CHAPTER 2

To extract bioactive compounds from fruits of Norabogori (*Prunus persica* L. Batsch) and their quantification and characterization

2.1 Introduction

Fruits are excellent providers of nutrients, and eating them has been linked to both favourable physical and psychosocial outcomes [1]. Fruit and vegetable quality is affected by a number of variables, including genotypic, agro-environmental, and postharvest ones [2-4]. The nutritional value, however, is insufficient. The so-called biologically active substances (BAS), or various sorts of components (dietary fibre, phenolic compounds, carotenoids, vitamins, etc.) that have an effect on how the human body functions, are what consumers most frequently favour in products. The most well known and sought-after of these are probably polyphenols. They have anti-cariogenic, anti-microbial, and antioxidant properties [5,6].

Stone fruits are renowned for their distinctive organoleptic and visual qualities. Acid level, sugar content, and appearance have historically been used as fruit quality indicators. However, fruits also contain a huge number of phytochemicals that, despite being present in very little amounts, are crucial to overall quality [4,5]. Their color and aroma may be significantly influenced by certain of these compounds [6]. Many of these substances have also been discovered to have preventive effects against specific disorders [7]. These metabolites can have observable long-term physiological impacts when consistently consumed in large amounts as part of a daily diet [8].

The abundance of organic acids, minerals, carbohydrates, and dietary fiber, which are some of the fruit's main elements, determines the nutritional profile of peaches [9]. Peaches are valued as a good source of beneficial compounds that, when consumed, can produce great health impacts in addition to their sensory appeal. Among these are phenolic compounds, which are made up of phenolic acids, flavonoids, and anthocyanins.

Modern extraction technologies (such as pulsed electric field extraction, enzymatic extraction, ultrasound-assisted extraction, supercritical fluid extraction, microwave-assisted extraction, etc.) emerge as promising alternatives to the more time-consuming traditional extraction methods (such as Soxhlet, maceration, percolation, solvent extraction, etc.), with a focus on the acquisition of high-quality extracts rich in bioactives. The high quality of the final extracts, the brief extraction time, the reduced volumes of organic solvents, their high selectivity and efficiency towards specific solutes, and their ability to use novel green extracting agents, like natural deep eutectic solvent (NADES), are their main advantages when compared to traditional extraction [10].

The most well-known non-conventional extraction methods include supercritical fluid extraction (SFE), microwave aided extraction (MAE), ultrasound assisted extraction (UAE), and MAE. The mechanisms underlying UAE and MAE, respectively, cause acoustic cavitation, which leads to the compression and decompression of microbubbles, heating from ionic conduction and dipole rotation, and the disruption of plant cells, increased solubility, and ultimately the release of the compounds of interest in the solvent [9,11].

Contrary to traditional extraction, the performance of non-conventional procedures is significantly influenced by a number of characteristics. The most important variables affecting extraction efficiency are the extraction solvent, extraction time, temperature, pressure, US or MW power, sonication duration, duty cycle, solvent/material ratio, and particle size [9,12]. In order to reduce the number of experiments and evaluate the interactions between the extraction variables, their optimization through the application of experimental design models (e.g., two-level designs, Plackett-Burman, central composite designs, and Box-Behnken designs) is crucial [13].

Nevertheless, norabogori is an underutilized peach variety of Assam and generally considered under subtropical fruits. Due to a lack of knowledge about its edible and therapeutic qualities, their intake is quite low. Because of this, it is crucial to promote the use of the underexplored, underutilized Assam peach from the perspectives of the environment and human health.

The extraction method, solvent polarity, and extraction time all have a substantial effect on effective polyphenols extraction[8,10]. Like other environmentally friendly extraction processes of bioactive compounds, using microwave-assisted extraction (MAE) as a green Eco technological technique has gathered an important research interest [11] recently. Ionic conduction (movement of charged ions) and dipole rotation (reversal of dipoles) are the two mechanisms which reduce solvent requirement and extraction time [9] in MAE and result in the reduction of the process environmental footprint. There is no data reported regarding the microwave-assisted extraction and analysis of phenolic compounds from the peach of Assam Region (India) and also their applications.

In the present study, the optimization of extraction of phytochemicals from norabogori fruit using response surface methodology was determined. The purpose of the current study is to identify the ideal optimized conditions of solvent type, microwave power, solvent concentration,

solid-liquid ratio and time for the extraction of phytochemicals from norabogori using response surface methodology. We also focused to assess the phytochemical composition of norabogori fruit extract by HR-LCMS and quantification of phenolic compounds by RP-HPLC. The current investigation is a step towards to the exploration of bioactive compounds present in the fruit.

2.2 Materials and methods

2.2.1 Chemicals

The analytical-grade chemicals and the standards utilized in the present study were obtained from Sigma, Merck, and Himedia.

2.2.2 Plant sample collection and preparation

Norabogori fruit was obtained from Sonitpur district of Assam Region (India) (Latitude: 26° 51' 44.226". Longitude: 92° 51' 27.7596") which is available during Rabi season. This peach fruit variety is having slight color difference from popular peach variety. It has been identified to be *Prunus persica* (L.) Batsch of Rosaceae family.

