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Phenolics Compounds and Antioxidant Activities from Optimized *Pheonix dactylifera* Extract using Response Surface Methodology

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Phoenix dactylifera has been documented to possess numerous health potentials as it contains a myriad of bioactive compounds. *P. dactylifera* was extracted in this study, and extraction conditions through Soxhlet extraction procedures were improved by adopting response surface methodology (RSM). The effects of extraction time (3 h, 4.5 h, and 6 h), solute-to-solvent ratio (1:10, 1:20, and 1:30 g/mL), and size of sample (1.00 mm, 2.87 mm, and 4.75 mm) on total phenolic content (TPC), total flavonoid content (TFC) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity were determined. The present study demonstrated the quadratic polynomial coefficients had a significant impact ($p < 0.05$) for all models, with a non-significant lack of fit at $p > 0.05$ and R^2 exceeded 0.90 respectively. The best extraction conditions obtained were as follows: time for extraction (6 h), solute-to-solvent ratio (1:10), and size of sample (4.75 mm). Under ideal conditions, the TPC, TFC, and DPPH radical scavenging activity were 50.25 mg GAE/g, 11.14 mg QE/g, and 79.22% respectively. The phenolic compounds in the extract were screened and recorded by using Ultra High-Performance Liquid Chromatography-Quadrupole Time of Flight-Mass Spectrometry (UHPLC-QTOF-MS). Eleven phenolic compounds of prime importance in *P. dactylifera* extract were identified. This work indicated that *P. dactylifera* has a unique phytochemical profile which has antioxidant potential to be an adjunct in preventing a variety of diseases.

Keywords: *P. dactylifera*; Soxhlet extraction; TPC; TFC; DPPH radical scavenging; response surface

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The market for halal products in the manufacturing sector has experienced rapid expansion in response to increasing demand. While halal considerations are present, the primary emphasis lies on the Halalan-Toyyiban components. Given the increasing demand for beauty products, it is imperative for cosmetic enterprises and industries to prioritize the safety of their products as a core obligation. Nevertheless, several cosmetic makers persist in including illicit substances and chemicals into their products, despite their knowledge of the potential negative consequences, owing to the affordability and efficacy of such compounds. The date extract has been identified as a potentially viable alternative source of safe plant-derived compounds that possess antioxidant characteristics and offer various benefits.

Dates, *Phoenix dactylifera* L. fruit, locally known as "*buah kurma*" in Malaysia, is the most regularly consumed fruit in the diet of various cultures. The plant in concern is classified within the Arecaceae family, which falls under the category of Angiosperms and monocotyledons. Specifically, it belongs to the genus Phoenix. The fruit of this plant has a coloration that ranges from purple to red, and possesses a distinctive elongated shape like the fingers of a fruit bunch. [1]. *P. dactylifera*, often known as the date palm, has a long history of cultivation dating back at least 5000 years in the regions of North Africa and the Middle East. Furthermore, it is extensively farmed in the arid and warm climatic regions of Asia, as well as the Arabian Peninsula [2, 3]. On records, more than 5000 date palm cultivars are distributed worldwide, varying in dietary, morphological, and genetic characteristics, although the number of commercial cultivars is small [4]. Dates output in the world has climbed from 4.60 million tons in 1994 to 8.52 million tons in 2018 [5].

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P. dactylifera is highly nutritious and contains significant amounts of carbohydrates, minerals, salts, unsaturated fats, proteins, and fibres. [3, 6]. Previous studies have indicated that P. dactylifera contains many active ingredients such as flavonoids, steroids, phenols, and saponins, which have been shown to have antioxidant properties through their ability to scavenge radicals [7–9]. Additionally, Moreover, the antioxidant capacity of *P. dactylifera* is believed to be derived from its diverse array of phenolic compounds, such as p-coumaric, procyanidins, ferulic acid, and sinapic acid. Previous research has demonstrated that *P. dactylifera* contains a total of thirteen flavonoid glycosides, namely luteolin, quercetin, and apigenin. [10, 11].

The method of extraction is the first step in extracting phytochemicals from plant materials. Soxhlet extraction (SE) was chosen because it offers more benefits and is more adequate for screening compounds. The benefits of SE include the following: (1) The material is brought into contact repeatedly with fresh extractant; (2) After a leaching phase, no filtration is necessary; and (3) SE is suitable for both initial and bulk extraction [12, 13]. SE is a commonly employed technique in the extraction of phenolic compounds, demonstrating superior efficiency compared to alternative conventional procedures [14].

The present study utilised Response Surface Methodology (RSM) as an optimisation technique to enhance the efficiency of the SE system, hence maximising the associated benefits. Response Surface Methodology (RSM) is a collection of statistical and mathematical techniques aimed at enhancing, maximising, and optimising responses in situations where a multitude of variables exert impact on the outcome. By employing RSM, it becomes possible to optimise the response mentioned earlier [15, 16]. Box– Behnken design (BBD) is one type of RSM that was chosen for this study because it is easy to set up and explain experiments [17]. The base peak chromatogram of the polyphenol of dates was achieved by UHPLC-QTOF-MS.

EXPERIMENTAL

Sample Preparation and Chemicals Sample Preparation and Chemicals

Fruits of *P. dactylifera* were purchased from Madinah, Arab Saudi through a local supplier. The fruits and pits were separated manually and subsequently dried in a laboratory oven under 60 °C for 2 weeks. After drying, the flesh of the samples was crushed into smaller sizes, sieved into several sizes, and stored in sealed containers for subsequent processes. The following chemicals and reagents were purchased from Sigma-Aldrich: Folin–Ciocaltue's reagent, gallic acid, quercetin, aluminum chloride ($AlCl_3$), sodium carbonate (Na_2CO_3), and methanol. All chemicals were of analytical grade.

Extraction of Samples using Soxhlet Technique

The extraction of Ajwa dates was conducted using the Soxhlet extraction method [18] with few modifications. Samples of *P. dactylifera* were measured and placed into the extraction device utilising a Soxhlet extractor thimble. The samples were subjected to weighing procedures according to the specified solute-to-solvent ratios (1:10, 1:20, and 1:30), using samples of varying sizes (1.00 mm, 2.87 mm, and 4.75 mm). A volume of 200 mL of distilled water was measured and subsequently transferred into a conical flask with a capacity of 250 mL. The mixture underwent reflux for multiple extraction cycles with a heating mantle. The duration of the extraction process encompassed time intervals of 3 hours, 4.5 hours, and 6 hours. The supernatant obtained was subjected to freeze-drying process until the extract was fully desiccated. The crude extracts were placed in a refrigeration unit set at a temperature of 4 °C in order to facilitate further analysis.

