



Original scientific paper

DOI: 10.17508/CJFST.2022.14.1.02

High pressure and ultrasound-assisted extraction of bioactive compounds from *Santolina chamaecypatissus* L.

Zorana Mutavski^{1*}, Jelena Vladić¹, Senka Vidović¹, Nataša Nastić¹, Milica Aćimović²¹Faculty of Technology Novi Sad, Novi Sad, Serbia²Institute of Field and Vegetable Crops, Novi Sad, Serbia

ARTICLE INFO

Article history:

Received: July 6, 2021

Accepted: October 13, 2021

Keywords:

Santolina chamaecypatissus
supercritical extraction
ultrasound-assisted extraction
antioxidant activity

ABSTRACT

For maximal utilization of bioactive components from *Santolina*, a combination of extraction techniques, supercritical extraction with carbon dioxide (SFE-CO₂), followed by ultrasound-assisted extraction (UAE), was used. SFE-CO₂ was done at a pressure of 300 bar and a temperature of 40 °C. This extraction targeted to extract non-polar components from investigated plant material. The raw material remaining after extraction was subjected to the UAE, using ethanol solutions as an extraction solvent in concentrations of 30, 50, and 70% (v/v), at temperatures of 30, 50, and 70 °C, for a period of extraction of 10, 20 and 40 min. The yield, achieved by the application of SFE-CO₂, was 3.98% (w/w). The highest UAE yield (28.48%) was obtained using 50% ethanol as an extraction solvent, at the temperature of 50 °C during a period of extraction of 40 min. The lowest yield (22.15%) was obtained when 30% ethanol was applied at 50 °C for 20 min of extraction. The highest measured concentration of total phenols was 2.45 mg GAE/mL of extract, achieved at the following extraction conditions: extraction time 20 min, temperature of 50 °C, extraction solvent 70%.

Introduction

Santolina chamaecypatissus L., commonly known as cotton lavender, belonging to the family *Asteraceae*, is an aromatic dwarf fragrant dense mound with attractive grayish silver foliage, native to the Mediterranean area (Chirane et al., 2019). It is widely used in Mediterranean folk medicine for its analgesic, anti-inflammatory, antiseptic, antispasmodic, bactericidal, fungicidal, digestive, and vulnerable properties, and is also used in phytotherapy for different kinds of dermatitis (Chirane et al., 2019). Several components detected in the Tunisian *S. chamaecyparissus* L. flowerhead essential oil have been reported as efficient antibacterial or antifungal agents. These are 1,8-cineole, α -terpineol, terpinen-4-ol, α -pinene, β -pinene, α -phellandrene, and p-cymene (Tirillini et al., 1996; Pattnaik et al., 1997; Dorman and Deans, 2000; Tzakou et al., 2001; Cimanga et al., 2002). Artemisia ketone (38.1%), camphor (11.7%),

β -phellandrene (9.2%), α -bisabolol (6.6%), and myrcene (4.3%) are considered to be the main compounds found in the *S. chamaecypatissus* L. (Demirci et al., 2000).

Generally, the health beneficial properties of the medical plants can be used directly through the application of raw plant material (usually in the form of herbal tea or powdered material filled into the capsules), or through the isolation of their bioactive compounds and the production of more concentrated product extracts, in liquid or powder form. There are several techniques, conventional and modern, which can be applied for the preparation of herbal extracts. However, today more attention has been paid to the green extraction techniques. Green extraction of natural products is a new concept that meets the challenges of the 21st century which are to protect both the environment and consumers, and to enhance the competition of the industries to be more ecological,

*Corresponding author E-mail: mutavski.4.19.d@uns.ac.rs

economic, and innovative (Chemat et al., 2012; Rombaut et al., 2014). Some of the most applied green extractions technologies nowadays are supercritical fluid extraction by carbon dioxide (SFE-CO₂) and ultrasound-assisted extraction (UAE).

