Journal of Functional Food and Nutraceutical

EXPERIMENTAL DESIGN-ASSISTED OPTIMIZATION OF CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS QUANTITATION OF PHENOLIC COMPOUNDS IN DRIED FLOWERS EXTRACT

Mira Thorvaldsson[1]
Nurul Mutmainah[2]
Asadin Briliantama[2]
Sitti Rahmawati[2]
Ash Priyanto[2]
Pargiyanti[2]
Widiastuti Setyaningsih[2*]

[1]Department of Biology and Biological Engineering, Food and Nutrition Science, Chalmers University of Technology, Kemivägen 10, SE-412 96 Gothenburg, Sweden,
[2]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, Jalan Flora, Bulaksumur, Sleman, 55281, Yogyakarta, Indonesia,

ABSTRACT

This research aimed to develop and validate a reversed phase-high performance liquid chromatography method to determine phenolic compounds in dried flowers extract simultaneously. The research was divided into two parts: (1) optimization of the separation condition employing a Box Behnken design, and (2) validation test including assessment for the precision, accuracy, and method applicability of a High-Performance Liquid Chromatography (HPLC) coupled with Diode Array Detector (DAD). The studied factors for the optimization of the separation condition were flow rate (0.8−1.2 ml min^{-1}), percentage of the mobile phase at the beginning (0−20% phase B), and end (70−100% phase B) of the gradient program. It was statistically evinced that the chromatographic resolutions (*Rs*>1.0) indicated acceptable separation for protocatechuic acid, p-hydroxybenzoic acid, protocatechuic aldehyde, vanillic acid, *p*-coumaric acid, and ferulic acid. A fast separation method (8.00 min) was achieved by applying the optimum condition of a flow rate of 1 mL min^{-1}, mobile phase composition of 20% acidified methanol at the beginning, and 100% acidified methanol at the end of the gradient program. The validation was then performed for the developed method assuring high precision and accuracy. Additionally, the HPLC-DAD method was successfully applied to determine the phenolic compounds in three dried flower extracts revealing that the method was reliable for routine analyses.

Keywords: bnox-behnken design; edible flowers; method applicability; method validation

ABSTRAK

Penelitian ini bertujuan untuk mengembangkan dan validasi metode kromatografi cair kinerja tinggi untuk mengidentifikasi komponen fenolik pada ekstrak bunga kering secara bersamaan. Penelitian dibagi menjadi 2 bagian: (1) optimasi kondisi pemisahan menggunakan metode desain Box Behnken, dan (2) uji validasi yang meliputi pengujian presisi, akurasi, dan aplikabilitas metode pada Kromatografi Cair Kinerja Tinggi (HPLC) yang dilengkapi dengan detektor *Diode Array* (DAD). Faktor yang dianalisis untuk optimasi kondisi pemisahan adalah laju aliran (0.8-1.2 ml min^{-1}), persentase fase gerak pada awal (0-20% fase B), dan pada akhir (70-100% fase B) program gradien. Dibuktikan secara statistik bahwa resolusi kromatografi (*Rs*>1.0) mengindikasikan pemisahan yang baik untuk asam protokatecuat, asam *p*-hidroksi benzoat, aldehid protokatecuat, asam vanilat, asam *p*-kumarat, dan asam ferulat. Metode separasi yang cepat (8.00 min) didapatkan dengan mengaplikasikan kondisi optimum dari laju aliran yaitu 1 mL min^{-1}, komposisi fase gerak 20% metanol ter-asidifikasi pada tahap awal, dan 100% metanol ter-asidifikasi pada tahap akhir program gradient. Validasi dilakukan terhadap metode yang dikembangkan untuk memastikan tingginya presisi dan akurasi. Metode HPLC-DAD berhasil diaplikasikan untuk mengidentifikasi komponen fenolik dari tiga ekstrak bunga kering dan membuktikan bahwa metode tersebut dapat digunakan untuk analisis rutin.

Kata kunci: aplikabilitas metode; bunga yang daat dimakan; desain box-behnken; validasi metode.

