Optimized extraction of polyphenols from leaves of Rosemary (*Rosmarinus officinalis* L.) grown in Lam Dong province, Vietnam, and evaluation of their antioxidant capacity

Abstract: In the present study, the optimized solvent extraction conditions with regards to the total polyphenol content (TPC) and antioxidant capacity of rosemary leaf extract (RLE) were determined. The one-factor-at-a-time method was used to independently investigate the effect of several extraction parameters, including ethanol concentration (0–100% v/v), extraction temperature (50–80°C), extraction period (15–60 min), material–solvent ratio (1:5–1:10 g/mL), and extraction cycles (1, 2, and 3 times) on polyphenol content. Response surface methodology (RSM), in combination with a central composite design, was used to perform optimization. The following optimal conditions that gave maximal TPC were determined and experimentally verified: ethanol concentration of 65% (v/v), extraction temperature of 65°C, material–solvent ratio of 1:7.5 g/mL, extraction time of 15 min, and 2 cycles of extraction. These parameters corresponded with the TPC yield of 87.42 ± 0.25 mg gallic acid equivalent/g dried feed material (mg GAE/g DW). The optimal conditions gave a high extraction yield (337 ± 6 mg dried extract/g dried feed material) with 197.28 ± 3.11 mg GAE/g dried extract. The estimated models were strongly significant \((p < 0.05)\) for TPC values with significant regression coefficients \((R^2)\) of 0.9979. The obtained RLE was supposed to be the top grade of natural antioxidant with the IC\(_{50}\) (DPPH assays) value of 9.4 ± 0.1 µg/mL, which is higher than that of the vitamin C by just three times \((IC_{50} = 3.2 ± 0.1 µg/mL)\). Current results justify RLE as a potential agent in food preservation applications.

Keywords: maceration extraction, *Rosmarinus officinalis*, optimization, polyphenol content, antioxidant activity

1 Introduction

Various examinations have shown that herbs have powerful antioxidant properties, because of the amount and nature of phenolic compounds present in them [1]. Rosemary (*Rosmarinus officinalis* L.) is a restorative herb that is broadly utilized throughout the world. Among natural antioxidant plants, rosemary has been acknowledged as the most noteworthy cancer prevention plant [2]. These antioxidant properties were identified with the presence of phenolic contents, accounting for the most majority rosmarinic acid (RA), carnosic acid (CA), and carnosol (COH) [3,4].

Rosemary finds extensive use in various applications. In various Western countries, rosemary leaf extract (RLE) has been commercialized as an antioxidant and flavorant [5]. RLE has also been proved to exhibit hepato-protective properties, suggesting the potential use in medicinal applications for treatment of maladies [6], Alzheimer’s malady [7], and angiogenesis-related diseases [8]. In addition,
they have been used in product preservation due to their ability to restrict oxidation and microbial defilement [9–12]. In this manner, RLE could be valuable for supplanting or indeed diminishing synthetic preservative in foods.

Techniques for extraction of antioxidants from herbal plants have been reported in several studies. For example, it has been reported that ultrasound-assisted extraction (UAE) of marjoram using methanol solvent could afford cancer prevention agents, including RA, luteolin-7-O-glucoside, apigenin-7-O-glucoside, caffeic acid, CA, and COH [13]. For rosemary, developed extraction methods for recovery of antioxidant compounds included UAE [14], solvent extraction [15], pressurized green dissolvable solvent extraction [16], and CO₂ supercritical liquid extraction [17,18].

Although there have been several studies on rosemary extraction methods, the optimal production conditions for solvent extraction process have not been adequately reported. One exception is the report of Hosseini et al. (2018) [19] where optimization of heat-assisted extraction (HAE) and UAE conditions was carried out, but lacked a single-factor investigation to narrow the survey amplitude. This resulted in limited selectivity, insignificant influence of response, and wide amplitude of the surveyed parameters (e.g., extraction time and liquid-solid ratio), causing waste or deviation from the optimal point.

