CHAPTER 4

To optimize the phytochemical extraction process from an edible flower using novel techniques

4.1. Introduction

Edible flowers are rich natural source of nutritional properties such as minerals, vitamins, phytochemicals or different bioactive compounds also along with providing aesthetic appearance, variety of fragrance and flavor to dishes. Different cultures cuisines such as Indian, Chinese, Romans, Middle Eastern, North American and European etc. use edible flowers in cooking (Fernandes et al., 2017). Though edible flowers have such health beneficial properties but due to consumers limited idea regarding its properties still most people are neophobic to eat flowers. Also, due to its high perishability, the market viability of edible flowers is limited.

Phlogacanthus thyrsiflorus also known as Nongmangkha flower is a common edible flower in North East India. Red brick color Nongmangkha flowers belong to *acanthaceae* family and an important medicinal plant which blooms in the month of February to April. These flowers were reported to deliver beneficial health effects on hyperlipidemia (Chakravarty et al., 2014), also showed antioxidant and radical scavenging activities (Nongthombam et al., 2018a), hypoglycemic and hypolipidemic properties (Ahmed et al., 2016) etc. Nongmangkha flower were believed to cure pox; prevent skin disease like sore, scabies, anti-allergic effect etc. to treat wounds, tumours growth and as a blood purifier (Koushik et al., 2020), kidney stones and liver disorders (Das et al., 2017). Nongthombam et al. (2018b) found that Nongmangkha flower contained steroids, terpenoids, flavonoids and phenol etc.

Novel extraction techniques are employed due to its advantage over conventional extraction methods as conventional extraction methods such as maceration, decoction, percolation, infusion, digestion, serial exhaustive extraction, and soxhlet extraction etc. which had been practiced for extraction of phytochemicals were proved to be labourious, time consuming, and usage of high amount of organic solvents etc. High hydrostatic pressure, ultrasound, pulsed electric field, supercritical fluid, microwave-assisted extraction etc. were considered as novel extraction techniques and these techniques provide advantages over conventional extraction techniques by many research studies.

Supercritical fluid extraction (SFE) has emerged as a highly efficient and environmentally friendly technique for extracting bioactive compounds from plant materials. Supercritical fluid extraction has been used for extraction of many bioactive compounds and oil etc. (Kazan et al., 2014; Santos et al., 2012; Ghafoor et al., 2012 & Akanda et al., 2012). Supercritical fluid carbon dioxide is widely used as it has many advantages of using carbon dioxide is as it is nontoxic, non-corrosive, non-flammable, such as low viscosity, high diffusivity and easy handling and supercritical operation can be done at low pressure and around room temperature. This technique employs fluids in their supercritical state, where they exhibit properties of both gases and liquids. Supercritical CO_2 is the most commonly used solvent in SFE, owing to its mild critical temperature (31.1 °C) and pressure (73.8 bar), making it particularly suitable for the extraction of heat-sensitive compounds. The superiority of SFE lies in its ability to tune the solvent's density by altering pressure and temperature, thereby enabling selective extraction of target compounds. This is especially advantageous in the extraction of phenolic compounds and antioxidants, which are often sensitive to harsh chemical solvents or high temperatures used in conventional extraction methods.

Microwave assisted extraction is regarded as green and novel efficient extraction technique which provide advantages over conventional extraction methods by higher extraction rate, less capital cost, good performance under atmospheric condition (Filip et al., 2017), lower consumption of solvent and sample preparation time than conventional methods (Sun & Lee, 2003) etc. But extraction time, liquid-to-solidratio, extraction power, and type of extraction solvent etc. influence on the efficiency of microwave assisted extraction technique (Liu et al., 2016). Microwave assisted extraction technique utilizes microwave energy to migrate active compounds or target compounds from the sample matrix into the solvent. Microwave is electromagnetic field having frequency range from 300 MHz to 300 GHz (Ridlo et al., 2019). Due to ion conduction and bipolar rotation heat is generated and as a result cell wall disruption and release of target compounds occurs in solvent from plant samples or sample matrix (Mandal et al., 2015). There were researches which conducted on optimization of microwave extraction from fruits (Ridlo et al., 2019), leaves (Filip et al., 2017), flowers (Bonomini et al., 2018) etc.

In case of ultrasound extraction, the waves of ultrasound causes cavitation and bubbles are formed due to pressure and finally they are collapsed when reaching peaks of

compression and expansion and resulted sonoporation in the disruption of the plant cell matrix (Rocha & Noreña, 2020). Disruption of cell wall is most important for the release of phenolic compounds from plant materials in both cases of UAE and MAE treatment. The deformation of cell matrix is more in combined UAE and MAE treatment leading to an effective and higher yield of phenolic compounds then solely UAE treatment. There were several successful researches conducted on ultrasonic pretreatment prior to the microwave assisted extraction for the higher extraction yield of pectin from grapefruit (Bagherian et al., 2011), isoflavones from soybean (Pananun et al., 2012) and phenolics from wheat dried distiller's grain (Izadifar, 2013), glucosinolates from cabbage outer leaves (Pongmalai et al., 2013) etc. Ethanol is less toxic, environment friendly and provides good potency for extraction of phenolic compounds, here in this study 80% ethanol was used as sample solvent for extraction process. Solvent concentration and solvent polarity affects the extraction and purification of phytochemical. Based on chemical nature of target compound to be extracted, the solvents of different polarity are selected for better extraction (Nawaz et al., 2020).

