

Optimization of extraction process for the recovery of antioxidants from *Morus nigra* leaves

UMER YOUNAS, SHAHID IQBAL* & JAMSHED AKBAR

Department of Chemistry, University of Sargodha, Sargodha, 40100, Pakistan

ARTICLE INFORMATION

Received: 05-07-2019
Received in revised form:
16-09-2019
Accepted: 18-10-2019

*Corresponding Author:

Shahid Iqbal:
umer0608analyst@gmail.com

ABSTRACT

In current work, effects of different drying procedures as well as of different solvents on extraction of antioxidants from *M. nigra* leaves have been studied. Initially, samples were dried using different drying procedures (shade, oven and microwave drying) and extracted using different solvents (ethyl acetate, acetone and methanol) in combination with microwave-assisted extraction technique. Antioxidant potentials of extracts were determined in terms of total phenolics, total flavonoids and ascorbic acid contents. In addition, radicals (DPPH[•] & ABTS^{•+}) scavenging potentials were also determined. Results revealed that drying methods have no significant influence on recovery of antioxidants from sample matrix. However, methanolic extracts exhibited highest antioxidant potential that confirmed superiority of methanol as extraction media over the other solvents used. In addition, microwave-assisted extraction technique for the recovery of antioxidants from *M. nigra* leaves was optimized using response surface methodology. For the purpose, oven dried samples were further extracted using methanol under different sets of extraction conditions (microwave intensity and extraction time). A central composite design was employed to determine the optimum conditions for the maximum recovery of antioxidant components from leaf matrix. Significant effects of different extraction conditions were recorded on recovery of antioxidant components and radical scavenging potential. On the basis of results, optimum conditions for the recovery of antioxidant components from *M. nigra* leave have been prescribed.

Keywords: Mulberry leaves, Optimization, RSM (response surface methodology), Antioxidants, Microwave-assisted extraction

Original Research Article

INTRODUCTION

Regular and continuous generation of free radicals, reactive oxygen (O₂^{•-}, HO[•], H₂O₂) and nitrogen (NO[•], ONOO⁻) species is the result of normal cellular metabolic functions in aerobics (Perron & Brumaghim, 2009). Being unstable and reactive, these species cause oxidative damages to biomolecules such as DNA, protein and lipids. In human body, built-in antioxidant defensive system resists harmful activities of these species by neutralizing or scavenging them. But, sometimes, excessive generation of these species couldn't be proportionally countered or scavenged by built-in antioxidant defense system of the body, which results in state of oxidative stress in human body (Iqbal *et al.*, 2012). In addition to this, issue of oxidative stress has become a threat for lipid containing foods due to risks associated with

oxidative deterioration. Therefore, use of antioxidants as food supplements for human body and as preservatives in food products has become mandatory nowadays (Park *et al.*, 2011). Natural antioxidants *i.e.* phenolics, flavonoids, carotenoids, anthocyanins, and vitamins, have received significant attention of researchers and consumers due to reports published about safety of synthetic antioxidants (Saha *et al.*, 2011). Different parts (roots, shoots, leaves and fruits) of medicinal plants have been reported as potent sources of antioxidants, which are frequently available at low cost and more efficient as compared to synthetic antioxidants.

Exploitation of medicinal plants, as sources of antioxidant components, mainly depends upon three major processes including drying, extraction and quantification of bioactive components followed by determination of their antioxidant potential

(Wijekoon *et al.*, 2011). In first step, drying of botanical materials is carried out to inhibit microbial growth, moisture mediated enzymatic degradation and changes in organoleptic characteristics (Hossain *et al.*, 2010). Drying of plant materials must be conducted with great care as drying may cause considerable quality changes (taste, colour and nutrition) (Fan *et al.*, 2012), as well as availability of solutes from internal parts of plant materials (Maskan, 2001). Under shade and hot air drying are commonly preferred methods due to their low cost and ease to handle. Among the advanced techniques, microwave drying is rapid, more uniform, and energy efficient.

