engineering proceedings

Proceeding Paper

Comparative Study of Microwave-Assisted Extraction and Ultrasound-Assisted Extraction Techniques (MAE vs. UAE) for the Optimized Production of Enriched Extracts in Phenolic Compounds of *Camellia japonica* var Eugenia de Montijo †

Antia G. Pereira [1,2], Luis Cruz [3], Lucia Cassani [1,2], Franklin Chamorro [1], Catarina Lourenço-Lopes [1], Victor Freitas [3], Paz Otero [1], Maria Fraga-Corral [1,2], Miguel A. Prieto [1,2,*], Jesus Simal-Gandara [1] and Rosa Perez-Gregorio [1,3,*]

1 Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Faculty of Science, Universidade de Vigo, 32004 Ourense, Spain; antia.gonzalez.pereira@uvigo.es (A.G.P.); luciavictoria.cassani@uvigo.es (L.C.); franklin.noel.chamorro@uvigo.es (F.C.); c.lopes@uvigo.es (C.L.-L.); paz.otero@uvigo.es (P.O.); mfraga@uvigo.es (M.F.-C.); jsimal@uvigo.es (J.S.-G.)
2 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
3 LAQV-REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal; luis.cruz@fc.up.pt (L.C.); vfreitas@fc.up.pt (V.F.)
* Correspondence: mprieto@uvigo.es (M.A.P.); mariarosa.perez@uvigo.es (R.P.-G.)
† Presented at the 2nd International Electronic Conference on Processes: Process Engineering—Current State and Future Trends (ECP 2023), 17–31 May 2023; Available online: https://ecp2023.sciforum.net/.

Citation: Pereira, A.G.; Cruz, L.; Cassani, L.; Chamorro, F.; Lourenço-Lopes, C.; Freitas, V.; Otero, P.; Fraga-Corral, M.; Prieto, M.A.; Simal-Gandara, J.; et al. Comparative Study of Microwave-Assisted Extraction and Ultrasound-Assisted Extraction Techniques (MAE vs. UAE) for the Optimized Production of Enriched Extracts in Phenolic Compounds of *Camellia japonica* var Eugenia de Montijo. *Eng. Proc.* **2023**, *37*, 124. https://doi.org/10.3390/ECP2023-14615

Academic Editor: Antoni Sánchez

Published: 17 May 2023

Abstract: *Camellia japonica* is an underexplored medicinal plant with associated bioactivities. Innovative approaches are proposed in regard to the large-scale application of *C. japonica*, being one of the main routes for the extraction of phenolic compounds. The optimum conditions for the extraction of phenolic compounds from the flowers of *C. japonica* var. Eugenia de Montijo were determined using the response surface methodology (RSM). A five-level experimental design was carried out and analyzed via RSM using, as variables, temperature (T), time (t) and solvent (S), in the case of microwave-assisted extraction (MAE), and power (P), t and S in the case of ultrasound-assisted extraction (UAE). The compounds were identified using HPLC–MS–MS. Two responses were studied: the extraction yield and concentration of phenolic compounds. The results showed that the maximum yields (80%) were obtained at high temperatures and low times (180 °C, 5 min) when using MAE. Lower yields (56%) were obtained using UAE (optimal conditions 62% amplitude, 8 min, 39% acidified ethanol). The main family of phenolic compounds were flavonols. Moreover, the present study contributes to the valorization of underused flower species commonly present in the North-West region of Spain, by obtaining extracts rich in phenolic compounds that can be potentially applied as ingredients in different industrial fields.