Statistical Analysis and RSM and Box-Behnken Design

The process of optimising the extraction of phyto-chemicals from P. dactylifera extract was carried out utilising Response Surface Methodology (RSM). The experimental design employed by RSM involved the utilisation of the Box-Behnken concept. The adoption of BBD for this research was based on its notable efficiency in handling a reduced number of trials connected with three variables, hence offering a more productive and cost-effective method [19]. Table 1 presents the coded values and levels of three independent variables, namely extraction times, solute-to-solvent ratio, and size of sample, that were chosen for the study. The experiment was conducted using Design-Expert software version 11.1.0.1, developed by Stat-Ease in Minnesota, USA. A cumulative number of 17 experiments were conducted, as indicated in Table 2. The data of responses, including DPPH radical scavenging activity, TPC, and TFC contents, were analysed using the answer surface regression approach. A second-order poly-nomial equation was employed to establish a match between the data and the extraction parameters. The equation was expressed as in Equation 4:

$$Y = \beta_{\circ} + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j+1}^{3} \beta_{ij} x_i x_j \ (\mathrm{i} \neq \mathrm{j}) \quad (1)$$

Table 1. Experimental independent variables and their levels for Box-Behnken design.

Independent Variables	Level		
	-1	0	1
Extraction time (h)	3	4.5	6
Solute-to-solvent ratio	1:10	1:20	1:30
Size of sample (mm)	2.00	2.80	4.75

Table 2. The results of a BBD with three variables and the antioxidant of *P. dactylifera*.

Run Order	X_1 (extraction time, h)	X_2 (Liquid/solid ratio, g/mL)	X_3 (Size of sample, mm)	TPC (mg GAE/g)		TFC (mg QE/g sample)		DPPH (%)	
				Observed	Predicted	Observed	Predicted	Observed	Predicted
1	-1	-1	0	49.83	50.51	8.29	9.26	66.01	67.10
2	1	-1	0	46	46.64	9.85	10.72	70.01	70.23
3	0	-1	1	51.67	50.79	5.65	4.67	72.94	72.77
4	0	1	-1	32.22	33.10	13.98	14.96	78.49	78.66
5	1	0	-1	37.5	37.30	20.55	20.54	64.8	65.73
6	0	1	1	39.83	40.27	7.41	8.27	52.19	53.33
7	0	-1	-1	39	38.56	7	6.14	60.32	59.18
8	-1	0	-1	36.83	36.59	7.6	7.49	58.61	58.66
9	0	0	0	40.25	40.82	6.05	6.11	53.95	51.28
10	0	0	0	41.83	40.82	5.09	6.11	46.89	51.28
11	0	0	0	39	40.82	6.19	6.11	48.07	51.28
12	1	0	1	47	47.24	14.23	14.34	60.08	60.03
13	-1	0	1	45.83	46.03	5.53	5.54	53.55	52.62
14	1	1	0	44.17	43.49	27.38	26.41	75.45	74.36
15	0	0	0	40.33	40.82	7.5	6.11	57.57	51.28
16	-1	1	0	38.33	37.69	6.87	6.00	63.22	63.01
17	0	0	0	42.67	40.82	5.72	6.11	49.91	51.28

Statistical analyses and ANOVA were conducted using Design Expert Software. Three levels were created using the BBD method, namely X_1 (3 h, 4.5 h, and 6 h), X_2 (1:10, 1:20, and 1:30), and X_3 (1.00 mm, 2.87 mm, and 4.75mm).

Determination of Total Phenolic Content (TPC)

The evaluation of total phenolic components in the extract of P. dactylifera was conducted utilising the Folin-Ciocalteau method [20], with slight adaptations.

The preparation of samples involved the dilution of 5 mg of extract in 1 mL of distilled water. In this experiment, 50 µL of Folin solution was combined with 100 µL of sample extract. Prior to this, the sample extract was diluted with 7.9 mL of distilled water. The mixture was then left at room temperature for a specified duration. Subsequently, a volume of 1.5 mL of a sodium carbonate solution with a concentration of 7.5% was carefully dispensed into each individual vial. The combination was incubated in a light-restricted environment for a duration of 120 minutes. The absorbance readings of the samples were analysed at a wavelength of 765 nm using a UV-VIS microplate reader. The analysis was conducted three times for each sample. A standard solution of gallic acid was generated with varying concentrations ranging from 25 to 1000 µg/L in order to establish a standard calibration curve. Total phenolic compounds (TPCs) are determined by the utilisation of a standard calibration curve and are quantified in milligrammes of gallic acid equivalent per gramme (mg/g GAE) of the extracted sample.

Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of the extract was assessed utilising the spectrophotometric technique developed by Stankovic [21]. The samples and quercetin, serving as the standard, were produced at specific dilutions. Each sample was combined with 100 µL and 2% AlCl3 in a 96-well plate. The resulting mixture was then incubated in a dark environment at room temperature for a duration of 15 minutes. The absorbance readings of the samples were recorded at a wavelength of 415 nm. The calibration curve was produced by repeating the experiment using a standard quercetin solution. The content of flavonoid was quantified by determining the absorbance and thereafter represented as quercetin equivalent (QE), (mg of quercetin/g of extract).

Determination of 2,2′-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The DPPH radical scavenging activity was conducted using the procedures outlined by Blois [22] with certain variations. In summary, a quantity of 5 mg of extract was measured and subsequently dissolved in 1 mL of distilled water within a centrifuge tube. A quantity of 10 milligrammes (mg) of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was solubilized in ethanol and subsequently diluted to a total volume of 100 millilitres (mL) using a volumetric flask, resulting in a concentration of 0.1 millimolar (mM). A mixture comprising 150 µL of DPPH and 50 µL of extract was combined and distributed into a 96-well microliter plate. Following that, the combination was incubated in a lightless environment for a duration of 30 minutes. The absorbance of the sample was measured at a wavelength of 515 nm using a UV-VIS microplate reader. A representative standard was generated by excluding the sample extract from the DPPH solution. The analyses were conducted in duplicate for each sample. The DPPH radical scavenging properties of the extracts were assessed utilising the following formulas:

$$\text{DPPH Scavenging Activity (\%)} = (A_a - A_b))/A_a \times 100, \quad (2)$$

where A_a is the absorbance of the control and A_b is the absorbance of the sample.

