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Microwave-assisted extraction of phenolic compounds from Carica papaya leaves: An optimization study and LC-QTOF-MS analysis

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ABSTRACT

Carica papaya is a well-known plant with diverse importance to human beings. The efficacy of microwave-assisted extraction (MAE) in the recovery of phenolic compounds from *C. papaya* leaf was investigated in this study. The MAE process variables were optimized using response surface method; at the optimized conditions, the embedded phenolic compounds in the extract were identified through LC-QTOF-MS analysis. The results showed that the best MAE conditions were: time of irradiation at 3 min; microwave power of 420 W; feed-to-solvent ratio of 1:12 g/mL; and solvent concentration of 56% ethanol/water to achieve 102.59 mg GAE/g d.w. of total phenolic content (TPC). At these conditions, an aggregate of 72 phenolic compounds was tentatively identified. These results infer that MAE as a technique can recover phenolic compounds from *C. papaya* leaf. Hence, the identified compounds can further be quantified with their respective pharmacological properties in future studies.

1. Introduction

Several clinical trials and epidemiological studies have outlined that the intake of plant-based foods especially nuts, fruits and vegetables can secure human system against oxidative-stress diseases, cardiovascular and gastrointestinal ailments (Joanne and Lloyd, 2012). The consumption of these plant-origin foods tends to build-up the body immune system in fighting against the attack of different diseases. Different kinds of phytochemicals that include glucosinolates, carotenoids, and polyphenols synergistically work to minimize oxidation and inflammation; and provide defense against the evolution and initiation of sicknesses (Shetty and Ao, 2004). Phenolic compounds are the main contributors in the polyphenol groups. These phenolic compounds donate hydrogen atoms or electrons to reactive radicals and prevent cellular damages or degradation; these show their antioxidant activity (Cheynier, 2012). Other than the antioxidant activity, phenolic compounds show several other biological activities such as anti-inflammatory, anti-fungal, antibacterial, anti-diabetic, anti-allergic, and several others (Cheynier, 2012; Działo et al., 2016). Moreover, phenolic compounds can take part in the enzymatic pathways that act as signaling molecules in the cells (Garcia-Salas et al., 2010).

Carica papaya is one of the plant enriched with diverse medical and nutritional values. Conventionally, different parts of this plant (roots, fruits (both unripe and ripe), barks, seeds, and leaves) are being employed to treat different kind of ailments such as dengue, jaundice, wound dressing, stomach problem, ringworm, urinary problem, high blood pressure, roundworm, weight loss, and removal for snake poison (Anaga and Onehi, 2010; Sultana et al., 2014; Zunjar et al., 2011). In addition, the leaf extract had been reported to comprise different phytochemicals which include tocopherol, papain, ascorbic acid, glycosides, flavonoids, cyanogenic, and glucosinolates; that can improve antioxidant activity in the blood and minimize lipid peroxidation level (Alara et al., 2020a, 2020b; Yadav et al., 2017). It should be noted that selection of adequate extraction technique is important to achieve desire results from plant materials.

Extraction involves the separation of a substance from a particular matrix; this can either be solid phase or liquid-liquid extraction. It entails the distribution of a solute between two phases at an equilibrium condition through partition theory. Moreover, extraction is a process used to recover phenolic compounds from plant materials. There are two main categories, that is, conventional and unconventional methods; the latter includes soxhlet, maceration and soaking while the unconventional methods include ultrasound-assisted, pressurized-assisted, extraction induced with electric field, supercritical fluid, microwave-assisted, and several others (Alara et al., 2018; Herrero et al., 2012). Amongst the methods, microwave-assisted extraction (MAE) is mostly employed due to its simplicity, cost effectiveness, reduction in time of

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Table 1
Experimental design and results for TPC yields obtained through FCCCD.

Standard deviation	Time of irradiation (min)	Microwave power (W)	Feed/solvent ratio (g/mL)	Solvent concentration (% v/v)	TPC (mg GAE/g extract)
1	2	400	0.07	40	79.39
2	6	400	0.07	40	80.70
3	2	600	0.07	40	80.04
4	6	600	0.07	40	81.34
5	2	400	0.1	40	81.12
6	6	400	0.1	40	79.95
7	2	600	0.1	40	83.55
8	6	600	0.1	40	78.82
9	2	400	0.07	60	95.85
10	6	400	0.07	60	95.57
11	2	600	0.07	60	90.48
12	6	600	0.07	60	84.33
13	2	400	0.1	60	98.27
14	6	400	0.1	60	93.91
15	2	600	0.1	60	86.55
16	6	600	0.1	60	79.27
17	2	500	0.09	50	97.23
18	6	500	0.09	50	96.54
19	4	400	0.09	50	101.65
20	4	600	0.09	50	96.32
21	4	500	0.07	50	96.52
22	4	500	0.1	50	93.35
23	4	500	0.09	40	87.03
24	4	500	0.09	60	98.78
25	4	500	0.09	50	97.73
26	4	500	0.09	50	101.91
27	4	500	0.09	50	101.28
28	4	500	0.09	50	101.05
29	4	500	0.09	50	101.15
30	4		0.09	50	101.30

recovering phenolic compounds from plant materials, and reduction in solvent consumptions (Alara et al., 2019; Alara and Abdurahman, 2019; Chan et al., 2011; Taamalli et al., 2012). In the extraction of phenolic compounds from *C. papaya* leaf, shaking water bath had previously been used to obtain total phenolic content (TPC) of 23.06 ± 1.06 mg GAE/g d.w. using water as the solvent (Vuong et al., 2013). In another study, TPC of 18.33 µg GAE/mg d.w. was recovered using maceration technique (Nariya and Jhala, 2017). Thus, the MAE of phenolic compounds from *C. papaya* leaf is yet to be reported. MAE is a method that is influenced by factors such as solvent concentration, time of extraction, temperature, feed/solvent ratio, and microwave power (Alara et al., 2018). In order to optimize the extraction process, response surface methodology (RSM) was considered. RSM is a statistical-based technique that employs quantitative data to evaluate and solve multifactor equations so as to optimize the required processes (Uma et al., 2010). It had been employed in the extraction of phenolic compounds from different kinds of plant samples (Akhbari et al., 2018; Alara et al., 2018, 2019).

