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***Viola odorata*: Influence of supercritical fluid extraction on the efficiency of ultrasound- and microwave-assisted extraction of bioactive compounds**

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ABSTRACT

This study aimed at examining the effect of supercritical carbon dioxide (ScCO₂) extraction on the subsequent extraction of the *Viola odorata* polar bioactive components. The raw material was first submitted to ScCO₂ extraction for the extraction of the lipophilic fraction. Then the exhausted raw material was subjected to ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) in order to extract the polar components. ScCO₂ extraction was performed under the pressure of 300 bar and at a temperature of 40 °C for 4 hours. In order to see the effect of ScCO₂, the UAE and MAE (50% aqueous ethanol solution as solvent) were conducted on both exhausted (residue-after the ScCO₂) and unexploited raw materials. Also, the impact of the various UAE and MAE conditions was tested. The UAE was conducted on two different temperatures (40 and 50 °C) at the constant extraction time (40 and 20 min). MAE was conducted on two different extraction powers (470 and 800 W) and at the constant extraction time (10 min). The results were focused on the content of total phenols, total flavonoids and antioxidant activity of the obtained extracts. The yield during the ScCO₂ process was 1.43% (w/w). It was noted that the extracts obtained by applying UAE and MAE after the ScCO₂ were noticeably richer in the content of total phenols. UAE conditions of 40 °C and 20 min showed the highest yield of total phenols, recording 70.38 mg GAE/g DE, while the MAE at the power of 470 W achieved 11.89% higher yield of polyphenols in residue extracts. The antioxidant activity has also been in correlation with the concentration of polyphenols.

Introduction

Native to Europe and Asia, *Viola odorata* L. is now a widely spread evergreen perennial herb, commonly known as sweet violet, wood violet or common violet. Sweet violet belongs to a flowering plant family *Violaceae*, and by having a number of pharmaceutical effects, it has a distinctive place within a family of about 800 species (Mittal et al., 2015). Extensive use in traditional medicine (especially in traditional Persian medicine), such as treating cough, fever, common cold, headache, insomnia and epilepsy led to further research in the field of pharmaceutical properties of sweet violet (Feyzabadi et al., 2017).

Modern pharmacy and phytotherapy state that sweet violet has an anti-inflammatory, antihypertensive, antidyslipidemic, antipyretic, antibacterial and hepatoprotective effect (Mittal et al., 2015; Koochek et al., 2003). For pharmaceutical purposes, sweet violet can be applied in a wide range of different preparations, such as medicinal oils for topical and nasal use, linctus or respiratory-targeted preparations, pills, syrups and extracts of sweet violet (Feyzabadi et al., 2017; Hamedi et al., 2013). *V. odorata* has a diverse chemical composition that includes: polysaccharides (galactose, glucose and galacturonic acid), phenylpropanoids (anthocyanins, flavonoids, flavonol glycosides), macrocyclic peptides (28-37

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amino acids), free sugars and mucilage (Drozdova and Bubenchikov, 2005; Herrmann et al., 2008; Karioti et al., 2011). Among these components, the main ones responsible for a sweet violet wide range of pharmacological activities are flavonoids, terpenes, tannins, glycosides and alkaloids. Obtaining these components is performed by conventional extraction methods such as organic solvent-based extraction or Soxhlet (Akhbari et al., 2012; Vishal et al., 2009). It has been proven that these conventional methods have many disadvantages, such as using large amounts of organic solvents and generation of toxic residue, the longer extraction time and the lower efficiency compared to the modern extraction methods like supercritical fluid extraction, microwave-assisted extraction (MAE) or ultrasound-assisted extraction (UAE) (Da Porto et al., 2013; Pan et al., 2002).

Supercritical fluid extraction uses fluids at temperatures and pressures higher than their critical temperatures and critical pressures. Carbon dioxide (CO₂) represents one of the most commonly used supercritical fluid in the extraction of natural materials. Supercritical carbon dioxide (ScCO₂) extraction is an efficient as green extraction technology and has many distinct advantages in extracting lipophilic components from various plant materials such as selectivity, attainment of solvent-free safe products, better mass transfer and the use of non-toxic solvents. In addition, CO₂ is cheap, available and safe and due to the mild supercritical conditions, it is adequate for thermosensitive compounds (Nadalin et al., 2014; Vidović et al., 2020; Vladić et al., 2016; Wheeler et al., 1989; Henning et al., 1994; Reverchon et al., 2003). UAE is one of the promising extraction techniques which can offer high reproducibility in a short period of time, simplified manipulation, reduced solvent consumption and temperature and lower energy input (Chemat et al., 2008). MAE is the extraction technique that combines microwave and traditional solvent extraction. The study by Ganzler et al. (1986) shows that MAE has many advantages, such as the shorter extraction time, less solvent demand, the higher extraction yield, the lower degradation of thermosensitive compounds and lower operational costs. The combination of supercritical extraction with other extraction techniques such as UAE has been shown to enhance the mass transfer of the components of interest from the matrix to the solvent (Balachandran et al., 2006). In order to achieve the most rational use of material and maximum extraction efficiency of *V. odorata*, bioactive components of different green extraction techniques were applied to separate the components of different polarity. Firstly, ScCO₂ extraction was performed to separate the lipophilic fraction, then the

material (after ScCO₂ extraction - residue) was subjected to UAE and MAE to extract the polar fraction. Also, to determine the effect of ScCO₂ on the extraction efficiency of the polar fraction, unexploited (raw) plant material was also used for UAE and MAE. Extraction efficiency was determined by measuring the content of total polyphenols and flavonoids in obtained extracts from unexploited material and residue after extraction and also the antioxidant activity of extracts was determined.