In this study, the extraction process of rosemary was optimized with respect to maximal recoveries of total polyphenol and antioxidant ability of the afforded extract. Response surface methodology (RSM) in conjunction with central composite design (CCD) were adopted to guide optimization experiments [20,21]. Experimental conditions that were taken into consideration for optimization consisted of ethanol concentration, extraction temperature, time of extraction, material–solvent ratio, and the number of extraction cycles. Obtained findings are expected to aid in improving the existing extraction processes and justifying the use of rosemary materials in production of phenolic-rich plant extracts.

2 Materials and methods

2.1 Plant sample preparation

The rosemary leaves were harvested at Lam Ha District, Lam Dong Province, Vietnam, in February 2020. The material was dried at 45°C by using forced air convection oven until it reached a moisture content of less than 12%. This temperature was based on the previous studies as the condition allowed high retention of important compounds in rosemary leaves [22,23]. Moisture content was monitored by a moisture meter (MA35, Sartorius, Göttingen, Germany). Dried rosemary leaves were preserved in zip bags and placed in a dry and ventilated place.

2.2 Extraction process

Dried rosemary leaves were extracted with ethanol using the maceration method. The mixture was stirred at 200 rpm and heated up at a specified temperature and then filtrated under vacuum. The remaining solid residue was then extracted for a second time under similar conditions. Finally, an aliquot of the filtered extract was dried under a vacuum drier at 60°C to dryness to remove the solvent and the yield of extraction was identified.

2.3 Quantification of total polyphenol contents (TPCs) based on Folin-Ciocalteu (FC) methodology

The polyphenol contents were determined following previously described methods [24] with certain modifications. Dried extract and 40 μL of DMSO were added to 200 μL of FC reagent, and the mixture was vibrated for 5 min with ultrasonic unit (Elma S 100 H, Elma, Singen, Germany). Then, 3,160 μL of H₂O and 600 μL of Na₂CO₃ were added to the mixture to dilute and support the reaction. The samples were vibrated for 30 min at room temperature and measured at λ = 760 nm by Thermo Scientific GENESYS 10S Series UV-Visible Spectrophotometer.

The TPC was expressed as a gallic acid equivalent (GAE) from the following calibration curve: Concentration (ppm) = 0.0012 × Absorbance – 0.0179 of GA standard solution ($R^2 = 0.9951$) and expressed as mg of GAE per gram of dry extract. The amount of TPC was calculated using the following equation:

$$\text{TPC} = \frac{\text{mg gallic acid}}{\text{g dried weight}} \times \frac{C_i m_1}{m_2}$$

where $C_i$ is the concentration obtained from gallic acid standard curve (mg GAE/mL DMSO), $C_j$ is the concentration of the dried extract (mg dried extract/mL DMSO), $m_1$ is the weight of the total dried extract (mg), and $m$ is the weight of the dried material (g).
2.4 Antioxidant evaluation with 1,1-diphenyl-2-picrylhydrazyl (DPPH)

DPPH assays were carried out following method of Brand-Williams [25]. DPPH was first blended in with methanol (Sigma-Aldrich) of 80% (with optical density at 517 nm of 0.80 ± 0.02) to form the solution with the concentration of 40 µg/mL. Afterwards, 180 µL of the as-prepared DPPH solution was added with 120 µL of another mixture containing the sample dissolved in 80% of methanol. The obtained solution was then shaken, taken into storage for 30 min at 30°C in dimness, and measured for optical density at 517 nm. Positive controls of different concentrations (0–100 µg/mL) were prepared by dissolving vitamin C (Sigma-Aldrich) in 80% of methanol. Moreover, the color solution was prepared including MeOH and the sample solution. The blank solution was also prepared using DPPH solution and MeOH solution. The percentage of DPPH radical scavenging ability was calculated as follows:

\[ \text{DPPH} \text{ (})%\text{ = } \frac{A_b - (A_s - A_c)}{A_0} , \]

where \( A \) is the optical density. The subscript b, c, and s denote the blank sample, the tested sample, and the color solution, respectively. \( IC_{50} \) value was calculated by graphing the concentration against % of inhibition.