Thus, the focus of this study was to examine the applicability of MAE in recovering phenolic compounds from *C. papaya* leaves through the optimization of process parameters using face-centered central composite design (a simplex form of RSM); and to identify the embedded bioactive compounds using LC-QTOF-MS analysis at the optimized conditions.

2. Materials and methods

2.1. Plant material, chemical materials and reagents

C. papaya leaves were harvested from the cultivars planted at Universiti Malaysia Pahang resident's garden located in Gambang, Kuantan, Malaysia. In order to achieve uniform plant sample for the phenolic extraction, the leaves were obtained from a single *C. papaya* tree, washed and dried in the dark at room temperature. Sodium carbonate, Folin-Ciocalteu's phenol reagent and gallic acid were purchased from Sigma Aldrich Sdn. Bhd., Selangor.

2.2. Preparation of leaf extract of Carica papaya

The dried leaves of *C. papaya* were ground and sieved to a fine powder (average particle size of 105 mm). Afterwards, 5 g of *C. papaya* leaf sample was extracted using different ethanol/water ratio (based on experimental design, 1:10–1:16) in an enclosed microwave extractor (1000 W, 2450 MHz, Milestone, Italy) at different microwave power (300–600 W), time of irradiation (2–8 min), solvent concentration (40–70% v/v), temperature (50–80 °C). The obtained extracts were filtered through HPLC 0.45 µm porosity into well-closed neat vials and stored in the dark at 4 °C before determining the total phenolic contents in the extracts.

2.3. Measurement of total phenolic content (TPC) in the leaf extract

TPC in the leaf extracts of *C. papaya* was determined colorimetrically, following the method outlined by Alara et al., 2018. Calculation of TPC was based on a generated calibration curve using gallic acid ($y = 0.0006x + 0.0169$, $R^2 = 0.9903$).

2.4. Screening and RSM studies

Firstly, the MAE factors including time of irradiation, microwave power, solvent concentration, feed/solvent ratio, and temperature were screened to understand the effect and significance of individual factors. These factors were varied to estimate the range of values for the optimization study: time of irradiation, 2–8 min; microwave power, 300–600 W; solvent concentration, 40–70% v/v; feed/solvent ratio, 1:10–1:16 g/mL; and temperature, 50–80 °C.

The significant MAE factors were further optimized using face central centered composite design (FCCCD). Four Factors (time of irradiation, microwave power, feed/solvent ratio, and ethanol concentration) were optimized to recover optimal TPC from *C. papaya* leaf. The independent factors are time of irradiation (X_1: 2–6 min), microwave power (X_2: 300–500 W), feed/solvent ratio (X_3: 1:10–1:14 g/mL), and ethanol concentration (40–60% v/v) with TPC (Y_1) as the response factor. Thirty