Though, edible flowers are abundantly available in all over the world but their consumption is not equally popular in worldwide and still most people neophobic to eat flowers. Many flowers are edible but proper identification is essential as not every species of flowers around us are edible. Because, in some species of flowers there may be the presence of unauthorized chemical compounds naturally, which might be poisonous to us also. Therefore, it is necessary to conduct more scientific study to list out edible flowers along with mentioning their health beneficial properties as a food. This could increases awareness, market growth of edible flowers, encourages more scientific innovative research work to conduct and innovation in food technology, pharmacology also. This research was mainly targeted to develop efficient phenolic compounds extraction technique from Nongmangkha flowers by employing supercritical fluid extraction technique; and ultrasound pretreatment, microwave assisted extraction technique. Presence of phenolic compounds were analyzed for Nongmangkha flowers extract obtained from one of the optimized extract by HP-HPLC were studied where various phenolic compounds standards (HPLC grade) were taken.

4.2. Materials and Methods

4.2.1. Chemicals and reagents

Nongmangkha flowers were procured from local market in Sivasagar (27.02124729648634, 94.67215867263882) (India). The fresh flowers were washed with distilled water and undergone to shade drying. Dried flowers sample were ground to powder and stored kept in airtight containers at 4 °C for further analysis. High purity analytical grade chemicals and reagents were employed and purchased from Sigma-Aldrich (USA) and Himedia.

4.2.2. Conventional sample extract preparation

5 g sample was mixed with 50 mL of 80 % ethanol and this sample mixture was kept in orbital shaker for 12 h. After that centrifuged at 6000 rpm for 30 min at 25 °C and the supernatant was collected. This sample extract was stored at refrigeration condition for further analysis.

4.2.3. Supercritical fluid extraction

4.2.3.1. Experimental design for supercritical fluid extraction (SFE)

Effects of parameters such as pressure, time and temperature of SFE were studied on extraction of phenolic compounds of Nongmangkha flowers were studied. A three-factor, three-level design of RSM BBD (Box-Behnken Design) was used to investigate the effects of the independent variables on the extraction efficiency. The chosen factors and their levels are mentioned in Table 4.1. The independent variables were time 60, 90, and 120 min; temperature 45, 50, and 55 °C and pressure 100, 150, and 200bar.

4.2.3.2. Supercritical fluid extraction

Supercritical fluid extraction (SFE) was used to extract phytochemicals from Nongmangkha flowers. The SFE chiller was set to 5 °C. Muslin cloth bags were prepared, and 5 g of dried flower powder was placed in each bag for every experimental run. These bags were then placed inside the extraction vessel of the SFE system (Waters, SCF100, USA).

The SFE system used in the experiment consisted of a chiller (Julabo, FL601, Germany), pressure valves, a supercritical fluid (carbon dioxide) input device, backpressure regulators, a heat exchanger, extraction chambers, and a computer setup with a system data logger that automatically controlled the entire process. The flow of supercritical carbon dioxide and the modifier fluid (ethanol) was regulated using a pump, while the temperature, pressure, and carbon dioxide flow rates were controlled by the system's data logger.

A total of 17 experimental runs were performed. After each run, the extracts were collected and air-dried at room temperature. Finally, the dried extracts were stored in a refrigerator for further analysis.

Table 4.1. Independent and dependent variables of SFE

S no.	Independent variables	Levels			Dependent variables
		-1	**0**	**+1**	
1	Time (min)	60	90	120	Total phenolic content and DPPH free radical scavenging activity
2	Temperature (^{0}C)	45	50	55	
3	Pressure (bar)	100	150	200	

4.2.4. Ultrasound pretreated microwave assisted extraction

4.2.4.1. Experimental design for Ultrasound pretreated MAE

In this research experiment, effects of microwave extraction process parameters such as microwave power and time on total phenolic content, total flavonoid content and antioxidant activity of extract from Nongmangkha flowers were studied. The central composite design (CCD) of Response Surface Methodology was used for design the experiments and a total of 13 experimental runs with 5 center points were obtained. The independent variables with their levels are shown in Table 4.2.

Table 4.2. Level of independent variables in Microwave assisted extraction

Sl no.	Independent variable	Unit	Level			Dependent variables
			-1	0	+1	TPC, TFC and DPPH radical scavenging activity
1	Power	W	300	500	700	
2	Time	min	2	4	6	

4.2.4.2. Ultrasound pre treatment

Dried powder of Nongmangkha flowers was dissolved in 80 % ethanol in a ratio of 1:10 in a 100 mL beaker. In this case 80 % ethanol was selected as solvent as water alcohol combination could be more helpful for extraction purpose (Filip et al., 2017). Also, to absorb microwave by solvent, dielectric properties of the solvent is important so that there would be successful generation of heat from microwave energy (Zhang et al., 2011). Based on earlier research conducted by Rodríguez-González et.al, (2018) and Lu & Yin (2018) and concerning the further effect of microwave power on it, the sample mixture was ultrasonicated (VCX500, Sonics, Vibra Cell, USA.) at 250 W for 15 min. A probe size of ¾ inch diameter was immersed in sample mixture beaker. This condition was constant for all the experimental runs. After the ultrasound pretreatment the treated sample mixture were transferred into a flat bottom flask for microwave assisted extraction.