SFE-CO₂ represents a valid alternative to the conventional techniques when it is necessary to guarantee thermal stability, and high-quality products (in terms of purity and yield without solvent traces) (Sovová et al., 1994). The low viscosity and high diffusivity of supercritical fluid allow the fluid to diffuse easily through the plant matrix (Bruno et al., 2019). Moreover, CO₂ is not reactive at low temperatures, and it is easily recovered after each extraction stage (Kitzberger et al., 2009). Process parameters that are influencing the SFE-CO₂ are pressure, extraction time, temperature, solvent flow rate, and co-solvent addition. The most important parameter affecting the extraction process is the pressure. Up to now, SFE-CO₂ has been applied for the extraction of carotenoids, tocopherols, essential oils, fatty acids, waxes, and cannabinoids (Aladić et al., 2015; Pavlić et al., 2016; Elgndi et al., 2017).

UAE is another key technology in achieving the objective of sustainable green chemistry and extraction (Chemat et al., 2017). Different mechanisms are involved during UAE (fragmentation, erosion, capillarity, denaturation, and sonoporation). Fragmentation is one of the main mechanisms of UAE. Fragmentation of friable solids resulting from ultrasonic cavitation has been identified by several authors (Kusters et al., 1993; Kusters et al., 1994; Suslick et al., 1999). Fragmentation can be due to interparticle collisions and shock waves created from collapsing cavitation bubbles in the liquid. A direct consequence of the reduction in particle size by ultrasound action is the increase of the surface area of the solid resulting in higher mass transfer and increased extraction rate and yield (Chemat et al., 2017). Parameters that influence the process of UAE are physical parameters (frequency, wavelength, and amplitude) and medium parameters (temperature, solvent, matrix parameters). Up to now, UAE has been applied for the extraction of carotenoids, natural colors, phenols, anthocyanins, volatile compounds, and oils (Ramić et al., 2015; Tomšik et al., 2016).

The focus of this study was the fractionating isolation of bioactive compounds from *S. chamaecyparissus* L. by applied two green extraction technologies, SFE-CO₂ and UAE. First, non-polar components were isolated from the dry plant material by SFE-CO₂ at a pressure of 300 bar and a temperature of 40 °C. Herbal material leftovers were then subjected to the UAE, using ethanol solutions as an extraction solvent in concentrations of 30, 50, and 70% (v/v), at

temperatures of 30, 50, and 70 °C, for a period of 10, 20 and 40 min. The main target of applied UAE were polar compounds present in the exhausted material left after the SFE-CO₂. According to this, the content of total phenols (TP) and flavonoids (TF) in the obtained extracts was determined, as well as antioxidant activity.

Materials and methods

Plant sample and chemicals

The plant material used in this research, Santolina, was introduced to the Medicinal plant collection garden Institute of Field and Vegetable Crops Novi Sadin 2017. It flourished for the first time in 2018 when the determination of *Santolina chamaecyparissus* (Vouch No. 2-1446) was confirmed and the plant was stored in the Herbarium BUNS. At the time of full flowering (in May 2018), the flower heads were cut off and dried in the shade at a place with adequate air circulation. After drying, the plant material was packed in paper bags and properly stored until further analysis.

Folin-Ciocalteu reagent and DPPH hydrate were purchased from Sigma-Aldrich (Steinheim, Germany). All other used chemicals were of analytical grade.

Supercritical CO₂ extraction

SFE-CO₂ was performed on the laboratory-scale high-pressure extraction plant (HPEP, NOVASwiss, Effertikon, Switzerland) given in detail elsewhere (Vidović et al., 2011). Applied process parameters were: pressure 300 bar, temperature 40 °C, and extraction time of 240 min. The extraction kinetics was observed in the time set of 30, 60, 90, 120, 180, and 240 min.

Ultrasound-assisted extraction

Plant material, left after the SFE-CO₂, was subjected to UAE. UAE was performed in a sonication water bath (EUP540A, EU instruments, France) with a fixed frequency at 40 kHz. In all experimental combinations, 3.0 g of plant raw material was mixed with 30 mL of ethanol (concentrations of solvent were 30, 50, and 70%) in 100 mL glass flasks (Erlenmeyer). Different extraction time (10, 20, and 40 min) and temperatures (30, 50, and 70 °C) were used and their effect on the extraction process was evaluated. After the extraction, extracts were immediately filtered through the filter paper under vacuum, collected into plastic bottles and stored at -4 °C until the analysis.

Total phenol content

Total phenolic content (TP) was determined using the Folin-Ciocalteu procedure (Singelot and Rossi, 1965). Gallic acid was used for the preparation of a standard curve. The absorbance of samples was measured at 750 nm (on 6300 Spectrofotometer, Jenway, UK). TP content was expressed as mg gallic acid equivalent (GAE) per mL of extract.