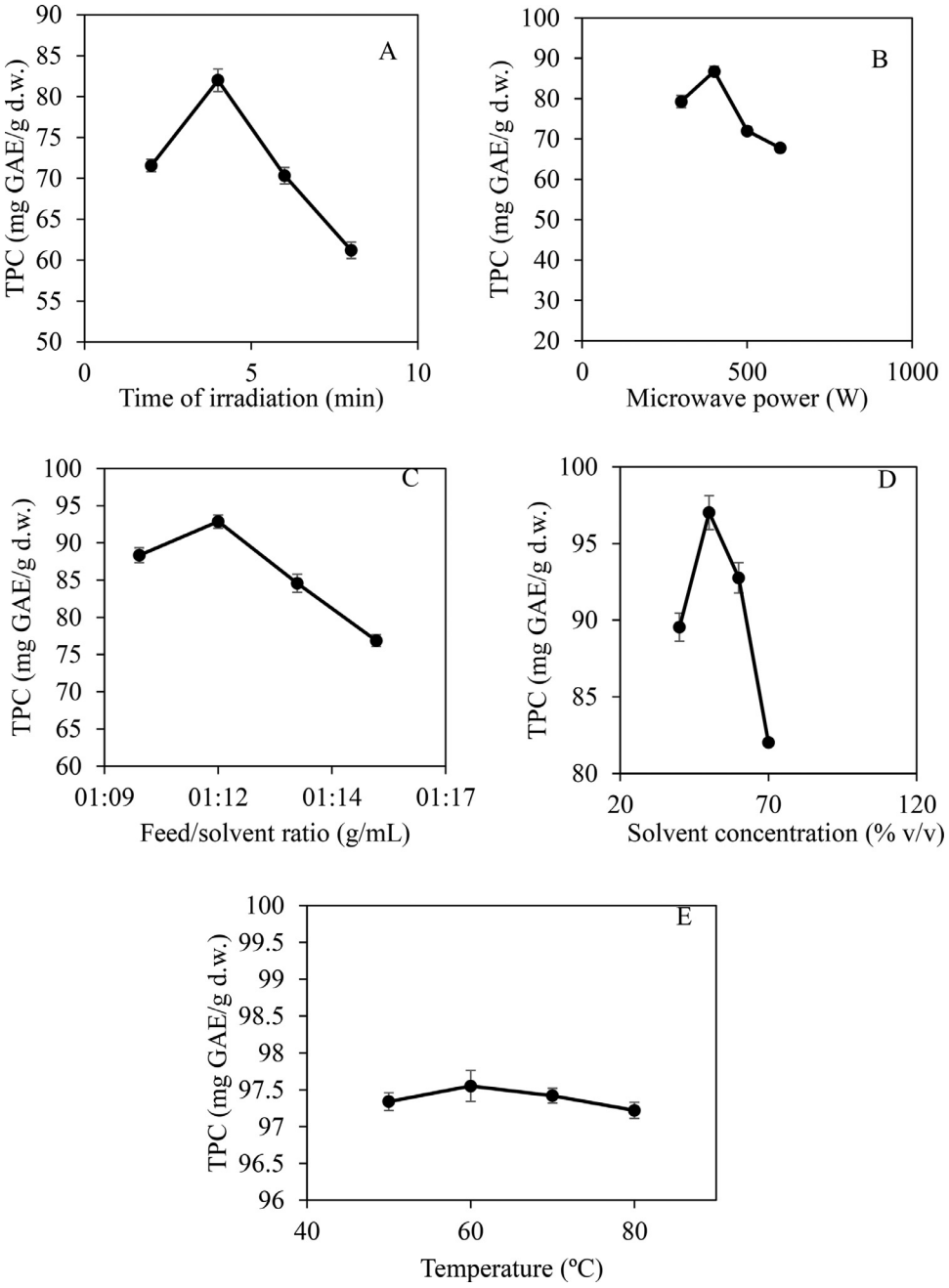

Fig. 1. The influence of time of irradiation (A), microwave power (B), feed/solvent ratio (C), solvent concentration (ethanol) (D), and temperature (E) on the yield of phenolic compounds from *C. papaya* leaf.

randomized experimental runs including six center points were obtained through FCCCD (Table 1). The regression analysis was performed to generate the quadratic models (Eq. (1)). Furthermore, the generated optimal value was verified by comparing the predicted and experimental values using a paired *T*-test analysis.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_1 X_1 + \sum_{\substack{i=1 \\ i<j}}^{k=1} \sum_{j=2}^{k} \beta_{ij} X_1 X_j + \sum_{i=2}^{k} \beta_{ii} X_i^2 \quad (1)$$

where Y represents response factor TPC, β_0 is a constant, β_i denotes the linear coefficient, β_{ii} is the second-order polynomial coefficient, β_{ij} represents the interaction coefficient between two factors, X_i and X_j are the independent factors.

2.5. LC-QTOF-MS conditions

The extract obtained at optimized MAE conditions was analysed using LC-QTOF-MS to identify the bioactive compounds. A Waters Acquity ultra-performance liquid chromatography (UPLC HSS TS, USA) machine that contains autosampler and binary pump was used. An analytical column C18 (100 mm × 2.1 mm × 1.8 µm; Waters, USA) at 30 °C was employed to separate the bioactive compounds. The operating conditions and gradient elution for negative and electrospray ionization source interface (ESI) that was previously reported by the authors were used (Alara et al., 2018). The identification of bioactive compounds in the leaf extract of *C. papaya* was carried out using the MS/MS fragmentation pattern in relative to the UNIFI Waters library reference standards.

2.6. Analysis of data

The experimental trials were repeated thrice, and average values for TPC yields were recorded as mean ± standard deviation using Microsoft Excel 2013®.

Table 2
ANOVA analysis for the response surface quadratic models.

Extraction parameters	Sum of squares	df	Mean square	*F*-value	*p*-value Prob > *F*
Model	2052.62	14	146.62	67.13	< 0.0001
X_1	27.01	1	27.01	12.37	0.0031
X_2	116.08	1	116.08	53.15	< 0.0001
X_3	4.94	1	4.94	2.26	0.1533
X_4	460.76	1	460.76	210.98	< 0.0001
$X_1 \times_2$	9.55	1	9.55	4.37	0.0540
$X_1 \times_3$	11.76	1	11.76	5.39	0.0348
$X_1 \times_4$	13.65	1	13.65	6.25	0.0245
$X_2 \times_3$	5.93	1	5.93	2.71	0.1202
$X_2 \times_4$	129.73	1	129.73	59.40	< 0.0001
$X_3 \times_4$	6.50	1	6.50	2.98	0.1050
X_1^2	19.01	1	19.01	8.71	0.0099
X_2^2	0.96	1	0.96	0.44	0.5173
X_3^2	56.24	1	56.24	25.75	0.0001
X_4^2	115.92	1	115.92	53.08	< 0.0001
Residual	32.76	15	2.18		
Lack of fit	21.46	10	2.15	0.95	0.5604
Pure error	11.30	5	2.26		
Cor total	2085.38	29			
R^2	0.9843				
Adj. R^2	0.9696				
Pred. R^2	0.9299				
C.V.%	1.62				
PRESS	146.23				
Adeq. precision	22.981				

3. Results and discussion

3.1. Influence of microwave extraction parameters on the recovery of TPC

The contributing influences of MAE parameters (time of irradiation, microwave power, feed/solvent ratio, solvent concentration, and temperature) on the yield of TPC from *C. papaya* leaf were investigated using one-factor-at-a-time experimental trials. Fig. 1 presents the outcome results from these investigations.

