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Intensification of Polyphenols Extraction from Sohiong (*Prunus nepalensis*) using Microwave-Assisted Extraction

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Microwave-assisted extraction (MAE) was investigated to extract polyphenols from sohiong (*Prunus nepalensis*) fruit. The effect of process variables (solvent concentration, solvent-solid ratio, microwave power and time) on yield and total phenol content (TPC) of fruit extract were studied using response surface methodology. The best conditions for extraction of polyphenolic extract were solvent concentration (55.17%), solvent-solid ratio (26.09%), microwave power (500 W) and time (238 s). Under optimized MAE conditions, the yield, TPC, DPPH and FRAP of sohiong extracts were 47.25%, 146.61 mgGAE/g, 89.02% and 0.58 mgAAE/g, respectively. Furthermore, MAE showed higher yield, TPC and antioxidant activity as compared to conventional solvent extraction (CSE) (28.13%, 96.56 mgGAE/g, 74.87% (DPPH) and 0.54 mgAAE/g (FRAP)), respectively. Thus, MAE is a potential alternative for polyphenols extraction from sohiong fruit and as a rich source of antioxidant compounds, it can be a potent ingredient for pharmaceuticals and food industries.

Keywords: Sohiong, *Prunus nepalensis*, Microwave extraction, Polyphenols, TPC, Antioxidant activity.

INTRODUCTION

Phenolic compounds derived from plants and animals show numerous health benefitting effects which include decreasing the risks of several diseases. The extraction of these polyphenols from natural products and their utilization serves to be one of the most significant research areas in the food, pharmaceutical and chemical industries. As consumer's preference for natural antioxidants is increasing, the studies are now being focused on polyphenols rich indigenous and underutilized crops [1]. Sohiong (*Prunus nepalensis*) belongs to Rosaceae family is an interesting wild fruit. The plant grows to a height of 15-20 m and fruits can be harvested after 7-8 years of planting. It is mainly found in *Khasi* and *Jaintia* Hills in Meghalaya, India [2]. The plant bears a dark purple fruit that has a unique flavour and odour. It also contains a rich amount of total phenols, vitamin C, flavonols and anthocyanin content [3]. It is consumed as fresh fruit among the native population and also being processed into several products like jams, squash, juices and wines etc. at small scale. The high antioxidant activity of the fruit is due to its high polyphenolic content [4,5]. In addition, sohiong can also be used for its astringent properties, its leaves may also be utilized for oedema and as a diuretic agent [6]. Various bioactive compounds like quercetin, quinic acid, rutin, catechin, resperine, kaemferol-3-rutinoside, caeffeoyl hexose, ascorbic acid, purpurin, gallic acid are present in *Prunus nepalensis* [7,8].

Extraction is the key step in restoring phenolic compounds from food wastes and in past few years, various techniques have been investigated for the extraction of polyphenols. Maceration and soxhlet extraction was used for polyphenols extraction from grape waste [9]. However, the conventional techniques use high temperatures with long exposure time and use of hazardous solvents, resulting in hydrolysis and oxidation of the polyphenols [10]. Green extraction technologies are better alternative to conventional methods of extraction [11]. Among various methods, microwave-assisted extraction is a useful green extraction technique which is fast, eco-friendly, cost-effective and much efficient due to its good extraction rate, the accelerated extraction time and exclusive quality of product at a reasonable cost [12,13]. The major reason for effective extraction is the presence of polar molecules such as water in the plant cell that is rapidly heated by a microwave owing to electromagnetic dipole rotation and evaporation generating significant cell wall stress that causes the plant cell to swell.

The high pressure pushes and stretches the cell wall leading to disruption of the cell wall and eases the discharge of these compounds from the cells, which increases the yield of extraction. The sample, sample size, temperature, solvent employed and concentration are the main factors that affect the microwave-assisted extraction (MAE) process [14,15].

Response surface methodology (RSM) is the statistical technique used for different engineering operations for enhancing and optimizing processes, evaluating the interlinkages amidst the dependent and the independent variables, and forecasting the results. Keeping all the above points in mind, the main target of the study was to optimize the processing conditions of MAE for the extraction of the polyphenols from sohiong fruit using RSM and compared it with conventional method (CSE). The optimized extract was characterized for its antioxidant activity with a view to utilize this in different food products.

