Erciyes Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
Cilt 38, Sayı 1, 2022

Erciyes University
Journal of Institue Of Science and Technology
Volume 38, Issue 1, 2022

Optimizing and Modeling of Microwave Assisted Extraction of Phenolics from Dandelion (*Taraxacum officinale*) by Response Surface Methodology

Nilay BEĞİÇ *1,

*1 Biruni Üniversitesi Eczacılık Fakültesi, İSTANBUL

(Alınış / Received: 04.02.2022, Kabul / Accepted: 07.04.2022, Online Yayınlanma / Published Online: 30.04.2022)

Keywords
Microwave-Assisted Extraction,
Dandelion,
Total Phenolic Content,
Response Surface Methodology

Abstract: Modelling microwave assisted extraction (MAE) of phenolics from dandelion (*Taraxacum officinale*) was carried out via response surface methodology in this study. Face-centered composite design was used to optimize the MAE factors as temperature and time of extraction, concentration of solvent and ratio between solid and solvent. Process factors of the MAE were optimized for maximum total phenolic content (TPC) of the dandelion extract. When the relationship between independent parameters and response was examined, the model calculated for TPC was found to be significant ($p<0.0001$). It was determined that the most significant factor is extraction temperature for the extraction of phenolics from the dandelion by MAE. The most insignificant parameter was determined as solid-to-solvent ratio parameter in MAE. Experimentally found and predicted data were found to be compatible with each other. It shows success of both the model and the optimization. The TPC yield was obtained as 1.26 mmol TR/g-dried sample in optimum MAE conditions. Consequently, the modeled method can be used for the extraction of phenolics from the dandelion in the pharmaceutical and food industries.

Yanıt Yüzey Metodolojisi ile Karahindiba'dan (*Taraxacum officinale*) Fenoliklerin Mikrodalga Destekli Ekstraksiyonunun Optimizasyonu ve Modellenmesi

Anahtar Kelimeler
Mikrodalga-Destekli Ekstraksiyon,
Karahindiba,
Toplam Fenolik İçerik,
Yanıt Yüzey Metodolojisi

Öz: Bu çalışmada, karahindibadan (*Taraxacum officinale*) fenoliklerin mikrodalga destekli ekstraksiyonunun (MAE) modellenmesi, yanıt yüzey metodolojisi ile gerçekleştirilmiştir. Ekstraksiyon sıcaklığı, ekstraksiyon süresi, çözücü konsantrasyonu ve katı ile çözücü arasındaki oran gibi MAE faktörlerini optimize etmek için yüz merkezli kompozit tasarım kullanıldı. MAE'nin işlem faktörleri, karahindiba ekstraktının maksimum toplam fenolik içeriği (TPC) için optimize edildi. Bağımsız parametreler ile yanıt arasındaki ilişki incelendiğinde TPC için hesaplanan model anlamlı bulundu ($p<0,0001$). MAE ile karahindibadan fenoliklerin ekstraksiyonunda en önemli faktörün ekstraksiyon sıcaklığı olduğu belirlenmiştir. MAE'de en önemsiz parametre olarak katı-çözücü oranı parametresi olduğu saptanmıştır. Deneysel olarak bulunan ve tahmin edilen verilerin birbiriyle uyumlu olduğu görülmüştür. Bu sonuç, hem modelin hem de optimizasyonun başarısını göstermiştir. TPC verimi, optimum MAE koşullarında 1.26 mmol TR/g kurutulmuş numune olarak elde edilmiştir. Sonuç olarak, modellenen yöntemin, farmasötik ve gıda endüstrilerinde karahindibadan fenoliklerin ekstraksiyonu için kullanılabilir bir yöntem olabileceği fikrine varılmıştır.

*Corresponding Author, email: nbegic@biruni.edu.tr

1. Introduction

As cells use oxygen to produce energy, reactive oxygen species (ROS) are formed as a result of the cellular redox process. The formation of reactive oxygen species in high concentrations causes oxidative stress [1, 2]. The oxidative stress is defined as imbalance between oxidant and antioxidant for living organisms [1]. Under oxidative stress conditions, excess ROS production may generate many health problems such as cardiovascular and neurodegenerative diseases, diabetes, hypertension and cancer [3]. Antioxidants are substances which can delay or inhibit oxidation by stabilizing or deactivating free radicals [4, 5]. The antioxidants are either produced in human body (endogenous) or taken through foods (exogenous). Food antioxidants both help in the production of endogenous antioxidants and play a direct role in scavenging reactive oxygen species [1, 6]. Therefore, dietary intake of food antioxidants is very important.