2.5 Optimization of polyphenol extraction by using RSM

The process was optimized following an RSM procedure with CCD. The statistical model took TPC of RLE as the response and three experimental parameters including ethanol concentration (\( X_1 \)), temperature (\( X_2 \)), and material–solvent ratio (\( X_3 \)) were taken into consideration as independent variables. To identify the suitable levels of parameters for CCD, a series of single-factor investigations were carried out by varying three parameters in the following order: ethanol concentration, temperature, and dry material–solvent ratio. Three different levels, which are low (−1), medium (0), and high (+1), were then assigned to the obtained set of optimum parameters (Table 1), which was then used in CCD. A total of 20 combinations consisting of eight factorial points, six axial points, and six center points were generated by CCD. Distance from center to point \( a = 2^{k/4} = 1.682 \) (with \( k = 3 \) conditions). Those combinations were then experimentally attempted to obtain the data of \( Y \), which were then fitted to a second-order polynomial equation [26]:

\[ Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \sum_{j=1}^{3} \beta_{ij} X_i X_j, \]

where \( Y \) indicates the dependent variable (total polyphenol), \( X \) indicates experimental conditions and \( \beta \) denotes model coefficients. The subscript 0, i, j, and \( ij \) represent the constant, linear terms, the quadratic terms, and interactive terms, respectively.

The estimated model was then tested with Analysis of variance (ANOVA) for determination of significant terms. Statistical significance was recognized at 5%. Post-estimation statistics, including \( R \)-squared, adj-\( R \)-squared, predicted-\( R \)-squared, \( F \)-value, and lack-of-fit were used to evaluate model adequacy.

### Table 1: Experimental conditions for the rates used in the trial

| Factor          | Symbol | Level |
|-----------------|--------|-------|
| EtOH concentration (%) | \( X_1 \) | 60 70 80 |
| Temperature (°C)     | \( X_2 \) | 50 60 70 |
| Material–solvent ratio (mL/g) | \( X_3 \) | 1:6 1:8 1:10 |

\[ Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \sum_{j=1}^{3} \beta_{ij} X_i X_j, \]

where \( Y \) indicates the dependent variable (total polyphenol), \( X \) indicates experimental conditions and \( \beta \) denotes model coefficients. The subscript 0, i, j, and \( ij \) represent the constant, linear terms, the quadratic terms, and interactive terms, respectively.

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3 Results and discussion

3.1 Single-factor investigations on TPC

3.1.1 Influence of ethanol concentration

Ethanol has been commonly used as the extraction solvent for recovery of naturally active compounds from plants due to its safety and health-benign nature. Figure 1 illustrates the TPC values of RLE obtained at different ethanol concentrations ranging from 0 to 99.5%. The TPC

![Figure 1: Polyphenol extraction affected by ethanol concentration (Operating conditions: temperature of 50°C; material–solvent ratio of 1:5 g/mL; operating time of 30 min; and 2 extraction cycles).](image-url)
recovered from the extract at 60% of ethanol (56.5 mg GAE/g DW) was significantly higher than those obtained at lower ethanol concentrations (30 and 0%). Rising the ethanol concentration from 60 to 70% slightly improved TPC yield to 59.0 (mg GAE/g DW) which was the peak of the investigation. At ethanol concentrations higher than 70%, obtained TPC was significantly lower than those at lower concentrations and minimum TPC yield, 27.6 (mg GAE/g DW), was reached at absolute ethanol.