Time of irradiation is an essential parameter during the extraction using microwave. Inadequate selection of time, either selection of lesser or enormous time of extraction can alter the recovery of phenolic compounds from plant materials. This implies that the underexposure or overexposure of plant matrix to microwave radiation belittle the yield intents to recover. Hence, the influence of time on TPC yield from *C. papaya* leaf was studied within the frame of 2 to 8 min as provided in Fig. 1A; other parameters including microwave power, feed/solvent ratio, solvent concentration, and temperature were fixed at 300 W, 1:10 g/mL, 40% v/v, and 50 °C, respectively. Increasing yield of TPC was clearly observed as the time of irradiation increased from 2 to 4 min with sharp decline as the time exceeded 4 min. It indicated that there was a total interaction between the extracting solvent and plant sample in 4 min, which initiated an increased in the yield of TPC from *C. papaya* leaf after an equilibrium state had been reached. Hence, the dissolution process of phenolic compounds into the solvent stopped and further exposure to microwave radiation caused the degradation (Alara et al., 2018; Dahmoune et al., 2014). The time of irradiation was then selected to be 4 min for the subsequent experimental trials.

Microwave power is another important parameter influencing the performance of MAE technique in the recovery of phenolic compounds from plant materials. During the MAE, this parameter is attributed to the dissemination of electromagnetic energy and activate the diffusion coefficient between the solvent and plant samples (Alara et al., 2018; Dahmoune et al., 2014). In view of this, microwave power was varied within 300 and 600 W at fixed 4 min of irradiation time, 1:10 g/mL of feed/solvent ratio, 40% v/v of solvent concentration, and 50 °C of extraction temperature. The highest yield of TPC (86.76 mg GAE/g d.w.) was achieved at 400 W of microwave power from *C. papaya* leaf (Fig. 1B). The reduction in phenolic compounds beyond 400 W can be associated with the influence of overexposure to microwave radiation emanated from increased power. Thus the range between 300 and 500 W was chosen for the optimization process and 400 W for subsequent OFAT experimental trials (Akhbari et al., 2018; Alara et al., 2018, 2019).

The influence of feed/solvent ratio on the recovery yield of TPC from *C. papaya* leaf was also studied. The highest yield of TPC from *C. papaya* leaf was determined by varying feed/solvent ratio between 1:10 and 1:16 g/mL at fixed 4 min of irradiation time, 400 W of microwave power, 40% v/v of solvent concentration, and 50 °C of extraction temperature. The obtained result is depicted in Fig. 1C; it was observed that the phenolic compounds improved with increasing volume of solvent, leading to the highest yield of TPC at 1:12 g/mL. This showed that the cell walls of *C. papaya* leaf optimally ruptured at the volume of the solvent used, causing the excessive swelling of the sample through the absorption of microwave radiation (Alara and Abdurahman, 2019; Muhd and Norashikin, 2018). Nevertheless, the yield declined beyond 1:12 g/mL; this was an indication of phenolic compounds degradation that occurred from excessive microwave time of exposure (Alara et al., 2018). Hence, the range between 1:10 and 1:14 g/mL was chosen for the next OFAT experimental runs and the optimization investigations.

The concentration of the extracting solvent is another essential parameters during the extraction of phenolic compounds from plant materials using microwave-assisted technique. This is because the yield of TPC is proportionally linked to the solubility of phenolic compounds in the extracting solvent (Lovrić et al., 2017). In food industrial processes, water and ethanol are mostly considered as safe solvents. On this note, the concentration of ethanol was varied between 40 and 70% against water as time of irradiation, microwave power, feed/solvent ratio, and temperature were fixed at 4 min, 400 W, 1:12 g/mL, and 50 °C, respectively. The outcome showed that the highest yield of TPC was achieved at 50% v/v of ethanol against water (97.02 mg GAE/g d.w.) as depicted in Fig. 1D. Thus, solvent concentration as a MAE parameter was varied between 40 and 60% v/v in the subsequent OFAT experimental trials and optimization study.

The last parameter considered during the OFAT experimental trials in this study was temperature. Excessive level of temperature can result in the degradation of thermolabile bioactive compounds from plant materials (Alara et al., 2018; Lovrić et al., 2017). It is important to choose an adequate temperature value to avert oxidation and degradation of