4.2.4.3. Microwave assisted extraction (MAE)

Microwave extractor (TW/MWEX/2/17/18, Twin Engineers, Pimpri-Chinchwad, India) were employed for optimization of extraction process to obtain phytochemicals from Nongmangkha flowers. Microwave power of 300 to 700 W and time 2 to 6 min were taken for the extraction process. The maximum power generated by microwave were 1000 W, therefore, ON/OFF time for getting 300 W, 500 W, and 700 W were 6 s/14 s, 10 s/10 s, and 14s/6s, respectively. In an experimental run, first the sample mixture was exposed to microwave for 20 seconds (total cycle time) and the sample flask took out from microwave extractor to cool down into room temperature by using cold water. After cooling down to room temperature the sample were again exposed to microwave and this process was repeated until the required microwave treatment was

provided. This same technique was also practiced by Hao et al. (2002). After each experimental run, the sample extracts were filtered through filter paper immediately and the extracts were kept at 4 °C for further study.

Here, for providing the microwave treatment Eq. 4.1. and 4.2 was followed.

1000 W× On Time = Microwave power (required) × Total run time (4.1)

As the microwave assisted extractor has the maximum of 1000W so this power was fixed for the formula.

Off time = Total time – On time (4.2)

4.2.5. Total phenolic content

The total phenolic content of the extracts were analyzed with slight modification of the method applied by Khatiwora et al. (2010). 0.2 mL of sample extract was taken and diluted upto 3 mL with distilled water. 0.5 mL of FCR (Folin Ciocalteu's phenol reagent) (1:10) were added into it and after few minutes 2 mL of Na_2CO_3 (20 %) were mixed into it. After incubation for 1 h the absorbance of the sample were studied at 650 nm. A gallic acid standard curve was prepared and the results were expressed in gallic acid equivalent (mg GAE/g).

4.2.6. Total flavonoid content

In a test tube, 1mL of extract and 4 mL distilled water were taken, and 0.3 mL of sodium nitrite (5 %) solution was added and kept for 5 min. Further, 0.3 mL of aluminium chloride ($AlCl_3$) (10 %) was poured into it. Again, after 5 min rest, 2 mL of 1M sodium hydroxide was added and diluted up to 10 mL with distilled water. This sample mixture in the test tube was mixed well and absorbance was taken at 510 nm. Standard curves were obtained by quercetin and expressed the results of quercetin equivalent (mg QE/g) (Panhekar et al., 2019).

4.2.7. DPPH radical scavenging activity

DPPH radical scavenging activity was determined by following the method of Tundis et al. (2015) with a slight modification. 0.5 mL sample extract was mixed with 2.5 mL of DPPH solution (0.1 mM DPPH) and incubate for 30 min in dark at room

temperature. The wavelength was read at 517 nm. Calculation was done by using Equation 4.3.

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (4.3)$$

4.2.8. HPLC analysis

The dried Nongmangkha flower extract that had been optimized was subjected to HPLC analysis along with dried conventional flower extract to search for phenolic compounds. Binary RP-HPLC system (Waters, USA; 1525, 2414, 2489) gradient elution was performed for this where column was used. The flow rate was 1mL/min for elution and detection achieved by ultraviolet detector and chromatograms read at 280 nm and 360 nm. The phenolic compounds present in the flower extract were determined by comparing their retention time and UV –vis spectra with phytochemical analytical standards. There were 16 phenolic compound standards were taken such as gallic acid, resorcinol, 3,4-dihydroxybenzoic acid, catechin, chlorogenic acid, epigallocatechin gallate, caffeic acid, syringic acid, rutin, salicyclic acid, ellagic acid, p-coumeric acid, quercetin, kaempherol, apigenin. Dried sample extract of optimized Nongmangkha extract were taken and dissolve it with HPLC grade methanol at a concentration of 1mg/mL and filtered with 0.2 µm Whatman syringe filter (nylon) in vials. Identification of phenolic compounds was achieved by comparing the retention time and UV-vis spectra with respective HPLC grade phenolic standards.

With a slight modification the reference of Muchahary and Deka (2021) acidified Milli-Q water (1% acetic acid) and HPLC grade methanol was used as mobile phase A and B respectively. Likewise, the gradient was set as 0 min – 80 % A, 7 min- 80 % A, 10 min- 65 % A, 11 min-65 % A, 16 min- 45 % A, 17 min- 45 % A, 24 min- 30 % A, 25 min- 30 % A, 30 min- 20 % A, 31min- 20 % A, 35 min- 10 % A, 36 min-10 % A, 40 min- 80 % A. An amount of 20 µl sample and standards were injected in the HPLC injector with the help of glass syringe and the elution was carried out at a solvent flow rate of 1 mL/min. Quantification of phytochemicals was obtained from peak area according to their respective standard calibration curves. The results were delivered as milligram per gram (mg/g) dry extract.

4.3. Results and Discussion

4.3.1. Model fitting for SFE

RSM was employed to obtain the optimized condition for supercritical fluid extraction parameters such as pressure, time and temperature. There were 17 experimental runs were obtained based on BBD to understand the influence of time, temperature and pressure of supercritical fluid extraction on total phenolic content and DPPH radical scavenging activity. This experimental design matrix with the responses is shown in Table 4.3.

The ANOVA results for TPC and DPPH radical scavenging activity are detailed in Table 4.4. providing insights into the contributions of individual factors. ANOVA was utilized for understanding the effect of process variables and to identify the model statistical validity with the 0.05 significance levels with a linear model.

