich, Universal 320R, Tuttlingen, Germany) at 4,500 g for 10 min. Extracts were kept in dark-colored bottles at -18 °C until the analysis.

#### Process Optimization

Process optimization was performed using response surface methodology (RSM) for obtaining the maximum amount of total phenolic, flavonoid compounds, anthocyanin content and antioxidant capacity. The design is composed of two factors for the CSE method, namely temperature and time of extraction process between 30-60°C and 10-30 min, respectively. Process factors in the RSM for microwave extraction were determined as microwave power and time, which were between 300-600 W and 1-3 min, respectively. Two-factor-3-level Central Composite Design including 3 center points were carried out in the study to optimize extraction process. TPC, AA, TFC and TAC were selected to be response of the corresponding established model. Eleven experimental points set by the use of the Design Expert software (Version 7 Stat-Easy Co., Minneapolis, MN, USA).

In the RSM, backward elimination procedure was carried out to remove insignificant model terms ( $p > 0.05$ ) to simplify the models to be established. A quadratic model type was used in response part of the design. Model acceptability was evaluated according to the  $R^2$  values. General model equation is as follows (Eq. 1):

$$y = \beta_0 + \sum_{i=1}^N \beta_i x_i + \sum_{i=1}^N \beta_{ii} x_i^2 + \sum_{i < j}^N \beta_{ij} x_i x_j \quad (1)$$

where  $y$  is response (total phenolic, flavonoid compounds, antioxidant activity and total anthocyanin content),  $\beta_0$  is the intercept,  $\beta_i$ ,  $\beta_{ii}$ ,  $\beta_{ij}$  are the constants of the corresponding parameters. The  $x_i$  and  $x_j$  represent the independent variables (while extraction temperature and time were for CSE and microwave power and time were for MAE. 3D surface plots were drawn with the software under the predetermined conditions. Optimization was performed by maximizing the total phenolic content, antioxidant capacity, total flavonoid and anthocyanin content of the extracts. For this aim, the single factor design was performed to determine maximum and minimum level of response variable.

#### Determination of Total Phenolic Content

TPC of purple basil leaves was determined according to the Folin-Ciocalteu method (Singleton *et al.*, 1999).

Firstly, distilled extracted samples were mixed with 2.5 mL 0.2 N Folin-Ciocalteu reagent. After 3 min, 2 mL of 7.5 % (w/v) Na<sub>2</sub>CO<sub>3</sub> was added into the tube. The mixture was stored for 45 min at room temperature in a dark place. The absorbance of the samples was measured at 760 nm using a spectrophotometer (Shimadzu UV-1800 spectrophotometer, Japan). Results for TPC was presented as mg GAE/g samples. The formula and  $R^2$  value for the gallic acid calibration curve were given below;  $y = 10.168x + 0.061$ ,  $R^2 = 0.9964$  respectively.

#### Determination of Antioxidant Capacity

CUPRAC method described by Apak *et al.* (2004) with some modifications was used to determine the antioxidant capacity (AA) of PBL extracts. 1 mL of each of CuCl<sub>2</sub> solution (0.01 M), neocuproine (7.5 mM), and 1 M ammonium acetate (NH<sub>4</sub>Ac) buffer (pH 7.0) solutions were added to tubes. After addition of 0.1 mL of diluted purple basil extracts into the tubes, the total volume was adjusted to 4.1 mL with distilled water. The absorbance was measured at 450 nm using spectrophotometer (Shimadzu UV-1800 spectrophotometer, Japan) after the incubation of 1 h at room temperature in the dark. Trolox was chosen as standard and the results were obtained as mg trolox equivalent per g sample. The formula and  $R^2$  value for the CUPRAC calibration curve were given below;  $y = 1.6168x + 0.0057$ ,  $R^2 = 0.9913$  respectively.