Total flavonoid content

Total flavonoid content (TF) was determined using the aluminum chloride colorimetric assay (Harborne, 1984). Catechin was used for the preparation of a standard curve. The absorbance of the samples was measured at 510 nm. TF content was expressed as mg catechin equivalent (CE) per mL of extract.

Antioxidant activity

The free radical-scavenging activity of samples was determined using the DPPH assay previously described by Espin et al. (2000). Different volumes of the diluted sample were mixed with 95% (v/v) methanol and 1 mL of 90 $\mu\text{mol/L}$ DPPH to obtain different final concentrations. After incubation at room temperature for 60 min, the absorbance at 515 nm was measured and the result expressed as radical-scavenging capacity (RSC) calculated using the following equation:

$$\text{RSC (\%)} = 100 - ((A_s \times 100) / A_b) \quad (1)$$

where A_s is the absorbance of the sample solution and A_b is the absorbance of the blank probe. Antioxidant activity was expressed as IC_{50} (mg/mL), which is the concentration of test solution required to obtain 50% RSC.

Statistical analysis

All analyses were run in triplicate and the results were expressed as means \pm standard deviation (SD). Mean values were considered significantly different at $p < 0.05$ confidence level, after the performance of the one-way ANOVA statistical analysis followed by Tukey's test.

Results and discussion

For more intensive extraction of Santolina bioactive compounds and more intensive utilization of the herbal material, this study applied the SFE-CO₂, suitable for the isolation of lipophilic constituents,

followed by UAE by ethanol, suitable for the isolation of medium-polar and polar constituents. Similar process pathway was previously efficiently applied on the several different raw materials, among microalgae from wastewater treatment (Martín Juárez et al., 2020).

The yield achieved by the application of the SFE-CO₂ of Santolina, at pressure of 300 bar and temperature of 40 °C, was 3.98% (w/w). Figure 1 is showing the kinetics of the extraction process, and the increase of the extraction yield in the time.

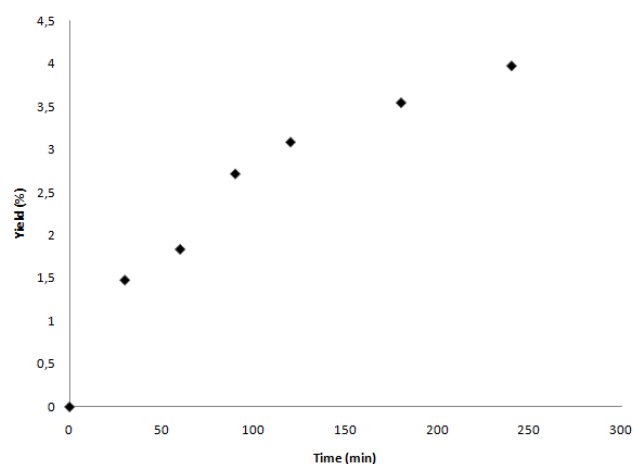


Figure 1. SFE-CO₂ kinetics.

Up to now, the SFE-CO₂ was used to isolate the volatile oil from *S. chamaecyparissus* L. in the study of Grosso et al. (2009), where applied extraction conditions were as following: pressure in the range of 80 to 90 bar, the temperature in the range of 40 to 50 °C, and flow rate (0.7, 0.8, 1.1, and 1.3 kg/h) (Grosso et al., 2009). The extraction yield achieved in the study of Grosso et al. was between 0.1 and 1.4% (w/w), depending on the conditions, which was lower than the one achieved in this research. Coelho et al. (2012) examined the efficiency of supercritical extraction in isolating volatile oil from *S. chamaecyparissus* L. under the similar conditions (pressure of 80 bar, the temperature of 40 °C, and flow rate of 1.08 kg/h). In this case, the SFE-CO₂ proved to be an effective method for the isolation of essential oil from *S. chamaecyparissus* L. Even the analysis of the obtained essential oil proved that higher concentrations of individual compounds were isolated by this method compared to hydrodistillation (1,8-cineole - 38.3% in essential oil versus 24.8%; camphor - 10.7% in essential oil versus 7.4%).