Yield, composition, purity, nature of extracted compounds (Cheng *et al.*, 2012) and antioxidant potential of extracts are strongly influenced by the polarity of solvent (Boulekbache-Makhlouf *et al.*, 2013). Water and organic solvents (acetone, ethanol, methanol, ethyl acetate, diethyl ether etc.) are commonly used for the extraction of bioactive compounds (Kallithraka *et al.*, 1995). Reports revealed that every solvent recovered specific class of bioactive compounds, (Cheng *et al.*, 2012) due to affinities developed between compounds and solvent molecules of specific polarity which led to the maximum recovery of targeted compounds (Hayouni *et al.*, 2007). Therefore, it is necessary to test different drying methods, extraction solvents for a selected extraction method and plant material, to obtain maximum amount of target compounds. (Wijekoon *et al.*, 2011). Keeping in view multiple extraction parameters, optimization of extraction process is focused by researchers, employing different statistical tools such as response surface methodology (RSM). RSM is exercised by the scientists to improve extraction efficiency of different methods with an aim to decrease extraction time, expenses, consumption of chemicals and to obtain maximum amount of target compounds (Şahin & Şamlı, 2013; Yim *et al.*, 2012).

Morus nigra L. is a commonly growing rustic plant, also found in gardens (Pérez-Gregorio *et al.*, 2011). It is used for the treatment of diabetes and rheumatis in Chinese folk medicine. Antioxidant activities have been reported for different parts of this species and in addition, separation and isolation of two new flavonoids have been achieved (Iqbal *et al.*, 2010). In continuation of our studies on *Morus* (Iqbal *et al.*, 2012), the present work was undertaken to find out the effect of different drying procedures, extraction solvents and extraction conditions on antioxidant potential of *M. nigra* leaves. Selected drying methods (Shade, Oven, and microwave) and different solvents (ethyl

acetate, acetone and methanol) were compared in terms of their effect on recovery of antioxidant components in leaf matrix. In addition, optimization of extraction parameters i.e. extraction time and microwave intensity were achieved employing response surface methodology in order to maximize the yield of antioxidants from *M. nigra* leaves.

MATERIALS AND METHODS

Chemicals

Analytical grade solvents were used in this work. Gallic acid, Epicatechin, Folin-Ciocalteu (FC) reagent and Ascorbic acid were purchased from Merck (Darmstadt, Germany). Trolox, Diammonium (2Z,2'Z)-2,2'-[(1Z,2Z)-1,2-hydrazinediylidene]bis(3-ethyl-2,3-dihydro-1,3-benzothiazole-6-sulfonate) (ABTS^{•+}) and 2,2'-diphenyl-picrylhydrazyl (DPPH[•]) stable radicals were procured from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Collection and drying of samples

Samples of *M. nigra* leaves were collected from peripheral areas of Lahore, Punjab, Pakistan. Leaves were washed with tap water and then rinsed with deionized water. Leave samples were chopped and then dried using three different methods i.e. microwave drying, hot air oven drying and shade drying.

For shade drying, leaves were placed in dark for one week. Hot air drying was carried out by placing samples in hot air oven and temperature was maintained at 40 °C for two days. Microwave oven drying of medicinal plant leaves was carried out in commercial Samsung microwave oven and drying was carried out for 3 to 9 minutes. The dried samples were cooled in a desiccator at room temperature. This procedure was repeated three times to ensure the stabilization and complete drying of samples. Powder of dried leaves was prepared using grinder and stored in sealed polyethylene bags at ambient conditions.

Extraction procedures

Extracts of powdered sample were prepared using three solvents including ethyl acetate (EtOAc), acetone and methanol (MeOH). For the purpose, microwave assisted extraction technique was used. Sample (10.0 g) was taken in round bottom flask having one of the solvents (50 mL) and extraction was carried out for 2 min at 100 watt microwave intensity. Sample was extracted three times and extracts were filtered with Whatman filter paper followed by centrifugation (Hettich, Germany) at 4000 rpm, for 15 minutes and the crude extracts were obtained. The extracts were

kept in dark glass bottles inside the freezer until further analysis.