Dandelion (*Taraxacum officinale* Weber) is a medicinal plant and is mostly used for its stimulant, anti-inflammatory, diuretic, mild laxative properties [7, 8, 9]. Dandelion contains phytochemicals such as triterpenes, terpenoids, and phenolics (apigenin, luteolin, caffeic acid, chlorogenic acid, and isoquercitrin) [8]. These bioactive components make dandelion an important source of natural antioxidants.

The extraction procedures are an important step in purification of the bioactive constituents in the plant matrices. Traditional methods such as Soxhlet extraction and maceration, and modern methods including microwave-assisted (MAE), ultrasound-assisted extraction (UAE), and supercritical fluid extraction (SFE) have been used in the literature [10]. The advantages of MAE over other extraction methods are high extraction efficiency, shorter contact time, energy savings, less production cost due to reduced solvent waste, adoption of renewable fuel use and automated operation [11]. Microwave-assisted extraction has become popular for the separation of bioactive compounds in the plant matrices due to these advantages. The specific features of the interaction between solvents and microwave radiation are quite supportive in this regard, therefore this method is widely used and increase the efficiency of extraction of bioactive compounds [12].

There are many factors which affect microwave extraction; temperature, type of solvent, time of application of microwave, power level of microwave, and contact surface area [12]. All these factors need to be optimized to maximize extraction efficiency. Traditionally, optimization is performed on an experimental response by changing one factor at a time. In traditional optimization, only one parameter is changed while other parameters are kept constant. This technique does not include the interactive effects between the variables studied. In addition, as the number of experiments to be done will increase, it causes loss in terms of time and cost. In order to avoid these problems, response surface methodology (RSM), one of the multivariate statistical techniques, is widely used for optimization [13].

In this study, a simple, rapid and cost-effective MAE method was proposed for extraction of phenolics from the dandelion and RSM technique was used for optimizing the parameters of the proposed method. Temperature and time of extraction, ratio between solid and solvent, and concentration of solvent were optimized respectively. In order to optimize all these parameters, dandelion extract was aimed to have maximum total phenolic content (TPC). The effects of these parameters on TPC were evaluated.

2. Material and Method

2.1. Materials

Copper (II) sulfate, Folin-Ciocalteau reagent, and ethanol (EtOH) were supplied from Sigma-Aldrich (USA). Copper (II) chloride dihydrate, sodium carbonate, potassium sodium tartrate tetra hydrate, and sodium hydroxide were purchased from Merck (Germany). Dandelion was purchased from the seller of medicinal herbs.

2.2. Instrumentations

The absorption measurements were recorded using a ultraviolet-visible spectrophotometer (Varian Cary 100, Australia). MAE of the phenolic compounds from *Taraxacum officinale* was performed with an closed vessel oven system (Ethos-One, USA), and oven temperature was checked via a fiber optic sensor.

2.3. Microwave assisted extraction

Raw *Taraxacum officinale* sample was air dried and then ground. Microwave-assisted extraction process of Taraxacum officinale was carried out under parameters such as temperature and time of extraction, concentration of solvent, and ratio between solid and solvent. The temperature of extraction was performed from 50 to 100 ℃, the time of extraction was performed from 1 to 10 min. The solvent concentration was varied from 20% to 80%, ethanol in water. The ratio between solid and solvent was varied from 0.1 g/20 mL to 0.4 g/20 mL. The microwave power (0-1500 W) of MAE system with closed vessels was adjusted automatically according to temperature. The extracts obtained by changing each parameter were cooled, filtered using 0.45 µm PTFE filters (Merck, Germany). Filtered samples kept at 4 ℃ until analysis.