Identification of Phenolic Compounds Using UPHLC QTOF-MS Analysis

The ultra-high-performance liquid chromatography (UHPLC) analysis was conducted using a Waters (Manchester, UK) ACQUITY UPLC I-Class instrument, comprising a binary pump, a vacuum degasser, an auto-sampler, and a column oven. The phenolic chemicals were separated by chromatographic techniques utilising a Waters column, specifically the ACQUITY UPLC HSS T3 (100 mm x 2.1 mm x 1.8 m). The temperature remains steady at 40°C. Mobile phases A and B consisted of a linear binary gradient of water containing 0.1% formic acid and acetonitrile, respectively. During the course of the experiment, adjustments were made to the mobile process composition as outlined below: at 0 minutes, the composition consisted of 1% B; at 0.5 minutes, the composition remained at 1% B; at 16.00 minutes, the composition was increased to 35% B; at 18.00 minutes, the composition was further increased to 100% B; finally, at 20.00 minutes, the composition was reverted back to 1% B. The volume of the injection was 1 µL, while the flow rate was 0.6 mL/min. The investigation employed Waters in conjunction with an electrospray ionisation apparatus operating in negative ion modes. The data were collected within a mass range of 50 to 1500 m/z, with a scanning time of 0.1 seconds each scan, using high-definition mass spectrometry increased energy (HDMSE) mode. During the experimental procedure, two separate scans were conducted using different collision energies (CE). These scans were performed in an alternating manner, while a low-energy (LE) scan was carried out at a constant collision energy of 4 eV. Additionally, a high-energy (HE) scan was conducted, where the collision energy was varied between 10 and 40 eV.

RESULTS AND DISCUSSION

Fitting Model

RSM and BBD were used to explain the association between the functions of the responses and process variables in all seventeen experiments, which used several combinations of independent variables. The independent variables specifically focused on the several aspects that affected the outcomes. The aspects that count in these experiments are the time of extraction, solute-to-solvent concentration, and size of sample. The selection of an effective extraction process has had a notable influence on the substantial

enhancement of total phenolic content (TPC), total flavonoid content (TFC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the extract derived from *Phoenix dactylifera*. The reaction was achieved by conducting RSM. The highest-order polynomial was used to choose the models in the current study. A quadratic polynomial model was chosen, as indicated by the algorithm, and well-fitted for the three separate variables and responses [23]. As seen in Equations (1) to (3), the final equations for estimation developed by the system were represented as a series of coded variables where the empirical association between extraction time (A), solute-to-solvent ratio (B), and size of sample (C) were established:

The BBD concept matrix in its entirety, with a total of 17 experiments was identified by using analysis of variance (ANOVA) and evaluating the F-value and p-value as shown in Table 3. The p-value, which can reveal the pattern of correlations between variables, was used to determine the significance of each coefficient. A strong coefficient of regression and a small p-value indicates a significant effect on the response variables. When the p-value was less than 0.05, a quadratic model of the experimental results was considered significant. The ANOVA for the second-order polynomial model of TPC ($p = 0.00020$, TFC ($p < 0.0001$), and DPPH radical scavenging ($p = 0.0015$) activity showed that the models were significant.

$$Y(TPC) = + 40.82 + 0.4813A - 3.99B + 4.85C + 2.42AB + 0.1250AC - 1.27BC + 2.44A^2 + 1.33B^2 - 1.46C \quad (3)$$

$$Y(TFC) = + 6.11 + 5.47A + 3.11B - 2.04C + 4.74\,AB - 1.06\,AC - 1.31BC + 5.23A^2 + 1.76B^2 + 0.6400C^2 \quad (4)$$

$$Y(DPPH) = +51.28 + 3.62A + 0.0087B - 2.39C + 2.06AB + 0.00850AC - 9.73BC + 5.33\,A^2 + 12.06B^2 + 2.65C^2 \quad (5)$$

Table 3. Analysis of variance (ANOVA) of regression equation for optimization of TPC, TFC, and DPPH radical scavenging activity in *P. dactylifera*.

	TPC					TFC				DPPH			
Variance Source	dF	p-value	Sum of Square	Mean Square	F-value	p-value	Sum of Square	Mean Square	F-value	p-value	Sum of Square	Mean Square	F-value
Model	9	0.0002	387.94	43.10	24.07	< 0.0001	587.50	65.28	45.81	0.0015	1387.34	154.15	12.69
A	9	0.3371	1.85	1.85	1.06	< 0.0001	238.93	238.93	167.69	0.0218	104.76	104.76	8.36
B	1	< 0.0001	127.60	127.60	< 0.0001	0.0002	77.19	77.19	54.17	0.9945	0.0006	0.0006	0.0001
C	1	< 0.0001	187.99	187.99	< 0.0001	0.0019	33.25	33.25	23.34	0.0489	68.80	68.80	5.67
AB	1	0.0081	23.38	23.38	0.0081	< 0.0001	89.78	89.78	63.01	0.2762	16.93	16.93	1.39
AC	1	0.8553	0.0625	0.0625	0.8553	0.1183	4.52	4.52	3.17	0.9625	0.0289	0.0289	0.0024
BC	1	0.0970	6.40	6.40	0.0957	0.0650	6.81	6.81	4.78	0.0008	378.69	378.69	31.18
A^2	1	0.0068	25.03	25.03	0.0068	< 0.0001	115.06	115.06	80.75	0.0164	119.83	119.83	9.87
B^2	1	0.0780	7.43	7.43	0.0780	0.0192	13.04	13.04	9.15	0.0002	612.37	612.37	50.43
C^2	1	0.0571	9.03	9.03	0.0571	0.3076	1.72	1.72	1.21	0.1630	29.51	29.51	2.43
Residual	7		12.22	1.75			9.97	1.42			85.01	12.14	
Lack of Fit	3	0.6358	3.90	1.30	0.6246	0.1645	6.84	2.28			6.86	2.29	0.1171
Pure Error	4		8.34	2.08			3.13	0.7837			78.15	19.54	
Total	16		400.16				597.47				1427.35		
R^2			0.9695										
Adj- R^2			0.9302										
Pre- R^2			0.8117										
Adeq. Pre			17.4512										
CV%			3.15										

Following this, the regression models' coefficient of determination (R^2) for the anticipated TPC, TFC, and DPPH radical scavenging activities were 0.9695, 0.9833, and 0.9423, respectively. This demonstrates the adequacy and precision of the model. Regression models' validity in determining the effects of independent variables on dependent variables can be reasonably assessed by considering strong coefficient of determination (R2) values of 70% and above [24]. Furthermore, the adjusted coefficient of determination (R_{adj}^2 =0.9302) indicated that the model was highly significant, and the coefficient of variation (C.V. = 3.15%) this finding suggest that the polynomial model exhibited a high degree of accuracy and reliability.