Determination of antioxidant potential

Total phenolic contents (TPC) extracts of *M. nigra* leaves were determined using FC reagent assay reported by Khan *et al.*, 2019 and TPCs were calculated as mg gallic acid equivalent per gram of dried sample (mg GAE/ g of dried sample). For the determination of total flavonoid content (TFC), a method reported by Hussain *et al.*, (2012) was followed and TFC were calculated as μg Epicatechin equivalent/ g of dried leaf samples (μg ECE/ g of dried sample). For estimation of ascorbic acid content, an already reported titrimetric method was used (Iqbal *et al.*, 2012). The content of ascorbic acid was calculated as mg ascorbic acid equivalent/ g of dried leaves using standard curve.

M. nigra extracts was evaluated for free radical scavenging potential following DPPH assay reported by Roja *et al.*, (2011). In addition, free radical scavenging potential of extracts was also determined employing ABTS radical cation scavenging assay reported by Yuan *et al.*, (2012). Results of both assays were calculated as % inhibition.

Response surface optimization

For microwave assisted extraction method, conditions for the maximum recovery of antioxidant components from *M. nigra* leaves were optimized employing response surface methodology. Mutual effect of two independent variables (X_1 = radiation power and X_2 = extraction time) on antioxidant potential of *M. nigra* leaves was investigated. Extracts of *M. nigra* leaves were prepared employing the selected extraction conditions of time employing different radiation power (watt). Analysis of extracts was carried in terms of determination of TPC, TFC, AAC and antiradical potential, as given above. Codes used in the response surface analysis and the corresponding parameter values are given in Table I.

Table I: Codes used in RSM and corresponding level of independent variables for the two level full factorial design

Variables	Code units	Coded variable levels		
		-1	0	1
Time (min)	X_1	3	6	9
Power (watts)	X_2	200	300	400

The regression equation model in un-coded units for each response was as follows:

$$Y = \beta_0 - \beta_1 X_1 - \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2$$

Where Y=response value, β =constant coefficients, and X=independent variable.

Regression analysis (R^2) was performed to predict the adequacy of the model. Using Minitab 17, analysis of variance (ANOVA) was calculated, while RSM was applied using Design Expert Software (Minitab 17) program. Applying 5% probability level ($\alpha = 0.05$), significance of the model was determined. Effect of independent variables on efficiency of extraction process was judged on the basis of selected response variables including TPC, TFC, AAC and radical scavenging potential. Response of selected dependent variables was plotted against two independent extraction conditions (extraction time and radiation power) and three-dimensional response surface plots were generated.

RESULTS AND DISCUSSION

Effect of drying conditions and extraction solvents

Some preliminary studies were carried out to investigate the effect of drying procedures and extraction media on recovery of antioxidants from *M. nigra* leaves. Extracts were analyzed for their contents of phenolics, flavonoids, ascorbic acid and radical scavenging potential (table II).

Table II. Effect of drying procedure and extraction solvent on antioxidant potential of *M. nigra* leaves

<i>Morus Nigra</i> Leaves	Shade drying			Oven drying			Microwave drying		
	Microwave assisted extraction								
	EtO Ac	Ace-tone	Me OH	EtO Ac	Ace-tone	Me OH	EtO Ac	Ace tone	Me OH
TPC (mg GAE/g)	8.79	34.14	50.56	7.84	20.04	51.62	7.44	25.15	58.08
TFC (μg ECE/g)	27.60	27.27	35.15	29.25	31.17	49.38	24.70	28.47	43.09
AAC (mg AAE/g)	7.78	15.27	22.19	8.44	14.43	22.26	8.44	14.68	22.63
DPPH' (% Inhibition)	28.34	40.46	42.46	19.85	32.06	46.66	11.45	34.26	60.84
ABTS** (% Inhibition)	39.86	56.43	71.86	16.00	55.29	81.14	21.43	55.71	81.57

High amount of antioxidant components was found in samples dried using oven or microwave drying procedures. However, drying procedures had no significant effect on antioxidant potential of leave sample. Among different tested solvents, extracts prepared using MeOH was found containing maximum amount of antioxidants. Therefore, it was decided to use hot air oven drying method and MeOH as extraction media for further studies *i.e.* response surface optimization of microwave assisted extraction technique.