Article Information

Article Type: Research Article
Journal Type: Open Access
Volume: 3 Issue 2

Manuscript ID
V3n2942-1

Received Date
16 December 2021

Accepted Date
22 February 2022

Published Date
01 March 2022

DOI: 10.33555/jffn.v3i2.88

Corresponding author:
Widiastuti Setyaningsih
Yogyakarta, Indonesia, 55281
Email:
widiastuti.setyaningsih@ugm.ac.id

Citation:
Thorvaldsson, M., Mutmainah, N., Briliantama, A., Rahmawati, S., Priyanto, A., Pargiyanti., Setyaningsih, W. 2022. Experimental design-assisted optimization of chromatographic method for the simultaneous quantitation of phenolic compounds in dried flowers extract. J. Functional Food & Nutraceutical, 3(2), pp.83-91

INTRODUCTION

Edible flowers are a class of harmless flowers that has gained attention in many sectors widely. It is preferred as traditional cuisine, traditional medicine, and primarily as ornaments. Edible flowers could enhance the food flavor, color, and aesthetic value. Consumption of flowers has started in ancient Rome and Greece, and the trend has recently increased [9]. In the recent market, edible flowers are used as salad, soup, and tea from dried ones [24]. Currently, the infusion has become the most common way to utilize edible flowers since it is a simple way to obtain the bioactive substance of the flower. Moreover, edible flowers can be transformed into sauces, jams, jelly, candy, wine, and food preservatives [14]. [13] reported that 97 families, 100 genera, and 180 species of edible flowers are used globally, and their demand is exceptionally high. Some are dianthus, osmanthus, chrysanthemum white, orange lily, calendula, and peach blossom.

The aforementioned edible flowers are well-known because they exhibit an excellent source of bioactive compounds, such as phenolic, flavonoid, anthocyanin, and alkaloid [6] [15]. The phenolic compounds found in the edible flowers include epicatechin, gallic acid, protocatechuic acid, and catechin. The total phenolic compounds were also relatively high [6][17]. The phenolic compounds of edible flowers are attractive to explore due to exhibit antioxidant power, whereas phenolic compounds positively impact several chronic diseases [5][24]. To obtain the advantage of edible flowers, therefore, identifying and quantifying phenolic compounds is necessary. The most frequently used method for determining bioactive compounds is liquid chromatography [5][11].

High-performance liquid chromatography (HPLC) is a separation method for chemical compounds and is the most commonly used method for the analysis of phenolic compounds [2][8][9]. The compound will be separated in the column depending on their polarity. When using the reverse phase column, the less polar compounds will retain in the column for a longer time than the more polar compounds, and hence, the more polar compounds have a shorter retention time. HPLC is a fast method to analyze compounds since it uses high pressure to force the solvents through the column [6].

Retention time is the amount of time a compound spends on the column after being injected. If a sample contains several compounds, each compound will spend a different amount of time on the column according to its chemical composition, i.e., each will have a different retention time. Retention times are usually quoted in units of seconds or minutes [7]. Retention time is used to measure the resolution, which measures how well separated two peaks are from each other. Resolution is defined as the difference in retention time between the peaks divided by the widths of the peaks [5]. Some factors such as gradient program and flow rate could affect the resolution and analysis time of the separation using HPLC [8].

Usually, the interaction among the separation factors may influence the resolution. The chemometric approach can be applied to assist the optimization of the chromatographic method by evaluating the factors concurrently. In evaluating the chromatographic parameters, factorial design is more worthwhile than the single-factor experimental [19]. Moreover, BBD is time efficient since it offers fewer runs for three factors over other factorial designs [7].

After developing an analytical method, validation testing must be performed by checking the linearity, detection and quantification limits, precision, and accuracy [3]. In this study, the ICH Guideline Q2 (R1) and suggestions in ISO 17025 outlined the assessment of the validation parameters. Henceforth, the objective of this study was to evaluate the robustness and other validation parameters of HPLC-DAD for the determination of phenolic compounds in some selected dried edible flowers (dianthus, chrysanthemum, and orange lily).