Materials and methods

Plant material and chemicals

Plant material was provided by the Institute of Field and Vegetable Crops, Novi Sad, Serbia. The dried plant material was ground in a domestic blender before the extraction and the mean particle size (0.32 mm) of ground material was determined using vibration sieve sets (CISA, Cedacteria, Spain). The carbon dioxide (Messer, Novi Sad, Serbia) with purity >99.98% (w/w) was used for the ScCO₂ extraction. Methanol of HPLC grade was purchased from J.T. Baker (the Netherlands), whereas Folin–Ciocalteu reagent, gallic acid and catechin (≥99.0% purity (HPLC)) were purchased from Merck (Germany).

Supercritical carbon dioxide (ScCO₂) extraction

For the ScCO₂ extraction of *V. odorata*, high-pressure laboratory-grade system (NOVA, Swiss, Effertikon, Switzerland) was used. The extraction was conducted at a constant pressure of 300 bar and a constant temperature of 40 °C for 4 hours. The CO₂ flow was also constant at 0.194 kg/h, while the conditions in the separator were 15 bar and 23 °C. Kinetic of ScCO₂ was monitored during extraction.

Ultrasound-assisted extraction (UAE)

Extraction was conducted in a sonication water bath (EUP540A, Euinstruments, France) with a constant frequency of 40 kHz. Extraction conditions that were applied were the same for both residue and raw materials. Amount of 3 g *V. odorata* plant material was mixed in 200 mL Erlenmeyer flasks with 60 mL of 50% aqueous ethanol solution (solid/liquid ratio 1:20). Flasks were fixed with condensers in order to minimize solvent evaporation. Two different sets of experiments were conducted: extraction at constant temperature of 40 °C and two different times of 20 and 40 min, and also extraction at a constant time of 20 min and two different temperatures of 40 and 50 °C. After the UAE, extracts were filtered on vacuum pump

and stored at a temperature of 4 °C until further analysis.

Microwave-assisted extraction (MAE)

The extraction was conducted on a modified home microwave oven at a constant extraction time of 10 min. Extraction time of 10 min was selected based on the preliminary conducted experiments where it was noted that with longer extraction time there was a significant decrease in the extraction yield. The amounts of 3 g of both residue and raw material were mixed in different round flasks with 60 mL of 50% aqueous ethanol solution (1:20 w/v). The round flask was positioned in a way so the condenser could be fixed on the top of the flask in order to reduce solvent evaporation. Powers of 470 and 800 W were applied. After the extraction, the extracts were filtered on a vacuum pump and stored at a temperature of 4 °C.

Determination of extraction yield

The extraction yield obtained by UAE and MAE was determined by evaporating solvent using a vacuum rotary evaporator. After the evaporation, drying for 3 hours at 105 °C was performed, and mass was measured using an analytical scale. All experiments were performed in three replicates and the results are expressed as mean values.

Determination of total phenols

The content of total phenols in *V. odorata* extracts was determined using the Folin-Ciocalteu method (Singleton et al., 1965). The absorbance was measured at 750 nm using a single beam UV/VIS spectrophotometer (6300 Spectrophotometer, Jenway, UK). The results were expressed as gallic acid equivalents per gram of dried extract. All experiments were performed in three replicates and the results are expressed as mean values.

Determination of total flavonoids

Aluminium chloride colourimetric assay was used for the determination of the content of total flavonoids (Harborne et al., 1989). The absorbance was measured at 510 nm using a single beam UV/VIS spectrophotometer as described in a previous method. The results were expressed as equivalents of catechin per gram of a dried extract. All experiments were performed in three replicates and the results are expressed as mean values.

Determination of antioxidant activity

The free radical scavenging ability of the *V. odorata* extracts was determined by a colourimetric method, measuring the change in purple colour of the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical solution (Espín et al., 2000). The test solution was made by mixing different volumes of extracts obtained under the same extraction conditions with 95% methanol and 90 µM DPPH radical in order to determine the effect of different extract concentrations on scavenging of the DPPH radicals. After 1 h of incubation, absorbance was measured at 515 nm using DPPH solution as blank and methanol for calibration of the spectrophotometer. The results were reported as radical scavenging capacity of the extracts (%RSC). The %RSC value was calculated by measuring the absorbance of DPPH in a methanol solution (A_{blank}) and the same solution with an extract added (A_{sample}) by the Eq. (1):

$$\%RSC = 100 - ((A_{\text{sample}} \times 100) / A_{\text{blank}}) \quad (1)$$

Referring to the RSC%, the antioxidant activity was expressed as inhibition concentration, which represents the concentration of the extract that scavenges 50% of free radicals present in the test solution (IC_{50}). All experiments were performed in three replicates and the results are expressed as mean values.