#### Determination of Total Flavonoid Content

Total flavonoid content (TFC) of extracted samples was determined according to the method described by Zhishen *et al.* (1999). First, 1 mL purple basil extract was mixed with 4 mL distilled water, and 0.3 mL NaNO<sub>2</sub> (5% w/v) was placed in test tube. After 5 min, 0.3 mL of AlCl<sub>3</sub> was added and after 6 min, 2 mL of 1 M NaOH solution was added. The mixture was completed to 10 mL by adding distilled water. The absorbance of the samples was measured at 510 nm using a spectrophotometer (Shimadzu UV-1800 spectrophotometer, Japan). The results were expressed as mg catechin equivalent per g samples. The formula and  $R^2$  value for the catechin calibration curve were given below;  $y = 3.4378x + 0.011$ ,  $R^2 = 0.9966$ .

#### Determination of Total Anthocyanin Content

Total anthocyanin content analysis of purple basil extracts was performed according to the pH differential method (Yildirim *et al.*, 2017). The extracts were diluted with buffers of pH 1 (0.025 M KCl) and pH 4.5 (0.4 M CH<sub>3</sub>COONa) and extracts were incubated at room temperature in the dark for 30 min. The absorbance of the samples was measured at 510 and 700 nm using a spectrophotometer (Shimadzu UV 1800 spectrophotometer, Japan). Results were calculated depend on the following equation. The total anthocyanin content of purple basil samples was expressed as mg of cyanidin-3-glucoside per kg sample.

$$A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5} \quad (2)$$

$$TAC \left( \frac{\text{mg}}{\text{L}} \right) = \frac{(A \times MW \times DF \times 1000)}{\epsilon} \quad (3)$$

where  $MW$  is molecular weight of Cyanidin-3-glucoside (449.2 g/mol),  $DF$  is dilution factor, and  $\epsilon$  is extinction coefficient (26 900 L/cm mol),  $A$  is absorbance and  $TAC$  is total anthocyanin content of samples. Please insert a continuous break at the end of the manuscript so both columns end at approximately the same height.

#### Statistical Analysis

All analysis was performed triplicate and values expressed as mean. Design expert software, v7 (Stat-Ease, Minneapolis, MN), was used to specified regression and variance analysis (ANOVA) of experimental points. The effects of the dependent variables on the responses were assessed by quadratic models and response surface plots. The model practically was determined by Regression coefficient ( $\beta$ ), coefficient of determination ( $R^2$ ) and  $F$  test value of predicted, the lack of fit test, and model  $p$ -value ( $p < 0.05$ ).

### III. RESULTS

#### A. Effect of CSE Factors (Time and Temperature) on Response ( $TPC$ , $AA$ , $TFC$ , $TAC$ )

Table 1 presented  $TPC$ ,  $AA$ ,  $TFC$  and  $TAC$  values obtained from CSE methods. The  $TPC$  varied from 12.68 to 33.54 mg g<sup>-1</sup> for CSE. As we can see in Fig. 1, the increase in temperature with time led to an increase of  $TPC$  at all time and temperature range. When the time increased at the constant temperature, the extraction rate of phenolic compounds increased due to a positive linear quadratic effect. Similarly, by increasing temperature from 45 °C to 60 °C led to increasing in  $TPC$ .

The effect of extraction time and temperature on the bioactive properties of the purple basil leaf was previously studied by Pedro *et al.* (2016). They reported  $TPC$  value of the purple basil leaf as 272.54-688.22 mg/100g. They also reported that extraction time and temperature significantly affected the phenolic compounds extraction rate and yield. In a similar to our study, the  $TPC$  value increased with increasing temperature and time. The increase in the extraction rate of phenolic compounds can be explained by easily the penetration of the solvent into the plant matrix and resulted in a higher mass transfer rate (Pandey *et al.*, 2018; Al-Farsi and Lee, 2008). In addition, time is very important for conventional extraction

especially the presence of temperature due to the destruction of plant matrix and timely occurred high mass transfer with solvent. A similar result was obtained by Zuorro *et al.* (2015).  $AA$ ,  $TFC$ ,  $TAC$  value were 75.69- 160.34 mg TEAC/g, 4.65-11.89 mg CAE/g and 156.15 -329.05 mg Cy-3-glu/kg respectively for CSE given (Table 1). According to Table 1,  $AA$ ,  $TFC$  and  $TAC$  showed a similar trend with  $TPC$ . An increase in time and temperature increased  $AA$ ,  $TFC$ , and  $TAC$  value.