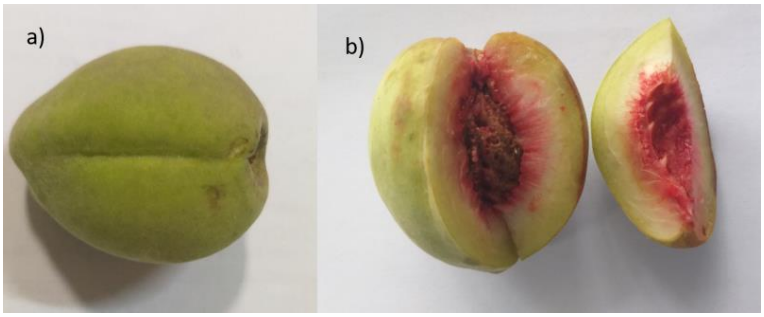


Fig.2.1 Various parts of norabogori (*Prunus persica* L. Batsch) fruit: a) Whole fruit b) Inner view of fruit cut

The harvested norabogori fruits were cleaned, the seeds were removed, and the fleshes were cut into thin slices for uniform drying. The slices were freeze-dried in a lyophilizer (Labtech, Korea) at -80°C overnight, kept in an airtight container, and protected from humidity and light exposure at 4°C until extraction and further analysis.

2.2.3 Chemical composition:

The moisture, crude protein, crude fiber, crude fat, and total ash content of norabogori fruit were estimated using AOAC (2005) [2] .

2.2.4 Extraction of phytochemicals

2.2.4.1 Conventional extraction of phytochemicals

For conventional solvent extraction, various solvents (ethanol, acetone, ethanol, hexane and chloroform) were used at a fixed concentration (70%). The dried norabogori fruit powder stored at 4°C was mixed with solvents of 70% concentration in 1:40 ratio (solid: solvent) at room temperature (30°C). The extraction was done in conventional extraction system in a shaking incubator (Innova 42R, Eppendorf, Germany) at 120-rpm agitation for 2 h. After the extraction period, the crude extract was centrifuged (5430R, Eppendorf, Germany) at 5000 rpm for 20 min. The dried extract was kept at -20°C until further use and the supernatant was dried in a hot air oven (advantage lab, AL01-05-100) at 40°C [14]. Considering important aspects such as yield and effect on health condition, the best extraction solvent will be selected.

2.2.4.2 Total phenolic content (TPC)

The TPC of the fruit extract was calculated using the Slinkard & Singleton [15] method, and the absorbance was measured using a spectrophotometer (AG22331, Eppendorf, Germany) at 765 nm. The reference used for the calibration curve was gallic acid, and the total phenolic content was thereafter represented as mg gallic acid equivalents (mg GAE) per 100 g of dried fruit extract.

2.2.4.3 Microwave-assisted extraction of phytochemicals

The norabogori fruit was extracted using a microwave extraction technique (NEOS GR, Milestone, Italy). The organic solvent that evinced the highest phenolic extract yield in the conventional extraction system was further used as a solvent for extracting phenolic compounds. A total of 3 g of dried norabogori powder was put into a beaker and mixed with different concentrations (50-100%) of the selected solvent and in varying liquid: solid ratios (20-40 mL/g). The dispersion was then subjected to a range of microwave power (400-900 W) for a stipulated time (5-15 min). The extraction parameter ranges were set after an extensive literature review and preliminary trials. Each experiment was performed 3 times, and control was used as a comparison for the outcomes (Conventional solvent extracted sample). After MAE, the samples were centrifuged at 6000 rpm for 10 min to elute the solid residue. The supernatant was filtered using

Whatman filter paper, the solvent was evaporated in a tray dryer at 40°C, and finally, the extracts were kept at -20°C for further analysis [16].

2.2.4.3.1 Optimization using Box-Behnken design (BBD)

A response surface methodology (RSM) was utilized to assess the performance of microwave power, solvent concentration, solid-liquid ratio and time on the total phenolic content of the norabogori extract using MAE. For each factor (independent variables) the upper and lower level values were determined and combined to form the 4 factor 3 level Box Behnken design (BBD) along with the response shown in Table 2.1 and it provides the coded value levels of factors. The following quadratic equation (Eq. 1) was employed to explain how variables affect each other using linear, quadratic, and cross terms.

$$Y = b_0 + \sum_i b_i x_i + \sum\sum b_{ij} x_i x_j + \sum b_{ii} x_i^2 \quad (1)$$

In this context, the dependent variable is Y, the constant coefficient is b_0, the linear, quadratic, and interaction coefficients are b_i, b_{ii}, and b_{ij} respectively, and the independent variables x_i and x_j are coded. The significance of model was evaluated using analysis of variance (ANOVA), checked the suitability of model using the values of R_2, and adjusted R^2.

Table 2.1 Range of independent variables with their corresponding levels

Coded variable levels	Independent variables	Levels		
		-1	0	1
A	Microwave power (W)	400	650	900
B	Ethanol concentration (%)	50	75	100
C	Liquid:Solid ratio (mL/g)	20	30	40
D	Time (min)	5	10	15

Table 2.2 BBD for extraction of phytochemicals using microwave-assisted extraction (MAE)

Sl no.	Microwave power (W)	Ethanol concentration (%)	Liquid:solid (mL/g)	Time (min)	TPC (mg GAE/100g)
1	650	75	30	10	4113.2
2	400	75	20	10	3810.0
3	650	50	30	15	4600.0
4	650	50	20	10	2543.0