MATERIALS AND METHOD

Materials

HPLC-grade methanol and acetic acid were

purchased from Merck (Darmstadt, Germany). Standard compounds of the highest available purity were used. Protocatechuic acid (PRO), Protocatechuic aldehyde (PRA), p-hydroxybenzoic acid (*p*-OHB), vanillic acid (VAA), *p*-coumaric acid (*p*-COU), and ferulic acid (FER) were obtained from Sigma Aldrich (St. Louis, MO, USA). Water (aqua pro injection) was obtained from PT Ikapharmindo Putramas (Jakarta, Indonesia). Stock standard solutions of studied compounds were prepared in aqueous methanol 50:50 (v/v) at 1000 mg L^{-1}.

Dried edible flowers

The separation method was developed using a mixture of three commonly used dried flowers for tisane: carnation or dianthus (*Dianthus caryophyllus*), chrysanthemum white (*Dendranthema x grandiflorum*), and orange lily (*Lilium bulbiferum*). The dried edible flowers were purchased from Elif Tea and Tisane (Cirebon, Indonesia). While for the real sample application, the method was utilized to determine the phenolic compounds in each dried flower.

Sample preparation

The dried flowers were firstly ground for 4.5 min with resting for 30 s in every 30 s milling process. The phenolic compounds in 1 g of dried flower samples were extracted using the Ultrasound-Assisted Extraction (UAE) method applying the conditions of 80% ultrasound power at ambient temperature using methanol:water (1:1) as an extraction solvent with a sample-to-solvent ratio of 1:5. The extraction process was performed in three cycles, each for 10 min. The obtained supernatants were separated with a centrifuge of 4000 × g for 10 min. Subsequently, the resulting extracts were collected, and the organic solvent was removed under a vacuum using a rotary evaporator until the remaining extract was 5 mL. The concentrated extract was then filtered with a 45 µm nylon filter prior to the injection to the HPLC-DAD system.

Chromatographic method

The compounds separation was performed by an HPLC Shimadzu prominence with a C18 column Shim-Pac GIST Shimadzu (150 mm, 4.6 mm, 5 µm) supported by a binary pump (LC-20AD) and an auto-sampler (SIL-HTC, Shimadzu, Japan). The diode array detector (DAD SPD M-20A) was set for compound identification using a three-dimensional (3D) scan mode in the wavelength range from 190 to 350 nm. While for compounds quantification, a channel of 260, 280, and 320 nm was selected. This chromatographic system was managed using LabSolutions software. The mobile phases consisted of phase A containing 2% acetic acid, 5% methanol in water, and mobile phase B containing 2% acetic acid, 88% methanol in water. Both phases were filtered using a 45 µm nylon filter then were sonicated in an ultrasound bath for 20 min before use.

Experimental design

A Box Behnken Design (Figure 1) was used to evaluate the effect of three studied independent variables: x_1, flow rate (min); x_2, mobile phase composition at the gradient start (%B initial), and; x_3, mobile phase composition at the end of gradient elution (%B end). Each variable was normalized, resulting in three levels coded as -1, 0, and 1 (Table 1). The composition of the mobile phases in this study was set by earlier studies of phenolic compounds with HPLC [8]. Standard solutions of the six studied phenolic compounds were used to assess the effect of the operating HPLC condition on the separation result. All standard compounds were mixed, achieving a concentration of 200 mg L^{-1} for each compound.