EXPERIMENTAL

Prunus nepalensis fresh fruits were collected from the Shillong fruits market, India. The seeds were physically removed and after extracting the juice, the fruit was dried at 40 ± 2 ºC in a tray drier. The dried sample was grounded and after passing through 50 mesh sieve, it was stored in airtight containers at 4 ºC.

Gallic acid, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and all other chemicals were obtained from Sigma-Aldrich (USA) and Merck (Germany). The analytical grade ethanol was used purchased from Merck, Germany.

Conventional solvent extraction (CSE): Conventional solvent extraction method was used to extract phenolic compounds from sohiong fruit following the method of Spingo *et al.* [16]. Sample (10 g) was mixed with 100 mL of 70% ethanol (v/v) and stirred at 50 ºC for 12 h. After extraction, the recovered supernatant was dried in rotary evaporator at 45 ºC and dried fruit extract was kept at 4 ºC till further use.

Microwave assisted extraction (MSE): Microwave assisted extraction was performed using a domestic microwave oven (2450 MHz, Samsung Model MS23F301TAK/TL, Malaysia). The microwave apparatus is having digital control system for time and microwave power (100 to 800 W). The modification of the oven was done to condense the vapours produced during extraction. The extractions were carried out using various combinations of solvent concentration (40-80%), solvent-solid ratio (10-30 mL/g), microwave power (300-600 W) and extraction time (90-240 s) (Table-1). The supernatant was collected after filtration and the solvent was evaporated under reduced pressure to obtain the dried extract [17].

Experimental design: RSM was practised to upgrade the extraction parameters for maximal yield and total phenolic content of fruit extract. The optimal MAE conditions were evaluated using Box-Behnken experimental design with four factors at three levels. Solvent concentration (%, X_1), solvent-

TABLE-1
EXPERIMENTAL DATA FOR YIELD AND TPC OF SOHIONG USING MICROWAVE ASSISTED EXTRACTION

Run	Extraction conditions				Responses	
	Solvent concentration (%) (X_1)	Solvent-solid ratio (mL/g) (X_2)	Microwave power (watt) (X_3)	Time (s) (X_4)	Yield (%)	Total phenolic content (TPC) (mgGAE/g)
1	40	20	600	165	37.42	134.15
2	60	20	600	240	47.18	140.90
3	60	20	450	165	43.14	145.23
4	80	20	600	165	34.64	124.74
5	40	10	450	165	33.43	139.48
6	60	20	450	165	43.13	144.93
7	80	30	450	165	33.72	131.13
8	60	20	450	165	42.49	145.03
9	60	20	300	240	43.62	142.09
10	60	10	600	165	38.74	139.91
11	40	20	300	165	35.83	137.87
12	40	30	450	165	36.27	147.07
13	60	20	450	165	42.35	144.22
14	40	20	450	90	34.47	131.99
15	60	20	600	90	40.19	133.13
16	60	20	300	90	38.80	137.53
17	40	20	450	240	39.73	142.96
18	60	30	300	165	41.50	143.65
19	60	10	450	240	40.84	144.84
20	80	20	300	165	31.42	126.40
21	60	10	300	165	36.26	143.22
22	60	30	450	240	46.91	146.30
23	80	20	450	240	36.60	127.84
24	80	10	450	165	28.00	127.86
25	60	30	600	165	43.62	141.16
26	60	30	450	90	39.71	139.60
27	60	20	450	165	41.99	146.26
28	80	20	450	90	30.73	126.94
29	60	10	450	90	36.20	140.10

solid ratio (mL/g, X_2), microwave power (Watt, X_3) and time (min, X_4) were the main four factors that are used in this study. Table-1 displays the decoded values of factors and their levels. There are 29 different runs with five replicates at center points that constitute the RSM design and these runs were examined using the software Design-expert 11.1.2.0 for statistical analysis of variance, regression coefficient and regression equation. The data were fit to quadratic model using second-order polynomial equation as follows:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{44}X_4^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{14}X_1X_4 + \beta_{23}X_2X_3 + \beta_{24}X_2X_4 + \beta_{34}X_3 X_4$$

where Y is the predicted response variable, β_0 is the intercept, β_1, β_2, β_3, β_4 are the linear coefficients, β_{11}, β_{22}, β_{33}, β_{44} are the squared coefficient and β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, β_{34} are the interaction coefficients of X_1, X_2, X_3 and X_4. All the experiments were performed in triplicates and the outcome were demonstrated as means.