4.3.2. Effect of SFE process parameters on TPC and DPPH radical scavenging activity

The ANOVA for TPC demonstrated that the model was highly significant ($p < 0.0001$) with an R^2 value of 0.845, indicating that 84.67 % of the variability in TPC can be explained by the model. The coefficient of determination (R^2) value was found to be 0.845 and the lack-of-fit for TPC indicates the adequacy of the model. Among the factors the extraction time ($F = 8.84$, $p = 0.0104$) significantly influenced TPC as the F value is 8.84. It was observed that the longer extraction time allowed for a higher release of phenolic compounds. The F value of temperature was 6.12 ($p = 0.0280$) which states that the effect of temperature was also significant, as higher temperature may enhance the solubility of phenolic compounds in solvent. The F value of pressure i.e., $F = 56.76$ ($p < 0.0001$) indicates that the pressure was the most influential parameter, highlighting the importance of high pressure in enhancing the solvation power of the supercritical fluid. Linear equations in terms of coded values was obtained in this model and shown in equations 4.4. Fig. 4.1(a) was illustrated the 3D effect of SFE process parameters on TPC.

$$Y_{TPC} = +26.68 + 1.27\ A + 1.05\ B + 3.19C \quad (4.4)$$

For DPPH activity, the ANOVA indicated moderate significance with an R^2 value of

0.7032, suggesting that 70.32 % of the variability was explained and the lack-of-fit tests p= 0.6352 for DPPH confirmed the adequacy of the models. Pressure (F = 30.29, p = 0.0003) was the only significant factor for DPPH activity radical scavenging activity, underlining its role in improving the extraction efficiency of antioxidant compounds. Time and temperature were not significant for DPPH radical scavenging activity, implying their limited contributions at these levels. Yi et al. (2009) also reported that increasing the pressure and temperature decreases the antioxidant activity and temperature but, TPC was increased.

Linear equations in terms of coded values was obtained in this model and shown in equations 4.5. The ANOVA results are shown in Table 4.4. Fig.4.1.(b) was illustrated the 3D effect of SFE process parameters on DPPH radical Scavenging Activity.

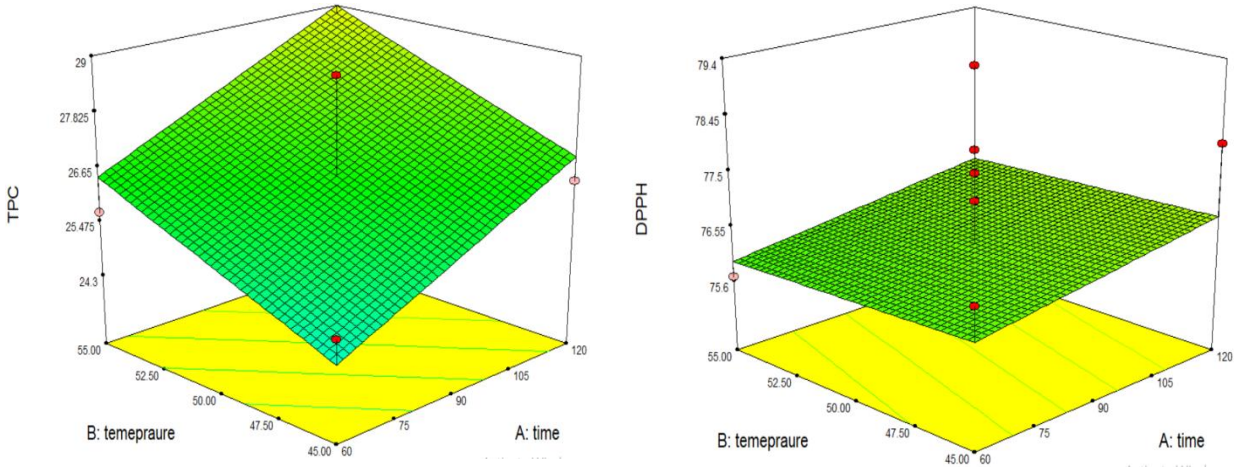
$$Y_{\text{DPPH Radical Scanvenging Activity}} = 76.34 + 0.31A - 0.087\ B + 2.47C \quad (4.5)$$

Table 4.3. Response surface methodology Box–Behnken design (RSM-BBD) with response data of Nongmangkha flower extract.

Run No	Time (min)	Temperature (°C)	Pressure (Bar)	TPC (mg GAE/g)	DPPH activity (%)
1	90	45	100	22.18	71.57
2	120	45	150	26.38	77.99
3	120	50	200	31.83	77.93
4	60	55	150	25.69	75.67
5	60	45	150	24.87	76.68
6	90	55	100	26.58	73.5
7	90	50	150	25.94	75.98
8	120	55	150	27	76.72
9	60	50	100	20.93	73.61
10	90	50	150	28.61	77.48
11	120	50	100	25.88	73.9
12	90	45	200	28.63	78.33
13	90	50	150	26.34	76
14	90	50	150	26.11	77
15	90	50	150	25.88	79.29
16	90	55	200	31.19	77.98
17	60	50	200	29.45	78.11