Analysis of Response Surface

Three main factors were taken into consideration in the effects of maximum TPC, TFC, and DPPH radical scavenging activity. Three-dimensional response surface-plot (3D) designed by the fitted models are shown in Figures 1 to 3. These surface plots offered a means to visualize the interactions of each parameter between responses and experimental stages [25]. The response surface plots were constructed by manipulating two variables within the specified experimental ranges, while keeping the remaining variables at their default values (0 levels) [26].

Response Surface Optimization of Total Phenolic Content, TPC

The volume of TPC derived from *P. dactylifera* extract ranged from 32.22 mg/g gallic acid equivalents to 51.67 mg/g gallic acid equivalents (GAE). Total *P. dactylifera* extracts obtained a mean value of 41.90 mg/g GAE. Experiment No. 4 exhibited the lowest total phenolic compound (TPC) content, as indicated in Table 2. Conversely, Experiment No. 3 displayed the highest TPC content, also documented in Table 2. The analysis of variance (ANOVA) conducted on the regression coefficient revealed that the solute-to-solvent ratio (B) and size of sample (C) exhibited statistical significance at a p-value of less than 0.0001 (refer to Table 1). The quadratic (A^2) and integrated interactions between extraction time and solute-to-solvent ratio (AB) were also significant ($p < 0.05$) on the yield of TPC.

There was a significant interaction between extraction time and solute-to-solvent ratio in Figure 1A. At lower solute-to-solvent ratios, TPC gradually increased as the extraction time increased. Meanwhile, higher solute-to-solvent ratios and longer extraction times resulted in lower TPC. TPC might increase in the early time range as a result of kinetic extraction.

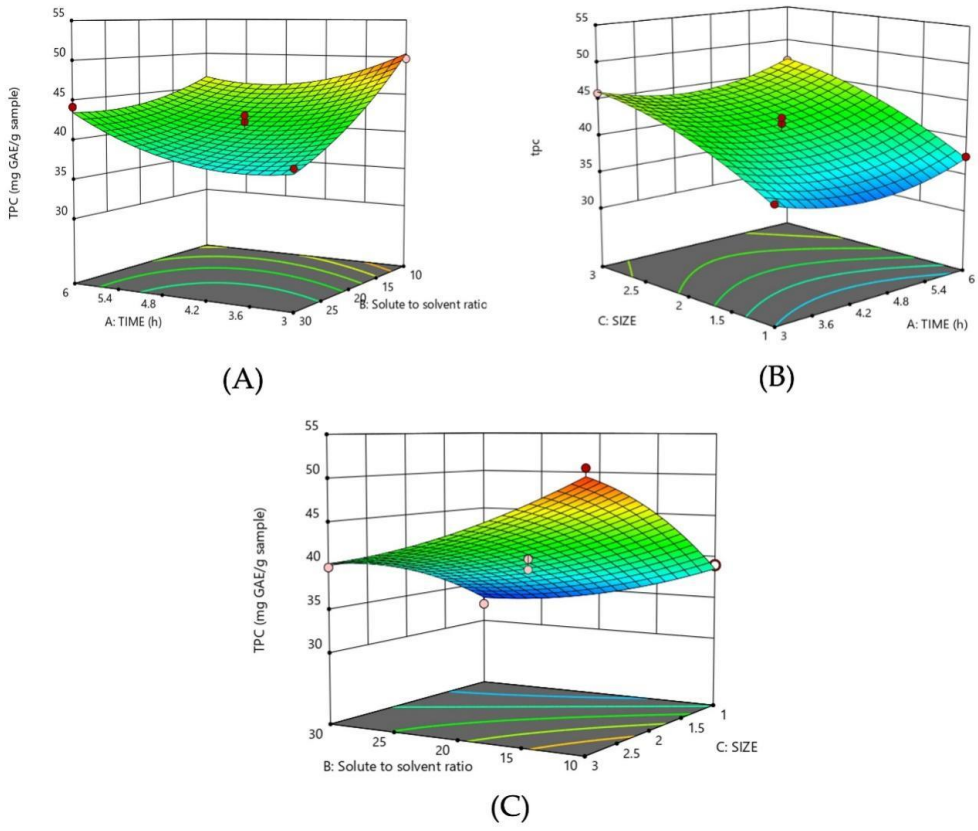

Figure 1. Response surface for effect of extraction time and solute to solvent ratio (A); the effect of extraction time and size of sample (B); and the effect of solute to solvent ratio and size on total phenolic content (C) in *P. dactylifera*. TPC: Total phenolic content; GAE: gallic acid equivalent.

In comparison to other ratios, a lower solute-to-solvent ratio indicates a higher rate of the sample. As a result, the antioxidant activity was high. Extending extraction time is unnecessary and could degrade compounds of interest [27]. The observed phenomenon can be elucidated by invoking the second Law of Fick's diffusion, which posits that a condition of equilibrium can be attained between the solute concentration within the solid matrix and the surrounding bulk solution after a specific duration of time [28]. Thus, prolonged extraction time is unnecessary for extracting phenolic compounds

Response Surface Optimization of Total Flavonoid Content, TFC

The mean experimental results showing TFC from *P. dactylifera* extract at various extraction conditions were reported at 9.70 mg QE/g extract, ranging from 5.09 to 27.38 mg QE/g extract. The highest TFC was found in Experiment 14 (Table 2) and the lowest yield was found in Experiment 10 (Table 2). The model has an F value of 45.81 and a p-value of 0.0001, indicating that it was acceptable. Meanwhile, all three linear (A, B, C), interaction parameter (AB), and quadratic parameters (A^2, B^2) were concluded as significant model terms (Table 3). The effects of the variables and their interactions on the responses are shown in Figure 2.

The surface reaction plot in Figure 2A reveals that solute-to-solvent had only a minor impact on flavonoid yield, whereas extraction time had a greater impact. A reduction in extraction time resulted in a significant increase in flavonoid yield at any level of solute-to-solvent ratio. In the present study, the optimum amount of flavonoid was revealed at a ratio of 1:20 g/mL at extraction times of 4.2 to 4.8 h. As its solid matrix was the main constraint of mass transfer in this range, a higher solute-to-solvent ratio boosted the effect [29]. The presence of excess solvent in the system corresponded to the diminished concentration of solute, resulting in a decrease in the occurrence of cavitation. This decrease was attributed to a lower number of nucleation sites, which ultimately led to a decline in the production of flavonoids [30]. Thus, the optimal solute-to-solvent ratio was chosen to be 1:20 g/mL.

The different extraction times and size of samples are shown in Figure 2B of the 3D response surface plots. The maximum flavonoid level in the *P. dactylifera* extract was obtained in the range of 3 h to 4.2 h and size 2.87 mm to 4.75 mm. The prolonged time of extraction led to a decrease in flavonoid content.