Figure 1 showed the effect of temperature and time on the  $AA$ ,  $TFC$ , and  $TAC$ . Fig 1 showed that the increase in temperature and time in the range of 30-60 °C and 10-30 min led to an increase in  $AA$ ,  $TFC$  and  $TAC$  value. The increase in the  $TAC$  value is more pronounced at high temperature and time values. This result can be explained by the positive effect of temperature and time interaction on the  $TAC$  value

The effect of extraction time and temperature on  $TPC$ ,  $TFC$ ,  $AA$  and  $TAC$  value of the purple basil samples was expressed by the quadratic model. Table 2 showed the quadratic model parameters. According to Table 2, the quadratic model successfully modeled the effect of temperature and time on the  $TPC$ ,  $AA$ ,  $TFC$  and  $TAC$  extraction yield with high  $R^2$  value (0.99) and lack of fit was insignificant. The linear effects of temperature and time on  $TPC$  were significant ( $p < 0.0001$ ). The quadratic effects of time on  $TPC$  was also significant ( $p < 0.001$ ). According to regression coefficient values,  $X_1$  value has the main effect followed by  $X_2$  and  $X_2^2$ , respectively. The effect of extraction temperature and time was significant on  $AA$  ( $p < 0.0001$ ). Temperature and time had a significant effect on  $AA$ , while the quadratic and interaction had a negative effect on  $AA$ .  $AA$  is more depend on  $X_2$  and followed by  $X_1$ . ANOVA results showed that temperature and time had a significant effect on  $TFC$  ( $p < 0.0001$ ).  $X_1$  value has a major effect on  $TFC$  followed by  $X_2$ . The model was successfully fitted ( $p < 0.05$ ,  $F$  value 0.036) and also demonstrated that an insignificant lack of fit was observed. Temperature and time had a significant effect on  $TAC$ .  $X_2$  value has a major effect on  $TAC$  followed by  $X_1$  and  $X_1X_2$ . The insignificant factors were removed and the polynomial equation for  $TPC$ ,  $AA$ ,  $TFC$  and  $TAC$  were presented below:.

Table 1. Total phenolic content ( $TPC$ ), antioxidant activity ( $AA$ ), total flavonoid content ( $TFC$ ) and total anthocyanin content ( $TAC$ ) of purple basil extracts obtained using conventional solvent extraction method.

| Extraction Conditions |                  |            | Experimental Results          |                               |                               |                                     |
|-----------------------|------------------|------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------------|
| Points                | Temperature (°C) | Time (min) | $TPC$ (mg GAE/g dry material) | $AA$ (mg TEAC/g dry material) | $TFC$ (mg CAE/g dry material) | $TAC$ (mg Cy-3-glu/kg dry material) |
| 1                     | 30               | 10         | 12.68                         | 75.69                         | 4.65                          | 156.15                              |
| 2                     | 30               | 20         | 18.18                         | 99.36                         | 5.98                          | 186.55                              |
| 3                     | 30               | 30         | 20.45                         | 119.39                        | 7.62                          | 218.80                              |
| 4                     | 45               | 10         | 18.41                         | 92.46                         | 6.35                          | 194.55                              |
| 5                     | 45               | 20         | 23.74                         | 123.26                        | 7.62                          | 220.45                              |
| 6                     | 45               | 20         | 24.29                         | 125.89                        | 7.19                          | 225.35                              |
| 7                     | 45               | 20         | 24.53                         | 126.85                        | 7.93                          | 230.45                              |
| 8                     | 45               | 30         | 26.49                         | 142.35                        | 9.49                          | 274.45                              |
| 9                     | 60               | 10         | 24.21                         | 117.34                        | 8.55                          | 211.75                              |
| 10                    | 60               | 20         | 31.98                         | 139.86                        | 9.94                          | 279.05                              |
| 11                    | 60               | 30         | 33.54                         | 160.34                        | 11.89                         | 329.05                              |