According to the published results, up to now, the SFE-CO₂ at a higher pressure, such is pressure of 300 bar, was not applied for the isolation of lipophilic

bioactive compounds from *S. chamaecyparissus* L. Another extraction technique which was previously used for the extraction of lipophilic compounds from the same material was the Soxhlet extraction. In the study by Giner et al. (1988), Soxhlet extraction of *S. chamaecyparissus* L. with different extraction solvents was performed. According to the results of the study, Soxhlet extraction, in which hexane was applied, enabled the yield of 2.98%, while chloroformic extract yielded 3.98%, ethyl acetic as extraction solvent 1.00%, and methanol 6.88% (w/w) (extraction conditions are unfamiliar). Compared to the results of this study, the extraction yield of the SFE-CO₂ was the same as the result obtained for chloroformic extract obtained by Soxhlet extraction (3.98% w/w).

In this study, after the SFE-CO₂ exhausted material was processed by UAE with the main aim, which was the isolation of moderate and polar bioactive compounds of *S. chamaecyparissus* L. According to the obtained results, the highest UAE extraction yield of material left after the SFE-CO₂ (28.48%) was obtained using 50% ethanol as extraction solvent, at the temperature of 50 °C during a period of 40 min, while the lowest yield (22.15%) was obtained when 30% ethanol was applied at 50 °C, and 20 min (Table 1).

Generally, the increase of ethanol concentration resulted in the higher concentration of TP in the prepared extracts (results presented in Table 2). The highest measured concentration of TP was 2.45 mg

GAE/mL of extract achieved at the following extraction conditions: extraction time 20 min, the concentration of extraction solvent 70% (v/v), and temperature of 50 °C. The lowest concentration of TP was 1.38 mg GAE/mL of extract achieved at the following extraction conditions: extraction time 20 min, the concentration of extraction solvent 50% (v/v), and temperature of 70 °C. A high amount of TP was extracted with extraction solvent of higher concentration of ethanol, which would mean that phenols present in the tested material were of moderate polarity. Chirane et al. (2019) examined polyphenolic components from the ethanolic extract obtained by Soxhlet extraction, using 95% ethanol as extraction solvent, while the extraction time was 6 h. The extraction yield achieved in Chirane et al. study was 18.44% (w/w), while the content of TP compounds and TF were 43.22 mg GAE/g SE and 27.41 mg CE/g SE, respectively (Chirane et al., 2019). Compared with these results, higher yields of TP and TF were obtained by application of UAE after SFE-CO₂.

Temperature performed a negative influence on the phenolic fraction content. According to the results in Table 2, contrary to the phenolics, higher content of TF was achieved with an increase in temperature and decrease in ethanol concentration of applied extraction solvent. No data have been found to explain this effect of temperature.

Table 1. Extraction yield obtained for *S. chamaecyparissus* L. performed in different UAE conditions.

| Samples | Time (min) | % ethanol | Temperature (°C) | Extraction yield (mg/mL) |
|----------|------------|-----------|------------------|--------------------------|
| Sample 1 | 10 | 50 | 50 | 22.86 |
| Sample 2 | 20 | 50 | 50 | 24.73 |
| Sample 3 | 40 | 50 | 50 | 28.48 |
| Sample 4 | 20 | 50 | 70 | 27.33 |
| Sample 5 | 20 | 50 | 30 | 24.12 |
| Sample 6 | 20 | 30 | 50 | 22.15 |
| Sample 7 | 20 | 70 | 50 | 27.17 |

Table 2. Content of total phenols and total flavonoid content.

| Samples | TP (mg GAE/mL extract) | TF (mg CE/mL extract) | IC ₅₀ (mg/mL) |
|----------|----------------------------|----------------------------|--------------------------|
| Sample 1 | 2.2877±0.0393 ^a | 1.2512±0.0106 ^a | 0.0048 |
| Sample 2 | 1.7683±0.0140 ^b | 0.9969±0.0225 ^b | 0.0084 |
| Sample 3 | 2.4168±0.0112 ^c | 1.1348±0.0166 ^c | 0.0084 |
| Sample 4 | 1.3752±0.0421 ^d | 1.3104±0.0071 ^a | 0.0079 |
| Sample 5 | 1.8441±0.0168 ^b | 0.8482±0.0095 ^d | 0.0062 |
| Sample 6 | 1.5661±0.0197 ^c | 1.0703±0.0189 ^c | 0.0053 |
| Sample 7 | 2.4477±0.0365 ^c | 0.7837±0.0213 ^d | 0.0235 |

*Different letters within a column indicates significant differences between samples ($p < 0.05$).