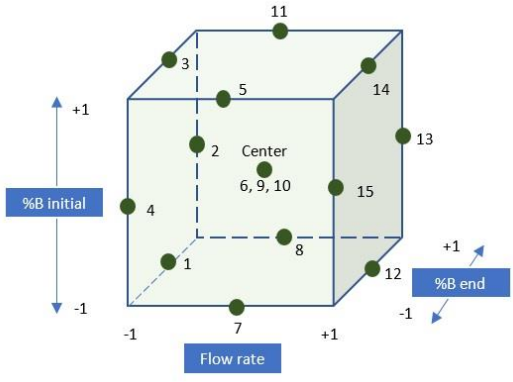

Figure 1. Fifteen run box-behnken design with 3 factors and 3 levels

Table 1. Selected variables and their levels

Factor	Coded			Unit
	-1	0	1	
Flow rate	0.8	1	1.2	mL min^{-1}
Phase $B_{initial}$	0	10	20	%
Phase B_{end}	70	85	100	%

The responses considered for the optimization were analysis time and the resolution (*Rs*) of chromatographic peaks. The analysis time was indicated by the retention time of the last eluted peak in the chromatogram. While the peak resolution (*Rs*) described the separation of two adjacent peaks in terms of their average baseline peak width and was measured using the following equation:

$$Rs = \frac{t2 - t1}{\frac{1}{2}(t2 - t1)} \quad (1)$$

where *Rs* is the peak resolution; t_1 and t_2 are the retention times of the first and second peaks, respectively; w_1 and w_2 are the corresponding widths at the bases of the pair of adjacent peaks.

Method validation

The validation criteria were based on ICH Guideline Q2 (R1) and suggestions in ISO 17025 for the linearity of the calibration curve, limit of detection, the limit of quantification, precision, and accuracy. Standard working solutions were prepared by dissolving the standard stock solution (1000 mg L−1) using aqueous methanol 50:50 (v/v), resulting in concentrations 1, 10, 30, 50, 75, and 100 mg L−1. Regression analysis was performed to measure the coefficient of determination (R^2). The limit of detection (LOD) and limit of quantification (LOQ) were then calculated using the regression result for each compound.

$$LOQ = 3.3\ x\ \frac{Standard\ error\ of\ the\ regression\ curve}{Slope\ of\ the\ regression\ curve} \quad (2)$$

$$LOD = 10\ x\ \frac{Standard\ error\ of\ the\ regression\ curve}{Slope\ of\ the\ regression\ curve} \quad (3)$$

The precision was done by comparing three HPLC-DAD runs intra-day analysis (repeatability, n = 9) and inter-day analysis (intermediate analysis, n = 3×3). Possible outliers were checked using the Q Dixon test. The precisions were indicated by the coefficient of variation (CV) values for each phenolic compound. The value of the CV should not exceed 15 % [1].

$$CV = \frac{Standard\ deviation}{Mean\ value}\ x\ 100\% \quad (4)$$

RESULTS AND DISCUSSION

Data acquisition for the responses

The peaks of phenolic compounds appeared in the order of polarity. As a reverse-phase column of C18 was used, the higher polarity, the faster the compound retained in the column. Therefore, protocatechuic acid (PRO) was first eluted, followed by *p*-OH benzoic acid (*p*-OHB), protocatechuic aldehyde (PRA), vanillic acid (VAA), *p*-coumaric acid (*p*-COU), and ferulic acid (FER). Hereafter, these order numbers indicate the corresponding compounds, as cited in the peak resolution. $R_{s1–2}$ means resolution between PRO and *p*-OHB, and so forth.

The collected extracts were analyzed using HPLC-DAD to identify the existing phenolic compounds in the sample. The compounds were identified based on the comparison of the retention times comparison of the peaks that appeared in the sample chromatogram to the peaks of the studied six standard compounds that were used as references.

Optimization of HPLC-DAD condition

Utilizing a Box-Behnken design (BBD) of three factors and three levels, 15 analyses were performed to assess the effect of HPLC-DAD factors on the separation of the studied phenolic compounds (Table 2). The resolution between each consecutive peak was higher than 1.0, confirming good separations applying different conditions suggested by the BBD. Henceforth, a flow rate change from 0.8 to 1.2 ml min^{-1} as well as altering the mobile phase composition slightly at the beginning (0 to 20% Phase B) and end (70 to 100%

Phase B) of the gradient program did not affect the separation performance of the method.