Results and discussion

Supercritical carbon dioxide extraction

In the scientific literature, different extraction yields of lipophilic components of sweet violet were reported. The significant differences in yields can be caused by different origins of the raw materials, climate conditions, as well as by the methods used for harvesting and drying. In addition, the extraction technique and the choice of the solvent have a very significant effect on the content of the lipophilic fraction. Akhbari et al. (2012) published that the essential oil yield obtained by applying a hydrodistillation technique was around 1.2%. Hamami et al. (2011) reported the essential oil yield of 2.3%, while a significantly higher yield was achieved by the Soxhlet extraction (7.88%) using methanol as a solvent for 72 h. These results are expected according to the application of different extraction techniques.

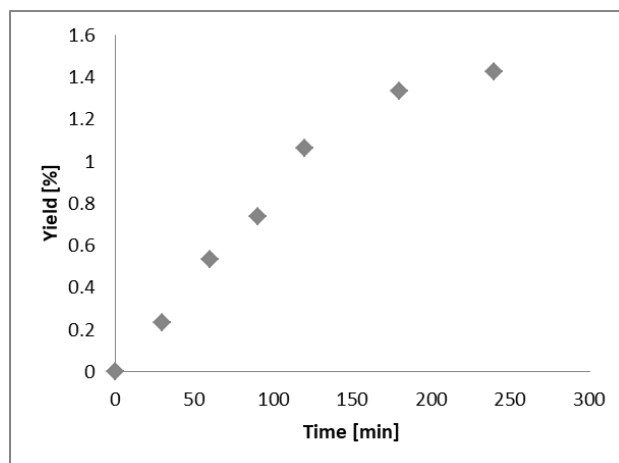


Fig. 1. Extraction kinetics of ScCO₂ extraction of *V. odorata* at temperature of 40 °C and pressure of 300 bar

The process of hydrodistillation extracts volatile components, while organic solvent-based Soxhlet extraction extracts other components of lipophilic nature, such as waxes and pigments (Danh et al., 2013; Vidovic et al., 2020). However, despite the high extraction yield that can be achieved by the Soxhlet extraction, this extraction method involves a purification process of the product, in order to remove solvents, which is time- and energy-consuming. An additional drawback of the Soxhlet extraction is the inability to adjust the quality of the product in terms of selective extraction, which can be achieved by using ScCO₂.

In order to extract the lipophilic fraction of *V. odorata* raw material, ScCO₂ extraction was performed at the conditions of 300 bar and 40 °C. During the process, kinetics was monitored at determined time intervals (Figure 1). A rapid extraction period was recorded at the beginning of the process. The first period of the extraction represents the constant extraction rate period, where the surface of the material particles is covered with an easily accessible solute. Moreover, convection is the dominant mass transfer mechanism in this period. The second extraction rate period is characterized by the failures in the external surface oil layer and the start of diffusion mechanisms combined with convection (Jokić et al., 2012). After 180 min, a yield of 1.33% was obtained, while after 240 min, a maximum yield of 1.43% was achieved. It has been reported that exposure of plant material to high pressure can have the effect of pretreatment and increase the efficiency of the subsequent extraction and/or improve the chemical or biological properties of the extracts. Vidović et al. (2014) found that the application of high-pressure pre-treatment increased the content of carvacrol in *Satureja montana* supercritical extract by 25%. The authors explained that the increase in carvacrol content may be caused

by higher solvent penetration in the cell structures of the plant material which is the result of high pressures. Therefore, the raw plant material after the extraction of the lipophilic fraction was used for further extractions of more polar components by applying UAE and MAE.

Ultrasound-assisted extraction *The content of total phenols*

The influence of temperatures 40 and 50 °C at the constant extraction time of 20 min, as well as the influence of extraction time 20 and 40 min at a constant temperature of 40 °C, was investigated. By applying different UAE conditions, the achieved yield of total polyphenols was in the range from 59.55 to 70.37 mg GAE/g (Figure 2, Table S1). Comparing these results with the results available in the scientific literature, it can be seen that the use of UAE achieves a higher yield than the traditional extraction methods, like the use of percolation (Ebrahimzadeh et al., 2010) where a yield of 35.4 mg GAE/g was recorded. The content of total phenols obtained from untreated plant material was in the range from 62.21 to 64.57 mg GAE/g, while the content of total phenols obtained from the residue material was in the range from 59.55 to 70.37 mg GAE/g. It was noted that an increase in the extraction time at the constant temperature of 40 °C resulted in the decrease of total phenol content in the extracts. Also applying the longer extraction time, at constant temperature 40 °C, 2.12% the higher phenol content was obtained from the raw material, while during the shorter extraction time, there was a more efficient extraction of residue with 8.24% higher yield. This could be explained by the fact that during ScCO₂ extraction, the cell structures are prone to damage due to the effect of high pressure on the material, which leads to the improvement of such extraction; however, prolonged UAE can potentially

lead to the degradation of active components, which might have happened in this experiment. The constant extraction time of 20 min showed that with the increase of temperature, the concentration of the total phenols decreases. Also, the temperature of 50 °C was more suitable for the extraction of raw material, where

s 7.36% higher yield was achieved. Therefore, the extraction conditions of 40 °C and 20 min and using ScCO₂ residue material proved to be the most efficient approach for the extraction of total phenols from *V. odorata* herbal material (Figure 2).