Experimental design for microwave-assisted optimized extraction

Codes and the corresponding values used for optimization purposes while extracting *M. nigra* leaves by microwave-assisted extraction (MAE) are given in Table I. The coded (independent variable), measured and predicted values (TPC, TFC, AAC and %age inhibition (DPPH[•] and ABTS^{•+})) are given in Tables III. Two level full factorial randomized regression equations are shown in Table IV and the validity of the model was established using the coefficients determination (R^2) and F and P values. The P value of any matter <0.05 with larger F were considered significant (Iqbal *et al.*, 2016).

Total phenolic content (TPC)

In order to optimize the microwave assisted extraction technique for the extraction of TPC, RSM has already been used (Prasad *et al.*, 2011). Total phenolic content in *M. nigra* leaves were found in range of 37.74 to 51.53 mg GAE. Highest TPC were obtained, when extraction was carried out at 300 watt for 6 min. The selected parameters (time and power) of microwave-assisted extraction technique were found effecting recovery of total phenolic content. As microwave power was increased, up to 20 % increase in TPC was recorded. However, data suggests that use of high intensity radiations for more time may not further increase content of total phenolics in extracts. Some previous studies have confirmed that degradation of phenolics that takes place due to prolonged exposure to radiations (Souza, *et al.*, 2018).

Three dimensional responses for the recovery of TPC against different power and time were recorded for microwave-assisted extraction technique under study and presented in Fig. 1. Statistical data (Table IV) revealed that the effect of extraction time and microwave power was linear quadratic and the interactive effect of time and power were significant for TPC recovery as value of

p was less than 0.05. Findings of our study are well in agreement with those reported by (Elik *et al.*, 2019) for RSM optimization of microwave assisted extraction of phenolics from blueberry. Measured and predicted TPC values (RSM) are given in Table III. Measured values for both response variables are in close agreement with the predicted values, indicating a satisfactory model.

Total flavonoid content (TFC)

Extracts of *M. nigra* leaves prepared applying different microwave intensity for different time intervals, were evaluated for total flavonoid content. Highest TFC (146.8 μg ECE/g) value was recorded for the extract prepared at 300 watt microwave intensity for 3 min. All the other extracts prepared at high microwave radiation power for longer time were found having lesser flavonoid content. Statistical data (Table IV) revealed that the mutual effect of radiation power and time was non-significant, as p value is greater than 0.05, the linear quadratic and interactive effect of power and time were also found to be non-significant. However, measured and predicted TFC values (Table III), for both response variables are in close agreement with the measured values, indicating a satisfactory model as reported for. Responses for the recovery of TFC using different extraction time and radiation power were recorded for microwave-assisted extraction techniques (Fig. 1).

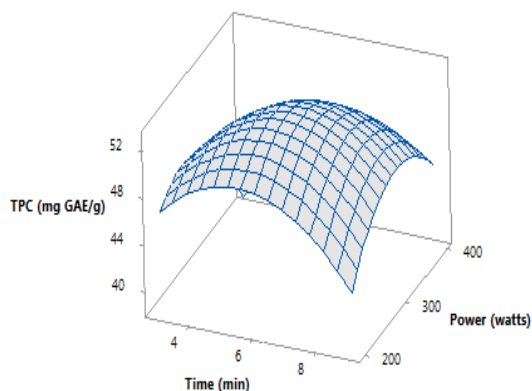
Ascorbic acid content (AAC)

Highest ascorbic acid content was obtained either by preparing extract by using low radiation power for longer time (2.94 mg AAE/g) or high radiation power for short time interval (2.96 mg AAE/g). Effect of extraction time and radiation power significantly affected the recovery of ascorbic acid content ($p << 0.05$) and the linear quadratic and interactive effect of radiation power and time were significant (Table IV). For microwave-assisted extraction technique, responses for the recovery of AAC from leave sample at different microwave power and extraction time were recorded (Fig. 1).

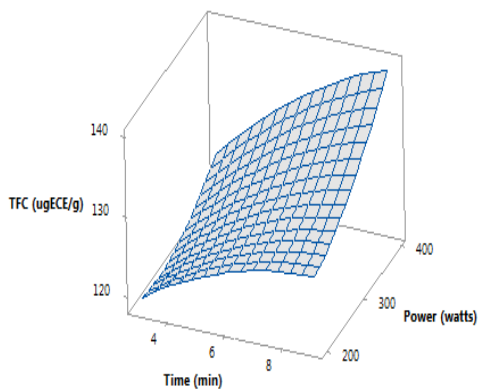
DPPH radical scavenging potential

Response surface methodology has already been used for the optimized extraction of antioxidant components with maximum DPPH radical scavenging potential (Vázquez *et al.*, 2012). In current study, radical scavenging activity of *M. nigra* leave's extract was evaluated by DPPH percent inhibition assay that ranged from 65 to 70.