2.4. Total phenolic content assay

The TPC of *Taraxacum officinale* extract was determined using Folin–Ciocalteau method [14]. According to Folin assay, solutions were prepared as follow: Lowry A solution: 2% Na_2CO_3 was prepared in 0.1 M NaOH. Lowry B solution: 0.5% $CuSO_4$ was prepared in 1% $NaKC_4H_4O_6$. Lowry A solution (50 mL) and Lowry B solution (1 mL) were mixed. This mixture solution is Lowry C reagent and should prepared freshly. Folin–Ciocalteau reagent diluted (1:3) with distilled water was used in the analysis.

x mL of the sample extract and (1 - x) mL of distilled water were added a test tube in this method. Lowry C solution (2.5 mL) was added to the test tube. After 10 min, Folin–Ciocalteau reagent (0.25 mL) was added to the test tube. The mixture was kept at room temperature for 30 min. The absorbance of the sample was measured against a reagent blank at 750 nm. The TPC was stated as Trolox (TR) equivalent and its unit is mmol TR per g-dried sample (DS).

2.5. Statistical analysis

Face Centered Composite Design (FCCD) was used in order to analyze and optimize the experimental data. Design-Expert Software Version 11 Trial (Minneapolis, USA) was used for this purpose. In this study, independent variables (four factors) were chosen as X_1: extraction temperature, X_2: extraction time, X_3: concentration of solvent, and X_4: solid-to-solvent ratio; and dependent variable (one response) was chosen as Y1: TPC.

The following relationship can be established between the independent factors and the responses:

$$Y = f(X_1, X_2, ..., X_k) + \varepsilon \tag{1}$$

If the quadratic polynomial model is applied to the experimental data, the equation can be written as [15]:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \cdots \sum_{j=2}^{k} \beta_{ij} x_i x_j + \varepsilon \tag{2}$$

f: the response function, X: the independent variable, Y: the response, β_0 : the constant regression coefficient, β_i, β_{ii}, β_{ij} : interaction coefficients, *k*: the factor number and ε: the experimental error.

The TPC analysis was performed for triplicate of the plant extract. We determined the interaction between the independent parameters and the TPC responses with Design-Expert program and analysis of variance (ANOVA) test.

3. Results

Table 1 shows the independent factors and their levels were inputted into Design-Expert Software. It also gives the responses calculated by experimental data.

Table 1. The independent variables and levels employed in RSM for optimization.

Independent variables	Symbol of the variables	Units	Levels		
			-1	0	1
Extraction temperature	X_1	°C	30	75	120
Extraction time	X_2	min	1	4	7
Ethanol concentration	X_3	%, v/v	20	50	80
Solid-to-solvent ratio	X_4	g/20 mL	0.10	0.25	0.40

The influences of the independent factors on the TPC results of dandelion extract obtained via MAE were summarized in Table 2. The TPC results of the dandelion extracts were determined ranged from 0.795 to 1.264 mmol TR/g-DS.

Table 2. FCCD of the operational factors (X_1, X_2, X_3, and X_4) for the TPC experimental results with the MAE.

Run No	Factors				TPC (mmol TR/g-DS)
	X_1	X_2	X_3	X_4	
1	30	1	20	0.10	0.795
2	120	1	20	0.10	1.033
3	30	7	20	0.10	0.944
4	120	7	20	0.10	1.049
5	30	1	80	0.10	1.019
6	120	1	80	0.10	1.088
7	30	7	80	0.10	0.988
8	120	7	80	0.10	1.176
9	30	1	20	0.40	0.817
10	120	1	20	0.40	1.040
11	30	7	20	0.40	1.026
12	120	7	20	0.40	1.136
13	30	1	80	0.40	0.903
14	120	1	80	0.40	1.045
15	30	7	80	0.40	1.053
16	120	7	80	0.40	1.187
17	30	4	50	0.25	0.988
18	120	4	50	0.25	1.137
19	75	1	50	0.25	1.100
20	75	7	50	0.25	1.170
21	75	4	20	0.25	1.175
22	75	4	80	0.25	1.239
23	75	4	50	0.10	1.157
24	75	4	50	0.40	1.220
25	75	4	50	0.25	1.264
26	75	4	50	0.25	1.261
27	75	4	50	0.25	1.247
28	75	4	50	0.25	1.260
29	75	4	50	0.25	1.258
30	75	4	50	0.25	1.261

3.1. Modeling and optimization of MAE with RSM

When the relationship between independent parameters and response was examined, the model calculated for TPC was found to be significant ($p<0.0001$). The most important factor in the TPC of dandelion extract was found as the extraction temperature. The quadratic models are fitted to the data and the results are given in Table 3. According to the ANOVA results, the contribution of the quadratic model is significant.

Table 3. The ANOVA for the TPC quadratic equation.