S. chamaecyparissus L. extract of the highest antioxidant activity was the one obtained in the shortest extraction time (10 min, 50 °C, and 50% ethanol), while the highest IC₅₀ value was measured in the extract obtained with 70% ethanol (20 min, and 50 °C).

The linear regression between the content of TP and the TF content in relation to the antioxidant activity of the extracts was examined. The examination was done in the R studio program. Based on the results of the summary model, it can be concluded that the linear model is appropriate and that there is a linear relationship between TP and TF content and antioxidant activity. Figure 2 shows a graph of the linear dependence between TP and IC₅₀ value. The value of the correlation coefficient of these two variables is 0.47, which proves a moderate correlation. From these data, it can be concluded that, in addition to isolated phenols, some other isolated components are also responsible for the antioxidant activity of the extracts.

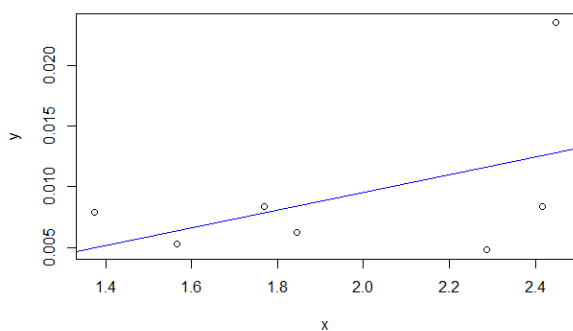


Figure 2. Graph of linear dependence of antioxidant activity to the content of total phenols: x – TP (mg GAE/mL extract), and y - IC₅₀.

Figure 3 shows a graph of the linear dependence between TF and IC₅₀ value. The value of the correlation coefficient of these two variables is 0.59, which proves a moderate correlation.

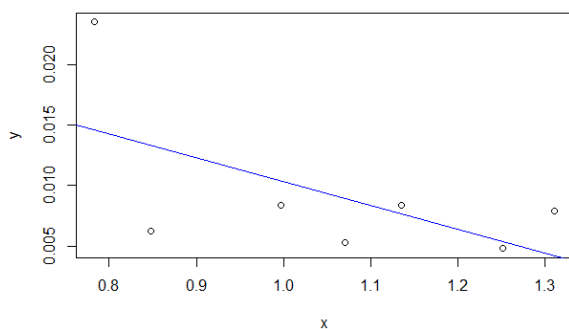


Figure 3. Graph of linear dependence of antioxidant activity to the content of total flavonoids:

x – TF (mg CE/mL extract), and y - IC₅₀.

In contrast to phenols, the flavonoids in the obtained extracts directly affect the antioxidant activity. As the TF content increases, it is assumed that the antioxidant activity of the extracts also increases.

Conclusions

UAE, applied after the SFE-CO₂, has been proven to be the efficient method for isolating polar and medium polarity components from *S. chamaecyparissus* L. Isolation of non-polar components from Santolina by supercritical extraction increases the possibility for better and easier solvent penetration to the isolation of polar components. In addition, the analysis of the obtained Santolina extracts showed that temperature and ethanol percentage have a significant influence on the concentration of polar and medium polarity components. This means that the optimization of the extraction process is required to obtain certain target polar compounds.

Addition, analysis of the obtained Santolina extracts showed that temperature and ethanol percentage have a significant influence on the concentration of polar and medium polarity components. This means that optimization of the extraction process is required to obtain certain target polar compounds.

Author Contributions:

Conceptualization: S.V. and J.V.; Methodology: J.V., N.N. and S.V.; Formal analysis: Z.M. and N.N.; Investigation: Z.M., M.A. and J.V.; Resources: M.A.; data curation, Z.M.; Writing—original draft preparation: Z.M.; Writing—review and editing: S.V., J.V., N.N., M.A. and Z.M. Visualization: M.A. and N.N.; Supervision: S.V. and J.V.; Funding acquisition: S.V. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: The authors are grateful to the Serbian Ministry of Education, Science and Technological Development (451-03-68/2020-14/200134).