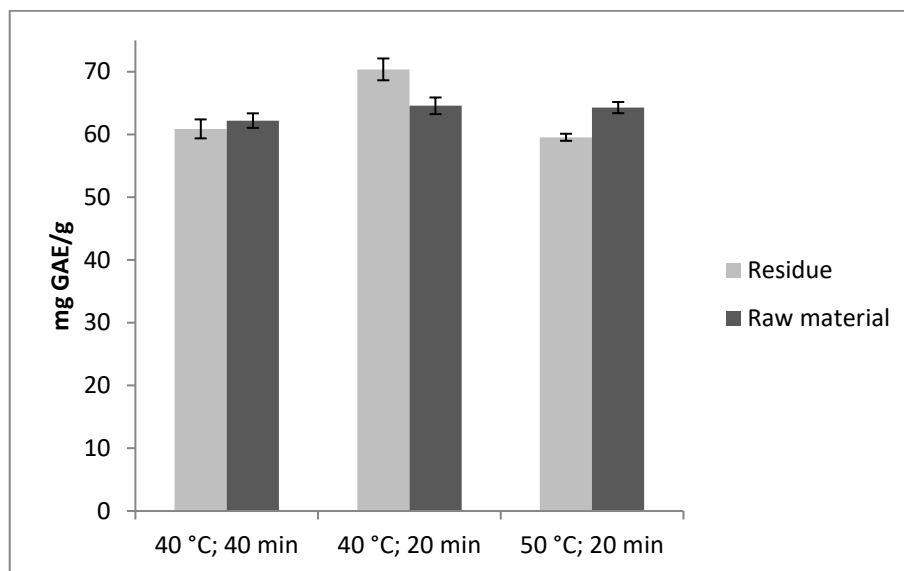


Fig. 2. The content of total phenols in the extracts obtained at different conditions of UAE extraction

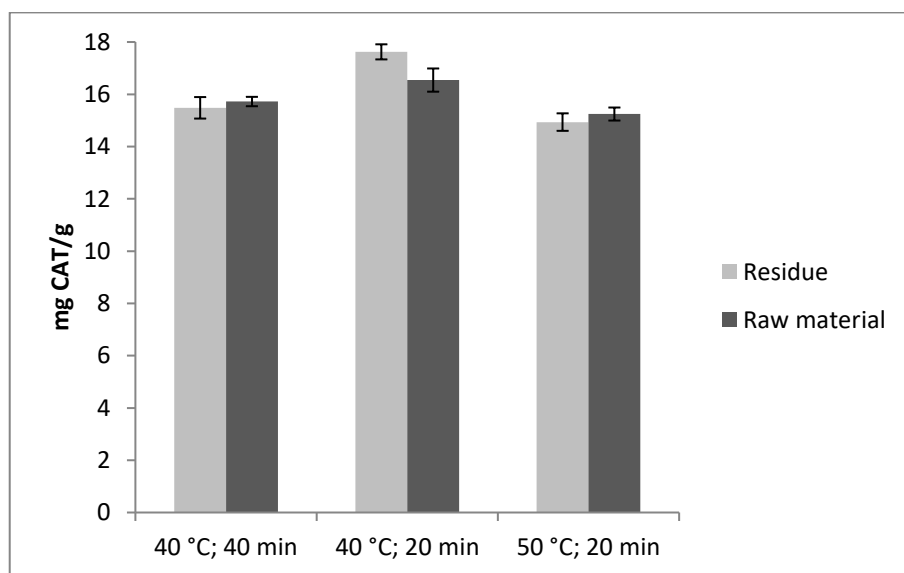


Fig. 3. The content of total flavonoids in the extracts obtained at different conditions of UAE