5	650	75	30	10	4613.2
6	650	100	20	10	3250.0
7	900	75	30	15	3749.0
8	650	100	40	10	2380.0
9	400	75	40	10	5370.0
10	650	75	40	5	3196.0
11	900	100	30	10	2132.5
12	650	75	40	15	6700.0
13	900	50	30	10	2975.0
14	400	75	30	15	4567.0
15	400	75	30	5	4967.5
16	650	75	20	5	4146.0
17	650	75	30	10	4513.2
18	900	75	40	10	2600.0
19	650	75	20	15	2654.0
20	650	75	30	10	4335.2
21	400	100	30	10	3256.0
22	650	100	30	15	4354.0
23	650	50	40	10	5135.0
24	900	75	20	10	3132.0
25	900	75	30	5	2787.0
26	650	75	30	10	4246.2
27	400	50	30	10	4665.0
28	650	100	30	5	4183.0
29	650	50	30	5	4786.0

2.2.4.3.2 Optimization and validation

Plazzotta et al. [17] approach was used to carry out optimization within the range of parameters under consideration. To concurrently achieve the maximum levels of TPC, the highest desirability represented the most adequate condition to verify the experimental design's prediction models under optimal conditions and a series of 29 experiments were conducted. The determination of the coefficient between the predicted and the experimental data was taken as an indicator of prediction accuracy (12.89%). Then, using a t-test for independent variables, differences between two procedures for extraction performances were compared for the extracts produced by MAE under optimal conditions ($p<0.05$).

2.2.5 Identification of major compounds in the norabogori extract by Hr-LCMS

The optimized crude extract of Norabogori extracted by microwave extraction system using ethanol as solvent was taken for Hr-LCMS analysis based on significant higher amount of phenolics. The UHPLC-PDA-Detector Mass spectrometer (HR-LCMS 1290 Infinity UHPLC System, 1260 Infinity Nano HPLC withChipcube, 6550 iFunnel GTOFs), Agilent Technolgies, USA) were used for the experiment and 0.1% of HCOOH mixed with Water and methanol separately were used as solvents. The solvents flow rate was kept at 0.4 mL/min in gradient elution program. Detection of various phytoconstituents were carried out by MS Q-TOF Mass Spectrometer (Agilent Technologies).

2.2.6 Phytochemicals analysis using Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)

RP-HPLC (Waters) system with Symmetry 300™ C_{18} (5 μm, 4.6 × 250 mm) column, a binary pump (Waters, 1525) and a UV–vis detector (Waters, 2489) was employed with an injection volume of 20 μL to determine the phenolic compounds in the extracts acquired under various extraction systems. The extracts obtained both by conventional solvent extraction and optimized microwave-assisted extraction system were subjected to the RP-HPLC analysis according to the method given by Muchahary et al. [18]. Acetic acid (0.1%) in distilled water with a mobile phase A of pH 3.2 and methanol as the mobile phase B were the mobile phases employed in the system. The gradient method consists of: 80% A for 0–8 min, 65% A for 9–12 min, 45% A for 13–16 min, 30% A for 17–20 min, 20% A for 21–30 min, 10% of A for 31–40 min and then, at last, the column was washed with 80% A for 41–45 min. The sample volume utilized was 20 μL. The UV-vis detector wavelength was 254 nm, and the flow rate was kept constant at 0.8 mL/min. Gallic acid, syringic acid, salicylic acid, sinapic acid, catechin, chlorogenic acid, caffeic acid, rutin, cyanidin glycoside, ellagic acid, ferulic acid, coumarin, quercetin, and kaempferol were the standards utilized for comparison and identification.

2.2.7 Statistical analysis

SPSS 16.0 was used for the data's statistical analysis. Every experiment was run at least three times, and the means were assessed using an analysis of variance (ANOVA) and multiple range tests by Duncan were used to identify significant variances. ($p<0.05$).

2.3 Results and discussion

2.3.1 Specimen identification: It was taxonomically authenticated by the Department of Botany, Guwahati University (Acronym: GUBH, reference no. Herb./GUBH/2020/141 and an accession 19001).

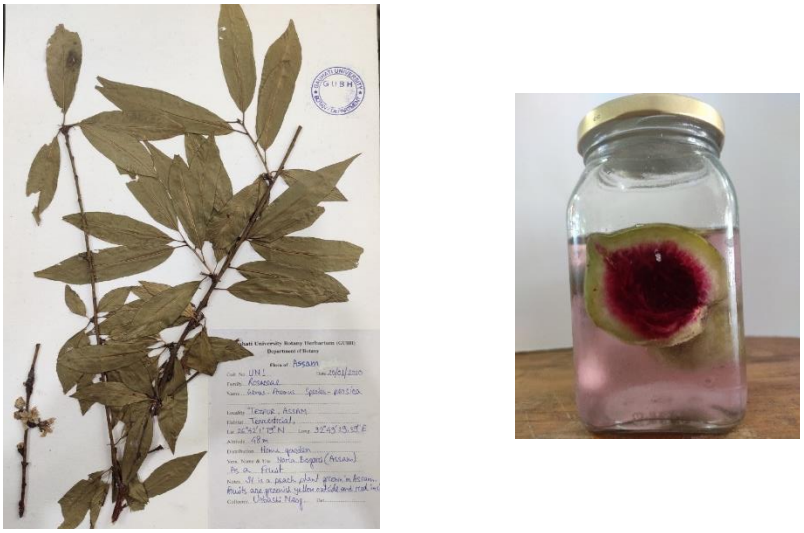


Fig 2.2. Herbarium for norabogori sample authentication

2.3.2 Chemical compositionof norabogori fruit

The proximate composition of the norabogori fruit was evaluated, i.e. moisture content, total ash and carbohydrate and crude protein, fat and fiber content. The moisture content of norabogori was found to be 84.45g/100g which puts it in the category of perishable agricultural product (moisture >70%). The amount is in agreement with the previously studied results in fresh, ripe, undamaged peach fruit from Bulgaria. The range of the values can be evaluated in 84%-89% which is a high amount for any agricultural product in general. From the study it can be seen that

varieties from the same locality having similar soil quality may give rise to differences in important physiochemical attributes [19,20]. Moisture content determines and affects the nutritional value and quality attributes of fruit like color, texture, volatiles, and nutrients. However, higher moisture content leads to enhanced microbial activity and reduced shelf life worsened food security. Drying processes with high heat treatment degrade the phytochemical content of the fruit and leads to deteriorated nutritional profile. To preserve its inherent quality, low temperature drying treatments are most efficient even though they are highly energy exhaustive methods. Peach is a seasonal fruit obtainable in bulk during May-July. So to proceed for further methodologies also to use it conveniently for the whole year around, the fruits were freeze dried for this study. Further it is stored in airtight container in 4^0C.