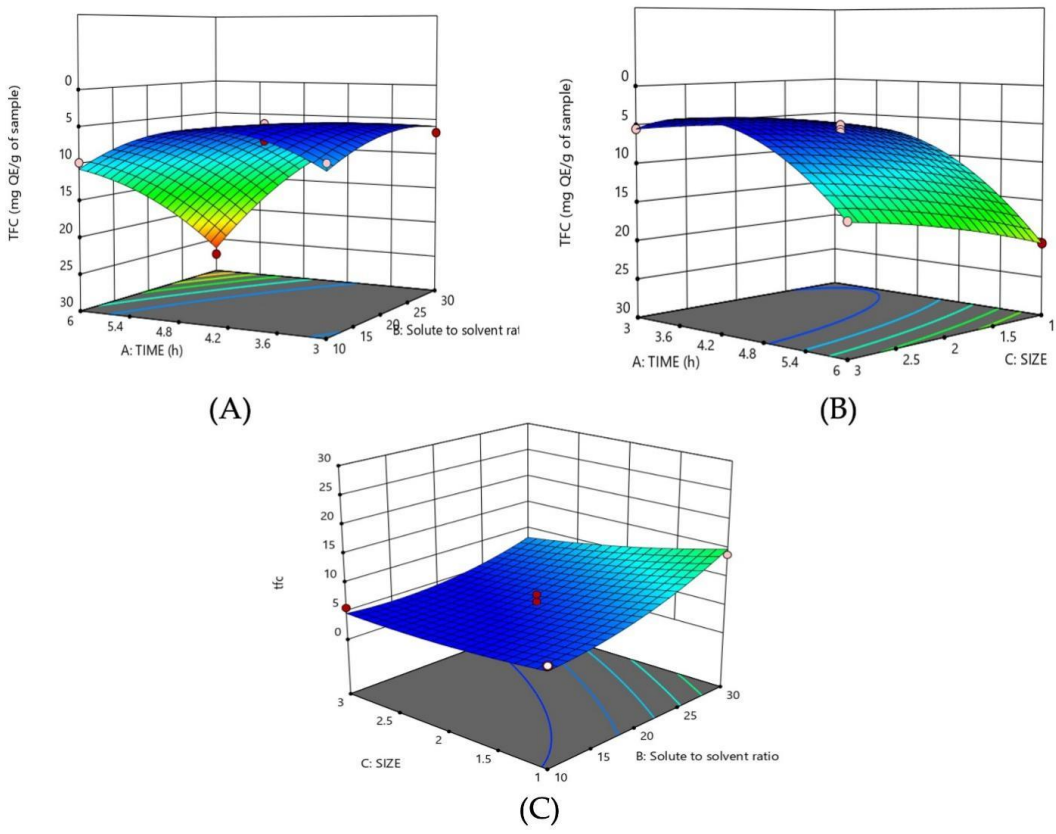

Figure 2. Response surface for the effect of extraction time and solute to solvent ratio (A); the effect of extraction time and size of sample (B); the effect of solute to solvent ratio and size on total flavonoid content (C) in *P. dactylifera*. TFC: Total flavonoid content; GAE: gallic acid equivalent.

According to *Mojzer* et al. [31], over-exposure in extension time on heating of sample caused degradation of phenolic compounds, suggesting that longer extraction time would cause a decline in the recuperation of phenolic compounds. According to the review, this was due to the oxidation of phenolic compounds caused by excessive heating of plant samples, implying that a suitable extraction period for greater recovery should be evaluated [32].

Response Surface Optimization of DPPH Radical Scavenging Activity

In this study, the DPPH radical scavenging activity of *P. dactylifera* extract ranged from 46.89% to 78.49%. The mean value recorded an average of 60.71%. The highest DPPH value was found in Experiment No. 10 (Table 5) while the lowest was found in Experiment No. 4 (Table 5). The ANOVA of the regression coefficient revealed that the model terms A, C, BC, A^2, and B^2 were significant ($p < 0.05$). Figure 3 depicts the effect of their variables and interactions on the response.

Figure 3A shows the response between the time of extraction (A) and solute-to-solvent (B). DPPH radical scavenging activity was observed to increase along with extraction time and solute-to-solvent particle at a constant size of 2.87 mm. The DPPH activity peaked at a range of 4.2 to 4.8 h time of extraction and 1:15 to 1:25 solute-to-solvent ratio. High DPPH radical scavenging activity could be achieved by increasing extraction time by enhancing the solute solubility and extraction coefficient [33]. Besides, the result showed that increasing the size of sample reduced DPPH activity.