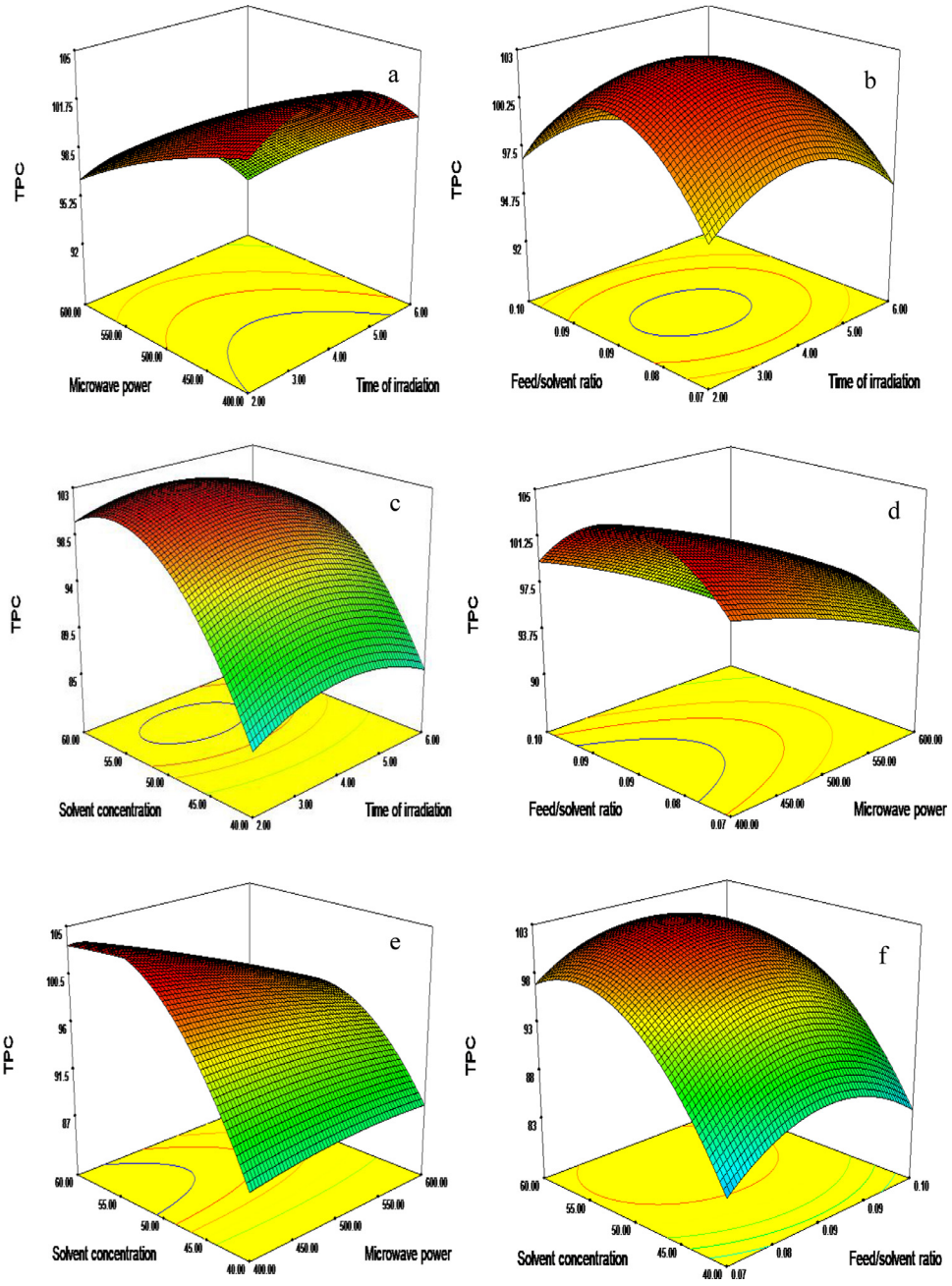

Fig. 2. Interactive effects of MAE parameters on the TPC yield from *C. papaya* leaf.

phenolic compounds. Therefore, temperature was varied between 50 and 80 °C as time of irradiation, microwave power, feed/solvent ratio, and solvent concentration were fixed at 4 min, 400 W, 1:12 g/mL, and 50% v/v, respectively. There was an insignificant influence of temperature on the yield of TPC from *C. papaya* leaf, causing this parameter not to be considered in the optimization study. However, the highest yield of 97.55 mg GAE/g d.w. was finally obtained at 60 °C (Fig. 1E).

3.2. Optimization study for extracting phenolic compounds from C. papaya leaf

Recently, several studies are being conducted on the utilization of medicinal plant as the alternative medicines especially because they are rich in different kinds of phenolic compounds responsible for diverse pharmacological activities. Thus, it seems important to establish a fast, simple, accurate, and rapid technique through the optimization of the process to extract phenolic compounds from these plant samples. In view of this, a face centered central composite design (FCCCD) with six center points was utilized to examine the efficacy of MAE technique in the recovering of TPC from *C. papaya* leaf. Table 1 depicts the FCCCD experimental design and TPC recorded from the experimental runs.

3.2.1. Fitting of RSM model

The regression analysis was carried out to investigate the actual data obtained from the experimental trials; leading to the generation of a quadratic regression model equation as presented in Eq. (2). This equation illustrates the relationship between independent parameter (TPC yield) and dependent parameters (time of irradiation, microwave power, feed/solvent ratio, and solvent concentration).

$$TPC = 100.17 - 1.23X_1 - 2.54X_2 - 0.52X_3 + 5.06X_4 - 0.77X_1X_2 - 0.86X_1X_3 - 0.92X_1X_4 - 0.61X_2X_3 - 2.85X_2X_4 - 0.64X_3X_4 - 2.71X_1^2 - 0.61X_1^2 - 0.61X_2^2 - 4.66X_3^2 - 6.69X_4^2 \quad (2)$$