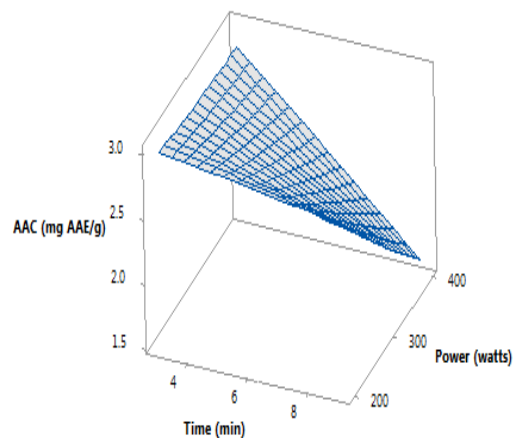
Highest DPPH percent inhibition was recorded for the extracts prepared using 300 watt radiation power for 6 min. Extracts prepared at high radiation power for long time duration were found having lesser DPPH percent inhibition. Trend of DPPH assay is same as observed for TPC indicating linear correlation among both the assays that confirms the degradation of antioxidant components upon extraction of *M. nigra* leaves at high microwave radiation power for long time. Statistical data (Table IV) confirmed significant effect ($p < 0.05$) of selected variable (radiation power and extraction time) for microwave-assisted extraction technique on radical scavenging potential of *M. nigra* leaves. It also confirms the interactive effect of both the independent variable (Fig. 1). Predicted DPPH % inhibition values (RSM) are given in Table III for both response variables are in close agreement with the measured values, indicating a satisfactory model as reported for recovery of DPPH radical scavenging compounds from *Marsilea quadrifolia* L (Chowdhury *et al.*, 2018).



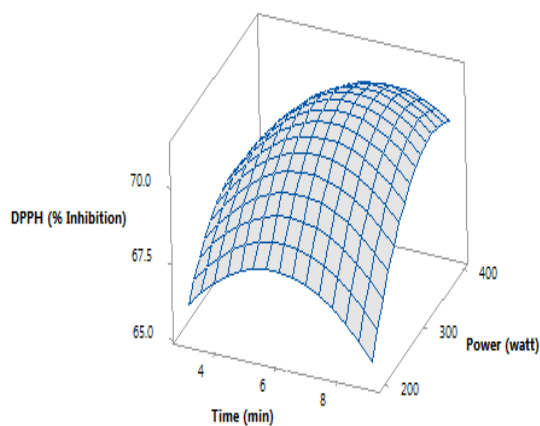
(a)



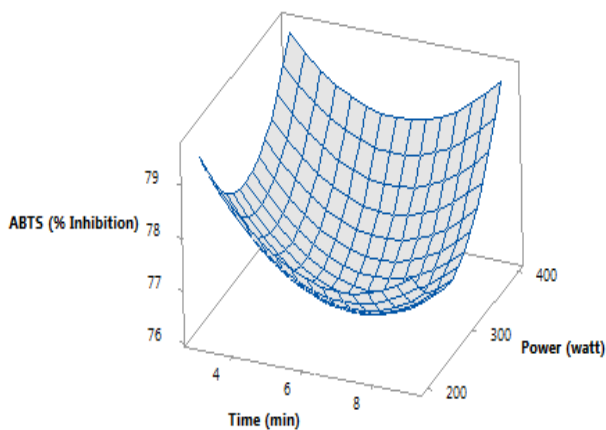
(b)



(c)



(d)



(e)

Fig. 1a, b, c, d & e: Response Surface plots showing the effect of microwave-assisted extraction parameters on TPC, TFC, AAC and %age inhibition (DPPH^{•+} and ABTS^{•+}) of 70 % MeOH extracts of *M. nigra* leaves