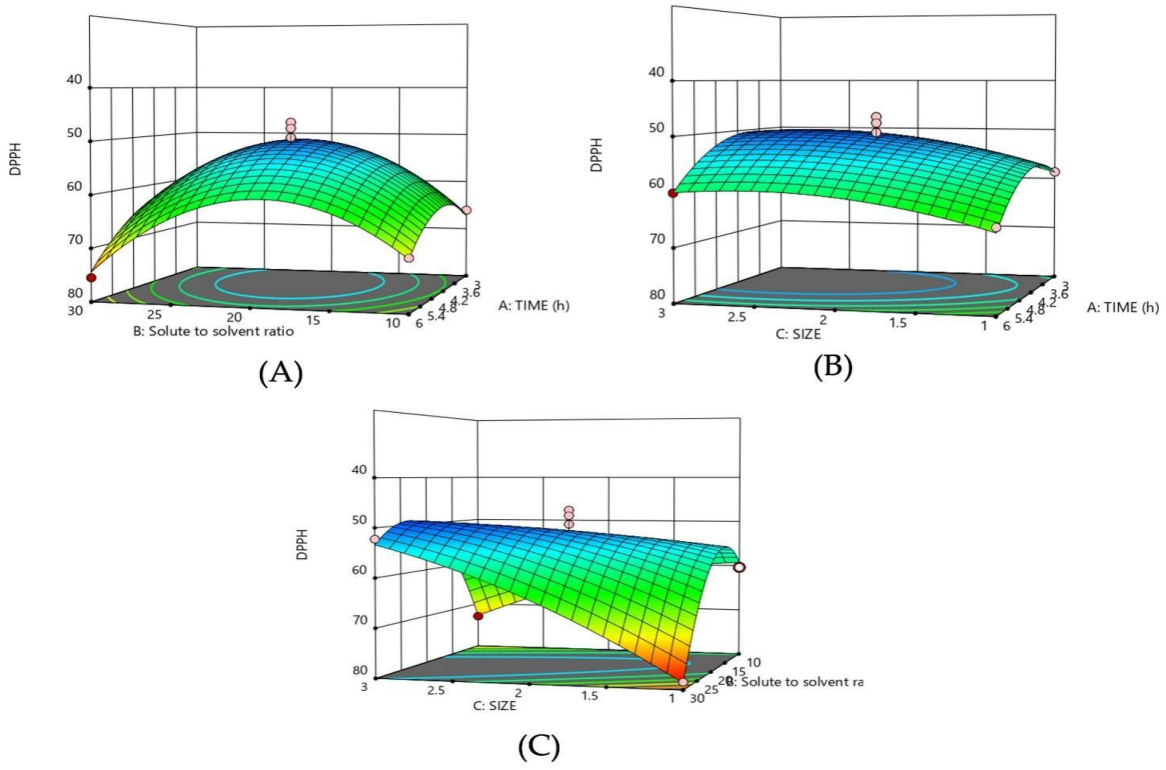

Figure 3. Response surface for the effect of extraction time and solute to solvent ratio (A); the effect of extraction time and size of sample (B); and the effect of solute to solvent ratio and size on DPPH (C) in *P. dactylifera*. DPPH: 2,2-diphenyl-1-picrylhydrazyl.

Table 4. Values predicted and observed for responses on TPC, TFC and DPPH activity in *P. dactylifera.*

1. Values	2. TPC 3. (mg GAE/g Sample)	4. TFC 5. (mg QE/g sample)	6. DPPH 7. (mg ascorbic acid/g sample)
Predicted	8. 50.93	9. 9.64	10. 78.17
Experimental	11. 50.25 ± 0.68	12. 11.14 ± 1.50	13. 79.22 ± 1.05

RSM Optimization in Extracting Conditions for TPC, TFC, and DPPH

The adequacy of the model equation in predicting the optimal response value was assessed under specific experimental settings, including a 6-hour extraction duration, a solute-to-solvent ratio of 1:10, and a sample size of 4.75 mm. Three parallel tests were done under the specified conditions. The observed result closely approximated the theoretical forecast, suggesting that employing this model for optimising the extraction process was a prudent decision. The results of the optimisation process are presented in Table 4, which displays the expected and observed values.

Identification of Phenolic Compounds in Dates Fruit Extract

The present study employed the ultra-high-performance liquid chromatography combined with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF/MS) technique to identify the phenolic compounds present in the extract of P. dactylifera. This analysis was conducted under optimal conditions of Soxhlet Extraction. The Ajwa cultivar of dates holds considerable importance and is widely recognised for its favourable characteristics. It is widely believed that the antioxidant activity exhibited by medicinal plants can be attributed to their phenolic content. Table 5 displays the roster of phenolic compounds that have been found, organised according to their respective retention times. The compounds were categorised based on the interpretation of their mass spectra, which were obtained using a QTOF mass analyzer in negative mode. This classification took into account the available information in the literature as well as pertinent databases. The retention times or mass spectra of the compounds associated with the Mass Waters library, as well as existing literature, were used to validate the detection of phenolic compounds. Phenolic compounds based on the Waters library were labeled as tentative, with errors below 5ppm and more than one theoretical fragment taken into account [34].

The present study showed that *P. dactylifera* extract possessed compounds that belonged to different phenolic groups. The superior compounds in any group of phenolic compounds are those that can eliminate free radicals and prevent other oxidation reactions [35]. Flavonoids were the most common phenolic compounds found in *P. dactylifera* extract, with a wide distribution. The health benefits of antioxidants and free radical scavenging properties are well known. It's mostly found in high concentrations within the skin of the fruit [36].

Arbutin is a hydroquinone and D-glucose compound that has been linked to skin depigmentation. Therefore, similar to other phenolic compounds found in the diet, arbutin has the ability to hinder signal transduction in melanocytes that is mediated by reactive oxygen species (ROS), consequently serving as a preventive measure against skin hyperpigmentation [37]. Compounds 3, 5, 6, 7, 8, and 9 are all variations of the class of compounds known as flavonoids.

Table 5. Identified phenolic compounds from date fruit extract with retention time (min), neutral mass (Da), Observed mass-to-charge ratio (m/z), and identification status.

No	Component name	Retention time (Min.)	Neutral Mass (Da)	Observed -to-charge ratio (m/z)	Identification status and label
1	Arbutin	2.56	272.0896	271.0819	Identified, tentative
2	6′-O-β-D-Glucosyl gentiopicroside	5.33	518.1632	517.1559	Identified, tentative
3	Quercetin-3-O-rutinoside	7.27	610.1543	609.1471	Identified, tentative
4	Leucocyanidin	7.63	306.0736	305.0663	Identified, tentative
5	3-O-[β-D-Glucopyra-nosyl-(1→2)]-β-D-glucopyranosyl-7-O-α-L-glucopyranosyl-kaempferol	7.66	756.21129	755.2037	Identified, tentative
6	Rutin	8.52	610.1534	609.1462 14.	Identified, tentative
7	6-Hydroxykaempferol-3-O-glucoside	8.70	464.09548	463.0885	Identified, tentative
8	Kaempferol-3-O-β-rutinoside	9.10	594.159	593.1517	Identified, tentative
9	Diosmin	10.45	608.1743	607.167	Identified, tentative
10	Homoplantaginin	10.36	462.1165	461.1092	Identified, tentative
11	Ciwujiatone	10.64	434.1575	433.1502	Identified, tentative
12	Sanleng acid	15.8	330.2405	329.2333	Identified, tentative

Both diosmin and rutin are well known as compounds that are effective in inhibiting the activity of tyrosinase [38,39]. In another scientific study, diosmin was found to be present in citrus fruits [40, 41]. It was also isolated from the leaves of *O. europaea*, which are utilized in the pharmaceutical industry [39]. Kaempferol is a natural flavonol found in dates that are best described as a cancer-fighting compound [35]. Figure 4 (A) shows UHPLC-QTOF/MS chromatogram of Quarcetion-3-O-rutinoside from dates fruit extract. Figure 4(B) is a mass spectra (MS/MS) of Quarcetion-3-O-rutinoside obtained with the minimal collision energy and Figure 4(C) is a mass spectra (MS/MS) of Quarcetion-3-O-rutinoside obtained at high collision energy. Figure 5(A) shows UHPLC-QTOF/MS chromatogram of Diosmin from dates fruit extract. Figure 5(B) is a mass spectra (MS/MS) of Diosmin rutinoside obtained with the minimal collision energy and Figure 5(C) is a mass spectra (MS/MS) of Diosmin obtained at high collision energy.