Keywords: *Camellia japonica*; flowers; phenolic profile; optimization; green technologies; response surface methodology

1. Introduction

Camellia spp. is a member of the Theaceae family, a group of perennial evergreen flowering plants that are widely commerzialized, which underlines their economic relevance [1]. This genus includes more than 250 plants with an intricate taxonomy, with *Camellia sinensis* L., *Camellia oleifera* Abel. and *Camellia japonica* L. being the most outstanding species. All these species have been traditionally used for multiple applications, including tea, the production of essential oils and ornamental purposes [2]. *C. japonica* and its hybrids are recognized as ornamentals since they have flowers of several colors and forms, long and varied blossoming seasons and different growth habitats [3]. According to many studies, *C. japonica* flowers possess several bioactive molecules that confer them several properties, such as anti-oxidant, antimicrobial, anti-inflammatory, and anti-cancer effects, among others [1,4–6]. These bioactivities are attributable to the occurrence of phenolic compounds (e.g., anthocyanins, flavan-3-ols, flavonols), polysaccharides, polyunsaturated fatty acids and pigments [1,7]. Despite these health-promoting activities, *C. japonica* flowers are still considered an underexploited resource at an industrial level [8]. However, in recent decades, several studies have disclosed the antioxidant activity, total phenolic contents, and specific phenolic acids of *C. japonica* flowers [9–11].

In recent decades, phenolic compounds are some of the compounds that have attracted the most attention due to the numerous beneficial properties attributed to them [12]. The extraction of phenolic compounds from plant matrices is complex and challenging since it includes a huge variety of molecules comprising very variable chemical properties [13]. So far, their extraction has been mainly performed using conventional methods; however, they require the use of large volumes of extraction solvents, extensive hands-on time and cannot be extracted using automated methods, so they are generally considered labour-intensive techniques [14]. These experimental drawbacks, along with the negative environmental impact they have, have led to the development of new extraction methods that are considered green technologies [15]. Among them, microwave-assisted extraction and ultrasound-assisted extraction techniques stand out for several reasons.

Microwave-assisted extraction (MAE) improves the extraction efficiency in comparison to conventional techniques due to the interaction among microwaves and the polar molecules in the extraction media, with an increase in the internal pressure of the solid material [13]. Moreover, in contrast to the conventional techniques, MAE reduces the thermal gradients and instant heating of the biomass, enhances the extraction yield, shortens extraction times and decreases solvent quantities [16]. MAE has been previously and succesfully applied to hassk or fruits from *Camellia* spp. [17–19].

Ultrasound-assisted extraction (UAE) improves the extraction efficiency in comparison to conventional techniques due to the implementation of high-frequency ultrasonic waves. These waves have the capacity to disrupt the plant cell walls, which facilitates the penetration of the solvent into the cells and therefore the extraction of molecules [20]. Several works have been recently published on the use of UAE applied to the extraction of phenolic compounds from *Camellia* spp. leaves [21,22].

To date, to the best of our knowledge, there is no study on the extraction of phenolic compounds from *C. japonica* flowers by using MAE or UAE. Hence, the aim of this study was to determine the extraction conditions required to best obtain extracts rich in phenolic compounds from *C. japonica* flowers by using these extraction methods.

2. Material and Methods

2.1. Chemicals and Reagents

Phenolic compound standards (cyanidin-3-glucoside, luteolin, quercetin, gallic acid, *p*-coumaric acid and resveratrol) were bought from Sigma (Saint Louis, MO, USA). Ethanol was bought from VWR (Radnor, PA, USA). All organic solvents used for the extraction and chromatographic analysis were HPLC-grade. High-purity water was obtained using Direct-Q 5UV Millipore equipment (Merck, Rahway, NJ, USA). Nylon syringe filters (0.22 µm pore size, 25 mm diameter) were acquired from Filter-Lab (Barcelona, Spain).

2.2. Sample Collection

C. japonica flowers (var. Eugenia de Montijo) were collected in Galicia (NW Spain) in the winter season of 2020. Samples were lyophilized (LyoAlfa10/15, Telstar, Thermo Fisher Scientific, Waltham, MA, USA), pulverized into a fine powder by a blender, and stored at −20 °C until extraction.