References

- Aladić, K., Jarni, K., Barbir, T., Vidović, S., Vladić, J., Bilić, M., Jokić, S. (2015): Supercritical CO₂ extraction of hemp (*Cannabis sativa* L.) seed oil. *Industrial Crops and Products* 76, 472-478. <https://doi.org/10.1016/j.indcrop.2015.07.016>
- Bruno, S. F., Ekorong, F. J. A. A., Karkal, S. S., Cathrine, M. S. B., Kudre, T. G. (2019): Green and innovative techniques for recovery of valuable compounds from seafood by-products and discards: A review. *Trends in*

- Food Science & Technology* 85, 10–22. <https://doi.org/10.1016/j.tifs.2018.12.004>
- Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A. S., Abert-Vian, M. (2017): Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics sonochemistry* 34, 540-560. <https://doi.org/10.1016/j.ultsonch.2016.06.035>
- Chemat, F., Vian, M. A., Cravotto, G. (2012): Green extraction of natural products: concept and principles. *International journal of molecular sciences* 13(7), 8615-8627. <https://doi.org/10.3390/ijms13078615>
- Chirane, M. S., Benchabane, O., Bousbia, N., Zenia, S. (2019): Antioxydant and antimicrobial activities of essential oil and ethanol extract of *Santolina chamaecyparissus* L. *Revue Agrobiologia* 9(2), 1660-1668.
- Cimanga, K., Kambu, K., Tona, L., Apers, S., De Bruyne, T., Hermans, N., Vlietinck, A. J. (2002): Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. *Journal of ethnopharmacology* 79(2), 213-220. [https://doi.org/10.1016/S0378-8741\(01\)00384-1](https://doi.org/10.1016/S0378-8741(01)00384-1)
- Coelho, J. P., Cristino, A. F., Matos, P. G., Rauter, A. P., Nobre, B. P., Mendes, R. L., Palavra, A. F. (2012): Extraction of volatile oil from aromatic plants with supercritical carbon dioxide: experiments and modeling. *Molecules* 17(9), 10550-10573. <https://doi.org/10.3390/molecules170910550>
- Demirci, B., Özek, T., Baser, K. H. C. (2000): Chemical composition of *Santolina chamaecyparissus* L. essential oil. *Journal of Essential Oil Research* 12(5), 625-627. <https://doi.org/10.1080/10412905.2000.9712173>
- Dorman, H. D., Deans, S. G. (2000): Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of applied microbiology* 88(2), 308-316. <https://doi.org/10.1046/j.1365-2672.2000.00969.x>
- Elgndi, M. A., Filip, S., Pavlič, B., Vladić, J., Stanojković, T., Žižak, Ž., Zeković, Z. (2017). Antioxidative and cytotoxic activity of essential oils and extracts of *Satureja montana* L., *Coriandrum sativum* L. and *Ocimum basilicum* L. obtained by supercritical fluid extraction. *The Journal of Supercritical Fluids* 128, 128-137. <https://doi.org/10.1016/j.supflu.2017.05.025>
- Espín, J. C., Soler Rivas, C., Wichers, H. (2000): Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. *Journal of Agric Food Chem* 48, 648–656. <https://doi.org/10.1021/jf9908188>
- Giner, R. M., Rios, J. L., Villar, A. (1988): CNS depressant effects, anti-inflammatory activity and anti-cholinergic actions of *Santolina chamaecyparissus* extracts. *Phytotherapy Research* 2(1), 37-41. <https://doi.org/10.1002/ptr.2650020106>
- Grosso, C., Figueiredo, A. C., Burillo, J., Mainar, A. M., Urieta, J. S., Barroso, J. G., Palavra, A. M. (2009): Supercritical fluid extraction of the volatile oil from *Santolina chamaecyparissus*. *Journal of separation science* 32(18), 3215-3222. <https://doi.org/10.1002/jssc.200900350>
- Harborne, B. (1984). *Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis* (2nd ed). Springer, Dordrecht, pp. 1–36.