Crude protein of the fruit was obtained to be 1.51g/100g, crude fat 0.04g/100g and crude fiber 1.49g/100g. Though the ranges are in agreement with the results obtained by altaf et.al. (2020) but the amount of crude fat and protein are found to be far less than reported by both altaf et.al. (2020) and Mihaylova, 2021 [19,20]. The crude fiber and total carbohydrate content (11.27g/100g) is in similar level with the peach gathered in Bulgaria and reported by Mihaylova, 2021.

Table2.3 The chemical composition of fruit determined by proximate analysis according to AOAC method

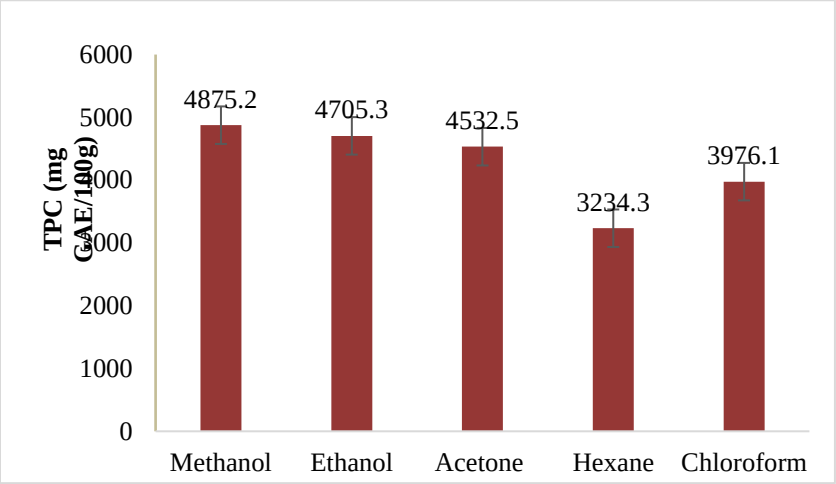
Test parameters	Content (g/100g) in wb
Moisture	84.45±0.44
Total Ash	1.28±0.13
Crude Protein	1.51±0.11
Crude Fat	0.04±0.02
Crude Fiber	1.49±0.17
Total Carbohydrate	11.27±0.21

Values are represented as mean of triplicates ±SD

2.3.3 Conventional extraction of phytochemicals

The solvent employed for extraction and the solubility of the phenolic compounds are the two key factors that affect phenolic compound extraction. In our investigation, it was observed that phenolic content of norabogori was impacted by the solvent used for extraction. According to the results presented in Fig.2.3, all the norabogori extracts contained phenolic compounds, and the amount of these compounds varied depending on the extraction solvent utilized. Among the tested

solvents, in 50% concentration methanol, ethanol and acetone presented with 4875.2, 4705.3 and 4532.5 mg GAE/100g, respectively from norabogori followed by hexane (3234.3 mg GAE/100g) and chloroform (3976.1 mg GAE/100g).



GAE= Gallic Acid Equivalent

Fig.2.3 Effect of various solvents on total phenolic content of the norabogori fruit

When employing various solvents the difference in polarity of various compounds present in the sample may be the cause of the variability in 'extract yields' from the samples employing various solvents [21,22]. Generally, aqueous methanol yields the highest recovery in the extraction of total extractable compounds [23]. As a solvent, acetone works best for proanthocyanidins and tannins extraction; whereas ethanol is efficient for flavonoids extraction and also for their tannins, glycosides and catechols; catechin and phenolic acids were efficiently extracted with methanol. These results are in line with the polarity of the solvent employed for the extraction and also the solubility of different phenolics in them since the methanol, acetone and ethanol has a polarity of 0.762, 0.355 and 0.654, respectively [24]. Consequently, any single solvent cannot simultaneously extract all of the different groups of phenolic compounds from a sample. However, from a toxicity point of view ethanol is the safest for human and its frequent use, and it is very crucial to look into the ethanolic extract's potential health benefits [25]. Ethanol is primarily considered as GRAS (generally recognized as safe) solvent makes it efficient for application in final food product. In the present study, methanol was discovered to be the most effective solvent for (TPC) extraction, followed by ethanol and acetone. On the contrary, as explained by Joshi and Adhikary [26], the methanol intoxication can result into metabolic acidosis and tissue injury if the norabogori extract contains any traces of remaining methanol. Also, the methanolic extract found out to be more toxic

on *in vivo* system than ethanolic extract [27]. So, taking both yield and health conditions into consideration, ethanol was finally selected to be the best extraction solvent for the subsequent work in the studies.