2.3. Sample Extraction Method

The process used to obtain the bioactive compounds was carried out via MAE and UAE. For MAE, the extraction was performed using the Multiwave 3000 (Anton Paar, Graz, Austria). The variables studied were temperature (T), time (t) and solvent (S), which were regarded as critical extraction parameters. Specifically, T varied between 50 and 180 °C, the t range was 5–25 min and the concentration of acidified ethanol was switched from 0 to 100% v/v. For UAE, the extraction was performed using the CY-500 (Optic Ivymen Systems). The variables studied were processing time (t, 5–45 min), power (P, 30–80%) and solvent (0–100% acidified ethanol), which were regarded as critical extraction parameters. Once the extractions were completed, the samples were centrifuged at 9000 rpm for 15 min and the supernatant was filtered. Extracts were stored in a freezer at −80 °C until their analysis.

The optimum conditions for the extraction of phenolic compounds from the flowers of *C. japonica* var. Eugenia de Montijo were determined using the response surface methodology (RSM) and using circumscribed central composite design (CCCD). As previously reported, this model allows one to identify the operating conditions that maximize the following responses: yield and phenolic compounds [23].

In addition, the extraction performance was calculated as follows (Equation (1)):

$$Y_1\ (\%) = (P_2 - P_1)/P_0 \times 100 \quad (1)$$

where P_0 is the mass of lyophilized flower prior to extraction (mg), P_1 is the mass of the empty crucible (mg), and P_2 is the mass of the dry extract in the crucible (mg).

2.4. Determination of Bioactive Compounds

The identification and quantification of phenolic compounds were carried out via HPLC–MS–MS (Thermo Scientific TSQ Quantis). Analytical separations were performed using a ThermoFisher C18 column (150 × 3.9 mm, 4 µm particle). The column was thermostated at 35 °C. The mobile phases used for the optimized analytical method were as follows: (A) milli-Q water acidified with 0.1% of formic acid; (B) acetonitrile acidified with 0.1% of formic acid. The flow rate was fixed at 0.350 mL/min. Briefly, 350 µL was pumped to the injection module in a C18 pre-concentration cartridge and further eluted by a loading pump following gradient conditions from 100% to 0% A. Tandem mass analysis was performed after optimizing the tube lens and the collision energy for each compound separately. Likewise, the ESI conditions were automatically adjusted to the set flow. Data acquisition and HPLC–MS–MS analysis interpretation were conducted by means of Xcalibur software (Version 4.3, Thermo Fisher Scientific, Waltham, MA, USA).

3. Results and Conclusions

The process was optimized by the RSM using a five-level central composite design, combining different independent variables. The RSM was performed in order to optimize the different responses associated with polyphenol production: the extraction yield and concentration of phenolic compounds. Theoretical models were fitted to experimental data, statistically validated, and used in the prediction and optimization steps.

Regarding the extraction efficiency of the two evaluated techniques, MAE provided better outcomes than UAE. For MAE, the results showed that the maximum yields (80%) were obtained using a combination of a high temperature and short time (180 °C, 5 min). Other approaches using the same temperature (180 °C) but longer incubation times (25 min) dropped the efficiency to 59%. Similarly, when using a combination of lower temperatures (50 °C) and short times (5 min), the extraction results were 52%. When applying longer times (25 min), recovery was around 50%. For UAE, the yields achieved under the optimal conditions (62% amplitude, 8 min, 39% acidified ethanol) showed a lower extractive efficiency, with values around 56%. Temperature does not have a significant impact on the results in UAE extraction. On the other hand, higher amplitudes provided yield efficiencies of up to 58%.

With regards to the phenolic composition of the extracts, the main family of phenolic compounds identified were flavonols, the major compounds of this family being quercetin-3-*o*-arabinose and kaempferol 3-*o*-acetyl-glucoside. These molecules possess a few associated biological activities, such as anti-oxidant, anti-microbial, anti-inflammatory and anti-viral effects [24–26].

Therefore, the present study underlines that MAE is a sustainable and effective technique that can be used to recover phenolic compounds from *C. japonica* flowers. The application of the optimized conditions would contribute to the valorization of underused flower species, common in the North-West region of Spain, via the obtainment of extracts rich in phenolic compounds that could potentially be applied as ingredients in different industrial fields.