Table 4.4. ANOVA of TPC and DPPH of Nongmangkha flower extract from SFE

	TPC				DPPH			
Source	**df**	**Sum of Squares**	**F-value**	**p-value**	**df**	**Sum of Squares**	**F-value**	**p-value**
Model	3	103.17	23.62	<0.0001	3	49.68	10.27	0.001
A-Time	1	12.88	8.84	0.0104	1	0.76	0.47	0.5038
B-Temperature	1	8.82	6.06	0.028	1	0.061	0.038	0.8485
C Pressure	1	81.47	55.95	<0.0001	1	48.86	30.29	0.0001
Residual	13	18.93			13	20.97		
Lack of Fit	9	13.633	1.19	1.14	9	13.57	0.81	0.6352
Pure Error	4	5.3			4	7.4		
Cor Total	16	122.1			16	70.65		
R^2		0.845				0.7032		
Adjusted R^2		0.809				0.635		
Predicted R^2		0.718				0.525		



(a) (b)

Fig. 4.1.3D Response surfaces for total phenolic content (TPC) (a) and (b) DPPH Radical Scavenging Activity of SFE extract from Nongmangkha

4.3.3. Optimized conditions for SFE

The optimal conditions for SFE were determined to be 200 bar pressure, 53 °C temperature, and 120 min extraction time with a desirability of 0.985. Numerical optimization technique was adopted here and keeping time, temperature and pressure in range along with maximizing the TPC and DPPH responses. At these conditions TPC

predicted value of TPC was 31.83 mg GAE/g, while the experimental value was obtained was 29.91 ± 3.79 mg GAE/g. For, DPPH radical scavenging activity the predicted value was 79.86%, and the experimental value was 80.83 ± 4.13%. The significant effect of pressure aligns with the known role of supercritical fluids in enhancing compound solubility under high pressure. Furthermore, the higher TPC and antioxidant activities under optimal conditions reflect the synergistic effects of the variables.

4.3.4. Model fitting for MAE

RSM was used to obtain the optimized condition and investigate the different microwave extraction parameters such as microwave time and microwave power for extraction of phytochemicals from Nongmangkha flower. The experimental design comprises of 13 experimental runs which were obtained based on CCD to study the effect of microwave power and time on TPC, TFC and DPPH. Experimental design matrix with the responses is shown in Table 4.5.

Quadratic models were resulted in this experimental design and ANOVA was used to study effect of variables and to identify the model statistical validity with the 0.05 significance levels. Quadratic equations in terms of coded values was obtained in this model and shown in equations 4.6, 4.7 and 4.8 and the ANOVA results are shown in Table 4.6.

4.3.5. Effect of microwave process parameters on TPC

The coefficient of determination (R^2) value was 0.8424 and followed by F value 7.98 were implicated that the model fit was significant. The results were comprised of a p value of 0.0099 and a non significant value of residual lack of fit F-value of 3.38. These suggested that the model was a good fit for the experiment. Fig. 4.2(a) was illustrated the 3D effect of microwave power and extraction time on TPC.

$$Y_{TPC} = 31.83 + 1.93A + 1.05B - 1.71AB - 0.71A^2 - 1.72B^2 \quad (4.6)$$

Where, A = Power , B= Time and Power (A) ranges from 300 W to 700 W and Time (B) ranges from 2 to 6 min.

The results which are obtained were shown in Table 4.5. It was observed that

TPC content of Nongmangkha flower ranges from 23.62 to 33.01 mg GAE/g. The lowest content of TPC was found in the extract at obtained condition of 300 W microwave power for irradiation time 2 min and highest at 700 W for 4 min. Longer the time means greater the contact between sample and solvent. Here, extraction time was prolonged from 2 min to 6 min and it was observed that with time the TPC was also increasing but when microwave power increased the TPC was reduced at 700 W for 6 min. This may be due to the fact that higher power with longer time could damage the structure of the response compounds (Xiong et al., 2016). Based on power increment the longer time showed the decrease of TPC. Longer time exposure to microwave and solvent might influence the separation of other compounds such as minerals and carbohydrates from the plant matrix except polyphnenols (Pavlović et al., 2013).

Table 4.5. Response surface methodology-central composite design (RSM-CCD) with response data of Nongmangkha flower extract.

Run	Microwave power (w)	Microwave time (min)	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH (%)
1	300	6	29.51	20.53	76.36
2	500	4	32.57	23.14	79.03
3	700	6	30.87	25.38	80.97
4	500	4	31.23	21.56	81.12
5	700	4	33.01	23.93	82.07
6	300	4	30.97	19.29	78.79
7	500	4	30.45	22.85	79.5
8	700	2	31.82	20.37	81.91
9	500	2	30.30	19.70	78.91
10	500	6	31.67	23.58	78.66
11	500	4	32.10	22.45	80.36
12	500	4	31.02	22.23	80.18
13	300	2	23.62	17.69	74.31

Table 4.6. Results of ANOVA of Central Composite Design for the quadratic model for MAE of TPC, TFC and DPPH radical scavenging activity