2.3.4.1 Optimization and fitting of the model

The responses from 29 experimental trials were collected, explained to determine which parameters significantly impacted the extraction, and an equation expressing the relationship between the TPC of the norabogori extracts and the MAE parameters was developed (Table 2.4). Table 2.4 displays the ANOVA findings for the model responses. All the models resulted significant on the microwave power (W), ethanol concentration (%), liquid:solid ratio (mL/g) and time (min) indicating a positive interaction on total phenolic content (TPC). Moreover, all the four factors were found to exhibit a quadratic effect on the response (TPC). Similar results were also described by Alam et al. [28], indicating a significant effect of independent variables on total phenolic content while the TPC showed no sign of being significantly impacted by the interaction factors. Data were examined using multiple regression after non-significant covariates were eliminated. The developed quadratic models in terms of coded values of all the factors are as follows:

$$TPC = +4364.20 - 771.67\ x_1 - 429.04\ x_2 + 487.17\ x_3 + 213.21\ x_4 - 511.47\ x_1^2 - 480.41\ x_2^2 - 398.35\ x_3^2 + 323.34\ x_4^2 + 141.63\ x_1 x_2 - 523.00\ x_1 x_3 + 340.63\ x_1 x_4 - 865.50\ x_2 x_3 + 89.25\ x_2 x_4 + 1249.00\ x_3 x_4 \quad (6)$$

The correlation between the calculated regression model and the response was significant ($p<0.01$), with R^2 value 0.91 and R^2_{adj} value of 0.84. Further, the lack of fit for the model was non-significant ($p<0.05$), indicating the impact of the regression equation on the independent variables of the extraction process of TPC from norabogori extract.

Table2.4 ANOVA (Analysis of variance) result of the fitted model for the response variable

Variables	DF	Estimated Variables TPC	F value TPC
Model	14	4364.20	11.40 a
x_1	1	-771.66	39.89 a

x_2	1	-429.04	12.33
x_3	1	487.16	15.90
x_4	1	213.20	3.04
x_1^2	1	-511.47	9.47
x_2^2	1	-480.41	8.35
x_3^2	1	-398.35	5.74
x_4^2	1	323.33	3.78
x_1x_2	1	141.62	0.44
x_1x_3	1	-523.00	6.10
x_1x_4	1	340.62	2.59
x_2x_3	1	-865.50	16.72
x_2x_4	1	89.25	0.17
x_3x_4	1	1249.00	34.83
Lack of fit			0.052
R^2			0.91

(*p value, significance of difference a <0.001; b<0.01; c<0.05)

2.3.4.2 Microwave-assisted extraction performance

The efficacy of microwave power (W), ethanol concentration (%), liquid-solid ratio (mL/g) and time (min) on total phenolic content (TPC) of freeze-dried norabogori powder was evaluated through RSM, using BBD. Obtained data are summarized and presented in the 3D response plots in Fig. 2.4 (a-d).

It was observed from the response graph (Fig.2.4), that the applied microwave treatments resulted in an initial rise in liquid-solid ratio up to 30mL resulting in an increased TPC value but beyond 30 mL/g slight decrease was observed. However, due to the thermal effect of MAE, ethanol concentration had a higher positive impact than temperature. Microwave power boosts MAE-induced heating, which then transfers heat energy into the sample [24]. These two variables interactions had a positive impact on the response [29]. High microwave power had a slightly negative impact on the TPC of the extract, according to the response plot for factors (Fig.2.4). But the TPC level increased with ethanol concentration, rising gradually before dropping slightly.

It was shown from the response graph (Fig.2.4) that the first increase in microwave power up to 650 Ws led to an increase in TPC, but a minor decline after 775 Ws was observed. When compared to time, ethanol concentration showed a higher positive impact. The response was positively affected by the interaction effect between the two factors. Orthosiphon stamineus and citrus mandarin peel extract both showed a similar rise in TPC with MAE power and time [17]. On TPC, the independent variable had a positive impact. Increased TPC activity was evident as the time and ethanol concentration increased. Additionally in this instance, a positive interaction effect on TPC activity was found [30].