	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model (TPC)	0.4794	14	0.0342	21.51	< 0.0001
X_1	0.1025	1	0.1025	64.41	< 0.0001
X_2	0.0439	1	0.0439	27.60	< 0.0001
X_3	0.0259	1	0.0259	16.26	0.0011
X_4	0.0017	1	0.0017	1.05	0.3218
X_1X_2	0.0012	1	0,0012	0.7308	0.4061
X_1X_3	0.0013	1	0.0013	0.8052	0.3837
X_1X_4	5.235×10^{-6}	1	5.235×10^{-6}	0.0033	0.9550
X_2X_3	0.0009	1	0.0009	0.5781	0.4589
X_2X_4	0.0089	1	0.0089	5.58	0.0321
X_3X_4	0.0050	1	0.0050	3.12	0.0976
X_1^2	0.0502	1	0.0502	31.54	< 0.0001
X_2^2	0.0116	1	0.0116	7.26	0.0167
X_3^2	0.0001	1	0.0001	0.0381	0.8479
X_4^2	0.0005	1	0.0005	0.3062	0.5882
Residual	0.0239	15	0.0016		
Lack of Fit	0.0237	10	0.0024	62.10	0.0001
Pure Error	0.0002	5	0.0000		
Cor Total	0.5032	29			

The quadratic model for the TPC are summarized in Equation 3. The significance of each coefficient was established by p-value from Table 3 and F-test.

$$TPC = +1.23 + 0.0755X_1 + 0.0494X_2 + 0.0379X_3 + 0.0096X_4 - 0.0085X_1X_2 - 0.0090X_1X_3 + 0.0006X_1X_4 - 0.0076X_2X_3 + 0.0236X_2X_4 - 0.0176X_3X_4 - 0.1392X_1^2 - 0.0668X_2^2 + 0.0048X_3^2 - 0.0137X_4^2 \quad (3)$$

The model obtained for the TPC was found significant ($p>0.05$). Furthermore, the predicted R^2 of 0.7411 is in reasonable agreement with the adjusted R^2 of 0.9083 (i.e. the difference is less than 0.2) for the TPC. Adequate precision ratio of the TPC was found as 15.901. For maximum TPC values, we optimized independent variables of the MAE via Design-Expert program. The highest TPC yield was obtained as 1.26 mmol TR/g-DS (X_1 = 76 ℃, X_2 = 5 min, X_3 = 76%, and X_4 = 0.39 g/20 mL).

3.2. Effects of operational factors on MAE

The 3D graphs in Figures 1, 2, and 3, drawn according to Equation 3, estimate the relationship between various parameters and the impact of these parameters on TPC responses. In the Figure 1, the relationship between time and temperature of the extraction is showed for the TPC responses of the dandelion extract. As in all other extraction types, the temperature of extraction is one of the most considerable parameters for MAE. Concordantly, it was determined that the most significant factor is extraction temperature for the extraction of phenolics from the dandelion by MAE in this work. We used a closed system for the microwave assisted extraction. The extraction process can be performed beyond the boiling point of the solvent with the closed system. The high extraction temperature causes the target components in the from the active sites sample matrix to dissolve easily, thus increasing the extraction efficiency. Besides, high temperature decreases the viscosity and surface tension of the solvent and increases the solubility capacity of the solvent. Thus, the extraction yield is increased [16-18]. In the different studies, authors reported that antioxidant and polyphenolic compounds begin to decompose after 80 °C [19, 20]. In addition, it has been reported that at high temperatures, bioactive compounds react with other components of the plant, reducing the extraction yield [21]. As seen in Figure 1, the TPC values increased with temperature but decreased after 90 ℃. The optimum temperature is determined as 76 ℃. Therefore, the extraction process is not carried out at very high temperatures, considering the decrease in extraction efficiency.

Extraction times studied in the microwave assisted extraction are quite shorter than the other extraction procedures. According to the studies in the literature, the extraction efficiency increases with the increasing extraction time. But the increase is relatively lower after a certain period of time [22, 23]. The results obtained in terms of extraction time are consistent with the results of the studies in the literature. The extraction yield of phenolics increased depending on the extraction time however the extraction yield decreased after 5 minutes. So the optimum extraction time was determined as 5 min. In the MAE process, an additional 3 minutes is required to reach the extraction temperature. Ethanol and methanol are solvents with high dielectric constant and high heating rate. In MAE processes with such solvents, long-term microwave irradiation can cause heating and decomposition of the analytes. Consequently, long MAE times should be avoided, both to save time and to avoid the risk of degradation of the analytes.