Table 2. Results obtained in 15 runs performed for peak resolutions analysis time

Run	HPLC factors			Resolutions*					Analysis time
	Flow rate	%B initial	%B end	Rs_{1-2}	Rs_{2-3}	Rs_{3-4}	Rs_{4-5}	Rs_{5-6}	
1	-1	-1	0	6.83	4.90	3.84	7.55	1.34	13.34
2	-1	0	1	6.30	5.23	3.49	7.46	1.28	11.55
3	-1	1	0	6.62	5.73	3.80	9.66	1.76	11.38
4	-1	0	-1	6.74	5.83	4.51	9.59	1.82	13.14
5	0	1	-1	6.35	5.40	3.87	10.20	2.12	10.22
6	0	0	0	6.19	5.41	4.28	9.04	1.76	10.83
7	0	-1	-1	6.93	5.32	4.64	8.85	1.74	12.32
8	0	-1	1	6.25	4.56	3.51	7.13	1.30	10.65
9	0	0	0	6.16	5.44	4.26	9.10	1.78	10.81
10	0	0	0	6.12	5.46	4.30	9.09	1.78	10.81
11	0	1	1	6.56	5.22	3.37	8.51	1.49	9.06
12	1	-1	0	6.36	4.67	4.42	8.37	1.71	10.54
13	1	0	1	5.59	4.93	3.78	8.06	1.54	8.89
14	1	1	0	5.88	5.00	3.52	9.40	1.93	8.57
15	1	0	-1	5.75	4.86	4.23	9.34	1.89	10.39

* Rs1-2, resolution between protocatechuic acid and *p*-OH benzoic acid; Rs_{2-3}, resolution between *p*-OH benzoic acid and protocatechuic aldehyde; Rs_{3-4}, resolution between protocatechuic aldehyde and vanillic acid; Rs_{4-5}, resolution between vanillic acid and *p*-coumaric acid; Rs*5-6*, resolution between *p*-coumaric acid and ferulic acid.

Subsequently, the effects of flow rate and mobile phase composition throughout the gradient program were calculated to optimize the HPLC-DAD factors with the target to minimize the analysis time. The statistical significance for each studied factor was measured (Figure 2). The corresponding bar crossing the vertical line showed the significant factors influencing the analysis time ($p<0.05$). All the main (x_1, x_2, and x_3) and their quadratic effects (x_1x_1, x_2x_2, and x_3x_3), in addition to the interaction of the mobile phase composition at the initial and end of the gradient program (x_2x_3), significantly influenced the analysis time.

The aforementioned significant effects were then used to construct a mathematical model to predict the optimum HPLC-DAD factors. The resulting model for the proposed chromatographic method was as follows:

$$Y = 10.82 - 1.38\,x_1 - 0.95\,x_2 - 0.74\,x_3 + 0.28\,x_1x_1 - 0.14x_2x_2 - 0.11\,x_3x_3 + 0.13x_2x_3$$

where y was the analysis time (min), *xi* were the studied factors (x_1, flow rate; x_2, mobile phase composition at the gradient start (%B initial), and; x_3, mobile phase composition at the end of gradient elution (%B end).

As suggested by the model, a fast separation (8.00 min) was achieved by applying a flow rate of 1 ml min^{-1} with 20% and 100% phase B for the mobile phase composition at the initial and end of the gradient program, respectively.

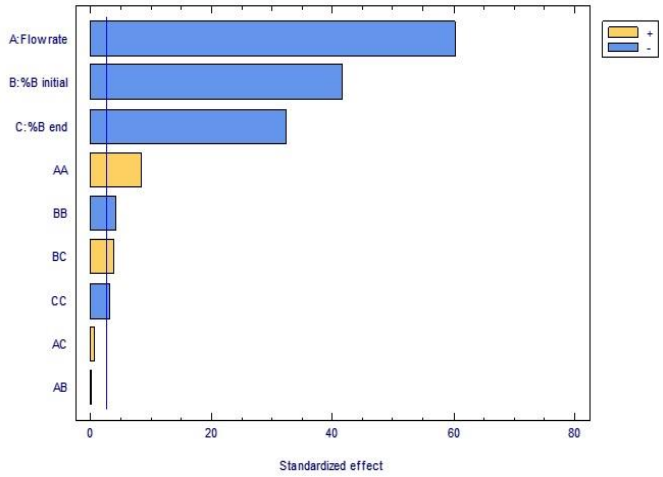

Figure 2 Standardized values of main, interaction, and quadratic effects of HPLC-DAD factors on the analysis time

