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

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Abstract: *Camellia japonica* is an underexplored medicinal plant with associated bioactivities [1]. Innovative approaches are proposed in regard to the large-scale application of *C. japonica*, being one of the main lines the extraction of phenolic compounds [2]. The optimum conditions for the extraction of phenolic compounds form the flowers of *C. japonica* var. Eugenia de Montijo were determined using response surface methodology (RSM). A 5-level experimental design was carried out and analyzed by 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 by HPLC-MS-MS. Two responses were studied: 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) in MAE. Lower yields (56%) were obtained with 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 the obtainment of 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 Theaceae family a group of perennial evergreen flowering plants widely commerzialized which underline their economic relevance [3]. This genus includes more than 250 plants with an intricate taxonomy, being *Camellia sinensis* L., *Camellia oleifera* Abel. and *Camellia japonica* L. the most outstanding species. All these species have been traditionally used for multiple applications, including tea, essential oils production and ornamental purposes [4]. *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 [5]. According to many studies, *C. japonica* flowers possess several bioactive molecules which confer them several properties such as antioxidant, antimicrobial, anti-inflammatory, or anticancer, among others [1,6]. These bioactivities are attributable to the occurrence of phenolic compounds, anthocyanins, polysaccharides, polyphenols, 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 the last 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 is one of the families of compounds that has attracted the most attention due to the numerous beneficial properties attributed to them [12]. The extraction of phenolic compounds from any plant matrices is complex and challenging since it includes a huge variability 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, long hands-on time and does not allow automatation so they are eventually considered labour-intensive techniques [14]. These experimental drawbacks along with the negative environmental impact they have has led to the development of new extraction methods considered green technologies [15]. Among them, microwave assisted extraction and ultrasounds assisted extraction techniques stand out for several reasons.

Microwave assisted extraction (MAE) improves the extraction efficiency in comparation 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 thermal gradients and instant heating of the biomass, as well as it enhances 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].

Ultrasounds assisted extraction (UAE) improves the extraction efficiency in comparation to conventional techniques due to the implementation of high frequency ultrasonic waves. They have the capacity to disrupt the plant cell walls which facilitates the penetration of the solvent into the cells and so the molecules extraction [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].

Up to date, to the best of our knowledge there is no study on extraction of phenolic compounds from *C. japonica* flowers by using MAE or UAE. Hence, the aim of this study was to determine the best extraction conditions to 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 compounds standards (cyanidin, 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 from a Direct-Q 5UV Millipore equipment (Merck, Rahway, NJ, USA). Nylon syringe filters (0.22 μm pore size, 25 mm diameter) were 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 for obtaining bioactive compounds was carried out by MAE and UAE. For MAE, the extraction was made using Multiwave 3000 (Anton-Paar, Graz, Austria). The variables studied were temperature (T), time (t) and solvent (S), as critical extraction parameters. Specifically, T varied 50–180 °C, 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 made 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), 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 form the flowers of *C. japonica* var. Eugenia de Montijo were determined using response surface methodology (RSM) using circumscribed central composite design (CCCD). As previously reports, this model allows to identify the operating conditions that maximize the responses: yield and phenolic compounds [23].

Besides, 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), 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 by 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. Mobile phases used for the optimized analytical method were 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 car-tridge 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 collision energy for each compound separately. Likewise, ESI conditions were automatically adjusted to the set flow. Data acquisition and HPLC-MS/MS analysis interpretation were conducted by means of Xcalibur software.

3. Results and Conclusions

The process was optimized by RSM using a five-level central composite design, combining different independent variables. Exactly, RSM was accomplished to optimize different responses associated with polyphenol production: 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, results showed that the maximum yields (80%) were obtained using a combination of 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), extraction results were 52%, while applying longer times (25 min) recovery was around 50%. For UAE, 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 in UAE extraction. On the other hand, higher amplitudes provided yield efficiencies up to 58%.

In regards of the phenolic composition of the extracts, the main family of phenolic compounds identified were flavonols, being the major compounds of this family quercetin-3-*o*-arabinose and kaempferol 3-*o*-acetyl-glucoside. These molecules have associated few biological activities such as antioxidant, antimicrobial, anti-inflammatory or antiviral [24–26].

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

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References

1. 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.
2. 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.
3. 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.
4. 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.
5. Mondal, T.K. Camellia. In *Wild Crop Relatives: Genomic and Breeding Resources*; Springer: Berlin/Heidelberg, Germany, 2011; pp. 15–39.
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.
7. Haro Bailón, A.d.; Obregón, S.; Río Celestino, M. del; Mansilla, P.; Salinero, M.C. Variability in seed storage components (protein, oil and fatty acids) in a Camellia germplasm collection. In Proceedings of International Camellia Congress, Pontevedra, Spain, 11–15 March 2014; pp. 302–309.
8. Teixeira, A.M.; Sousa, C. A review on the biological activity of camellia species. *Molecules* **2021**, *26*, 2178.
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.
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.
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.
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.
14. Alara, O.R.; Abdurahman, N.H.; Ukaegbu, C.I. Extraction of phenolic compounds: A review. *Curr. Res. Food Sci.* **2021**, *4*, 200–214.
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.
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.
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.
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.
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.
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.
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.
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.
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.
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.
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.