Table 2. Regression coefficient ( $\beta$ ), coefficient of determination ( $R^2$ ) and  $F$  test value of predicted second-order polynomial models (CCD) for phenolic compound by using CSE.

| CSE                                 |           |           |           |           |
|-------------------------------------|-----------|-----------|-----------|-----------|
| Regression Coefficients ( $\beta$ ) | TPC       | AA        | TFC       | TAC       |
| Intercept $\beta_0$                 | 24.32     | 123.96    | 7.61      | 228.14    |
| <b>Linear</b>                       |           |           |           |           |
| $X_1$                               | 6.4***    | 20.52***  | 2.02***   | 43.06***  |
| $X_2$                               | 4.2***    | 22.76***  | 1.58***   | 43.31***  |
| <b>Quadratic</b>                    |           |           |           |           |
| $X_1^2$                             | 0.57      | 2.30      | 0.32      | 0.57      |
| $X_2^2$                             | 2.06**    | 4.50      | 0.28      | 2.27      |
| <b>Cross Product</b>                |           |           |           |           |
| $X_1X_2$                            | 0.39      | 0.17      | 0.092     | 13.66***  |
| $R^2$                               | 0.9962    | 0.9917    | 0.9928    | 0.9906    |
| <b>F values (Model)</b>             | 261.76*** | 118.84*** | 137.63*** | 105.07*** |
| <b>F (value Lack of Fit)</b>        | 0.3329    | 0.2074    | 0.9886    | 0.3204    |

A= Temperature, B= Time TPC=Total polyphenolic content, AA=Antioxidant Activity TFC=Total flavonoids content, TAC=Total anthocyanin content. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.0001$ .  $X_1$ : represents temperature and microwave power for CSE and MAE respectively,  $X_2$  shows extraction time for CSE and MAE.