Validation of optimization

The analytical HPLC-DAD method was validated following the ISO 17025 and ICH Guidelines (R1) (ICH, 2005; ISO, 2005). The linearity of calibration curves for protocatechuic acid, *p*-OH benzoic acid, protocatechuic aldehyde, vanillic acid, *p*-coumaric acid, and ferulic acid was validated with a high coefficient of determination (R^2, higher than 99.69%). The limit of detection (LOD) ranged from 5.07 (*p*-coumaric acid) to 7.60 (ferulic acid) mg L^{-1}, while the limit of quantification (LOQ) ranged from 15.37 (*p*-coumaric acid) to 23.04 (ferulic acid) mg L^{-1}.

Table 3. Concentration of phenolic compounds in dried edible flowers

	Phenolic compounds (µg g^{-1})				
Samples	PRO	PRA	VAA	*p*-COU	FER
Dianthus caryophyllus	169.50±1.64	ND	148.96±0.54	ND	ND
Dendranthema x grandiflorum	ND	4.19± 0.01	ND	ND	ND
Lilium bulbiferum	ND	ND	ND	296.29±2.28	267.96±1.88

Note. PRO, protocatechuic acid; PRA, protocatechuic aldehyde; VAA, vanillic acid; *p*-COU, *p*-coumaric acid; FER, ferulic acid. ND, not detected as the value was lower than the limit of detection.

A Q-Dixon test was performed to evaluate the data for the precisions and showed that there were no outliers in the data set. Two levels of precision of the developed HPLC-DAD method, namely repeatability and intermediate precision, were evaluated. The precision, indicated as CV, of the HPLC-DAD method was ranged from 0.23 (protocatechuic aldehyde) to 0.97% (protocatechuic acid) for repeatability (n=9) and 0.56 (*p*-hydroxybenzoic acid) to 1.50% (ferulic acid) for intermediate precision (n=3×3). Since both CV values for repeatability and intermediate precision were below 1.50%, the developed HPLC-DAD has been proved as a precise separation method.

Real sample application

The compounds were identified based on the comparison of the retention times of the peaks that appeared in the sample chromatogram to the peaks of six standards compounds that were used as references (protocatechuic acid, *p*-OH benzoic acid, protocatechuic aldehyde, vanillic acid, *p*-coumaric acid, and ferulic acid). Five phenolic compounds were detected in the dried flower samples, namely protocatechuic acid, protocatechuic aldehyde, vanillic acid, *p*-coumaric acid, and ferulic acid. These identified compounds were in common to those that have been reported to be presented in edible flowers namely dianthus, chrysanthemum white and orange lily. Chen at al. (2015) reported protocatechuic acid (719.27 µg g^{-1}) and vanillic acid (555.18 µg g^{-1}) were found in *Dianthus caryophyllus*, while in *Dendranthema x*

grandiflorum showed different level of protocatechuic acid content (31.32 µg g^{-1}) in addition to protocatechuic aldehyde. Other report by Sim et al. (2020) revealed the identification of *p*-coumaric acid and ferulic acid in *Lilium lancifolium* with a concentration of 1.14 and 1.46 mg g^{-1} respectively. On the other hand, *p*-OH benzoic acid was not detected in the studied samples as the concentration was lower than the detection limits of the validated HPLC-DAD method.

CONCLUSION

An HPLC-DAD was developed and validated to determine phenolic compounds in selected dried flowers. This method offers the advantage of using a short run time of 8 min to separate six studied phenolic compounds on the C18 column. Sufficient separation of the compounds was achieved (*Rs* > 1.0), applying a mobile phase composition of 20% and 100% at the beginning and end of the gradient program, respectively, at a flow rate of 1 ml min^{-1}. Results from validation of the method proved satisfactory linearity, accuracy, and precision; therefore, we conclude that the method is suitable for routine quantification of individual phenolic compounds in dried edible flowers.