	TPC				TFC				DPPH			
Source	df	Sum of Squares	F-value	p-value	df	Sum of Squares	F-value	p-value	df	Sum of Squares	F-value	p-value
Model	5	54.95	7.48	0.0099	5	53.55	42.67	<0.0001	5	50.93	12.07	0.0025
A-Power	1	22.43	15.27	0.0058	1	24.68	98.35	<0.0001	1	39.99	47.40	0.0002
B-time	1	6.64	4.52	0.0712	1	22.93	91.37	<0.0001	1	0.12	0.15	0.7136
AB	1	11.70	7.96	0.0257	1	1.18	4.69	0.0670	1	2.24	2.65	0.1476
A^2	1	1.40	0.96	0.3608	1	1.53	6.11	0.0427	1	3.259×10^4	3.862×10^4	0.9849
B^2	1	8.15	5.55	0.0507	1	1.41	5.63	0.0495	1	7.38	8.74	0.0212
Residual	7	10.28			7	1.76			7	5.91		
Lack of Fit	3	7.38	3.38	0.1349	3	0.28	0.25	0.8559	3	3.31	1.70	0.3046
Pure Error	4	2.91			4	1.48			4	2.60		
Cor Total	12	65.24			12	55.31			12	56.84		
Adjusted R^2		0.73				0.946				0.822		
Predicted R^2		0.014				0.822				0.366		

Increase in microwave power increase the TPC but increase in extraction time might affect the extraction of TPC negatively. This fact was in harmony with study of Simsek et al. (2012) for extraction of phenolic compounds from sour cherry pomace at the optimum conditions of 700 W microwave power, 12 min of microwave irradiation time, ethanol-water mixture was used as solvent and solvent to solid ratios 20 mL solvent/ g solid. In their study beyond 12 min at 700 W microwave power TPC content were decreased. Increasing the microwave power enhanced the extraction of TPC but in case of increment of extraction time due to overexposure in microwave some phenolic compounds of the sample might get destructed (Table 4.6). Also, microwave power produced heat in sample matrix which could degrade the bioactive compounds of plant matrix. Time was required for the penetration of solvent into plant matrix but increasing extraction time might lead to over exposure to light and oxygen which could cause the oxidation of phenolic compounds (Tay et al., 2014). In Equation 4.6 and Fig.4.2(a) it was seen that TPC was increased with increasing independent variables but decreased at 700 W for 6 min microwave irradiation time.

4.3.6. Effect of microwave process parameters on TFC

TFC showed the co-efficient of determination (R^2) was 0.9682, which signified that the model was good fit for the experiment. A significant model fit was indicated by the F vaule was 42.67. In this model the p value of coefficient of regression were found to be < 0.0001 and the residual lack of fit F value was showed 0.25 which meant that the lack of fit is not significant. The values of R^2, Adj-R2 and Pred-R2 for TFC were 0.9682, 0.9455 and 0.9109 respectively which were close to 1 and signified that there is a good co relatioon between model predicted and actual results.

$$Y_{TFC} = 22.42 + 2.03A + 1.95B + 0.54AB - 0.75A^2 - 0.72B^2 \quad (4.7)$$

Where, A = Power , B= Time

Power (A) ranges from 300 W to 700 W and Time (B) ranges from 2 to 6 min.

The highest TFC content was 25.38 mg quercetin/g in the condition of 700 w microwave power for 6 min and lowest content of TFC was 17.69 mg quercetin /g and lowest at microwave power 300 W for extraction time 2 min. Extraction time and microwave power positively affect the TFC extraction from Nongmangkha flower. The

TFC content was increasing with increase in time. Similar interaction of microwave power and extraction time was shown by Weremfo et al. (2020). Tran et al. (2022) recommended that at a radiation time of 70 min and power of 700 W could give a better extraction of bioactive compounds from Coffee Pulp (*Coffea canephora*) waste. From Fig..4.2(b) it was seen that TFC content of flowers extract was increased with increase in extraction time and microwave power (shown in Equation 4.4 and Fig 4.2.(b)) till microwave power 700W for 6 min irradiation time.

4.3.7. Effect of microwave process parameters on DPPH

Coefficient determination (R^2) of DPPH was resulted 0.8961 which was good indication of model fitting for the experiment. Also, F value was found to be 12.07 which implied that the model was significant. The p value of coefficient of regression were found to be 0.0025 and the residual lack of fit p value was showed 0.3046 and also the lack of fit F-value was 1.70 which meant that the lack of fit is not significant. 1.16 % CV showed that the model is reliable (Table 4.5.).

DPPH of Nongmangkha flower in different process conditions varied from 74.31 % to 84.07 %. Lowest DPPH content was found at 300 W microwave power for 2 min of extraction time, where the highest value was found to be 700 W microwave power for 4 min of extraction duration. However further increment of time at same irradiation power reduced the DPPH capacity of the extract. Microwave power and the time of extraction showed a positive impact on DPPH separation from sample matrix (Equation 4.8 and Fig 4.2. (c)).