The relationship could be explained as follows. At ethanol concentration of around 70%, the presence of water in solvent causes the rosemary leaf matrix to be moderately swelled, thus facilitating the permeation of solvent into the material [27]. Therefore, as the ethanol concentration increased, lower water content in solvent led to weaker swelling of material, negatively affecting the extraction efficiency. By contrast, the high quantity of water in the solvent leads to excessive swelling of the material and unfavorable solvent polarity, both of which contributed to the lowered extraction yield.

### 3.1.2 Influence of temperature

Figure 2 illustrates the TPC of RLE obtained at different extraction temperatures. Significant yield improvement was observed as the temperature was elevated from 50 to 60°C, achieving the peak TPC of 42.4 (mg GAE/g DW). Further temperature elevations past 60°C seemed to cause negligible improvements in TPC obtained. Moreover, TPC diminished marginally to the point of 40.5 (mg GAE/g DW) when the temperature was increased to 80°C. This might be due to the thermal degradation of polyphenol. In addition, the mechanism through which increasing temperatures improve extraction recovery could be explained by increased diffusion of the solvent, increased mass transfer, and improved solubility of phenolic compounds in the solvent [28]. The temperature of 60°C seemed to give the highest TPC in RLE and was chosen for the next investigation. This result is in line with a previous report where 70°C was determined as the extraction temperature at which TPC of ethanolic RLE began to decline [29].

### 3.1.3 Influence of dry material–solvent ratio

In real scale production processes, the amount of used solvent significantly affects variable costs and the product quality. Therefore, an investigation has been carried out in order to determine the minimum amount of used solvent possible to recover majority of the active compound.

Figure 3 shows that the TPC rose from 63.4 to 69.9 (mg GAE/g DW) and then peaked at 72.2 (mg GAE/g DW) as the material–solvent proportion increased from 1:6 to 1:8 g/mL and then to 1:10 g/mL. At the equilibrium state, where no extractable polyphenols are present in the material, further addition of ethanol would not advance the TPC abdicate any longer. Improvement in TPC when rising the ratio from 1:6 to 1:8 could be due to the enhanced penetration of solvent through cell membrane and oil bags under thermal treatment, which help pushing active compound out [30]. The difference in yield of polyphenol extraction between solvent ratios 1:8, 1:9, and 1:10 was modest and unnoticeable, while the amount of additional solvent required to achieve such improvement was large (10%). Therefore, the raw material–solvent proportion of 1:8 was more temperate and chosen for consequent tests.

### 3.1.4 Influence of extracting time

The longer the extracting time was, the higher the efficiency would be. However, until a certain time, lengthening the extracting time could not raise up the efficiency.

**Figure 2:** Polyphenol extraction affected by temperature (Operating conditions: ethanol concentration of 70% (v/v); material–solvent ratio of 1:5 g/mL; operating time of 30 min; and 2 extraction cycles).

**Figure 3:** Polyphenol extraction affected by material–solvent ratio (Operating conditions: ethanol concentration of 70% (v/v); temperature of 60°C; operating time of 30 min; and 2 extraction cycles).
On the other hand, it caused wastage of energy and solvent. In accordance with Figure 4, prolonging the extraction time from 15 to 45 min caused the TPC to slightly increase, implying that time did not significantly affect the TPC obtained. The process achieved equilibrium rapidly, which might be due to small material size, high porosity of materials, high dispersion due to stirring, and high employed temperature. However, inconsiderable change in TPC between 15 and 45 min is not justifiable by significantly higher required time and energy consumption. Our result is in accordance with a previous report where antioxidant of RLE saw unnoticeable improvements after 15 min of extraction [31]. Therefore, 15 min should be applied for the extracting step.

### 3.1.5 Influence of number of extraction cycles

From Figure 5, it was suggested that TPC could not be thoroughly recovered by only one extraction cycle. It could clearly be seen that the cumulative yield after the second extraction cycle was significantly higher compared to the previous one, totaling 67.4 mg GAE/g DW (over 52% increase). After the third extraction cycle, cumulative TPC value increased by merely 8.8%, from 67.4 to 76.2 mg GAE/g DW, proposing that most of the TPC has been exhausted after the second cycle. Given that the TPC improvement achieved after carrying out the third extraction cycle was not large and that the amount of used solvent and energy consumption for one extraction cycle was considerably high, two repeated extraction cycles were considered for application in the process.