Author Contributions: Conceptualization, A.G.P., L.C. (Luis Cruz), L.C. (Lucia Cassani), F.C., C.L.-L., V.F., P.O., M.F.-C., J.S.-G., M.A.P. and R.P.-G.; methodology, A.G.P., L.C. (Luis Cruz), L.C. (Lucia Cassani), F.C., C.L.-L., V.F., P.O., M.F.-C. and R.P.-G.; software, A.G.P. and L.C. (Lucia Cassani); validation, L.C. (Lucia Cassani), J.S.-G. and M.A.P.; formal analysis, L.C. (Luis Cruz), L.C. (Lucia Cassani) and M.F.-C.; investigation, A.G.P., L.C. (Luis Cruz) and R.P.-G.; writing—original draft preparation, A.G.P. and M.F.-C.; writing—review and editing, A.G.P. and M.F.-C.; visualization, L.C. (Lucia Cassani), A.G.P. and M.F.-C.; supervision, L.C. (Lucia Cassani), R.P.-G., J.S.-G. and M.A.P. All authors have read and agreed to the published version of the manuscript.

Funding: The research leading to these results was supported by MICINN supporting the Ramón y Cajal grant for M.A. Prieto (RYC-2017-22891). R. Pérez-Gregorio would like to thank the Ramón y Cajal contract RYC2021-033224-I, funded by the Ministry of Science and Innovation and the National Agency for Research (0000421S140.06) from the Spain Government (MCIN/AEI/10.13039/501100011033) and the European Union under the frame of "NextGenerationEU/PRTR" funds; Xunta de Galicia for supporting the program EXCELENCIA-ED431F 2020/12, and the post-doctoral grants of M. Fraga-Corral (ED481B-2019/096) and L. Cassani (ED481B-2021/152). The authors thank the program BENEFICIOS DO CONSUMO DAS ESPECIES TINTORERA-(CO-0019-2021), which supports the work of F. Chamorro. The authors are grateful to the Ibero-American Program of Science and Technology (CYTED—AQUA-CIBUS, P317RT0003), and to the Bio-based Industries Joint Undertaking (JU) under grant agreement No 888003 UP4HEALTH Project (H2020-BBI-JTI-2019), which supports the work of P. Otero and C. Lourenço-Lopes. The JU receives support from the European Union's Horizon 2020 research and innovation program and the Bio-based Industries Consortium. The project SYSTEMIC Knowledge Hub on Nutrition and Food Security received funding from national research funding parties in Belgium (FWO), France (INRA), Germany (BLE), Italy (MIPAAF), Latvia (IZM), Norway (RCN), Portugal (FCT), and Spain (AEI) in a joint action of JPI HDHL, JPI-OCEANS and FACCE-JPI, launched in 2019 under the ERA-NET ERA-HDHL (n 696295).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author. The data are not publicly available due to privacy.