Yield determination: The yield was determined as the ratio of the weight of dried sohiong fruit extract to the original dried fruit weight and reported in percentage as:

$$\text{Yield (\%)} = \frac{W_e}{W_f} \times 100$$

where, W_e is the weight of dried fruit extract; W_f is the weight of dried fruit.

Total phenol content: Folin & Ciocalteu's phenol reagent method was used to evaluate the TPC of the *Prunus nepalensis* fruit extract [18]. The calibration curve was made with gallic acid as a standard to express the results in mgGAE/g of extract.

Antioxidant activity

DPPH assay: The antioxidant activity of sohiong fruit extract was assessed by DPPH assay according to the method of Brand-Williams *et al*. [19]. The antioxidant activity (AA) was calculated as:

$$\text{Antioxidant activity (\%)} = \frac{A - B}{A} \times 100$$

where, A = Absorbance of control and B = Absorbance of sample

Ferric reducing antioxidant power (FRAP) assay: Ferric reducing antioxidant power of *Prunus nepalensis* fruit extract was examined using colorimetric method [20]. An extract (0.1 mL) was combined with 3 mL of FRAP reagent and allowed to stand for 30 min in dark and then the absorbance was measured at 593 nm. The standard curve was performed with ascorbic acid results were expressed as mgAAE/g DM of extract.

Statistical analysis: The analysis of variance (ANOVA) was by Duncan's test using Statistica-7 (M/s. Stat Soft Inc., USA) software at a significance level of 0.05. The optimum conditions of microwave assisted extraction (MSE) and conventional solvent extraction (CSE) were compared using an independent sample t-test.

RESULTS AND DISCUSSION

Model fitting: Table-1 showed the data on the yield and total phenol content (TPC) obtained from the 29 experimental runs using microwave assisted extraction and were analyzed by ANOVA and R^2, which were statistically acceptable at a 95% confidence level ($p < 0.05$) (Table-2). The significance and adequacy of the generated data were tested by fitting the experimental data in second-order polynomial equation. All four linear variables showed a significant quadratic effect on both response variables. Moreover, solvent concentration-solvent-solid ratio, solvent concentration-microwave power, solvent-solid ratio-time and microwave power-time had significant interaction effects on yield of extract from sohiong

TABLE-2
ANOVA DATA SHOWING THE EFFECTS OF THE MAE CONDITIONS ON THE RESPONSES OF SOHIONG EXTRACT

Source	Yield			TPC		
	Sum of squares	F-value	p-Value	Sum of squares	F-value	p-Value
Model	640.42	383.08	< 0.0001	1232.85	477.12	< 0.0001
X_1	36.86	308.64	< 0.0001	305.93	1657.54	< 0.0001
X_2	71.35	597.48	< 0.0001	2.53	13.71	0.0024
X_3	17.02	142.51	< 0.0001	23.55	127.58	< 0.0001
X_4	100.34	840.3	< 0.0001	105.67	572.54	< 0.0001
$X_1 X_2$	0.8836	7.4	0.0166	0.714	3.87	0.0693
$X_1 X_3$	0.6724	5.63	0.0325	1.07	5.8	0.0303
$X_1 X_4$	0.093	0.779	0.3923	25.35	137.35	< 0.0001
$X_2 X_3$	0.0324	0.2713	0.6106	0.164	0.8887	0.3618
$X_2 X_4$	1.64	13.72	0.0024	0.9702	5.26	0.0379
$X_3 X_4$	1.09	9.15	0.0091	2.61	14.13	0.0021
X_1^2	374.62	3137.21	< 0.0001	689.48	3735.62	< 0.0001
X_2^2	32.35	270.94	< 0.0001	1.19	6.43	0.0238
X_3^2	0.8622	7.22	0.0177	92.07	498.82	< 0.0001
X_4^2	0.8136	6.81	0.0206	43.17	233.92	< 0.0001
Lack of fit	0.6546	0.2574	0.9628	2.18	2.15	0.2403
R-squared		0.9974			0.9979	
Adjusted R^2		0.9948			0.9958	
Predicted R^2		0.9917			0.9893	
C.V (%)		0.8965			0.3107	

whereas solvent concentration-microwave power, solvent concentration-time, solvent-solid ratio-microwave power and solvent-solid ratio-time had significant interaction effects on total phenol content of the extract. In addition, the coefficient of multiple regression (R^2) for yield and TPC was 0.9974 and 0.9979, respectively showing a strong relationship between observed and predicted values. The lack of fit testing was used to confirm the validity of the model as shown in Table-2. ANOVA for lack of fit for two responses was insignificant ($p > 0.05$) indicating the model was adequately fitted with the experimental data.