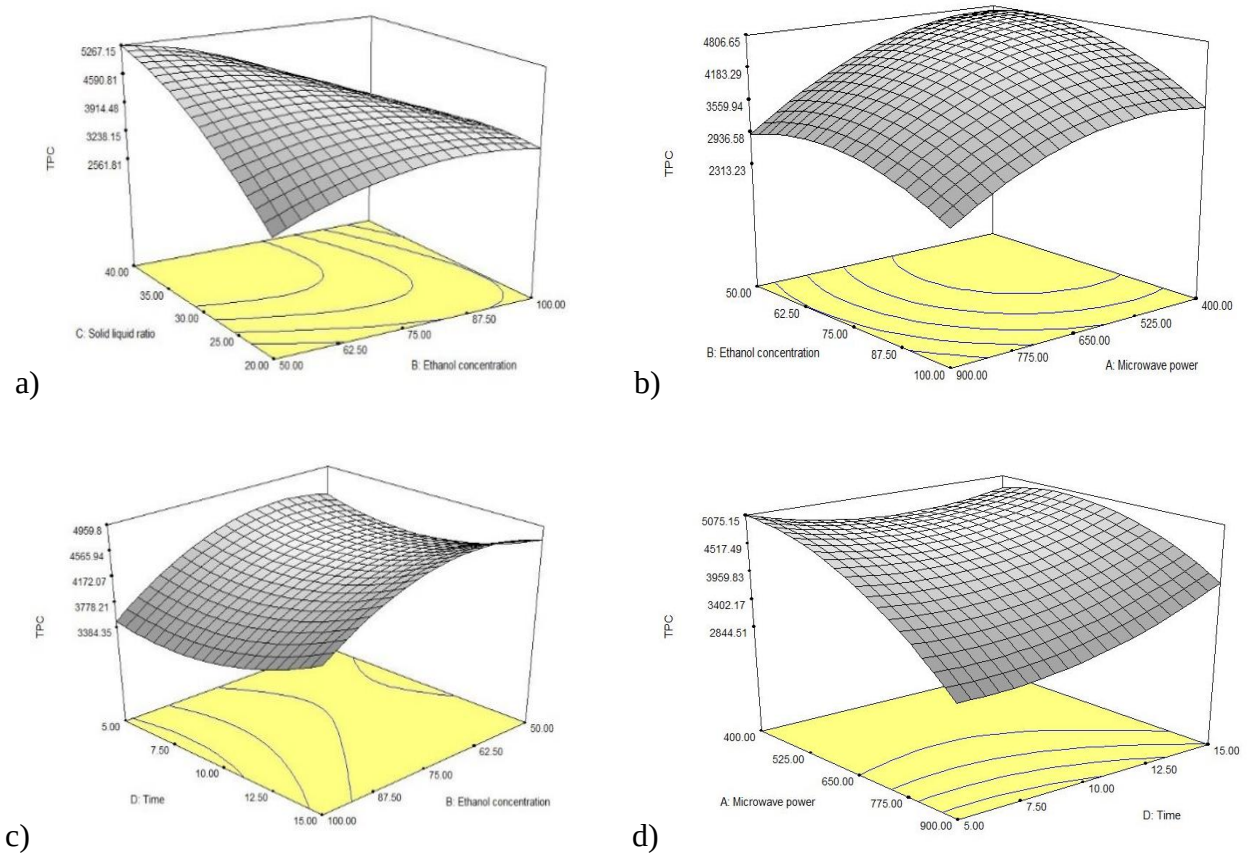


Fig.2.4 The effects of microwave power (Watt), ethanol concentration (%), liquid-solid ratio (ml/g) and time (min) on the extraction of total phenolic content; (a) Liquid-solid ratio-ethanol concentration (BC) for TPC, (b) Microwave power-ethanol concentration (AB) for TPC, (c) Ethanol concentration-time (BD) for TPC, (d) Microwave power-time (AD) for TPC

2.3.4.3 Validation of the optimized conditions

The optimal treatment conditions for the extraction of TPC from norabogori extract, exhibiting the RSM model results are shown in Table 2.5. In the case of norabogori extract, the optimum conditions were found at microwave power at 571.82 W, ethanol concentration 66.55 %, liquid-solid ratio 39.48 (mL/g), and time 14.79 min on total phenolic content (TPC) 6701.58 g GAE/100g. The derived models and 3D response graphs were validated using these data. Particularly, a higher-than-expected correlation between the predicted values and observed values showed that the equations and models correctly predicted the outcomes of the experiment. There was no discernible difference between the extraction parameters of optimal MAE treatments.

2.3.4.4 Verification of the predictive model

The optimal conditions were 1 g sample in 40 mL, 67% ethanol extracted with a microwave power of 570 Ws for 15 min and resulted in the maximum yield (6824.38 g GAE/100g), which was used to assess the adequacy of the model for the responses. The results revealed that the experimental values for the reactions were very much comparable and concurred with the expected value (Table 2.5).

Table2.5 Optimized solution obtained using the response optimizer

	Microwave power (W)	Ethanol concentration (%)	Liquid-Solid ratio (mL/g)	Time (min)	TPC (g GAE/100 g)
Predicted values	571.82	66.55	39.48	14.79	6701.58
Experimental value	570.00	67.00	40.00	15.00	6824.38

2.3.5 Identification of major compounds in the norabogori extract by Hr-LCMS

Hr-LCMS study was employed to detect the phytochemicals present in the optimized norabogori extract. The identified compounds along with their mass, chemical formula, retention time, concentration and m/z ratio are listed in (Table 2.6). Both positive and negative ionization was used to get MS data. The Hr-LCMS chromatogram for the ethanolic norabogori is shown in Fig.2.5.

Phenolic compounds were the predominant constituents found and primarily the m/z values fall between 118 and 610. It was found that the norabogori extract contain no. of phenolic acids like 4-Hydroxycoumarin, gallic acid, ferulic acid, chlorogenic acid, syringic acid etc.; polyphenols like caffeic acid, ellagic acid; flavonoids like furaneol, kaempferol, rutin etc. Many of these bioactive compounds are extensively studied and found to be effective for degenerative diseases and symptoms like antioxidant, anti-inflammatory, antidiabetic and anti-cancerous activity [31,32]. A LCMS analysis of peach pulp gathered from Greece has been carried out in a recent study and some similar compounds have been identified. Chlorogenic acid, ferulic acid, kaempferol, catechin are some major phytochemicals among them. In another study by Gedük et al. 2022, identification of fumaric acid, Quinic acid, protocatechuic acid, salicylic acid, rutin, chlorogenic acid, isoquercitrin, astragalin, hesperidin, nicotiflorin, quercetin etc was resulted [33]. These compounds detection enabled them to assess the antidiabetic, antioxidant, and antibacterial activity of peach extract in both ethanol and methanol as extraction solvent. Similarly, presence of these bioactive compounds in our tested samples opens up avenue for using it as anti-inflammatory agents to cure diseases.