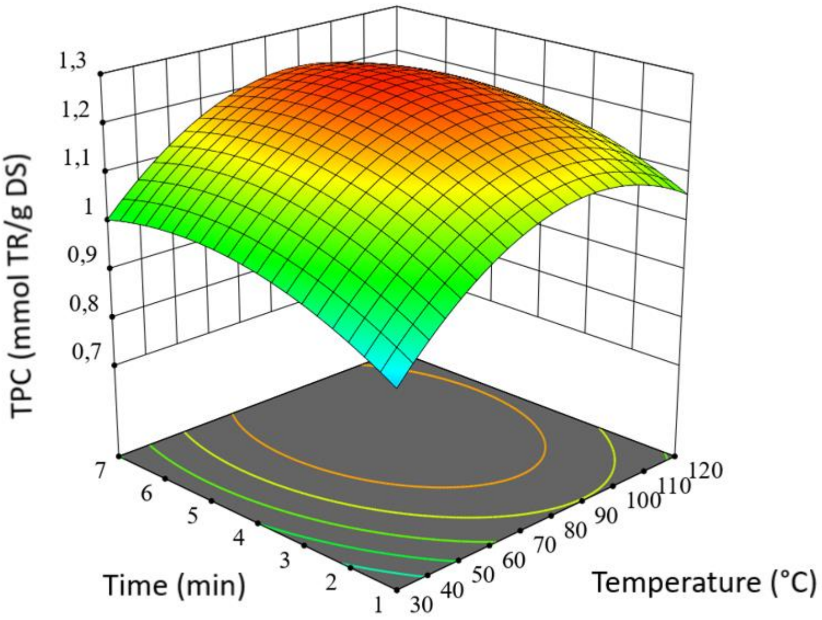

Figure 1. The responce surface plot (3D) for the TPC of the dandelion extract as a function of extraction time to extraction temperature.

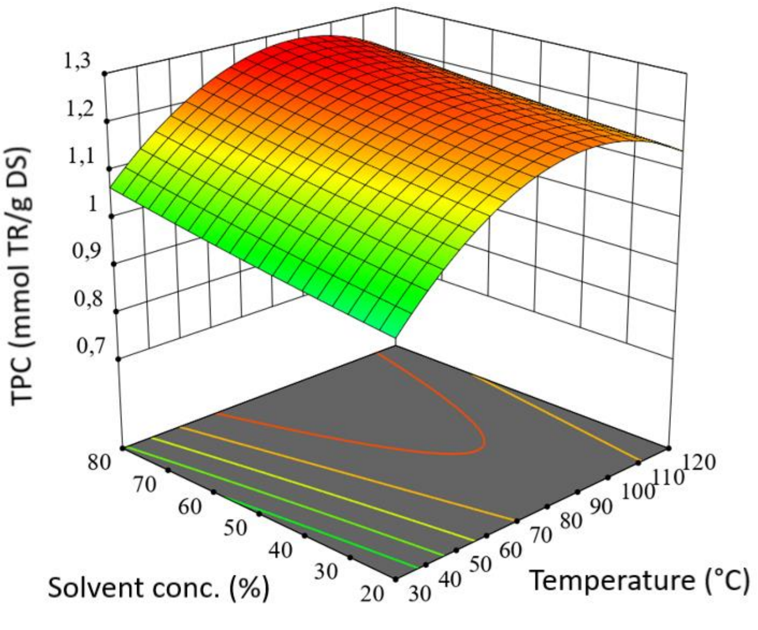

Figure 2. The responce surface plot (3D) for the TPC of the dandelion extract as a function of solvent concentration to extraction temperature.

Figure 2 shows the relationship between temperature of extraction and concentration of solvent for the TPC responses of the dandelion extract. For the extraction of polyphenolic compounds from plant matrices, polar solvents are often used. The most commonly used solvents are water, methanol, ethanol, and acetone [24]. Ethanol is a good solvent for the extraction of polyphenolic compounds due to its eco-friendly and safe for human consumption [25]. It is thought that ethanol in the solvent mixture breaks the bonds between plant matrices and analytes, thereby enabling the analytes to dissolve, while water plays a role as the plant swelling agent [26]. For this reason, water and ethanol were chosen as solvent components in the study. For optimum polyphenolic compound extraction, the ethanol concentration in the solvent mixture was determined as 49% ethanol in water.