Figure 4 (A)

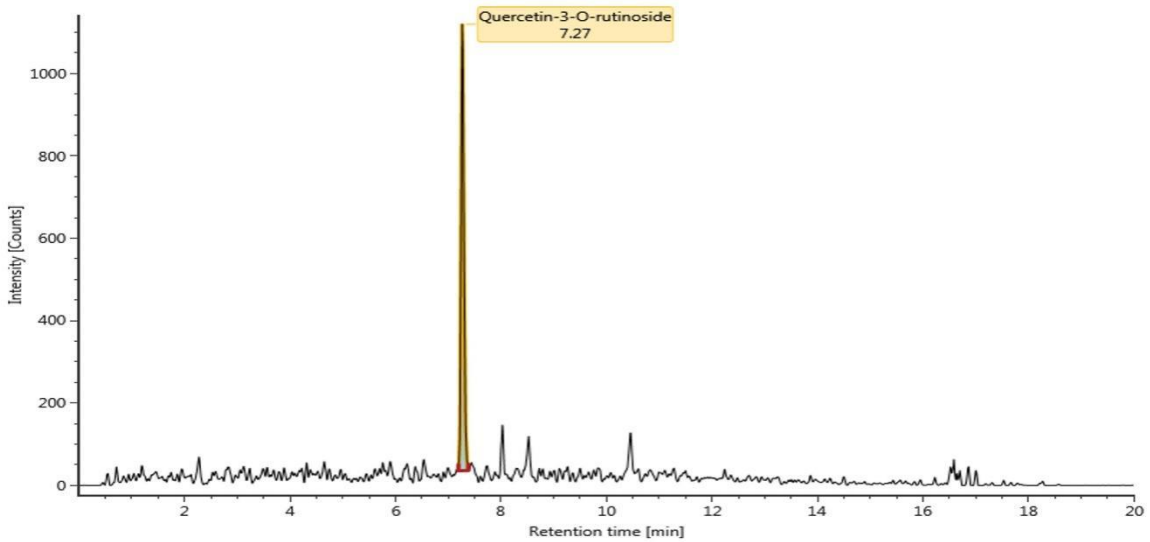

Figure 4 (B)

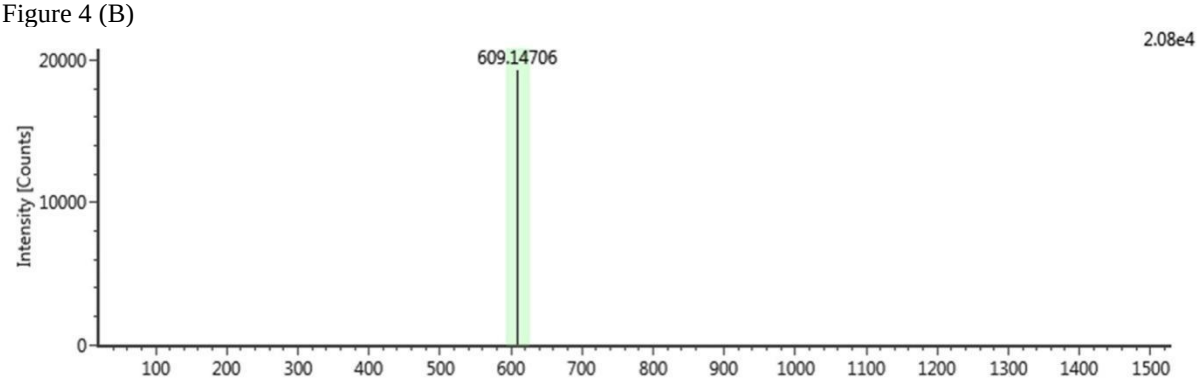

Figure 4 (C)

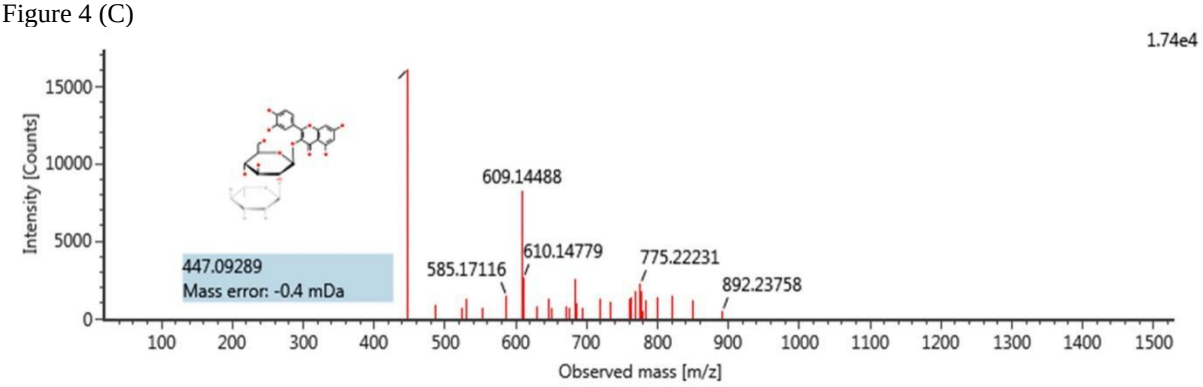

Figure 4. UHPLC-QTOF/MS chromatogram of Quarcetion-3-O-rutinoside from dates fruit extract. (A) Mass spectra (MS/MS) of Quarcetion-3-O-rutinoside obtained with the minimal collision energy; (B) Mass spectra (MS/MS) of Quarcetion-3-O-rutinoside obtained at high collision energy (C).

Figure 5 (A)