Table2.6 Identification of phytochemical compounds by HR-LCMS

Sl. No.	Retention Time [min]	Name	Formula	Molecular Weight	Area (Max.)
1	1.05	Muramic acid	C9 H17 N O7	251.10	1542005.9
2	1.07	L-Aspartic acid	C4 H7 N O4	133.04	768611.36
3	1.08	Pipecolic acid	C6 H11 N O2	129.08	2469983.6
4	1.12	Adipic acid	C6 H10 O4	146.06	771047.32
5	1.12	Furaneol	C6 H8 O3	128.05	1190444.9
6	1.12	Linamarin	C10 H17 N O6	247.10	53955.509
7	1.12	Arecoline	C8 H13 N O2	155.09	485048.51
8	1.14	D-(-)-Quinic acid	C7 H12 O6	192.06	2542597.4
9	1.15	(±)-Malic Acid	C4 H6 O5	134.02	462977.65
10	1.19	Nicotinic acid	C6 H5 N O2	123.03	223327.74
11	1.28	Citric acid	C6 H8 O7	192.03	4997949.7
12	1.28	trans-Aconitic acid	C6 H6 O6	174.02	525959.16
13	1.52	citramalic acid	C5 H8 O5	148.04	1202251.2
14	1.68	Kojic acid	C6 H6 O4	142.03	723528.09
15	1.95	Gallic acid	C7 H6 O5	170.02	732580.17
16	2.01	Succinic acid	C4 H6 O4	118.03	374787.07
17	8.67	Chlorogenic acid	C16 H18 O9	354.09	1891617.1
18	8.67	Ferulic acid	C10H10O4	194.18	1891617.1

19	8.90	propiomazine	C20 H24 N2 O S	340.16	1534369.1
20	15.46	Ellagic acid	C14H6O8	302.19	5499069.3
21	16.55	Rutin	C27H30O16	610.50	4117864.1
22	17.14	Coumarin	C9H6O2	146.14	133056.87
23	17.24	Syringic acid	C9H10O5	198.17	554446.42
24	20.04	Caffeic acid	C9 H8 O4	180.04	407542.82
25	20.04	4-Hydroxycoumarin	C9 H6 O3	162.03	250207.26
26	20.39	Asiatic acid	C30 H48 O5	488.35	1694958.9
27	20.54	(E)-parinaric acid	C18 H28 O2	276.21	270223.61
28	21.12	α-Eleostearic acid	C18 H30 O2	278.22	146506.9
29	21.47	Maslinic acid	C30 H48 O4	494.34	2617050.3
30	21.52	Hederagenin	C30 H48 O4	472.35	2369347.9
31	22.94	Oleanolic acid	C30 H48 O3	438.35	1282551.1
32	22.99	Ursolic acid	C30 H48 O3	456.36	699053.94
33	23.74	Kaempferol	C15H10O6	286.24	515592.35

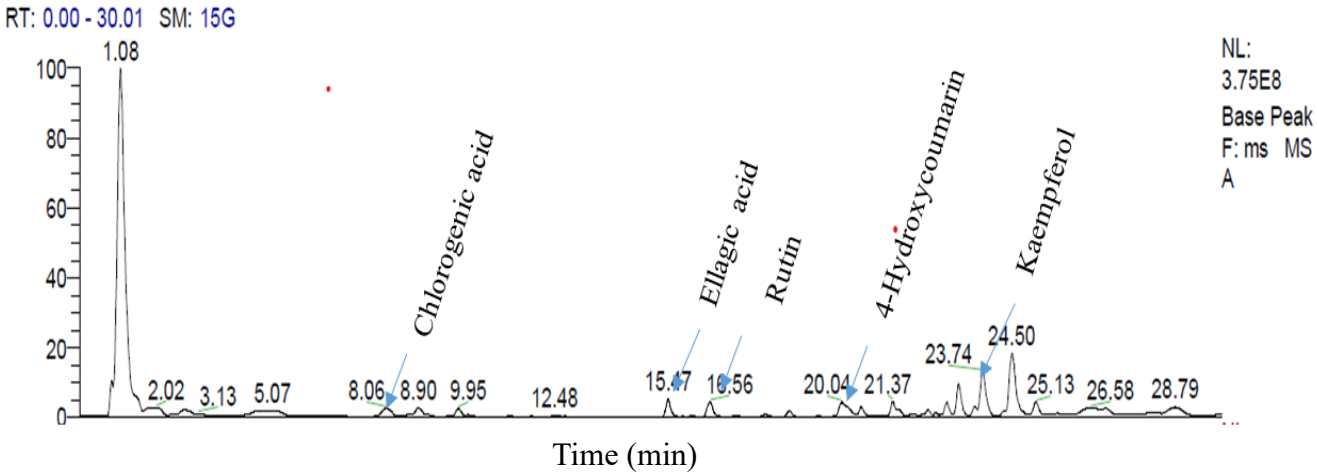


Fig. 2.5: Compounds showing peaks in HR-LCMS Chromatogram

2.3.6 Phytochemical analysis of norabogori fruit extract using RP-HPLC

In RP-HPLC, 14 phytochemical HPLC grade standards (viz., caffeic acid, syringic acid, gallic acid, ferulic acid, sinapic acid, catechin, chlorogenic acid, salicylic acid, rutin, cyanidin glycoside, ellagic acid, coumarin, quercetin, kaempferol) were run first and at a wavelength of 254nm and their retention time is reported (Table 2.7).