Influence of process variables on yield of extract: The experimental design with corresponding response data and ANOVA analysis is shown in Tables 1 and 2, respectively. All linear parameters and their quadratic parameters are highly significant ($p < 0.05$). The final predictive equation with R^2 = 0.9974 obtained was as follows:

$$\text{Yield} = 42.56 - 1.74X_1 + 2.44X_2 + 1.18X_3 + 2.90\,X_4 - 7.59X_1^2 - 2.21X_2^2 - 0.35X_3^2 - 0.37X_4^2 + 0.47X_1X_2 + 0.40X_1X_3 + 0.65X_2X_4 + 0.55X_3\,X_4 \quad (3)$$

Three-dimensional response surfaces were plotted to study the interactive effect and mutual interaction of independent variables on the yield of extract from sohiong (Fig. 1a-d). The response variable was plotted on *z*-axis against two independent variables while keeping other independent variables at their zero level [21,22]. In Fig. 1a, the optimum yield of extract obtained around 47.19% with an increase in solvent concentration and solvent-solid ratio until 55% and 26 mL/g and further increase in the solvent concentration results in negative effects. This might be due to easy accessibility of cells to water and low concentration of ethanol, however, at high ethanol concentration protein denaturation occurs which influences the extraction rate. In addition, water is also used to enhance the swelling of plant material while disturbance between solute and plant matrices bonding occurred due to ethanol. Hence, based on this observation, the extraction of phenolic compounds with the help of water and ethanol helps to increase the permeability of plant tissues by mass transfer using diffusion process [23,24]. More so, Fig. 1b reflected that the increase in solvent concentration and microwave power gradually increased the