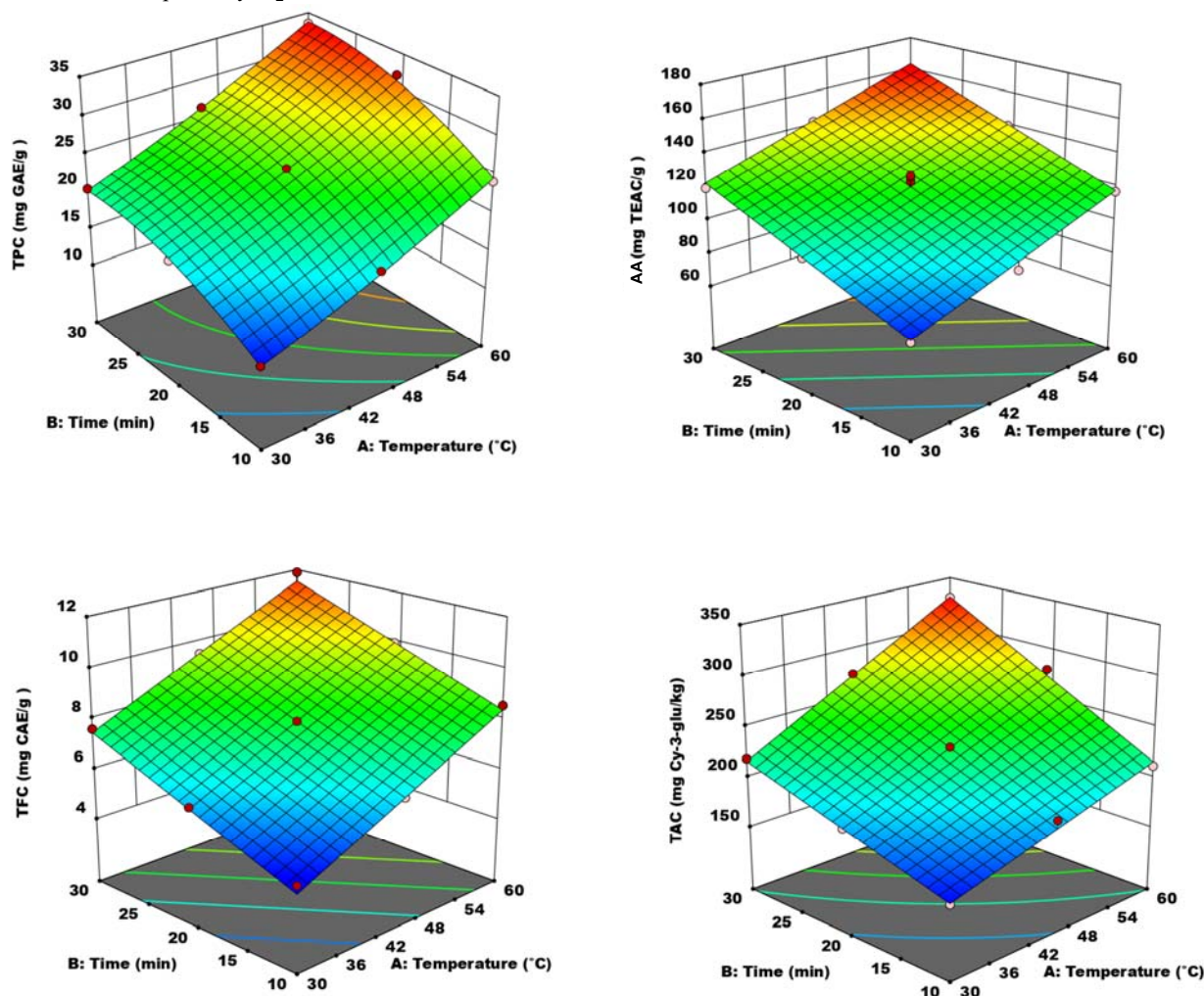


Figure 1: The effect of Time and Temperature on the TPC, AA, TAC, TFC of Purple Basil Leaf.

$$TPC = 24.32 + 6.4X_1 + 4.2X_2 - 2.06X_2^2 \quad (4)$$

$$AA(CUPRAC) = 123.96 + 20.52X_1 + 22.76X_2 \quad (5)$$

$$TFC = 7.61 + 2.02X_1 + 1.58X_2 \quad (6)$$

$$TAC = 228.14 + 43.06X_1 + 43.41X_2 + 13.66X_1X_2 \quad (7)$$

**B. Effect of MAE Factors (Microwave Power and Time) on Response (TPC, AA, TFC and TAC)**

The TPC, AA, TFC and TAC results obtained by MAE methods are presented in Table 3. Also, the variation of response in microwave power and time by MAE is shown in Fig. 2. TPC ranged from 10.88 to 62.72 mg g<sup>-1</sup> for MAE. As can be seen from Fig. 2, TPC, AA and TFC and TAC tend to increase with microwave power and time.

Table 3. Regression coefficient ( $\beta$ ), coefficient of determination ( $R^2$ ) and  $F$  test value of predicted second-order polynomial models (CCD) for phenolic compound by using MAE.

|                              | MAE       |           |         |           |
|------------------------------|-----------|-----------|---------|-----------|
|                              | TPC       | AA        | TFC     | TAC       |
| Intercept $\beta_0$          | 41.59     | 138.06    | 10.58   | 1566.41   |
| <b>Linear</b>                |           |           |         |           |
| $X_3$                        | 9.76***   | 40.21***  | 4.37**  | 847.88*** |
| $X_4$                        | 16.64***  | 51.07***  | 6.35*** | 977.77*** |
| <b>Quadratic</b>             |           |           |         |           |
| $X_3^2$                      | 2.63*     | 15.28*    | 0.26    | 251.45    |
| $X_4^2$                      | 2.47*     | 4.46      | 0.85    | 140.63*   |
| <b>Cross Product</b>         |           |           |         |           |
| $X_3X_4$                     | 0.59      | 6.83      | 1.54*   | 532.90**  |
| $R^2$                        | 0.9962    | 0.9909    | 0.9814  | 0.9876    |
| <b>F values (Model)</b>      | 259.94*** | 108.80*** | 52.74** | 79.70***  |
| <b>F (value Lack of Fit)</b> | 0.6769    | 0.4969    | 0.2398  | 0.1293    |

A= Temperature, B= Time TPC=Total polyphenolic content, AA =Antioxidant Activity TFC=Total flavonoids content, TAC=Total anthocyanin content. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.0001$ .  $X_1$ : represents temperature and microwave power for CSE and MAE respectively,  $X_2$  shows extraction time for CSE and MAE.