- Kitzberger, C. S., Lomonaco, R. H., Michielin, E. M., Danielski, L., Correia, J., Ferreira, S. R. (2009): Supercritical fluid extraction of shiitake oil: curve modeling and extract composition. *Journal of Food Engineering* 90(1), 35-43. <https://doi.org/10.1016/j.jfoodeng.2008.05.034>
- Kusters, K. A., Pratsinis, S. E., Thoma, S. G., Smith, D. M. (1993): Ultrasonic fragmentation of agglomerate powders. *Chemical engineering science* 48(24), 4119-4127. [https://doi.org/10.1016/0009-2509\(93\)80258-R](https://doi.org/10.1016/0009-2509(93)80258-R)
- Kusters, K. A., Pratsinis, S. E., Thoma, S. G., Smith, D. M. (1994): Energy-size reduction laws for ultrasonic fragmentation. *Powder technology* 80(3), 253-263. [https://doi.org/10.1016/0032-5910\(94\)02852-4](https://doi.org/10.1016/0032-5910(94)02852-4)
- Pattnaik, S., Subramanyam, V. R., Bapaji, M., Kole, C. R. (1997): Antibacterial and antifungal activity of aromatic constituents of essential oils. *Microbios* 89(358), 39.
- Pavlič, B., Đurković, A. V., Vladić, J., Gavarić, A., Zeković, Z., Tepić, A., Vidović, S. (2016): Extraction of Minor Compounds (Chlorophylls and Carotenoids) from Yarrow–Rose Hip Mixtures by Traditional versus Green Technique. *Journal of Food Process Engineering* 39(4), 418-424. <https://doi.org/10.1111/jfpe.12242>
- Ramić, M., Vidović, S., Zeković, Z., Vladić, J., Cvejin, A., Pavlič, B. (2015). Modeling and optimization of ultrasound-assisted extraction of polyphenolic compounds from *Aronia melanocarpa* by-products from filter-tea factory. *Ultrasonics Sonochemistry* 23, 360-368. <https://doi.org/10.1016/j.ultsonch.2014.10.002>
- Rombaut, N., Tixier, A. S., Bily, A., Chemat, F. (2014): Green extraction processes of natural products as tools for biorefinery. *Biofuels, Bioproducts and Biorefining* 8(4), 530-544. <https://doi.org/10.1002/bbb.1486>
- Singleton, V. L., Rossi, J. A. (1965): Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture* 16(3), 144-158.
- Sovová, H. (1994): Rate of the vegetable oil extraction with supercritical CO₂-I. Modelling of extraction curves. *Chemical Engineering Science* 49(3), 409-414. [https://doi.org/10.1016/0009-2509\(94\)87012-8](https://doi.org/10.1016/0009-2509(94)87012-8)
- Suslick, K. S., Price, G. J. (1999): Applications of ultrasound to materials chemistry. *Annual Review of Materials Science* 29(1), 295-326. <https://doi.org/10.1146/annurev.matsci.29.1.295>
- Tirillini, B., Velasquez, E. R., Pellegrino, R. (1996): Chemical composition and antimicrobial activity of essential oil of *Piper angustifolium*. *Planta Medica* 62(04), 372-373. <https://doi.org/10.1055/s-2006-957911>
- Toma, M., Vinatoru, M., Paniwnyk, L., Mason, T. J. (2001): Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. *Ultrasonics sonochemistry* 8(2), 137-142. [https://doi.org/10.1016/S1350-4177\(00\)00033-X](https://doi.org/10.1016/S1350-4177(00)00033-X)

- Tomšik, A., Pavlić, B., Vladić, J., Ramić, M., Brindza, J., Vidović, S. (2016): Optimization of ultrasound-assisted extraction of bioactive compounds from wild garlic (*Allium ursinum* L.). *Ultrasonics sonochemistry* 29, 502-511. <https://doi.org/10.1016/j.ultsonch.2015.11.005>
- Tzakou, O., Pitarokili, D., Chinou, I. B., Harvala, C. (2001): Composition and antimicrobial activity of the essential oil of *Salvia ringens*. *Planta Medica* 67(01), 81-83. <https://doi.org/10.1055/s-2001-10627>
- Juárez, J. M., Vladic, J., Rodríguez, S. B., Vidovic, S. (2020): Sequential valorization of microalgae biomass grown in pig manure treatment photobioreactors. *Algal Research* 50, 101972. <https://doi.org/10.1016/j.algal.2020.101972>