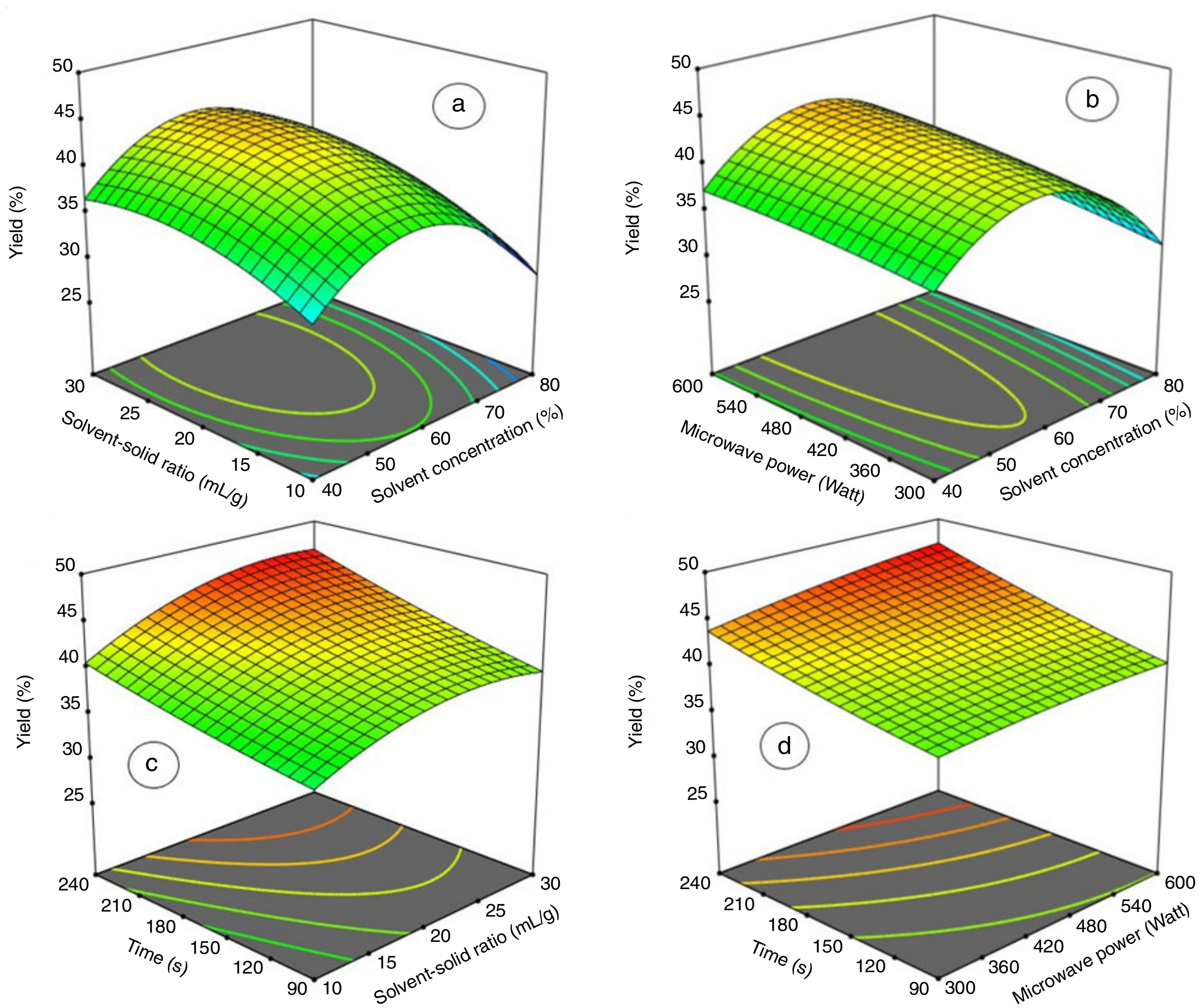

Fig. 1. Response surface plots of yield of extract from sohiong as a function of significant interactions between factors: (a) solvent concentration and solvent-solid ratio (b) solvent concentration and microwave power (c) solvent-solid ratio and time (d) microwave power and time

yield of fruit extract but after a certain solvent concentration, the recovery of extract declined. Whereas an increase in microwave power leads to an increase in the yield of extract. This might be due to the reason that with a gradual increase in microwave power the exposed material absorbed more amount of microwave energy, which lead to increased disruption of the cell and enhanced leaching of the active ingredients [25]. A significant increase in the yield of extract was obtained with an increase in both solvent-solid ratio and time and maximum yield (47.19%) was achieved when solvent-solid ratio was 26 mL/g and time was 238 s (Fig. 1c). High solvent-solid ratio acts as the driving force to speed the rate of mass transfer of phenolic compounds [21]. Fig. 1d showed that as time and microwave power were increased, the yield of extract was gradually enhanced. Higher power and longer exposure time caused the hike in the temperature of the extraction process which assisted in getting better yield. Longer exposure time also increases the solubility of phenolic compounds and decreases the viscosity of solvent used for extraction. This might have speed up the release and dissolution of the compounds while the risk of the deterioration of bioactive compounds was also higher [26].

Influence of process variables on TPC of extract: Total phenol content (TPC) and ANOVA analysis of obtained data from sohiong fruit extract are presented in Tables 1 and 2. Regression analysis was performed to optimize the MAE conditions for TPC of extract and obtained quadratic polynomial equation was as follows:

$$\text{Yield} = 144.98 - 5.05X_1 + 0.46X_2 - 1.40X_3 + 2.97X_4 - 10.31X_1^2 + 0.43X_2^2 - 3.77X_3^2 - 2.58X_4^2 + 0.52X_1X_3 - 2.52X_1X_4 + 0.49X_2X_4 + 0.81X_3X_4 \quad (3)$$

The effect of process variables on the TPC of sohiong fruit extract was shown in three-dimensional response surface plots (Fig. 2a-d). Fig. 2a showed the TPC increased with solvent concentration and microwave power at the beginning followed by a decrease after optimized conditions (solvent concentration (55%) and microwave power (500 W)). With the addition of water in ethanol, its polarity increased which may lead to greater affinity of phenolic compounds in an aqueous ethanol mixture [27]. Moreover, adding water also increased the mass transfer of phenolic compounds by disrupting the linkage between plant matrix and solutes. Furthermore, added water increased not