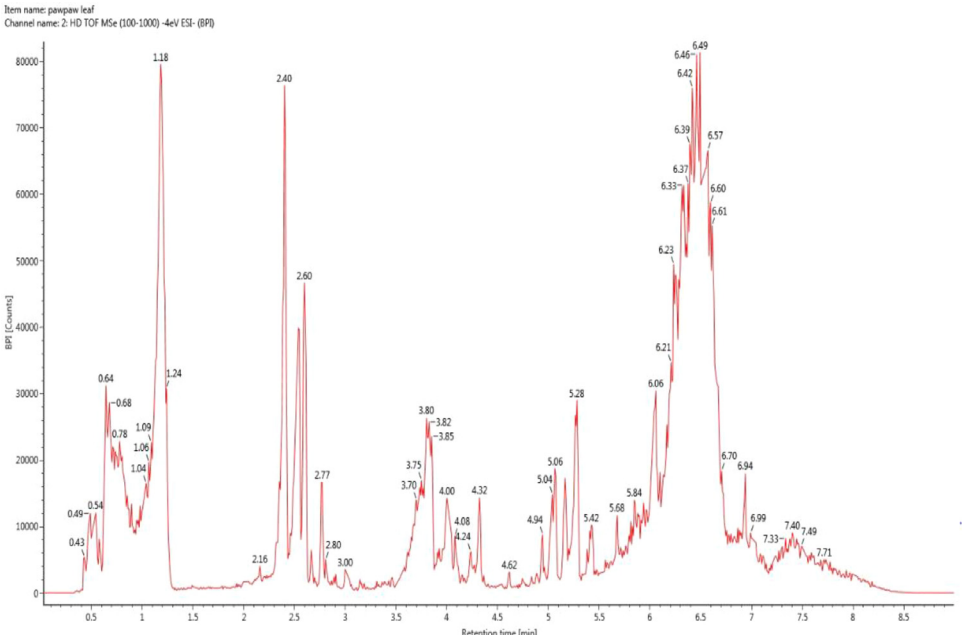

Fig. 3. Chromatograph of phenolic compounds from *C. papaya* leaf.

In order to evaluate the significance of individual MAE parameter to the recovery yield of TPC from *C. papaya* leaf for the model fitting, the analysis of variance (ANOVA) was used. The generated results are summarized in Table 2. The probability of error value (*p*-value) was used to checkmate the significance of each parameter; implying that a *p*-value < 0.05 shows that the model is significant and suitable to the extraction process. In this regard, the generated model was significant to predict the extraction of phenolic compounds from *C. papaya* leaf (Table 2). *F*-value of 67.13 with *p*-value < 0.0001 was an indication of higher significance of the generated model. Moreover, the obtained results in Table 2 depicted that all the four considered MAE parameters were significant except feed/solvent ratio (*p*-value > 0.05). The insignificance of feed/solvent ratio is relative to the fact that the TPC yield improved with decreasing feed/solvent ratio; this might be because of increasing driving force generated from the extractable phenolic compounds that were transferred to the diluted solution (Alara and Abdurahman, 2019). Only the interactions between time of irradiation and microwave power, time of irradiation and feed/solvent ratio, as well as microwave power and solvent concentration were significant. The second-order effects of solvent concentration, feed/solvent ratio and time of irradiation were also significant. There was a good correlation between the TPC yield and the MAE parameters with coefficient of determination (R^2) of 0.9843; there exist a close agreement between R^2 and adjusted R^2 as well. Additionally, coefficient of variance (CV) lesser than 10% provided the good reproducibility of generated model. An insignificant lack-of-fit verified the model validity (*p*-value of 0.5604); this shows that the generated model accurately fitted the experimental results (Alara et al., 2019; Özbek et al., 2019). Based on the achieved outcomes in Table 2, it can be inferred that the generated model can adequately present the experimental results.

3.2.2. Effects of MAE conditions on TPC through RSM

In order to identify the optimum data of the independent parameters for achieving the optimum yield of TPC as a response, the interactive effects of MAE parameters on the yield of TPC from *C. papaya* leaf were studied through three-dimensional profiles (it illustrates the interrelationship between each parameter and the response, interactions between any two considered parameters, optimum value of the independent parameters to achieve maximum response) of the generated model. Fig. 2 (a–f) presents the impacts of individual MAE parameters on the TPC from *C. papaya* leaf. The effect of microwave power and time of irradiation on TPC is presented in Fig. 2a; by increasing microwave power from 400 to 450 W, the TPC tends to increase with increasing time of irradiation until optimum values (microwave power of 420 W and 3.04 min time of irradiation) was reached and then declined. There exist a strong relationship between feed/solvent ratio and time of irradiation with a significant effect as presented in Fig. 2b and Table 2. By increasing the feed/solvent ratio to 1:12 g/mL and time of irradiation to 3.04 min, the optimum TPC of 102.60 mg GAE/g d.w. was obtained from *C. papaya* leaf. In the similar manner, the outcomes from other interactions are related with Fig. 2a and b, accordingly. From these figures, the predicted optimal MAE conditions are 3.04 min time of irradiation; 420 W of microwave power; feed/solvent ratio of 1:12 g/mL; and solvent concentration of 55.94% v/v to achieve 102.60 mg GAE/g d.w.

3.2.3. Accuracy of RSM model

The accuracy of the generated model was tested by comparing the predicted results from the model with the experimental results. This was carried by utilizing the values of optimized MAE parameters with marginal modifications; the considered values were 3 min time of irradiation, microwave power of 420 W, feed/solvent ratio of 1:12 g/mL, and solvent concentration of 56% v/v. After the experimental verification in triplicate, the mean value of TPC obtained was 102.59 ± 0.02 mg GAE/g d.w. which was closed to the predicted TPC of 102.60 mg GAE/g d.w. The adequate consistency between the experimental and predicted results showed that the generated model was appropriate in expressing the optimization of phenolic compounds from *C. papaya* leaf.

3.2. Identified bioactive compounds in the recovered extract

The extract obtained at the optimal conditions of MAE was analysed using LC-QTOF-MS; this was done to separate the phenolic compounds. Mass-to-charge ratio (m/z) of the characteristic ions and molecular ions were used to identify the composition for individual peak. Fig. 3 presents the chromatogram of the obtained phenolic compounds from *C. papaya* leaf. As shown in Table 3, a total number of 72 phenolic compound were observed. Each chromatogram peak was tentatively identified through retention time and reference to the mass spectra in literature data and UNIFI Waters library. The presence of these phenolic compounds in *C. papaya* leaf can be associated with different pharmacological effects this

Table 3
Identified phenolic compounds in *C. papaya* leaf extract.