Table III. Measured and predicted TPC, TFC, AAC, %age inhibition (DPPH[•] and ABTS^{•+}) values for *M. nigra* leaves extract prepared using microwave-assisted extraction technique

Design point	Independent variables		Response (TPC, mg GAE/g)		Response (TFC, µg ECE/g)		Response (AAC, mg AAE/g)		Response (DPPH [•] , % Inhibition)		Response (ABTS ^{•+} , % Inhibition)	
	Sr. No.	X ₁	X ₂	Measured	Predicted	Measured	predicted	Measured	predicted	Measured	predicted	Measured
1	-1	-1	47.09	49.75	133.5	125.10	2.86	2.77	65.48	65.57	75.58	77.55
2	-1	0	50.80	48.41	146.8	139.67	2.82	2.89	70.01	69.69	80.01	79.50
3	-1	1	43.49	43.07	127.5	119.83	2.94	2.55	69.30	68.20	79.30	79.58
4	0	-1	50.54	46.74	124.3	124.23	1.80	1.58	65.26	66.02	75.20	77.08
5	0	0	48.80	46.56	111.8	119.53	1.92	2.24	70.16	70.14	78.16	78.39
6	0	1	51.53	52.68	126.2	127.40	1.84	2.16	70.00	70.87	80.02	79.43
7	1	-1	42.07	44.88	126.3	126.96	2.96	2.92	66.25	66.59	77.54	77.85
8	1	0	37.74	38.73	126.6	133.80	2.96	2.99	69.30	69.53	79.35	77.97
9	1	1	45.85	47.09	124.8	131.26	2.84	2.88	68.65	67.80	78.50	76.31

X₁= Coded units for speed, X₂=coded units for time

Table IV. Polynomial equations and statistical parameters for *M. nigra* leave using microwave-assisted extraction technique, leaves dried under hot air oven

Response variables <i>M. nigra</i>	2 nd order polynomial equation (Microwave-assisted extraction)	R ²	F	P
TPC (mg GAE/g)	$10.7 + 4.38 X_1 + 0.203 X_2 - 0.548 X_1^2 - 0.000444 X_2^2 + 0.00803 X_1X_2$	0.9831	1.74	0.035
TFC (μ g ECE/g)	$126.0 + 2.4X_1 - 0.119X_2 - 0.206X_1^2 + 0.000205X_2^2 + 0.0067X_1X_2$	0.5039	0.61	0.707
AAC (mg AAE/g)	$2.76 + 0.161X_1 + 0.0012X_2 - 0.0015X_1^2 + 0.000002X_2^2 - 0.000900X_1X_2$	0.9750	2.46	0.025
DPPH* % Inhibition	$46.24 + 2.01X_1 + 0.1079 X_2 - 0.2226 X_1^2 - 0.000190 X_2^2 + 0.00295 X_1X_2$	0.9920	4.96	0.019
ABTS** % Inhibition	$97.4 - 2.02 X_1 - 0.1021 X_2 + 0.129 X_1^2 + 0.000166 X_2^2 + 0.00115 X_1X_2$	0.8148	0.43	0.811

ABTS radicalcation scavenging potential

Effect of radiation power and extraction time was non-significant on ABTS radical cation scavenging potential as *p* value was greater than 0.05 for linear quadratic and two way interactive effects for ABTS. Extract prepared applying different conditions were found having same ABTS radical scavenging potential i.e. antioxidant components having ABTS radical scavenging potential are extractable on all the conditions of radiation power and extraction time.

Response surface methodology was employed to find out the optimized conditions (Table V) for the maximum recovery of antioxidant components. Two parameters, such as radiation power and extraction time potentially influenced the microwave assisted extraction process. It was observed that with the change of extraction time (6 min to 9 min) and radiation power (200 to 400 watt) significantly influenced the antioxidant potential of *M. nigra* leaves. A significant increase in TPC, AAC and DPPH radical scavenging activity was recorded. Generally, it was noted that extraction carried out using medium radiation power for almost 6 min produced maximum amount of antioxidant components. However, variation in TFC and ABTS radical cation scavenging activity was not found significant.