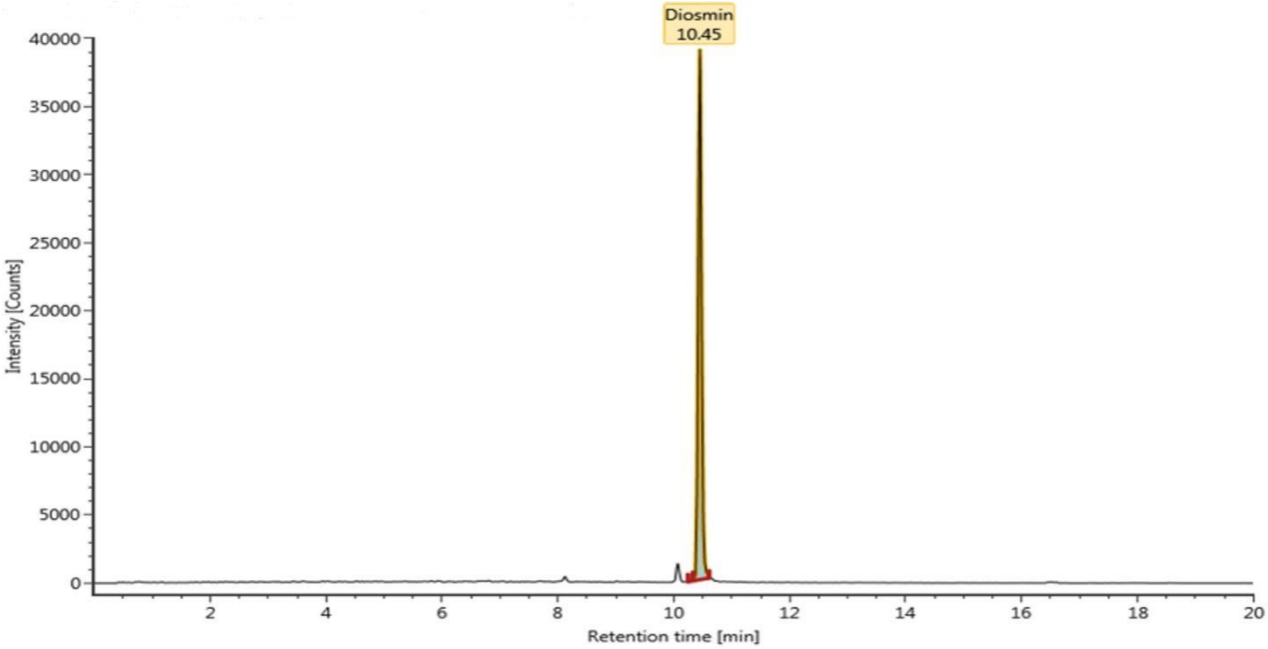

Figure 5 (B)

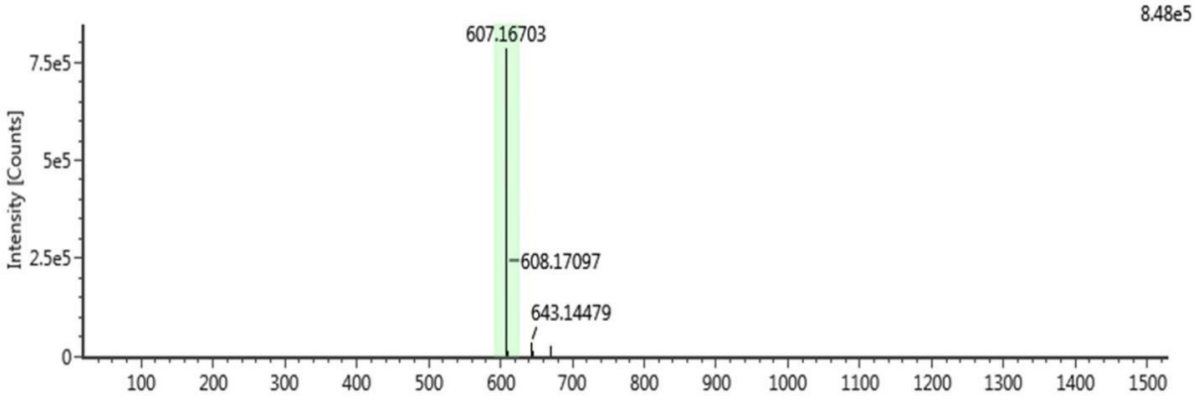

Figure 5 (C)

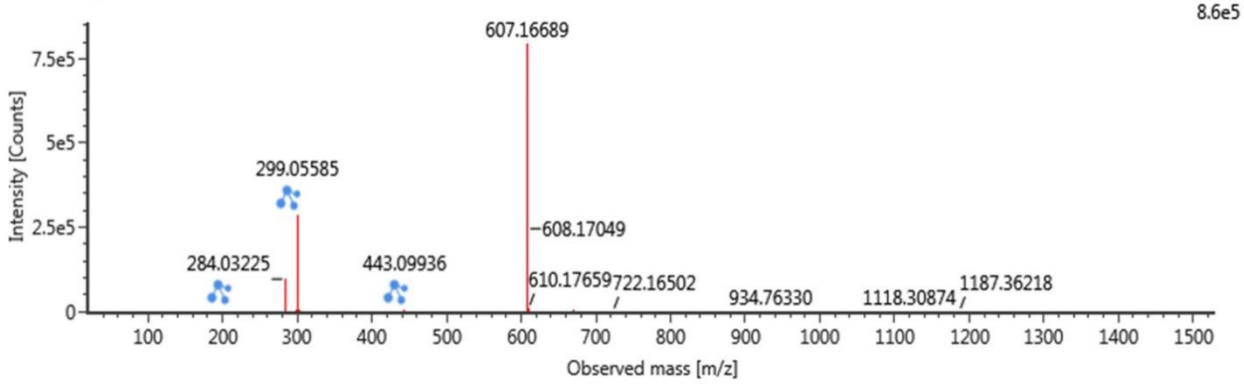

Figure 5. UHPLC-QTOF/MS chromatogram of Diosmin from dates fruit extract. (A) Mass spectra (MS/MS) of Diosmin rutinoside obtained with the minimal collision energy; (B) Mass spectra (MS/MS) of Diosmin obtained at high collision energy (C).

The compound iridoid 6′-O-β-D-Glucosyl gentiopicroside6′-O—D-Glucosyl gentiopicroside, which has also been found in fenugreek seed extract has been reported to have biological and health benefits [42]. Their importance in pharmaceutical chemistry research has been demonstrated by their prominent effects on various diseases. As one of the active ingredients in traditional Chinese medicine and natural medicine

[43, 44]. Besides, Ciwujiatone is a compound that performed exceptionally well in an in vitro test of DPPH's ability to measure antioxidant potential [45]. Sanleng acid is an organic acid found in date flesh extract, but no specific mechanism of action has been reported. According to Lu et al. (2021) [45], sanleng acid is one of the main active ingredients in the *Sparganii rhizoma* treatment of gastric cancer.

CONCLUSION

The determination of optimal conditions and second order polynomial models for predicting responses was conducted utilising Response Surface Methodology (RSM) through the Box-Behnken design (BBD). Significant total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging activity were observed in the extract of *P. dactylifera* when subjected to an extraction time of 6 hours, a solute-to-solvent ratio of 1:10, and a sample size of 4.75 mm. The identification of phenolic components in the extract of *P. dactylifera* was accomplished by the utilisation of UHPLC-QTOF-MS. The findings indicate that the extract derived from *P. dactylifera* exhibits significant antioxidant properties in relation to DPPH. This suggests that the extracts from *P. dactylifera* have promise as a natural antioxidant for incorporation into cosmeceutical formulations.

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