Table2.7 Retention time of phytochemical standards in RP-HPLC

Compound	Retention time (min)
Gallic acid	3.811
Catechin	7.883
Caffeic acid	11.086
Syringic acid	12.101
Ferulic acid	14.901
Sinapic acid	15.059
Chlorogenic acid	15.278
Salicylic acid	16.114
Rutin	16.537
Cyanidin glycoside	16.650
Ellagic acid	16.686
Coumarin	17.053
Quercetin	18.987
Kaempferol	20.518

Sharp phytochemical chromatograms with several derivatives can be seen between the wavelengths 230 and 360 nm [34]. Likewise, at various retention times, a strong phytochemical peak was observed at 254 nm. HPLC chromatograms revealed the detailed phytochemical analysis of MAE and conventional extraction method of norabogori extracts and are presented in Fig.2.6. Each peak's retention time was taken and compared with the standard phytochemicals. Both the extracts showed major peaks at 254 nm for kaempferol, gallic acid, rutin, coumarin and ellagic acid in their chromatograms (Fig.2.6). When compared to the conventional system extract, phytochemicals distinguished from the MAE extract had higher peaks (higher intensities) at 254 nm. Kaempferol with a retention time of 20.52 min was identified giving the highest concentration (211.09 ± 0.01 mg/100g) in the MAE extract when compared to the conventional technique during the analysis of phytochemicals (Table 2.8).

Table2.8 Phytochemical contents detected by RP-HPLC in norabogori extracts by microwave-assisted extraction and conventional extraction method

Phytochemicals detected by HPLC	MAE (mg/100g)	Conventional extraction system (mg/100g)
Kaempferol	211.09	193.81

Gallic acid	20.89	25.28
Ellagic acid	57.98	22.44
Rutin	83.06	29.34
Coumarin	34.31	5.41
Syringic acid	44.40	ND
Ferulic acid	7.13	ND
Salicylic acid	ND	10.16
Catechin	ND	44.53

ND = Not Detected

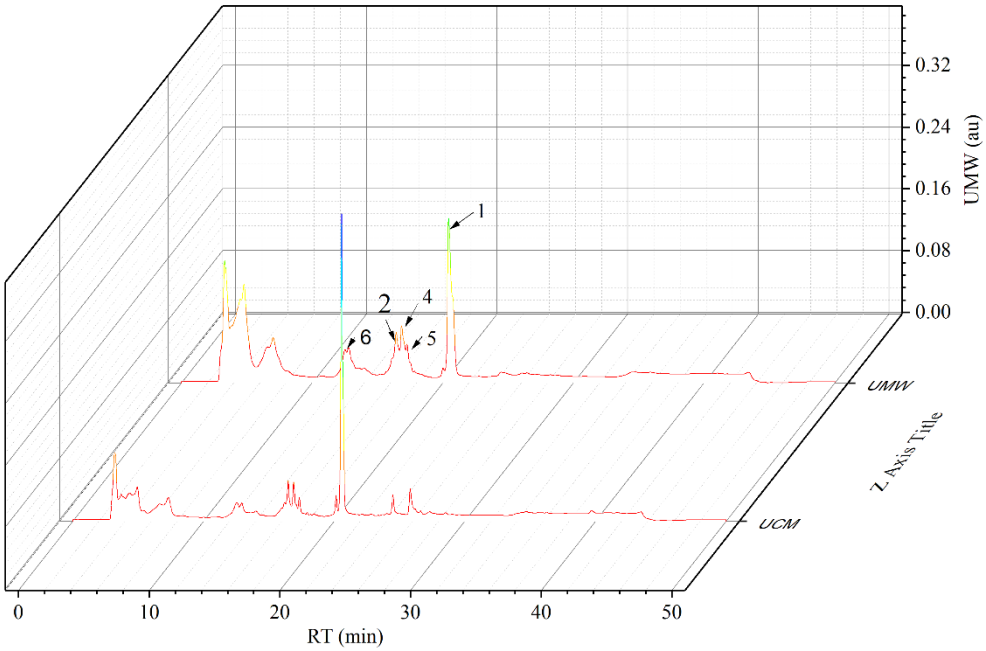


Fig.2.6 HPLC chromatogram of phytochemical compounds detected from norabogori fruit extract by microwave-assisted extract (MAE) and conventional system extract at 254nm (1-kaempferol, 2-gallic acid, 4-rutin, 5-coumarin, 6-syringic acid)

Our results are in line with those of Szwajgier et al. [35] who isolated quercetin, rutin and kaempferol as primary cholinesterase inhibitor compounds from peach. Guo et al. [5] in their studies showed a variety of compounds as commonly present throughout different varieties of Chinese peaches and nectarines. Catechin, neochlorogenic acid, and chlorogenic acid were among them; 3-p-coumaroyl quinic acid, myricetin, 1-caffeoylquinic acid, and kaempferol-3-o-rutinoside

were found to be the essential phenolic markers. Neochlorogenic acid, catechin, and chlorogenic acid were among the most prominent polyphenols.

2.3.7 Conclusions

Results of the present study revealed that ethanol concentration, microwave power, and time affect the efficiency of the total phenolic content extraction of the norabogori fruit. The maximum yield (6824.38 g GAE/100 g) is recorded in the combined interaction at ethanol concentration of 67%, 1 g sample in 40 mL ethanol extracted with a microwave power of 570 W for 15 min. It was found from Hr-LCMS that the norabogori extract contain no. of phenolic acids like 4-Hydroxycoumarin, gallic acid, ferulic acid, chlorogenic acid, syringic acid etc.; polyphenols like caffeic acid, ellagic acid; flavonoids like furaneol, kaempferol, rutin etc. RP-HPLC results of the crude extract confirmed the presence of kaempferol and 8 distinct polyphenols. Rutin was present in sufficient amounts in the crude extract, along with ellagic acid and syringic acid and catechin.

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