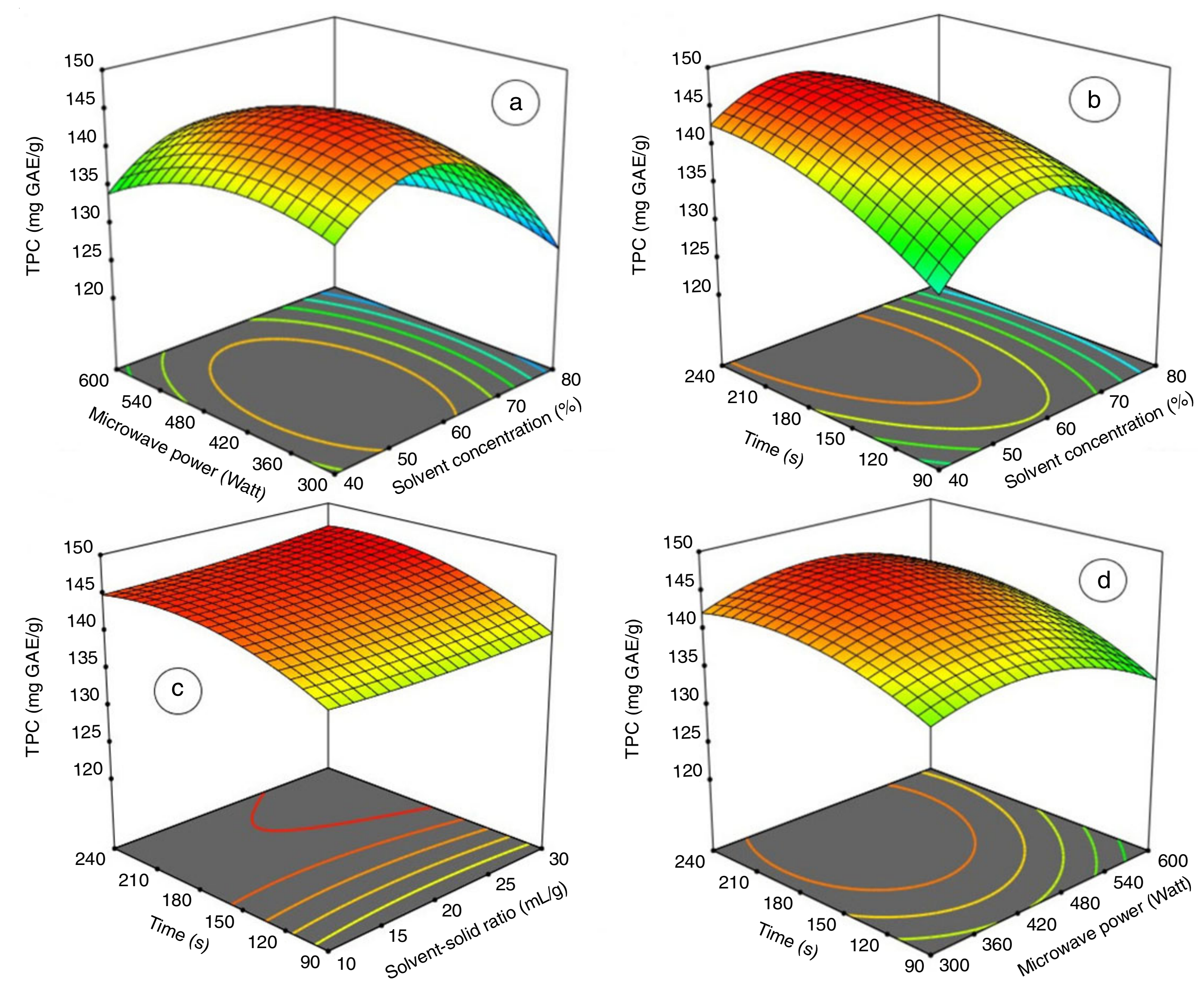

Fig. 2. Response surface plots of TPC of extract from sohiong as a function of significant interactions between factors: (a) solvent concentration and microwave power (b) solvent concentration and time (c) solvent-solid ratio and time (d) microwave power and time