S/N	Component name	Neutral mass (Da)	Observed neutral mass (Da)	Observed *m/z*	Mass error (mDa)	Mass error (ppm)	Observed RT (min)	Response	Adducts
1	Coniferol	180.07864	180.0783	179.071	−0.3	−1.9	0.43	1813	-H
2	Moupinamide	313.13141	313.1328	358.131	1.4	3.9	0.46	316	+HCOO
3	Agrimol D	654.26763	654.2694	653.2621	1.8	2.7	0.46	300	-H
4	Forsythoside D	478.16864	478.1683	523.1665	−0.3	−0.7	0.46	337	+HCOO
5	Feroxin A	356.14712	356.1454	401.1436	−1.7	−4.4	0.47	404	+HCOO
6	Mulberrofuran B	392.19876	392.198	437.1962	−0.8	−1.8	0.47	1073	+HCOO
7	Tribulusamide B	638.22643	638.2244	637.2172	−2	−3.1	0.48	8633	-H
8	Blestrianol B	588.2148	588.2165	587.2092	1.7	2.9	0.48	511	-H
9	Moracin C	310.12051	310.1188	355.117	−1.7	−4.7	0.57	433	+HCOO
10	Moracin H	338.11542	338.1139	337.1066	−1.6	−4.6	0.58	15,541	-H
11	Scroside D	830.28446	830.2847	829.2774	0.2	0.3	0.65	235	-H
12	Asebotin	450.1526	450.1536	495.1518	1	2.1	0.67	496	+HCOO
13	Neosappanone A	600.16316	600.1629	645.1611	−0.2	−0.4	0.68	271	+HCOO
14	(1R,2S,3R,6′R,7′R)−3,7′-Bis(3,4-dihydroxy-phenyl)−1,1′,2,2′,3,3′,4,4′-octahydro-1,1′-binaphthyl-2,2′,4′,6,6′,8-hexaol	574.1839	574.1829	619.1811	−1	−1.7	0.68	420	+HCOO
15	Protosappanin E-2	586.14751	586.1469	631.1451	−0.6	−1	0.74	950	+HCOO
16	Mulberrofuran M	590.1213	590.1197	635.1179	−1.6	−2.5	0.77	1367	+HCOO
17	Kuzubutenolide A	460.13695	460.1356	459.1283	−1.4	−3	0.77	237	-H
18	Polydatin	390.13147	390.1317	389.1244	0.2	0.6	0.82	292	-H
19	Cistanoside A	800.27389	800.2727	799.2654	−1.2	−1.5	0.82	781	-H
20	Agrimol E	626.23633	626.2367	625.2294	0.3	0.6	0.9	264	-H
21	Mulberrofuran N	392.19876	392.1974	437.1956	−1.4	−3.1	0.96	851	+HCOO
22	Mulberrofuran O	646.22028	646.2193	691.2175	−1	−1.4	1.06	588	+HCOO
23	Anthranol	194.07316	194.0739	193.0666	0.7	3.8	1.09	558	-H
24	Xanthohumol	354.14672	354.1485	399.1467	1.8	4.5	1.17	412	+HCOO
25	3,3′-Dihydroxy-5-methoxy-2,5′,6-tri(4-hydroxyphenyl)bibenzyl	562.23554	562.234	607.2322	−1.5	−2.5	1.32	2093	+HCOO
26	Cistanoside B	814.28954	814.2883	813.2811	−1.2	−1.5	1.33	412	-H
27	Torachrysone-8-O-*β*-D-glucopyranoside	408.14203	408.1422	453.1404	0.1	0.3	1.38	480	+HCOO
28	(3R,4R)−3,4-trans-7,2′-Dihydroxy-4′-methoxy-4-[(3R)−2′,7-*dihydroxy*-4′-*methoxy*-*isoflavan*-5′-*yl*]-isoflavan	542.19407	542.1945	587.1927	0.5	0.8	1.43	304	+HCOO
29	Koaburaside	332.11073	332.1119	377.1101	1.2	3.2	1.48	633	+HCOO
30	Scroside A	830.28446	830.2857	829.2784	1.2	1.5	1.57	588	-H
31	Forsythoside B	756.24768	756.2487	801.2469	1	1.3	1.61	260	+HCOO
32	Echinacoside	786.25824	786.2571	785.2498	−1.2	−1.5	1.66	521	-H
33	2′-Hydroxy-3′,4′-dimethoxy-isoflavan-7-O-*β*-D-glucoside	464.16825	464.1685	509.1667	0.3	0.6	1.89	305	+HCOO
34	3‴-O-Methylcrenatoside	636.20542	636.2062	681.2044	0.7	1.1	1.98	485	+HCOO
35	Dendrocandin F	544.20972	544.2098	543.2026	0.1	0.2	2.05	450	-H
36	Erianin	318.14672	318.1479	317.1406	1.2	3.7	2.05	361	-H
37	(-)-Suspensaside B	696.26294	696.2636	741.2618	0.7	0.9	2.09	743	+HCOO
38	4-(4′-Hydroxy-3′,5′-dimethoxyphenyl)-3-buten-2-one	222.08921	222.0905	267.0887	1.3	4.8	2.1	874	+HCOO
39	Cinchonain Ia	452.11073	452.1117	497.1099	1	2	2.11	564	+HCOO
40	Albaspidin AA	404.14712	404.149	449.1472	1.9	4.3	2.22	252	+HCOO
41	N-trans-Coumaroyltaramine	283.12084	283.1204	328.1186	−0.4	−1.3	2.29	516	+HCOO
42	Agrimol C	668.28328	668.2831	667.2758	−0.2	−0.3	2.34	365	-H
43	Mulberrofuran K	628.20972	628.2116	627.2043	1.8	2.9	2.39	331	-H
44	Chalcomoracin	648.23593	648.236	647.2287	0.1	0.1	2.63	387	-H
45	Dihydrocurcumin	370.14164	370.1413	415.1395	−0.3	−0.8	2.71	247	+HCOO
46	Kukoamine A	530.31044	530.3119	529.3046	1.5	2.8	2.76	586	-H
47	Mulberrofuran D	446.24571	446.2466	491.2448	0.9	1.8	2.79	467	+HCOO
48	Dendrocandin G	530.19407	530.1942	575.1924	0.1	0.3	2.81	455	+HCOO
49	2-((3R,4R)−7-Hydroxy-4-(4-hydroxy-5-((*R*)-7-hydroxychroman-3-yl)-2-methoxyphenyl)chroman-3-yl)-5-methoxycyclohexa-2,5-diene-1,4-dione	556.17333	556.1718	555.1645	−1.6	−2.8	2.81	254	-H
50	3,7-Dihydroxy-2,4-dimethoxyphenanthrene-3-O-glucoside	432.14203	432.1418	431.1345	−0.2	−0.6	2.82	257	-H
51	Thannilignan	330.14672	330.148	329.1407	1.3	3.9	2.86	351	-H
52	Octahydrocurcumin	376.18859	376.1893	421.1875	0.7	1.8	2.9	235	+HCOO
53	Obovatol	282.12559	282.1262	327.1244	0.6	1.9	2.91	367	+HCOO
54	2′-Acetylacteoside	666.21599	666.2152	711.2134	−0.8	−1.1	2.97	459	+HCOO
55	(3R,4R)−3,4-trans-7,2′,3′-Trihydroxy-4′-methoxy-4-[(3R)−2′,7-*dihydroxy*-4′-*methoxy*-*isoflavan*-5′-*yl*]-isoflavan	558.18898	558.1888	603.187	−0.2	−0.3	3.02	949	+HCOO
56	3′,4′-Dimethoxy-isoflavan-7,2′-di-O-*β*-D-glucoside	626.22107	626.2201	625.2128	−1	−1.6	3.09	234	-H
57	Verbascoside	624.20542	624.2064	669.2046	1	1.5	3.1	266	+HCOO
58	Tubuloside E	650.22107	650.2224	649.2152	1.4	2.1	3.15	382	-H
59	2,6-Bis(4-hydroxyphenyl)−3′,5-dimethoxy-3-hydroxybibenzyl	470.20932	470.2089	469.2017	−0.4	−0.8	3.55	1057	-H