### 3.2 Optimization of extraction condition from rosemary

#### 3.2.1 Model fitting using RSM

The responses (TPC) of experimental runs guided by CCD are summarized as in Table 2. Among the examined variables in single-factor investigations, extraction time and extraction cycle were fixed at their respective optimal levels (15 min and 2 cycles, respectively). Variations in three experimental parameters for these runs were as follows: ethanol concentration $X_1$ (60–90% v/v), extraction temperature $X_2$ (50–70°C), and material–solvent ratio $X_3$ (1:8–1:11.3 g/mL) and operating time 15 min.

#### Table 2: CCD of actual factors and responses based on actual and predicted values

| Run | $X_1$ | $X_2$ | $X_3$ | $Y_{TPC}$ (actual) | $Y_{TPC}$ (predicted) |
|-----|-------|-------|-------|--------------------|-----------------------|
| 1   | 60    | 50    | 1:6   | 66.58              | 66.59                 |
| 2   | 80    | 50    | 1:6   | 63.01              | 63.02                 |
| 3   | 60    | 70    | 1:6   | 82.61              | 82.30                 |
| 4   | 80    | 70    | 1:6   | 79.62              | 79.19                 |
| 5   | 60    | 50    | 1:10  | 80.89              | 80.86                 |
| 6   | 80    | 50    | 1:10  | 72.12              | 71.96                 |
| 7   | 60    | 70    | 1:10  | 83.98              | 83.74                 |
| 8   | 80    | 70    | 1:10  | 76.01              | 75.53                 |
| 9   | 53.2  | 60    | 1:8   | 83.21              | 83.32                 |
| 10  | 86.8  | 60    | 1:8   | 72.68              | 73.22                 |
| 11  | 70    | 43.2  | 1:8   | 63.01              | 62.79                 |
| 12  | 70    | 76.8  | 1:8   | 78.59              | 79.24                 |
| 13  | 70    | 60    | 1:4.6 | 74.01              | 74.35                 |
| 14  | 70    | 60    | 1:11.3| 82.95              | 83.27                 |
| 15  | 70    | 60    | 1:8   | 88.12              | 87.63                 |
| 16  | 70    | 60    | 1:8   | 87.25              | 87.63                 |
| 17  | 70    | 60    | 1:8   | 86.89              | 87.63                 |
| 18  | 70    | 60    | 1:8   | 87.69              | 87.63                 |
| 19  | 70    | 60    | 1:8   | 88.01              | 87.63                 |
| 20  | 70    | 60    | 1:8   | 87.95              | 87.63                 |

*Figure 4: Polyphenol extraction affected by extracting time (Operating conditions: ethanol concentration of 70% (v/v); temperature: 60°C; material–solvent ratio of 1:8 g/mL; and 2 extraction cycles).

*Figure 5: Polyphenol extraction affected by number of extraction (Operating conditions: ethanol concentration of 70% (v/v); temperature: 60°C; material–solvent ratio of 1:8 g/mL; and operating time 15 min).
ratio $X_3$ (1:6–1:10 g/mL). Generally, TPC of RLE ranged from 62.79 to 87.63 (mg GAE/g DW). The highest predicted response was 87.63 (mg GAE/g DW), which was obtained at the center point.