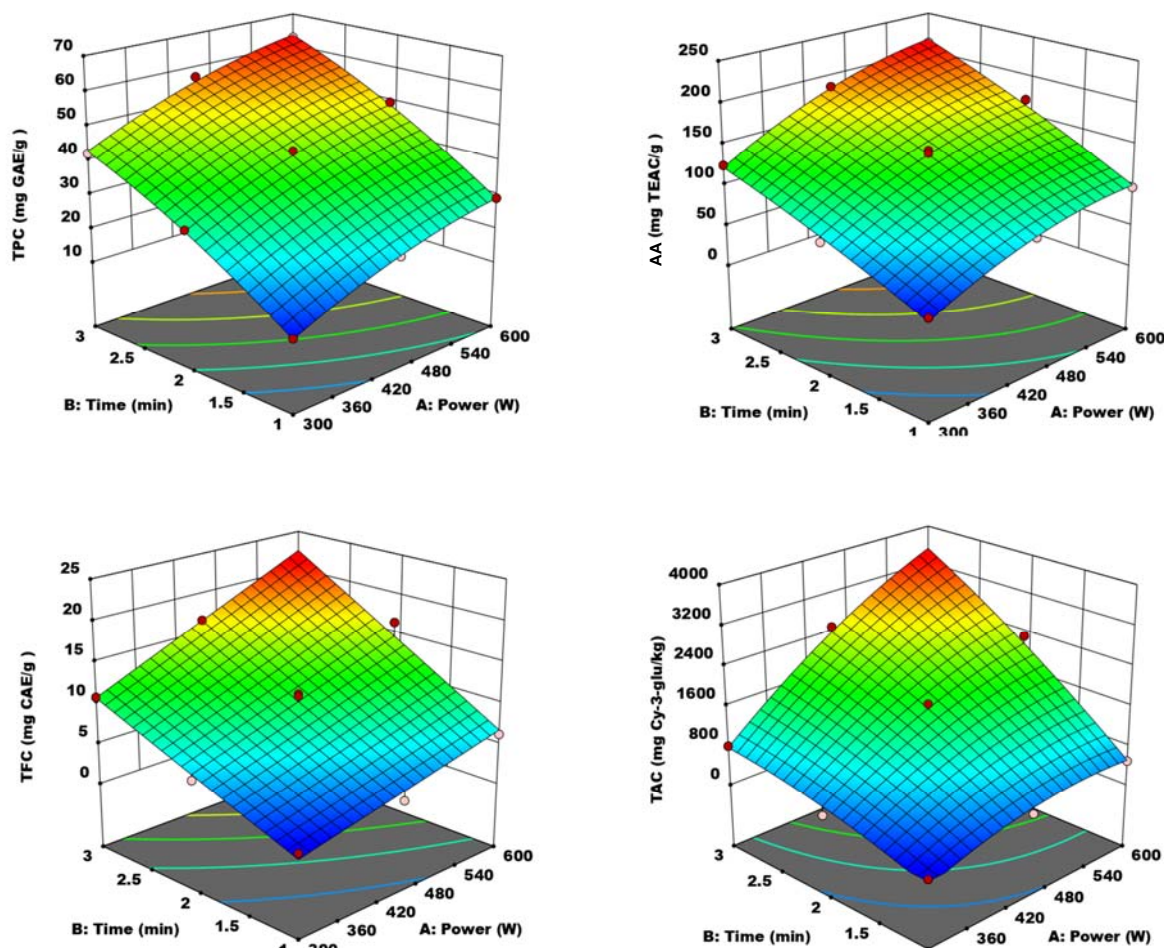


Figure 2: The effect of Time and Microwave Power on the TPC, AA, TAC and TFC of Purple Basil Leaf.