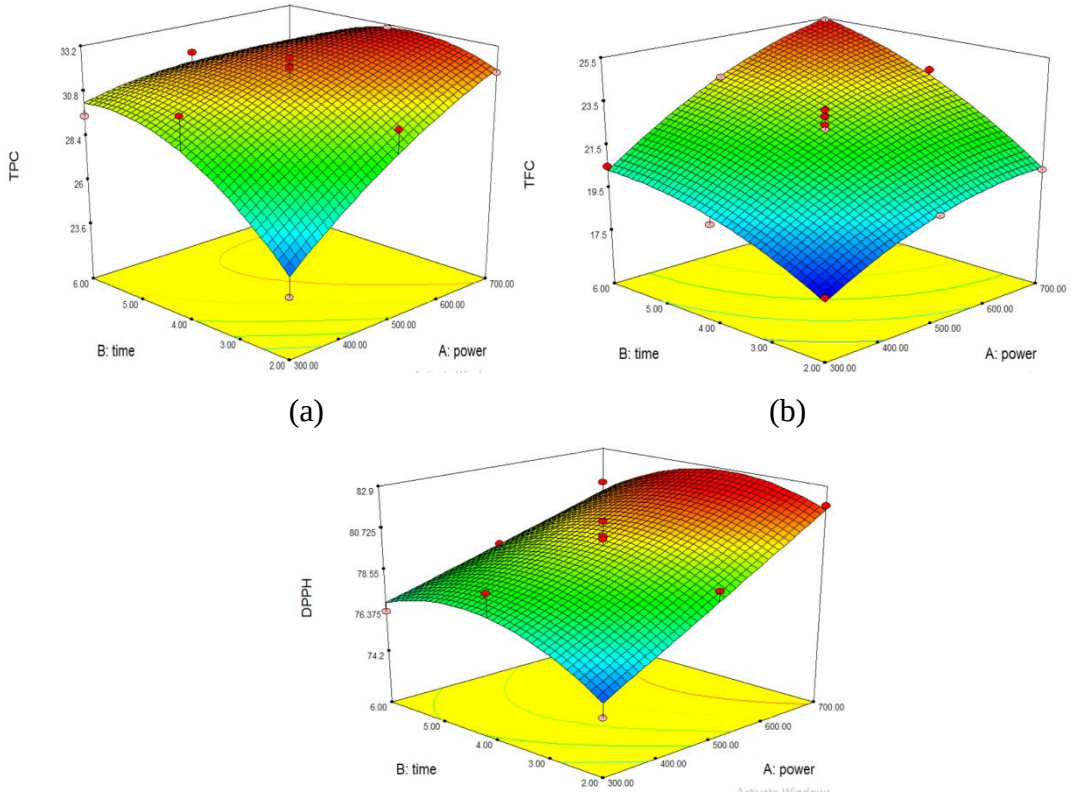
Microwave power and irradiation time together played a great role on DPPH capacity of flowers extract. DPPH percentage was shown to be increased with increase in microwave power. However further increment of irradiation time at same irradiation power reduced the DPPH percentage of the extract. This could be due to the fact that, the longer time in microwave irradiation in a specific microwave power could destruct the phenolic content of flowers such as total phenolic content, total flavonoid content etc. and as a result the antioxidant capacity of the extract turned to be decreasing (Dahmoune et al., 2014). Microwave-assisted extraction of natural antioxidants from the exotic *Gordonia axillaris* fruit: Optimization and identification of phenolic compounds. Also, Simsek et al., (2012) was found that in shorter irradiation time for microwave power at

700 W and 400 W was best suitable for antioxidant capacity and after 14 and 12 min at microwave power at 400 W and 700 W respectively the antioxidant capacity of sour cherry pomace extract was lowering down. Thus, in this model, microwave power 700 W for 4 min is suitable for getting highest DPPH capacity in this specific model condition.

$$Y_{DPPH} = 80.15 + 2.58A + 0.14B - 0.75AB + 0.011A^2 - 1.63B^2 \quad (4.8)$$

Where, A = Power , B= Time

Power (A) ranges from 300W to 700 W and Time (B) ranges from 2 to 6 min.



(a) (b)

(c)

Fig.4.2. 3-D Response surfaces for TPC (a), TFC (b) and DPPH (c) of microwave assisted extract from Nongmangkha as a function of extraction time (min) and microwave power (W).

4.3.8. Optimized conditions for MAE

To find the optimized ideal condition for microwave assisted extraction Design Expert software (Version 13, Stat-Ease, Inc., USA) were employed to get highest TPC, TFC and DPPH were predicted by maximizing the desirability of the responses.

Numerical optimization technique was used and power and time was selected in range along with maximizing the TPC, TFC and DPPH responses. The optimized condition was found to be at microwave power 600 W and time 5min. the predicted value for responses were obtained as TPC of 32.36 mg GAE/g, TFC of 24.72 mg QE/g and DPPH radical scavenging activity of 82.10 %. It was found that at microwave power 600 W for 5 min extraction time in our laboratory condition (experimental value) showed 30.204 (± 40.422) mg GAE/g of TPC mg QE/g, 81.26 % (± 0.288) of DPPH and 22.50 (±5.029) mg QAE/g of TFC in Nongmangkha flower extract.

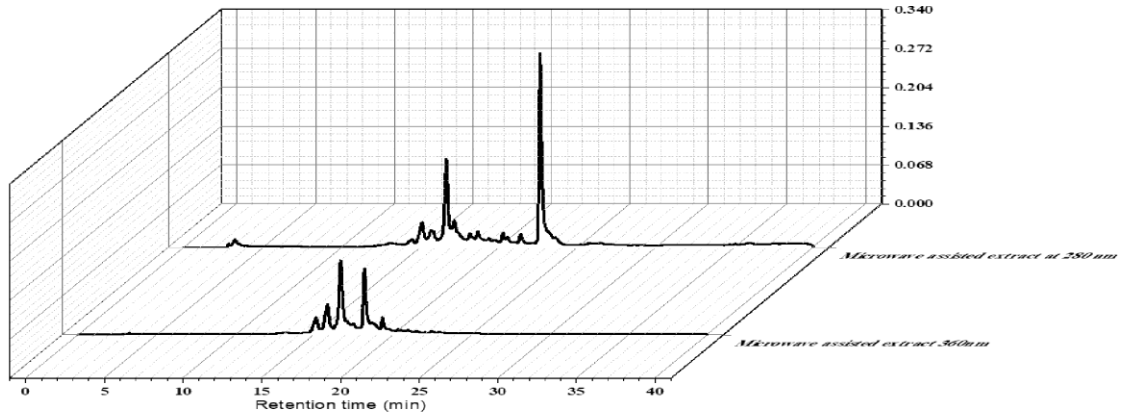
4.3.9. Detection of phenolic compounds by HPLC in optimized extract of MAE