only the dielectric constant of solvent but also the absorption of microwave energy, which lead to an increase in the sample temperature and cell breakage [27]. Fig. 2b-d showed the interaction effect between time of irradiation and other extraction parameters (solvent concentration, solvent-solid ratio and microwave power). In Fig. 2b, the increase in irradiation time and solvent concentration enhances the TPC but further increase in process variable results in negative effects. Longer exposure time of extraction enhanced the diffusion of plant compounds from inner plant matrix to the solvent thereby resulting in enhanced extraction yield. The higher solvent-solid ratio with increase in irradiation time decreases the TPC (Fig. 2c) of sohiong extract due to non-uniform distribution and consequently exposure to microwave heating [28]. Fig. 2d showed that TPC increased with increase in microwave power and irradiation time up to 146.61 mgGAE/g. However, the prolonged yield of TPC decreased with prolonged irradiation time with microwave power. Reduced recovery of polyphenols at higher microwave power and longer irradiation time could be due to thermal degradation of extracted compounds. Microwave heat with volumetric heating may be too powerful for the plant cells to break down the phenolic compounds that are not recovered at higher power levels [29]. Rodsamran & Sothornvit [30] also reported that TPC yield decreased with increase in microwave power.

Model verification and process optimization: To verify the predictive model validity, further experiments have been conducted for the MAE factors using the optimized data adjusted to nearest whole number *i.e.* solvent concentration (55.17%), solvent-solid ratio (26.09 Megh mL/g), irradiation time (238 sec) and microwave power (500 W). The experimental results showed that yield of extract and TPC were 47.25% and 146.61 mgGAE/g, respectively which was not significantly different ($p < 0.05$) from the predicted values using paired t-test. The strong correlation between predicted and experimental values showed that the response of the regression model was adequate to reflect the optimized conditions. In addition, this model also assisted to find optimized operating conditions for the extraction of phenolic compounds from sohiong fruit that could not be found with each factor analysis.

Comparison between CSE and MAE: Sohiong fruit was extracted and compared to CSE to evaluate the extraction efficiency and verify the MAE technique for rich extracts of polyphenol. The quantity of yield of extract, TPC and antioxidant activity (DPPH and FRAP) of sohiong fruit from conventional solvent extraction was 26.73%, 74.70 mgGAE/g, 68.98% and 0.54 mgAAE/g, respectively and optimized microwave assisted extract was 47.25%, 146.61 mgGAE/g, 89.02% and 0.58 mgAAE/g, respectively. These findings show that microwave aid substantially improved extract efficiency (TPC and antioxidant activity) as compared with CSE. These results indicate that microwave assistance enhanced the efficiency of yield of extract, TPC and antioxidant activity significantly ($p < 0.05$) as compared to CSE. The microwaves ability to enter the cell-matrix and interact with polar molecules, results in volumetric biomaterial heating, which would lead to an increase of pressure within the plant cells. This rise of pressure results in cell walls breaking down and phenolic compounds being released. This could be attributed to higher TPC and yield of microwave assisted polyphenolic extract from sohiong fruit.

Antioxidant activity of the extract is directly correlated to the quantitative presence of polyphenolic compounds in the extract. Higher TPC in MAE of sohiong fruit extract corresponds to its higher antioxidant activity. In addition, MAE leads to ionic conduction and dipole rotation which facilitate leaching out of phenolic compounds from plant matrix through molecular interaction [31]. Therefore, MAE is better extraction as compared to CSE because it requires less energy and ethanol percentage with shorter extraction time and gives higher yield, TPC as well as antioxidant activity [32,33].

Conclusion

The results obtained allowed the optimizing conditions of polyphenols extraction from sohiong fruit (*Prunus nepalensis*) using microwave assisted extraction (MSE), which was considered as an environmentally friendly technique and a good alternative to conventional extraction with a higher yield of fruit extract, TPC and antioxidant activity. The MAE allows faster extraction of natural antioxidants from sohiong and displays the efficiency of this process as compared to conventional solvent extraction (CSE). The optimal conditions of ultrasonic-assisted extraction (UAE) were solvent concentration (55.17%), solvent-solid ratio (26.09 mL/g), microwave power (500 W) and time (238 s) obtained through desirability function methodology. Factorial ANOVA has been performed to examine the effect of process variables on yield, TPC and antioxidant activity of extract and the results showed that solvent concentration was the most significant variable for TPC and DPPH and FRAP activity followed by solvent-solid ratio, amplitude and time whereas in case of yield solvent-solid ratio was the most significant variable. The results showed that predicted and experimental data were not significantly different. MAE method showed good antioxidant activity. These results have therefore, shown that sohiong fruit (*Prunus nepalensis*) can be used in the food and cosmetic sectors, as a promising source of antioxidants.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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