As shown in Table 3, microwave power and time had a significantly ( $p < 0.05$ ) effect on TPC. The B value is a major effect on TPC followed by  $X_3$ ,  $X_3^2$  and  $X_4^2$ . The linear( $X_3$ ,  $X_4$ ) has a positive significant effect on TPC, while quadratic ( $X_3^2$ ,  $X_4^2$ ) negative effect on TPC. The non-significant factors were removed and the polynomial equation for TPC comes as:

$$TPC = 41.59 + 9.76X_3 + 16.64X_4 - 2.63X_3^2 - 2.47X_4^2 \quad (8)$$

The non-significant value of lack of fit demonstrated the model was successfully ( $p < 0.05$ ,  $F$  value 0.59) fitted and showed good prediction ( $R^2 = 0.9962$ ). The decrease in time from 3 to 2 and 1 min led to a decrease in TPC, then further increase in time from 1 and 2 to 3

min, increase in *TPC*. In addition, by increasing microwave power and time led to an increase in *TPC*. A similar result was reported by Bhuyan *et al.* (2015).

The increase in microwave power will cause an increase in temperature. Therefore, increase the intracellular pressure, and that the transitions between the solvent and the cell. As a result of the decomposition of the cell surface due to the pressure and the extraction efficiency will increase (Hayat *et al.*, 2009).

Similar to *TPC*, *AA* extraction was significantly ( $p < 0.05$ ) effected by microwave power and time. The total antioxidant capacity from purple basil leaf (*PBL*) was found to range from 38.04 to 212.03 mg TEAC/g for *MAE* are given in Table 3. As shown in Table 4, the microwave power, time ( $X_3$ ,  $X_4$ ) and interaction ( $X_3X_4$ ) had a positive significant effect on *AA*, while the quadratic ( $X_3^2$ ,  $X_4^2$ ) negative effect on *AA*. *AA* is more depend on  $X_4$  followed by  $X_3$  and  $X_3^2$ . The non-significant factors were removed and the polynomial equation for *AA*:

$$AA(CUPRAC) = 138.06 + 40.21X_3 + 51.07X_4 - 15.28X_3^2 \quad (9)$$

The model was successfully fitted ( $p < 0.05$ ,  $F$  value 1.15) and showed a non-significant lack of fit. By decreasing the time from 3 to 1 min led to a decrease in *TPC*. Further increase in time from 1 to 2 and 3 min led to an increase in *AA*. In addition, an increase in time and microwave power increase in *AA* due to forming internal pressure and decomposition of the cell surface.

This result is compatible with previous studies. The antioxidant activity of *Pistacia lentiscus* was more effective than the other (conventional solvent, supercritical extraction, and ultrasound-assisted extraction techniques) (Bampouli *et al.*, 2014). The antioxidant capacity obtained by the microwave-assisted method is higher than the *CSE* (Alara *et al.*, 2018).

Similar to *TPC* and *AA*, obtained for *TFC* extraction. Microwave power and time was significantly ( $p < 0.05$ ) influence on *TFC* extraction. The *TFC* was found between 1.77- 21.35 mg CAE/g for *MAE* and are presented in Table 3. The time has a major effect on *TFC*, followed by  $X_3$ ,  $X_3X_4$ ,  $X_4^2$ , and  $X_3^2$ . In addition,  $X_3$ ,  $X_4$ , and  $X_3X_4$  have a significant positive effect on *TFC*. The non-significant factors were removed and the polynomial equation for *TFC*

$$TFC = 10.58 + 4.37X_3 + 6.35X_4 + 1.54X_3X_4. \quad (10)$$

The model is successfully and showed a non-significant lack of fit. By decreasing the time from 3 to 1 min led to a decrease in *TPC*. Further increase in time from 1 to 2 and 3 min led to increasing in *TFC*. In addition, an increase in time and microwave power increase in *TFC* due to forming internal pressure and decomposition of the cell surface. *MAE* is one of the advanced solvent-based extraction methods applied extensively for flavonoid extraction and is often preferred for both polar and non-polar flavonoids with different solvent combinations. In this study, we obtained a high rate of flavonoid concentration due to microwave energy of the water which is using as a solvent and it is an excellent solvent, especially for polar flavonoids. In addition, it is possible

to obtain pure polar flavonoids extracts by using microwave and aqueous and alcoholic solvents with the least possible extraction time and to achieve success with various solvent combinations for non-polar or less polar flavonoids (Chebrolu *et al.*, 2011, Rostagno *et al.*, 2007, Terigar *et al.*, 2009).