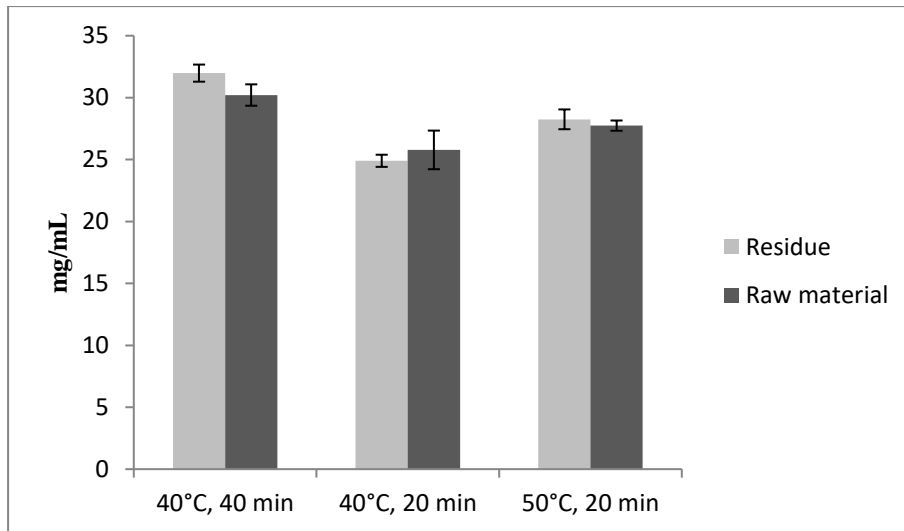


Fig. 4. The antioxidant activity of *V. odorata* extracts obtained at different UAE conditions

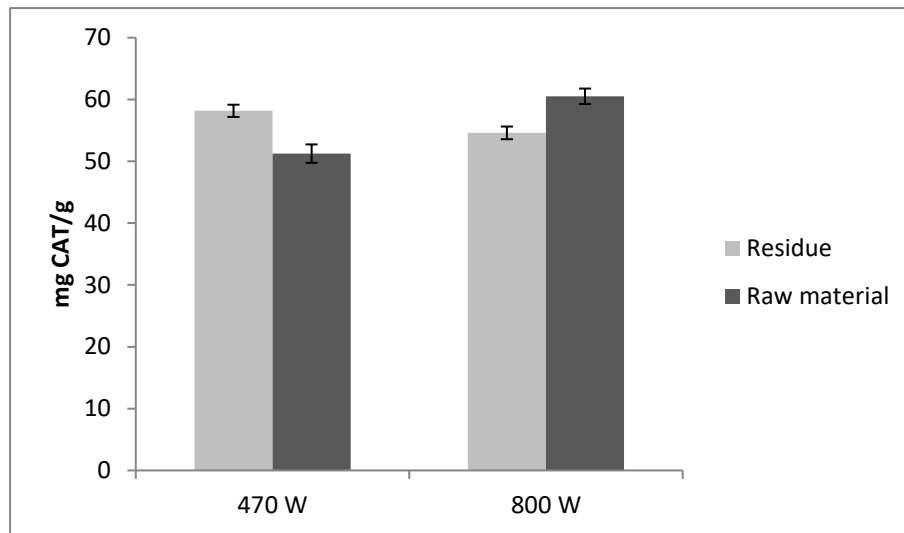


Fig. 5. The content of total phenols in the extracts obtained at different MAE conditions

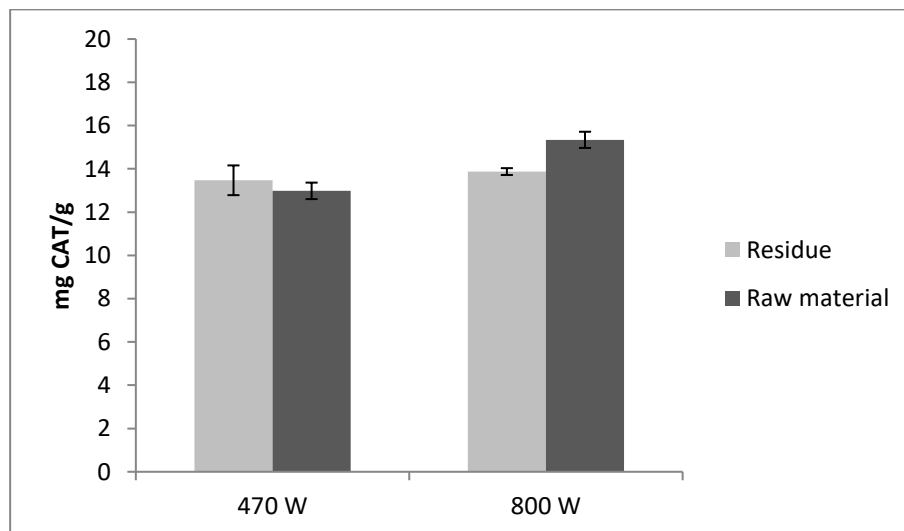


Fig. 6. The content of total flavonoids in the extracts obtained at different MAE conditions