(*continued on next page*)

Table 3 (*continued*)

S/N	Component name	Neutral mass (Da)	Observed neutral mass (Da)	Observed *m/z*	Mass error (mDa)	Mass error (ppm)	Observed RT (min)	Response	Adducts
60	Darendoside A	432.16316	432.1626	431.1553	−0.6	−1.3	4.01	864	-H
61	Laevigatin A	802.08649	802.0859	801.0787	−0.6	−0.7	4.83	229	-H
62	1,2,3,4,6-Penta-O-galloyl-β-D-glucopyranoside	940.11818	940.1194	939.1122	1.3	1.3	5.21	280	-H
63	3-(4-Hydroxyphenyl)-4-methoxy-2,7-dihydroxy-9,10-dihydrophenanthrene	348.13616	348.1359	393.1341	−0.2	−0.6	5.4	1037	+HCOO
64	Mallotinic acid	786.09157	786.0914	785.0842	−0.1	−0.2	5.49	232	-H
65	Isoarundinin II	350.15181	350.1507	395.1489	−1.1	−2.7	5.68	291	+HCOO
66	1,2,3,6-Tetra-O-galloyl-β-D-glucopyranoside	788.10722	788.1064	787.0991	−0.9	−1.1	5.8	710	-H
67	Cyclopseudohypericin	502.06887	502.0697	547.0679	0.8	1.5	5.88	349	+HCOO
68	2,3,5,4′-Tetrahydroxystilbene-2-O-β-D-glucopyranoside	406.12638	406.1272	451.1254	0.8	1.8	5.92	265	+HCOO
69	Tellimagrandin II	938.10253	938.1023	937.095	−0.2	−0.3	6.69	417	-H
70	Pedunculagin	784.07592	784.0761	783.0688	0.2	0.2	6.94	498	-H
71	6′-O-Galloyl-homoarbutin	438.11621	438.1164	437.1091	0.2	0.4	7.41	863	-H
72	Apocynin B	468.10565	468.1051	513.1033	−0.6	−1.1	8.01	249	+HCOO

plant possesses. The tentative identified phenolic compounds from *C. papaya* leaf can further be investigated in future studies.

4. Conclusion

This study verified the optimization of MAE in the recovery of TPC from *C. papaya* leaf and identification of phenolic compounds at the optimized conditions through LC-QTOF-MS analysis. The highest recovery of TPC corresponded to the following MAE conditions: 3 min of irradiation time; 420 W of microwave power; 1:12 g/mL of feed-to-solvent ratio; and solvent concentration of 56% ethanol/water to achieve TPC of 102.59 mg QE/g d.w. A sum of 72 phenolic compounds were identified at these conditions. The achieved outcomes have shown that *C. papaya* leaf is an embodiment of diverse phenolic compounds; showing that this plant material can be further studied to understand the embedded pharmacological features and quantifications of the identified phenolic compounds.

Declaration of Competing Interest

The authors declare no conflict of interest.

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