Acknowledgments: The authors are grateful to the Ibero-American Program of Science and Technology (CYTED—AQUA-CIBUS, P317RT0003) and to the Bio-based Industries Joint Undertaking (JU) under grant agreement No 888003 UP4HEALTH Project (H2020-BBI-JTI-2019), which supports the work of P. Otero and C. Lourenço-Lopes. The JU receives support from the European Union's Horizon 2020 research and innovation program and the Bio-based Industries Consortium. The project SYSTEMIC Knowledge Hub on Nutrition and Food Security received funding from national research funding parties in Belgium (FWO), France (INRA), Germany (BLE), Italy (MIPAAF), Latvia (IZM), Norway (RCN), Portugal (FCT), and Spain (AEI) in a joint action of JPI HDHL, JPI-OCEANS and FACCE-JPI, launched in 2019 under the ERA-NET ERA-HDHL (n° 696295). L.C. (Luis Cruz) acknowledges the FCT research contract DL57/2016/CP1334/CT0008.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sharma, T.S.K.; Selvakumar, K.; Hwa, K.Y.; Sami, P.; Kumaresan, M. Biogenic fabrication of gold nanoparticles using *Camellia japonica* L. leaf extract and its biological evaluation. *J. Mater. Res. Technol.* **2019**, *8*, 1412–1418. [CrossRef]
2. Garcia-Jares, C.; Sanchez-Nande, M.; Lamas, J.P.; Lores, M. Profiling the fatty acids content of ornamental camellia seeds cultivated in Galicia by an optimized matrix solid-phase dispersion extraction. *Bioengineering* **2017**, *4*, 87. [CrossRef] [PubMed]
3. Mondal, T.K. Camellia. In *Wild Crop Relatives: Genomic and Breeding Resources*; Springer: Berlin/Heidelberg, Germany, 2011; pp. 15–39.
4. Pereira, A.G.; Garcia-Perez, P.; Cassani, L.; Chamorro, F.; Cao, H.; Barba, F.J.; Simal-Gandara, J.; Prieto, M.A. *Camellia japonica*: A phytochemical perspective and current applications facing its industrial exploitation. *Food Chem. X* **2022**, *13*, 100258. [CrossRef]
5. Cho, J.; Ryu, H.; Ji, S.; Moon, J.; Jung, K.; Park, K. Phenolic Compounds from the Flower Buds of *Camellia japonica*. *Food Sci. Biotechnol.* **2009**, *18*, 766–770.
6. Way, T.D.; Lin, H.Y.; Hua, K.T.; Lee, J.C.; Li, W.H.; Lee, M.R.; Shuang, C.H.; Lin, J.K. Beneficial effects of different tea flowers against human breast cancer MCF-7 cells. *Food Chem.* **2009**, *114*, 1231–1236. [CrossRef]
7. Haro Bailón, A.d.; Obregón, S.; del Río Celestino, M.; Mansilla, P.; Salinero, M.C. Variability in seed storage components (protein, oil and fatty acids) in a Camellia germplasm collection. In Proceedings of the International Camellia Congress, Pontevedra, Spain, 11–16 March 2014; pp. 302–309.
8. Teixeira, A.M.; Sousa, C. A review on the biological activity of camellia species. *Molecules* **2021**, *26*, 2178. [CrossRef] [PubMed]
9. Kanth, B.K.; Lee, K.Y.; Lee, G.J. Antioxidant and radical-scavenging activities of petal extracts of *Camellia japonica* ecotypes. *Hortic. Environ. Biotechnol.* **2014**, *55*, 335–341. [CrossRef]
10. Lee, S.Y.; Hwang, E.J.; Kim, G.H.; Choi, Y.B.; Lim, C.Y.; Kim, S.M. Antifungal and Antioxidant Activities of Extracts from Leaves and Flowers of *Camellia japonica* L. *Korean J. Med. Crop Sci.* **2005**, *13*, 93–100.
11. Lee, H.H.; Cho, J.Y.; Moon, J.H.; Park, K.H. Isolation and identification of antioxidative phenolic acids and flavonoid glycosides from *Camellia japonica* flowers. *Hortic. Environ. Biotechnol.* **2011**, *52*, 270–277. [CrossRef]
12. Tungmunnithum, D.; Thongboonyou, A.; Pholboon, A.; Yangsabai, A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines* **2018**, *5*, 93. [CrossRef]