ANOVA results and estimated coefficients were reported as in Table 3. Most of the model terms, except for the $X_1X_2$ interaction, were statistically significant ($p < 0.05$). High coefficient of determination ($R^2$) of 0.9979 suggests that most of the variance in the response could be predicted by estimated variables. The close agreement between predicted and actual responses in Table 2. The reduced second-order polynomial function that describes the relationship between TPC and parameters is as follows:

$$Y_{TPC} = 87.63 - 3X_1 + 4.82X_2 + 2.65X_3 - 1.27X_1X_3 - 3.21X_2X_3 - 3.13X_1^2 - 5.83X_2^2 - 3.12X_3^2$$

### Table 3: Analysis of Variance (ANOVA) results for the quadratic model for optimization

| Factors | TPC | Sum of squares | $F$-value | $p$-value |
|---------|-----|----------------|-----------|-----------|
| Model  |     | 1308.19        | 524.60    | <0.0001   | Significant |
| $X_1$  |     | 123.14         | 444.44    | <0.0001   | —          |
| $X_2$  |     | 317.25         | 1144.97   | <0.0001   | —          |
| $X_3$  |     | 96.04          | 346.60    | <0.0001   | —          |
| $X_1X_2$ |   | 0.2380        | 0.8591    | 0.3758    | Not significant |
| $X_1X_3$ |   | 12.95         | 46.75     | <0.0001   | Significant |
| $X_2X_3$ |   | 82.30         | 297.04    | <0.0001   | —          |
| $X_1^2$ |   | 157.77        | 569.41    | <0.0001   | —          |
| $X_2^2$ |   | 490.64        | 1770.78   | <0.0001   | —          |
| $X_3^2$ |   | 140.25        | 506.17    | <0.0001   | —          |
| Residual |   | 2.77          | —         | —         | —          |
| Lack of Fit | | 1.59        | 1.35      | 0.3753    | Not significant |
| Pure Error | | 1.18        | —         | —         | —          |
| Cor Total | | 1310.96     | —         | —         | —          |
| Coefficient of Variation | | 0.6641     | —         | —         | —          |
| PRESS  |     | 13.74         | —         | —         | —          |
| $R^2$  |     | 0.9979        | —         | —         | —          |
| $R^2$ Adjusted | | 0.9960   | —         | —         | —          |
| $R^2$ Predicted | | 0.9895    | —         | —         | —          |
| Adequate precision | | 66.7504  | —         | —         | —          |

#### 3.2.2 Effect of process variables on total phenolic compounds

As shown by the estimated model, the effect of the ethanol concentration and extraction temperature on the production of phenolic compounds was more pronounced than that of material–solvent ratio. To further explore the interaction effects of parameters on the response, three-dimensional response surface plots (Figure 6) were plotted. In each plot, two variables were allowed to vary and the other variable was kept at its central point.

At constant material–solvent ratio (1:8), the impact of ethanol concentration and extraction temperature on TPC seemed to follow a plateau shape (Figure 6a). As seen, in ethanol concentration range of 60–70% (v/v) and temperature range of 60–70°C, a higher amount of phenolic compound was obtained. TPC gradually mounted with a rise in the concentration and temperature of ethanol and reached an optimum value at around 65% (v/v) and 65°C until it began to decline. In fact, with the addition of water to ethanol, the polarity of the mixture would increase continuously in order to extract more polar phenolic compounds. Therefore, phenolics derived using 70% of ethanol might be shown to be better than those of 80% of ethanol.

Figure 6b demonstrates the obtained TPC with respect to varying ethanol concentration and material–solvent ratio under a fixed extraction temperature of 60°C. Concentration of ethanol demonstrated a strong linear and quadratic effect on TPC (Table 3). Greater solvent quantity exerted positive effects on TPC. The increase in yields with the rise in solvent quantity was compatible with the principles of mass transfer. To be specific, when a lower solid to solvent ratio is used, the gradient of the concentration difference is expected to be higher and thus, might lead to promoted diffusion. On the other hand, using less solvent for extraction while still maintaining high yield is desirable from an economic point of view.