Similar to *TPC*, *AA*, and *TFC*, *TAC* extraction was effected by microwave power and time significantly ( $p < 0.05$ ). The *TAC* was found to range from 172.83 to 379.58 mg Cy-3-glu/kg and are presented in Table 3. As shown in Table 3, the *TFC* is more depend on  $X_4$  followed by  $X_3$  and  $X_3X_4$ . In addition, linear and interaction has a positive significant effect on *TFC*. The non-significant factors were removed and the polynomial equation for *TAC*:

$$TAC = 1566.41 + 847.88X_3 + 977.77X_4 + 532.90X_3X_4. \quad (11)$$

The model was successfully fitted ( $p < 0.05$ ,  $F$  value 6.89) and showed a non-significant lack of fit. By decreasing the time from 3 to 1 min led to a decrease in *TAC*. Further increase in time led to an increase in *TAC*. In addition, an increase in microwave power and time led to an increase in *TAC* at all levels of microwave power and time. In our study, the anthocyanin ratio is proportional to the extraction time. This results in agreement with a previous study (Zheng *et al.*, 2013).

### C. Determination of Optimum Point

Optimum extraction points were selected based on the maximum *TPC*, *AA*, *TFC* and *TAC* values. Optimum point for *CSE* and *MAE* was found to be 60°C and 30 min and 591.83 W and 2.98 min respectively. *TPC*, *AA*, *TFC* and *TAC* was obtained as 33.81mg/g, 160.27 mg/g, 11.89 mg/g and 331.01 mg/kg for *CSE* and 62.99 mg/g, 214.62 mg/g, 21.80 mg/g and 3462.93 mg/kg for *MAE* respectively. As can be seen, *MAE* showed higher bioactive compounds than that of the *CSE*. In considering the *TPC* and *TAC*, *MAE* showed 1.86 and 10.46 times higher *TPC* and *TAC* than *CSE* respectively.

Dahmoune *et al.* (2014) studied the optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves. The *MAE* found more effective than the other extraction techniques in terms of total phenolic compounds, total flavonoid, and antioxidant activity. In another study, Ince *et al.* (2013) studied the extraction of phenolic compounds from *Melissa*. They reported that at the optimum point, *MAE* found that more effective than ultrasound assisted extraction methods in terms of antioxidant activity and total phenolic compounds. Mandal *et al.* (2017) found that the *MAE* technique was more effective than the conventional extraction technique. These results are compatible and support our findings. The results reported that the microwave-assisted extraction method reduced extraction time and achieved a high rate of flavonoid production without degradation compared with soxhlet and ultrasound-assisted extraction methods (Xiao *et al.*, 2008). The results of this study support our findings.

#### IV. CONCLUSIONS

In this study, polyphenols of purple basil leaves were extracted by using conventional solvent, and microwave-assisted extraction methods. The optimization study was performed by the response surface methodology and results of the *TPC*, *AA*, *TFC* and *TAC* were chosen as the dependent variables. Both two extraction methods, all parameters significantly affected *TPC*, *AA*, *TFC* and *TAC* ( $p < 0.05$ ). Higher *TPC*, *AA*, *TFC* and *TAC* values were obtained at the point of higher temperature- time and higher microwave power-time for *CSE* and *MAE* respectively. When comparing the *CSE* and *MAE*, *MAE* showed higher *TPC*, *AA*, *TFC* and *TAC* than those of the *CSE*. It was determined that the *MAE* decreased the extraction time by 90% compared to the *CSE*. This study recommended that the *MAE* should be used for the extraction of *PBL* due to efficient, environmentally friendly and time-saving properties.

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