13. Ince, A.E.; Sahin, S.; Sumnu, G. Comparison of microwave and ultrasound-assisted extraction techniques for leaching of phenolic compounds from nettle. *J. Food Sci. Technol.* **2014**, *51*, 2776–2782. [CrossRef] [PubMed]
14. Alara, O.R.; Abdurahman, N.H.; Ukaegbu, C.I. Extraction of phenolic compounds: A review. *Curr. Res. Food Sci.* **2021**, *4*, 200–214. [CrossRef] [PubMed]
15. Pereira, A.G.; Jimenez-Lopez, C.; Fraga, M.; Lourenço-Lopes, C.; García-Oliveira, P.; Lorenzo, J.M.; Perez-Lamela, C.; Prieto, M.A.; Simal-Gandara, J. Extraction, Properties, and Applications of Bioactive Compounds Obtained from Microalgae. *Curr. Pharm. Des.* **2020**, *26*, 1929–1950. [CrossRef] [PubMed]
16. Georgiopoulou, I.; Tzima, S.; Louli, V.; Magoulas, K. Process Optimization of Microwave-Assisted Extraction of Chlorophyll, Carotenoid and Phenolic Compounds from *Chlorella vulgaris* and Comparison with Conventional and Supercritical Fluid Extraction. *Appl. Sci.* **2023**, *13*, 2740. [CrossRef]
17. Lu, J.; Zhou, C.; Rong, O.; Xu, Y.; Zhou, B.; Li, Z. Optimization of microwave-assisted extraction of flavonoids from cryptotaenia japonica hassk using response surface methodology. *Adv. J. Food Sci. Technol.* **2013**, *5*, 310–317. [CrossRef]
18. Zhang, L.; Wang, Y.; Wu, D.; Xu, M.; Chen, J. Microwave-assisted extraction of polyphenols from *Camellia oleifera* fruit hull. *Molecules* **2011**, *16*, 4428–4437. [CrossRef] [PubMed]
19. Kim, J.K.; Lim, H.J.; Kim, M.S.; Choi, S.J.; Kim, M.J.; Kim, C.R.; Shin, D.H.; Shin, E.C. Responsive surface methodology optimizes extraction conditions of industrial by-products, *Camellia japonica* seed cake. *Pharmacogn. Mag.* **2016**, *12*, 184–187. [PubMed]
20. Aourach, M.; González-De-peredo, A.V.; Vázquez-Espinosa, M.; Essalmani, H.; Palma, M.; Barbero, G.F. Optimization and comparison of ultrasound and microwave-assisted extraction of phenolic compounds from cotton-lavender (*Santolina chamaecyparissus* L.). *Agronomy* **2021**, *11*, 84. [CrossRef]
21. Liu, Y.; Luo, X.; Lan, Z.; Tang, J.; Zhao, P.; Kan, H. Ultrasonic-assisted extraction and antioxidant capacities of flavonoids from *Camellia fascicularis* leaves. *CYTA-J. Food* **2018**, *16*, 105–112. [CrossRef]
22. Horžić, D.; Jambrak, A.R.; Belščak-Cvitanović, A.; Komes, D.; Lelas, V. Comparison of Conventional and Ultrasound Assisted Extraction Techniques of Yellow Tea and Bioactive Composition of Obtained Extracts. *Food Bioprocess Technol.* **2012**, *5*, 2858–2870. [CrossRef]
23. Taofiq, O.; Silva, A.R.; Costa, C.; Ferreira, I.; Nunes, J.; Prieto, M.A.; Simal-Gandara, J.; Barros, L.; Ferreira, I.C.F.R. Optimization of ergosterol extraction from Pleurotus mushrooms using response surface methodology. *Food Funct.* **2020**, *11*, 5887–5897. [CrossRef]
24. Materska, M. Quercetin and its Derivatives: Chemical Structure and Bioactivity—A Review. *Pol. J. Food Nutr. Sci.* **2008**, *58*, 407–413.

25. Periferakis, A.; Periferakis, K.; Badarau, I.A.; Petran, E.M.; Popa, D.C.; Caruntu, A.; Costache, R.S.; Scheau, C.; Caruntu, C.; Costache, D.O. Kaempferol: Antimicrobial Properties, Sources, Clinical, and Traditional Applications. *Int. J. Mol. Sci.* **2022**, *23*, 15054. [CrossRef]
26. Michala, A.-S.; Pritsa, A. Quercetin: A Molecule of Great Biochemical and Clinical Value and Its Beneficial Effect on Diabetes and Cancer. *Diseases* **2022**, *10*, 37. [CrossRef]