ACKNOWLEDGEMENT

Setyaningsih W would like to thank the Faculty of Agricultural Technology, Universitas Gadjah Mada, Indonesia, for the financial support through the Innovative Research Grant No. 2720/UN1/FTP.1.3/SET-D/KU/2021.

REFERENCES

Akhtar, I., Javad, S., Ansari, M., Ghaffar, N., & Tariq, A. (2020). Process optimization for microwave assisted extraction of Foeniculum vulgare Mill using response surface methodology. Journal of King Saud University - Science, 32(2), 1451–1458. https://doi.org/10.1016/j.jksus.2019.11.041

Alara, O. R., Abdul Mudalip, S. K., & Olalere, O. A. (2017). Optimization of mangiferin extrated from Phaleria macrocarpa fruits using response surface methodology. Journal of Applied Research on Medicinal and Aromatic Plants, 5, 82–87. https://doi.org/10.1016/j.jarmap.2017.02.002

Alara, Oluwaseun Ruth, Abdurahman, N. H., & Olalere, O. A. (2018). Optimization of microwave-assisted extraction of flavonoids and antioxidants from Vernonia amygdalina leaf using response surface methodology. Food and Bioproducts Processing, 107, 36–48. https://doi.org/10.1016/j.fbp.2017.10.007

Alupului, A., Călinescu, I., & Lavric, V. (2012). Microwave Extraction of active principles from medicinal plants. UPB Scientific Bulletin, Series B: Chemistry and Materials Science, 74(2), 129–142.

Chen, G. L., Chen, S. G., Xie, Y. Q., Chen, F., Zhao, Y. Y., Luo, C. X., & Gao, Y. Q. (2015). Total phenolic, flavonoid and antioxidant activity of 23 edible flowers subjected to in vitro digestion. Journal of Functional Foods, 17, 243–259. https://doi.org/10.1016/j.jff.2015.05.028

Fernandes, L., Casal, S., Pereira, J. A., Saraiva, J. A., & Ramalhosa, E. (2017). Edible flowers: A review of the nutritional, antioxidant, antimicrobial properties and effects on human health. Journal of Food Composition and Analysis, 60(March), 38–50. https://doi.org/10.1016/j.jfca.2017.03.017

Ferreira, S. L. C., Bruns, R. E., Ferreira, H. S., Matos, G. D., David, J. M., Brandão, G. C., da Silva, E. G. P., Portugal, L. A., dos Reis, P. S., Souza, A. S., & dos Santos, W. N. L. (2007). Box-Behnken design: An alternative for the optimization of analytical methods. Analytica Chimica Acta, 597(2), 179–186. https://doi.org/10.1016/j.aca.2007.07.011

Fu, X. Q., Ma, N., Sun, W. P., & Dang, Y. Y. (2018). Microwave and enzyme co-assisted aqueous two-phase extraction of polyphenol and lutein from marigold (Tagetes erecta L.) flower. Industrial Crops and Products, 123(June), 296–302.

https://doi.org/10.1016/j.indcrop.2018.06.087

Guiné, R. P. F., & Florença, S. G. (2020). Edible Flowers, Old Tradition or New Gastronomic Trend: A First Look at Consumption in Portugal versus Costa Rica. Food Research International, 129(April 2019). https://doi.org/10.1016/j.foodres.2019.108868 \

Sim, W., Choi, S., Jung, T., Cho, B., Choi, S., Park, S., & Lee, O. (2020). Antioxidant and anti-inflammatory effects of Lilium lancifolium bulbs extract. https://doi:10.1111/jfbc.13176

Kataoka, H. (2019). Pharmaceutical analysis | sample preparation. In Encyclopedia of Analytical Science (3rd ed., Issue November 2017). Elsevier Inc. https://doi.org/10.1016/B978-0-12-409547-2.14358-6