Initially, 16 standards such as gallic acid, resorcinol, 3,4-dihydroxybenzoic acid, catechin, chlorogenic acid, epigallocatechin gallate, caffeic acid, syringic acid, rutin, salicyclic acid, ellagic acid, p-coumeric acid, quercetin, kaempherol, apigenin were run in high-performance liquid chromatography and retention time was recorded at 280 nm and 360 nm.

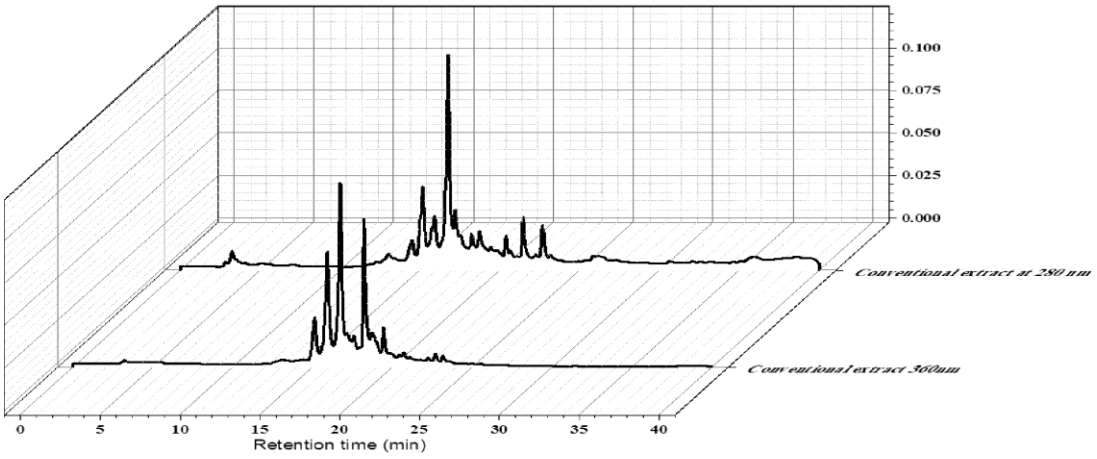
The HPLC chromatograms of MAE optimized Nongmangkha flower extract was presented in Fig.4.3. Phenolic compounds were detected were analyzed on the basis of retention time compared with the standard phytochemicals. Indeed, retention time of phenolic standards at 280 nm and 360 nm. There were different phenolic compounds were detected by HPLC in optimized extract of ultrasound pretreated microwave assisted extract of Nongmangkha flower. Resorcinol, rutin, ellagic acid, salicyclic acid, kaempferol and apigenin were present. Also, other phenolic compounds detected in microwave assisted extract such as caffeic acid, syringic acid, quercetin, ferulic acid. It can be said that microwave assisted extraction helped to extract various more phenolic compounds than conventional extraction process (mentioned in Chapter 3) of phenolic compounds. These results are shown in Table 4.7.

Table 4.7. Phenolic compounds present in Nongmangkha flower extracts: conventional and microwave-assisted extract.

Sl no.	Phenolic compound	Conventional flower extract (mg/g)	MAE optimized flower extract (mg/g)
1	Resorcinol	3.813	3.605
2	Caffeicacid	—	0.214
3	3,4dihydroxybenzoicacid	1.851	—
4	Syringicacid	—	0.0436
5	Rutin	8.142	12.864
6	Ellagicacid	4.112	7.085
7	Salicyclicacid	20.729	66.797
8	Quercetin	—	2.198
9	Ferulicacid	—	1.799
10	Kaempferol	0.386	1.118
11	Apigenin	0.387	1.139



(a) Microwave assisted extract



(b) Conventional extract

Fig.4.3. High-performance liquid chromatography (HPLC) chromatograms of phenolic compounds detected (a) microwave-assisted extract and (b) conventional extract.

4.4. Conclusions

Extraction of phenolic compounds such as TPC, TFC and DPPH capacity of Nongmangkha was done by using ultrasound pretreatment and microwave assisted extraction (MAE) technique and RSM, CCD showed successful and efficient application for this optimization of the selected MAE conditions. Both Microwave power and irradiation time showed impact on TPC, TFC and DPPH capacity. Supercritical fluid extraction also helped to extract TPC content from Nongmangkha flower. HPLC study also showed the presence of resorcinol, caffeic acid, syringic acid, rutin, ellagic acid, quercetin, ferulic acid, kaempferol and apigenin in the optimized MAE extract which is beneficial to our health. MAE extract showed better results of phenolic compounds in HPLC than conventional extract. This optimized condition could help to get maximum phenolic compounds from Nongmangkha and this is beneficial to pharmacology as well

as food industries. This phenolic compounds rich flower extract could pave a way to researchers for novel utilization of flowers extract. These results signified that the Nongmangkha flowers extract could be a useful in functional food product development.

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