Table V. Optimized conditions to recover maximum antioxidant components from *M. nigra* leaves

Antioxidant activity	Power (watts)	Time (min)
TPC = 52.6892	285.319	6.05
TFC = 139.280	307.055	7.93952
AAC = 2.98322	200.977	3.05190
DPPH % = 71.1618%	339.850	6.68272
ABTS % = 79.5346%	399.116	3.02058

CONCLUSION

Extraction of antioxidant components from *M. nigra* leaves was successfully optimized in this study. Preliminary studies show that drying procedures do not have significant effect on antioxidant potential of leaf samples. However, extraction conditions were found having strong influence on extraction of phenolics, flavonoids, ascorbic acid and those antioxidants that have DPPH radical scavenging potential. *Morus nigra* is a well-known medicinal plant and is being used for the preparation of different functional foods and nutraceuticals. The current study shows that use of almost 300 watt microwave radiation power for 3-6 min will be suitable for the maximum recovery of antioxidant components from leaf matrix. For the recovery of antiradical agents, radiation power of 400 watt will be good enough for 3-6 min. To secure maximum benefits associated with antioxidants available in *M. nigra*

leaves, microwave assisted optimized extraction of antioxidants using suggested conditions should be considered.

REFERENCES

- Boulekbatche-Makhlouf, L., Medouni, L., Medouni-Adrar, S., Arkoub, L., & Madani, K., 2013. Effect of solvents extraction on phenolic content and antioxidant activity of the byproduct of eggplant. *Ind. Crop. Prod.*, 49: 668-674.
- Cheng, V. J., Bekhit, A. E. A., McConnell, M., Mros, S., & Zhao, J., 2012. Effect of extraction solvent, waste fraction and grape variety on the antimicrobial and antioxidant activities of extracts from wine residue from cool climate. *Food Chem.*, 134(1), 474-482.
- Chowdhury, A., Panneerselvam, T., Kannan, S., Bhattachejee, C., Somasundaram, B., Sankaranarayanan, M., & Kunjiappan, S., 2018. Optimization of microwave-assisted extraction of bioactive polyphenolic compounds from *Marsilea quadrifolia* L. using RSM and ANFIS modelling. *Ind. J. Nat. Prod. Res.*, 9(3), 204-221.
- Elik, A., Yanik, D. K., & Gogus, F., 2019. Optimization of microwave-assisted extraction of phenolics from blueberry. *ROMANIAN BIOTECHNOLOGICAL LETTERS*, 24(1), 30-40.
- Fan, L., Li, J., Deng, K., & Ai, L., 2012. Effects of drying methods on the antioxidant activities of polysaccharides extracted from *Ganoderma lucidum*. *Carbohydr. Polym.*, 87(2), 1849-1854.
- Hayouni, A., Abedrabba, M., Bouix, M., & Hamdi, M., 2007. The effects of solvents and extraction method on the phenolic contents and biological activities *in vitro* of *Tunisian Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. *Food Chem.*, 105(3), 1126-1134.
- Hossain, M. B., Barry-Ryan, C., Martin-Diana, A. B., & Brunton, N. P., 2010. Effect of drying method on the antioxidant capacity of six Lamiaceae herbs. *Food Chem.*, 123(1), 85-91.
- Hussain, A. I., Chatha, S. A. S., Noor, S., Khan, Z. A., Arshad, M. U., Rathore, H. A., & Sattar, M. Z. A., 2012. Effect of extraction techniques and solvent systems on the extraction of antioxidant components from peanut (*Arachis hypogaea* L.) hulls. *Food Anal. Methods.*, 5(4), 890-896.
- Iqbal, M., Iqbal, N., Bhatti, I. A., Ahmad, N., & Zahid, M., 2016. Response surface methodology application in optimization of cadmium adsorption by shoe waste: a good option of waste mitigation by waste. *Ecol. Eng.*, 88, 265-275.
- Iqbal, S., Asghar, M. N., Khan, I. U., & Zia, I., 2010. Antioxidant potential profile of extracts from different parts of black mulberry. *Asian J. Chem.*, 22(1), 353.
- Iqbal, S., Younas, U., Chan, K. W., Sarfraz, R. Adil., & Uddin, Md., 2012. Proximate composition and antioxidant potential of leaves from three varieties of Mulberry (*Morus* sp.): a comparative study. *Int. J. Mol. Sci.*, 13(6), 6651-6664.
- Kallithraka, S., Garcia-Viguera, C., Bridle, P., & Bakker, J., 1995. Survey of solvents for the extraction of grape seed phenolics. *Phytochem. Anal.*, 6(5), 265-267.
- Khan, A. S., Arif, K., Munir, B., Kiran, S., Jalal, F., Qureshi, N., & Ghaffar, A., (2019). Estimating Total Phenolics in *Taraxacum officinale* (L.) Extracts. *Pol. J. Environ. St.*, 28(1), 497-501.
- Maskan, M., 2001. Drying, shrinkage and rehydration characteristics of kiwifruits during hot air and microwave drying. *J. Food Eng.*, 48(2), 177-182.
- Park, Y-S., Leontowicz, H., Leontowicz, M., Namiesnik, J., Suhaj, M., Cvikrová, M., Gorinstein, S., 2011. Comparison of the contents of bioactive compounds and the level of antioxidant activity in different kiwifruit cultivars. *J. Food Comp. Anal.*, 24(7), 963-970.
- Pérez-Gregorio, M. R., Regueiro, J., Alonso-González, E., Pastrana-Castro, L. M., & Simal-Gándara, J., 2011. Influence of alcoholic fermentation process on antioxidant activity and phenolic levels from mulberries (*Morus nigra* L.). *LWT-Food Sci. Technol.*, 44(8), 1793-1801.
- Perron, N. R., & Brumaghim, J. L., 2009. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. *Cell Biochem. Biophysics*, 53(2), 75-100.
- Prasad, K. N., Hassan, F. A., Yang, B., Kong, K. W., Ramanan, R. N., Azlan, A., & Ismail, A., 2011. Response surface optimisation for the extraction of phenolic compounds and antioxidant capacities of underutilised *Mangifera pajang* Kosterm. peels. *Food Chem.*, 128(4), 1121-1127.
- Roja, G., Vikrant, B. H., Sandur, S. K., Sharma, A., & Pushpa, K. K., 2011. Accumulation of vasicine and vasicinone in tissue cultures of *Adhatoda vasica* and evaluation of the free radical-scavenging