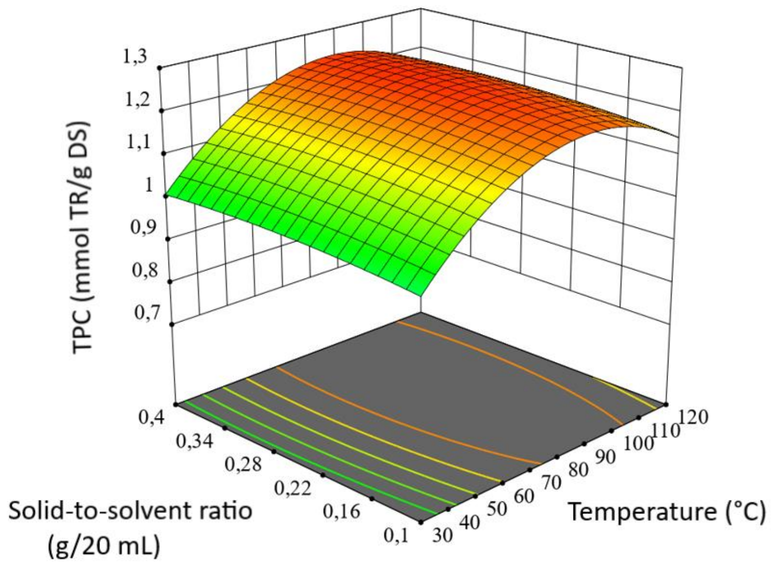

Figure 3. The responce surface plot (3D) for the TPC of the dandelion extract as a function of solid-to-solvent ratio to temperature of extraction.

Figure 3 shows the relationship between temperature of extraction and solid-to-solvent ratio for the TPC responses of the dandelion extract. In this work, the optimum ratio between solid and solvent for the extraction of antioxidant compounds from the dandelion extract with MAE was determined as 0.39 g /20 mL. A high or low solid-solvent ratio can cause some problems. Excessive solvent consumption is not preferred in terms of both environmental factors and cost.

4. Discussion and Conclusion

Dandelion contains phytochemicals such as phenolics, terpenoids, and triterpenes. Due to the presence of these bioactive constituents, dandelion is an important source of natural antioxidants. RSM using four different MAE parameters was proposed for the extraction of phenolics from dandelion and the model was optimized in this work. The investigated parameters are temperature and time of extraction, concentration of solvent, and solid-to-solvent ratio. The most important operational factor in the TPC of dandelion extract was determined as the extraction temperature. The high extraction temperature increases the extraction efficiency by causing the target components in the sample matrix taken from the active sites to be easily dissolved. However, the extraction efficiency decreases due to the decomposition of bioactive components at very high temperatures. Similarly, the optimum extraction temperature was determined as 76 °C in the study. On the other hand, the most insignificant parameter is ratio between solid and solvent. The Folin method is used in many studies to determine total phenolic content. In this study, we used Folin method to determine the total phenolic content of dandelion. The obtained TPC values indicate the antioxidant capacity of the dandelion extract. As a result, the proposed MAE model will be an alternative method for the fast, simple, highly efficient and low-cost extraction of antioxidants from dandelion in pharmaceutical and food industries.

Acknowledgment

The author thanks Istanbul University-Cerrahpaşa, Department of Analytical Chemistry for sharing their research infrastructures.

References

[1] Gupta, R. K., Patel, A. K., Shah, N., Choudhary, A. K., Jha, U. K., Yadav, U. C., ... & Pakuwal, U. 2014. Oxidative stress and antioxidants in disease and cancer: a review. Asian Pacific Journal of Cancer Prevention, 15(11), 4405-4409.

[2] Genestra, M. 2007. Oxyl radicals, redox-sensitive signalling cascades and antioxidants. Review. Cell Signal, 19, 1807-1819.

[3] Kondakçı, E., Özyürek, M., Güçlü, K., & Apak, R. 2013. Novel pro-oxidant activity assay for polyphenols, vitamins C and E using a modified CUPRAC method. Talanta, 115, 583-589.