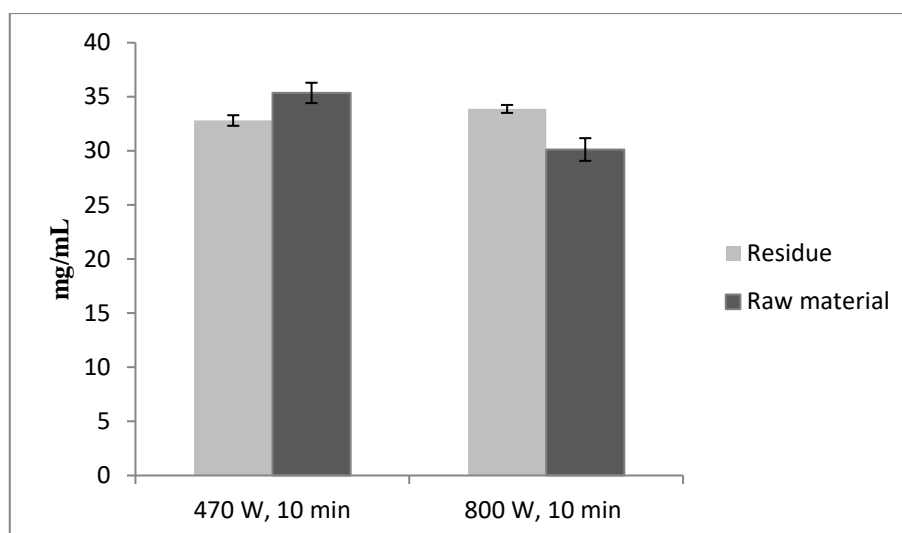


Fig. 7. The antioxidant activity of *V. odorata* extracts obtained at different MAE conditions

The content of total flavonoids

The extracts obtained by UAE had a flavonoid yield in the range of 15.48 to 17.62 mg CAT/g (Figure 3, Table S1). The highest yield of total flavonoids was obtained from the residue (17.62 mg CAT/g) at UAE conditions of 40 °C and 20 min, which was 6.12% higher than the content of flavonoids in the extract obtained from raw material (16.54 mg CAT/g) under the same conditions. During the extraction at the constant temperature of 40 °C, the time of 40 min was proved to be less effective than the shorter extraction time, while the raw material extraction was shown to be slightly more efficient (1.52%) than the extraction of the residue. Similar results were noted during the extraction at a constant time of 20 min, where the increase in temperature led to a decrease in the concentration of total flavonoids and also contributed to more efficient extraction of the raw material (2.01% higher yield was achieved). Therefore, it is safe to say that the content of total flavonoids followed the trend of total phenols, meaning that the longer extraction time and increased temperature proved to be a less efficient method for flavonoid extraction (Figure 3). Referring to the scientific literature, the yield of flavonoids in the extracts of sweet violet achieved by the use of UAE was lower than that obtained by a traditional method of extraction (percolation), where flavonoid yield of 22.8 mg CAT/g was reported (Ebrahimzadeh et al., 2010). These variations in the flavonoid content, as well as in the polyphenol content can also be attributed to differences in the raw materials themselves.

Antioxidant activity

Free radical scavenging activity was determined by the DPPH method and the results were reported as IC₅₀ value (Figure 4, Table S1). The analysis showed that the highest antioxidant activity was present in the extracts that had the highest content of total phenols and flavonoids. The highest antioxidant activity was 24.9 µg/mL and it was recorded in the residue extract obtained at UAE conditions of 40 °C and 20 min. At the same extraction conditions, raw material showed similar, but slightly less antioxidant activity of 25.7 µg/mL. UAE conditions of 40 °C, 40 min and 50 °C, 20 min had the same effect on the antioxidant activity as they had on total phenols and total flavonoid content, meaning the raw material showed higher activity than the residue, while in both cases the efficiency was lower than the extraction under conditions of 40 °C and 20 min. Antioxidant activity of extracts obtained at 40 °C and 40 min was 30.2 µg/mL for raw material and 31.9 µg/mL for residue, while conditions of 50 °C and 20 min recorded 27.7 and 28.2 µg/mL for raw material and residue respectively (Figure 4). Since the antioxidant activity followed the concentration trend of phenols and flavonoids, we can conclude that the main carriers of the antioxidant activity in the *V. odorata* extracts are phenolic components. Although these results do not show the high differences among themselves, it should be kept in mind that the antioxidant activity in extracts obtained using UAE showed significantly better results than the classical extraction methods recorded in the scientific literature. For example, aqueous extracts of *V. odorata* showed the

antioxidant activity of 140.7 and 163.6 $\mu\text{g}/\text{mL}$ (Stojković et al., 2011), which is over five times less than antioxidant activity in the extracts obtained using the UAE.

Microwave-assisted extraction

The content of total phenols

The content of total phenols in extracts obtained by MAE was in the range from 51.24 to 60.51 mg GAE/g (Figure 5, Table S2). Extracts obtained by MAE with a power of 470 W showed that the residue provides an 11.89% higher yield of total phenols (58.16 mg GAE/g) than the raw material (51.24 mg GAE/g). While residue proved to be a better source of polyphenols at extraction power of 470 W, extraction at higher power showed the opposite results: therefore at 800 W, the polyphenol content was 9.76% higher in the raw material (60.51 mg GAE/g) than in the residue (54.60 mg GAE/g). Although microwave extraction is less efficient than ultrasound extraction, we can still say that the results for the obtained polyphenol content are significantly higher than the results recorded by the earlier mentioned traditional extraction methods. Also, we should keep in mind that the extraction time was 10 min in the UAE, which can be a significant economic factor and can also have an impact on the selection of the extraction method.