- activities of the various crude extracts. *Food Chem.*, 126(3), 1033-1038.
- Saha, J., Biswas, A., Chhetri, A., & Sarkar, P. K., 2011. Response surface optimisation of antioxidant extraction from kinema, a *Bacillus* fermented soybean food. *Food Chem.*, 129(2), 507-513.
- Şahin, S., & Şamlı, R., 2013. Optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology. *Ultrasonics Sonochemistry*, 20(1), 595-602.
- Souza, M. M., Silva, B. DA., Costa, C. SB., & Badiale-Furlong, E., (2018). Free phenolic compounds extraction from Brazilian halophytes, soybean and rice bran by ultrasound-assisted and orbital shaker methods. *AN Acad. Bras. CIENC*, 90(4), 3363-3372.
- Vázquez, G., Fernández-Agulló, A., Gómez-Castro, C., Freire, M. S., Antorrena, G., & González-Álvarez, J., 2012. Response surface optimization of antioxidants extraction from chestnut (*Castanea sativa*) bur. *Ind. Crops Prod.*, 35(1), 126-134.
- Wijekoon, M. M., Bhat, R., & Karim, A. A., 2011. Effect of extraction solvents on the phenolic compounds and antioxidant activities of bunga kantan (*Etilingera elatior* Jack.) inflorescence. *J. Food Comp. Anal.*, 24(4), 615-619.
- Yim, H. S., Chye, F. Y., Koo, S. May., Matanjun, P., How, S. E., & Ho, C. W., 2012. Optimization of extraction time and temperature for antioxidant activity of edible wild mushroom, *Pleurotus porrigens*. *Food Bioprod. Process.*, 90(2), 235-242.
- Yuan, X., Gao, M., Xiao, H., Tan, C., & Du, Y., 2012. Free radical scavenging activities and bioactive substances of Jerusalem artichoke (*Helianthus tuberosus* L.) leaves. *Food Chem.*, 133(1), 10-14.