Li, A. N., Li, S., Li, H. Bin, Xu, D. P., Xu, X. R., & Chen, F. (2014). Total phenolic contents and antioxidant capacities of 51 edible and wild flowers. Journal of Functional Foods, 6(1), 319–330. https://doi.org/10.1016/j.jff.2013.10.022

Llompart, M., Garcia-Jares, C., Celeiro, M., & Dagnac, T. (2019). Extraction | Microwave-Assisted Extraction. In Encyclopedia of Analytical Science (3rd ed., Issue June). Elsevier Inc. https://doi.org/10.1016/B978-0-12-409547-2.14442-7

Lu, B., Li, M., & Yin, R. (2016). Phytochemical Content, Health Benefits, and Toxicology of Common Edible Flowers: A Review (2000–2015). Critical Reviews in Food Science and Nutrition, 56, S130–S148. https://doi.org/10.1080/10408398.2015.1078276

Pinakin, D. J., Kumar, V., Suri, S., Sharma, R., & Kaushal, M. (2020). Nutraceutical potential of tree flowers: A comprehensive review on biochemical profile, health benefits, and utilization. Food Research International, 127(September 2019), 108724. https://doi.org/10.1016/j.foodres.2019.108724

Purohit, S. R., Rana, S. S., Idrishi, R., Sharma, V., & Ghosh, P. (2021). A review on nutritional, bioactive, toxicological properties and preservation of edible flowers. Future Foods, 4(July), 100078. https://doi.org/10.1016/j.fufo.2021.100078

Rombaut, N., Tixier, A.-S., Bily, A., & Chemat, F. (2012). Perspective: Jatropha cultivation in southern India: Assessing farmers' experiences. Biofuels, Bioproducts and Biorefining, 6(3), 246–256. https://doi.org/10.1002/bbb

Rop, O., Mlcek, J., Jurikova, T., Neugebauerova, J., & Vabkova, J. (2012). Edible flowers - A new promising source of mineral elements in human nutrition. Molecules, 17(6), 6672–6683. https://doi.org/10.3390/molecules17066672

Segura-Carretero, A., Puertas-Mejía, M. A., Cortacero-Ramírez, S., Beltrán, R., Alonso-Villaverde, C., Joven, J., Dinelli, G., & Fernández-Gutiérrez, A. (2008). Selective extraction, separation, and identification of anthocyanins from Hibiscus sabdariffa L. using solid phase extraction-capillary electrophoresis-mass spectrometry (time-of-flight/ion trap). Electrophoresis, 29(13), 2852–2861. https://doi.org/10.1002/elps.200700819

Setyaningsih, W., Palma, M., & Barroso, C. G. (2012). A new microwave-assisted extraction method for melatonin determination in dried flower grains. Journal of Cereal Science, 56(2), 340–346. https://doi.org/10.1016/j.jcs.2012.02.012

Setyaningsih, W., Saputro, I. E., Carrera, C. A., & Palma, M. (2019). Optimisation of an ultrasound-assisted extraction method for the simultaneous determination of phenolics in dried flower grains. Food Chemistry, 288, 221–227. https://doi.org/10.1016/j.foodchem.2019.02.107

Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. Trends in Food Science and Technology, 17(6), 300–312. https://doi.org/10.1016/j.tifs.2005.12.004

Xiang, J., Yang, C., Beta, T., Liu, S., & Yang, R. (2019). Phenolic Profile and Antioxidant Activity of the Edible Tree Peony Flower and Underlying Mechanisms of Preventive Effect on H2O2-Induced Oxidative Damage in Caco-2 Cells. Foods, 8. https://doi.org/doi:10.3390/foods8100471

Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. Chinese Medicine (United Kingdom), 13(1), 1–26. https://doi.org/10.1186/s13020-018-0177-x

Zheng, J., Meenu, M., & Xu, B. (2019). A systematic investigation on free phenolic acids and flavonoids profiles of commonly consumed edible flowers in China. Journal of Pharmaceutical and Biomedical Analysis. https://doi.org/10.1016/j.jpba.2019.05.007