The content of total flavonoids

The obtained extracts showed flavonoid content in the range of 12.98 to 15.34 mg CAT/g (Figure 6, Table S2). The highest yield of total flavonoids was achieved using an extraction power of 800 W from raw material, where the content of total flavonoids was 9.58% higher than total flavonoids obtained by extracting the residue. In addition, the flavonoid content in extracts obtained from the residue at 470 W was higher than the content of flavonoids from the raw material. This was also the case with the content of total phenols. However, the extraction at 470 W was significantly less efficient compared to the higher applied power. Applying MAE, as well as applying UAE on *V. odorata* herbal material resulted in the lower content of total flavonoids than recorded in the scientific literature. Therefore, the application of residue for the purpose of obtaining flavonoids from *V. odorata* plant material can be justified if we take into account the production of lipophilic components in the process of supercritical extraction.

Antioxidant activity

The obtained results showed that the antioxidant activity of *V. odorata* MAE extracts follow the trend of total phenols obtained by MAE; therefore, antioxidant activity was higher in those extracts that had the higher content of total phenols (Figure 7, Table S2). The highest antioxidant activity was 30.13 $\mu\text{g}/\text{mL}$ and it was recorded in the extract obtained by the extraction of raw material at extraction power of 800 W which was 11.06% higher than the extracts obtained from residue at the same conditions, whereas the usage of the residue demonstrated to be more efficient at 470 W, where 7.21% higher antioxidant activity was achieved. Antioxidant activity in extracts obtained by MAE was found to be slightly lower than in extracts obtained by UAE, indicating that phenols were the most likely carriers of the antioxidant activity. However, the obtained results of the antioxidant activity are still significantly higher than the previously mentioned results recorded in the scientific literature: therefore, it can be said that both MAE and UAE have proven to be very effective methods for obtaining extracts rich in compounds that have the antioxidant activity.

Conclusion

In this study, the effect of pretreatment using ScCO_2 in the extraction of lipophilic components is investigated in order to improve the subsequent extraction of *V. odorata* polar bioactive components using UAE and MAE. It was concluded that the application of ScCO_2 contributed to an increase in the yield of polyphenolic components under certain conditions. The application of UAE on the residue after ScCO_2 extraction has shown to be the most effective in lower temperature and shorter extraction time, providing the extract richer in polar bioactive components and with the stronger antioxidant activity. Also, applying the ScCO_2 extraction had a positive effect on the MAE, leading to an increase in the content of polar components and antioxidant activity of the extracts obtained with lower extraction energy. ScCO_2 extraction did not show a positive effect on the improvement of a polyphenol yield under all examined conditions of UAE and MAE. However, this approach allows more adequate and efficient exploitation of material with regard to the extraction of lipophilic and polar fractions. Furthermore, the results of this study show a way to maximize the utilization of *V. odorata* material by applying extraction techniques that fit into the concept of green chemistry.