[4] Ozougwu, J. C. 2016. The role of reactive oxygen species and antioxidants in oxidative stress. International Journal of Research, 1-8.

[5] Percival, M. 1998. Antioxidants. Clinical Nutrition Insights. 1-4.

[6] Donaldson, M. S. 2004. Nutrition and cancer: a review of the evidence for an anti-cancer diet. Nutrition journal, 3(1), 1-21.

[7] Hu, C., Kitts, D. D. 2005. Dandelion (Taraxacum officinale) flower extract suppresses both reactive oxygen species and nitric oxide and prevents lipid oxidation in vitro. Phytomedicine, 12(8), 588-597.

[8] Saratale, R. G., Benelli, G., Kumar, G., Kim, D. S., & Saratale, G. D. 2018. Bio-fabrication of silver nanoparticles using the leaf extract of an ancient herbal medicine, dandelion (Taraxacum officinale), evaluation of their antioxidant, anticancer potential, and antimicrobial activity against phytopathogens. Environmental Science and Pollution Research, 25(11), 10392-10406.

[9] Grauso, L., Emrick, S., de Falco, B., Lanzotti, V., & Bonanomi, G. 2019. Common dandelion: a review of its botanical, phytochemical and pharmacological profiles. Phytochemistry Reviews, 18(4), 1115-1132.

[10] Azwanida, N. N. 2015. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants, 4(196), 2167-0412.

[11] Vinatoru, M., Mason, T. J., & Calinescu, I. 2017. Ultrasonically assisted extraction (UAE) and microwave assisted extraction (MAE) of functional compounds from plant materials. TrAC Trends in Analytical Chemistry, 97, 159-178.

[12] Routray, W., & Orsat, V. 2012. Microwave-assisted extraction of flavonoids: a review. Food and Bioprocess Technology, 5(2), 409-424.

[13] Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escaleira, L. A. 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta, 76(5), 965-977.

[14] Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in enzymology, 299, 152-178.

[15] Barani, H., & Maleki, H. 2011. Plasma and ultrasonic process in dyeing of wool fibers with madder in presence of lecithin. Journal of dispersion science and technology, 32(8), 1191-1199.

[16] Eskilsson, C. S., Björklund, E., 2000. Analytical-scale microwave-assisted extraction. Journal of Chromatography A, 902(1),227-250.

[17] Stadler, A., Pichler, S., Horeis, G., & Kappe, C. O. (2002). Microwave-enhanced reactions under open and closed vessel conditions. A case study. Tetrahedron, 58(16), 3177-3183.

[18] Chen, Y., Xie, M. Y., & Gong, X. F. (2007). Microwave-assisted extraction used for the isolation of total triterpenoid saponins from Ganoderma atrum. Journal of Food Engineering, 81(1), 162-170.

[19] Spigno, G., Tramelli, L., & De Faveri, D. M. 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. Journal of food engineering, 81(1), 200-208.

[20] Bener, M., Shen, Y., Xu, Z., & Apak, R. 2016. Changes of the anthocyanins and antioxidant properties of concord Grape (Vitis labrusca) pomace after acid hydrolysis. Records of Natural Products, 10(6), 794.

[21] González-Montelongo, R., Lobo, M. G., & González, M. 2010. The effect of extraction temperature, time and number of steps on the antioxidant capacity of methanolic banana peel extracts. Separation and Purification Technology, 71(3), 347-355.

[22] Sulaiman, I. S. C., Basri, M., Masoumi, H. R. F., Chee, W. J., Ashari, S. E., & Ismail, M. 2017. Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of Clinacanthus nutans Lindau leaves by response surface methodology. Chemistry Central Journal, 11(1), 1-11.

[23] Wang, L., & Weller, C. L. 2006. Recent advances in extraction of nutraceuticals from plants. Trends in Food Science & Technology, 17(6), 300-312.

[24] Naczk, M., & Shahidi, F. 2006. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. Journal of pharmaceutical and biomedical analysis, 41(5), 1523-1542.

[25] Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. Journal of food and drug analysis, 22(3), 296-302.

[26] Wang, Y., You, J., Yu, Y., Qu, C., Zhang, H., Ding, L., ... & Li, X. 2008. Analysis of ginsenosides in Panax ginseng in high pressure microwave-assisted extraction. Food Chemistry, 110(1), 161-167.