The relationship between extraction temperature and material–solvent ratio to TPC is shown in Figure 6c. The TPC of RLE increased rapidly with temperature up to 65°C, at which point TPC began to gradually decline thereafter. This could be rationalized by various phenomena that often occur under mild heating, including softened plant tissues, weakened cell wall, improved phenolic solubility, and hydrolysis of bonds that bind phenol with protein or with polysaccharide [32–34].
3.2.3 Effect of process variables on total phenolic compounds

Optimizing the estimated model with respect to maximal response yielded optimal conditions. These parameters were then slightly adjusted for convenience and attempted to verify the results. Table 4 shows the parameters and results of confirmation experiments. Mean TPC obtained after triplicate experiment was 87.42 (mg GAE/g DW), which approximates the predicted TPC. By applying the matched t-test, no critical change among real and anticipated qualities ($p < 0.05$) was recognized. Hence, the estimated model was satisfactory in predicting the TPC yield.

In optimum conditions, a high extraction yield (337 ± 6 mg dried extract/g dried feed material) and TPC (87.42 ± 0.25 mg GAE/g dried material) were achieved. This corresponded to TPC of 197.28 ± 3.11 mg GAE/g dried extract, which was calculated on the basis of dried extract and marginally higher than that calculated by Hosseini et al. [19]. Previously, the optimal conditions for HAE of dried rosemary included extraction time of 132.80 min, extraction temperature of 70°C, and solvent–material ratio of 20:1, giving extraction yield of 17.80%, which was equivalent to TPC of 147.56 mg GAE/g DW. Generally, when a longer extraction time is used, higher concentrations of polyphenols are obtained, whereas polyphenol degradation can also occur when longer treatment periods are correlated with high temperatures. Our results are also in accordance with the kinetic study of Jurinjak Tušek et al. (2016) [35] who examined solid-liquid extraction process of Asteraeae family plants and indicated that contents of bioactive compounds were exhaustive after the initial 10 min of the extraction process.

3.3 Evaluating the antioxidant ability

The antioxidant capacity of RLE was directly proportional to the concentration used. The RLE decreased the absorbance of DPPH solution to half of its initial value at a concentration of 9.4 ± 0.1 μg/mL, which is higher than that of the vitamin C by just three times (3.2 ± 0.1 μg/mL). This result was almost the same as the results of

**Table 4: The results of optimum condition experiment**

| EtOH Concentration (% v/v) | Extraction temperature (°C) | Material–solvent ratio (g/mL) | Predicted TPC (mg GAE/g DW) | Actual TPC (mg GAE/g DW) | Error with model (%) |
|---------------------------|-----------------------------|-------------------------------|-----------------------------|--------------------------|----------------------|
| 65                        | 65                          | 1:7.5                         | 88.64                       | 88.12                    | 0.41                 |
|                           |                             |                               |                             | 87.25                    | 1.10                 |
|                           |                             |                               |                             | 86.89                    | 1.39                 |

Average TPC ± standard deviation 87.42 ± 0.25
Klančnik et al. (2009), which illustrated the IC50 value of several commercial rosemary extract formulations to be 7.4–22.7 μg/mL [36]. The results clearly showed that RLE was among the top of natural antioxidants.

4 Conclusion

RSM was successfully used to determine the optimal conditions for the extraction of rosemary polyphenol. The second-order polynomial model provided a satisfactory description of experimental data. The optimum conditions for obtaining polyphenol of *Rosmarinus officinalis* L. involved an EtOH concentration of 65% (v/v), a material–solvent ratio of 1:7.5, and an extraction temperature of 65°C with corresponding TPC yield of 87.42 ± 0.25 (mg GAE/g DW). The optimum conditions produced a high extraction yield (337 ± 6 mg dried extract/g dried feed material) with 197.28 ± 3.11 mg GAE/g dried extract. The IC50 (DPPH assays) of the optimal extract (9.4 ± 0.1 μg/mL) was compared with the one recorded for vitamin C, confirming the antioxidant capacity of RLE.

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