References

- Akhbari, M., Batooli, H., Kashi, F. J. (2012): Composition of essential oil and biological activity of extracts of *Viola odorata* L. from central Iran. *Nat. Prod. Res.* 26 (9), 802-809. <https://doi.org/10.1080/14786419.2011.558013>.
- Balachandran, S., Kentish, S. E., Mawson, R., Ashokkumar, M. (2006): Ultrasonic enhancement of the supercritical extraction from ginger. *Ultrason. Sonochem.* 13 (6), 471-479. <https://doi.org/10.1016/j.ultrsonch.2005.11.006>
- Chemat, F., Tomao, V., Virost, M. (2008): Ultrasound-assisted extraction in food analysis. *Handbook of Food Analysis Instruments*, pp. 85-103.
- Da Porto, C., Porretto, E., Decorti, D. (2013): Comparison of ultrasound-assisted extraction with conventional extraction methods of oil and polyphenols from grape (*Vitis vinifera* L.) seeds. *Ultrason. Sonochem.* 20 (4), 1076-1080. <https://doi.org/10.1016/j.ultrsonch.2012.12.002>
- Drozdova, I. L., Bubenchikov, R. A. (2005): Composition and antiinflammatory activity of polysaccharide complexes extracted from sweet violet and low mallow. *Pharm. Chem. J.* 39 (4), 197-200. doi:0091-150X/05/3904-0197
- Ebrahimzadeh, M. A., Nabavi, S. M., Nabavi, S. F., Bahramian, F., Bekhradnia, A. R. (2010): Antioxidant and free radical scavenging activity of *H. officinalis* L. var. *angustifolius*, *V. odorata*, *B. hyrcana* and *C. speciosum*. *Pak. J. Pharm. Sci.* 23 (1), 29-34. doi:199557137
- Feyzabadi, Z., Ghorbani, F., Vazani, Y., Zarshenas, M. M. (2017): A critical review on phytochemistry, pharmacology of *Viola odorata* L. and related multipotential products in traditional Persian medicine. *Phytother. Res.* 31 (11), 1669-1675. <https://doi.org/10.1002/ptr.5909>
- Ganzler, K., Salgó, A., Valkó, K. (1986): Microwave extraction: A novel sample preparation method for chromatography. *J. Chromatogr. A* (371), 299-306. [https://doi.org/10.1016/S0021-9673\(01\)94714-4](https://doi.org/10.1016/S0021-9673(01)94714-4)
- Hamed, A., Zarshenas, M. M., Sohrabpour, M., Zargaran, A. (2013): Herbal medicinal oils in traditional Persian medicine. *Pharm. Biol.* 51 (9), 1208-1218. <https://doi.org/10.3109/13880209.2013.777462>
- Henning, J. A., Core, R. J., Gardea-Torresdey, J. L. (1994): Extracting volatile compounds from single plants using supercritical fluid extraction. *Crop Sci.* 34 (4), 1120-1122. <https://doi.org/10.2135/cropsci1994.0011183X00340040053x>
- Herrmann, A., Burman, R., Mylne, J. S., Karlsson, G., Gullbo, J., Craik, D. J., Göransson, U. (2008): The alpine violet, *Viola biflora*, is a rich source of cyclotides with potent cytotoxicity. *Phytochem.* 69 (4), 939-952. <https://doi.org/10.1016/j.phytochem.2007.10.023>
- Ignat, I., Volf, I., Popa, V. I. (2011): A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chem.* 126 (4), 1821-1835. <https://doi.org/10.1016/j.foodchem.2010.12.026>
- Jokić, S., Nagy, B., Zeković, Z., Vidović, S., Bilić, M., Velić, D., Simándi, B. (2012): Effects of supercritical CO₂ extraction parameters on soybean oil yield. *Food Bioprod. Process.* 90 (4), 693-699.
- Karioti, A., Furlan, C., Vincieri, F. F., Bilia, A. R. (2011): Analysis of the constituents and quality control of *Viola odorata* aqueous preparations by HPLC-DAD and HPLC-ESI-MS. *Anal. Bioanal. Chem.* 399 (4), 1715-1723. <https://doi.org/10.1007/s00216-010-4473-2>
- Koochek M, Pipelzadeh M, Mardani H. (2003): The effectiveness of *Viola odorata* in the prevention and treatment of formalin-induced lung damage in the rat. *J. Herbs. Spices. Med. Plants.* 10 (2), 95-103. https://doi.org/10.1300/J044v10n02_11
- Mittal, P., Gupta, V., Goswami, M., Thakur, N., Bansal, P. (2015): Phytochemical and pharmacological potential of *Viola odorata*. *Int. J. Pharmacogn.* Vol. 2(5): 215-220. <https://doi.org/10.5812/ans.81233>
- Nadalin, V., Lepojević, i., Ristić, M., Vladić, J., Nikolovski, B., Adamović, D. (2014): Investigation of cultivated lavender (*Lavandula officinalis* L.) extraction and its extracts. *CICEQ.* 20 (1), 71-86. <https://doi.org/10.2298/CICEQ120715103N>
- Pan, X., Niu, G., Liu, H. (2002): Comparison of microwave-assisted extraction and conventional extraction techniques for the extraction of tanshinones from *Salvia miltiorrhiza bunge*. *Biochem. Eng. J.* 12 (1), 71-77. [https://doi.org/10.1016/S1369-703X\(02\)00039-6](https://doi.org/10.1016/S1369-703X(02)00039-6)
- Reverchon, E., Volpe, M. C., Caputo, G. (2003): Supercritical fluid processing of polymers: composite particles and porous materials elaboration. *Curr. Opin. Solid. State. Mater. Sci.* 7 (4-5), 391-397. <https://doi.org/10.1016/j.cossms.2004.01.002>
- Stojković, D., Glamočlija, J., Čirić, A., Šiljegović, J., Nikolić, M., Soković, M. (2011): Free radical scavenging activity of *Viola odorata* water extracts. *J. Herbs. Spices. Med. Plants.* 17 (3), 285-290. <https://doi.org/10.1080/10496475.2011.603588>
- Vidović, S., Simić, S., Gavarić, A., Aćimović, M., Vladić, J. (2020): Extraction of sweet wormwood (*Artemisia annua* L.) by supercritical carbon dioxide. *Natural Medicinal Materials.* (40), 22-26. <http://dx.doi.org/10.5937/leksir2040022V>
- Vidović, S., Zeković, Z., Marošanić, B., Todorović, M. P., Vladić, J. (2014): Influence of pre-treatments on yield, chemical composition and antioxidant activity of *Satureja montana* extracts obtained by supercritical carbon dioxide. *J. Supercrit. Fluid.* (95), 468-473. <https://doi.org/10.1016/j.supflu.2014.10.019>
- Vishal, A., Parveen, K., Pooja, S., Kannappan, N., Kumar, S. (2009): Diuretic, laxative and toxicity Studies of *Viola odorata* aerial parts. *Pharmacologyonline.* 1, 739-748.
- Vladić, J., Zeković, Z., Jokić, S., Svilović, S., Kovačević, S., Vidović, S. (2016): Winter savory: Supercritical carbon dioxide extraction and mathematical modeling

of extraction process, *J. Supercrit. Fluid.* (117), 89–97. <https://doi.org/10.1016/j.supflu.2016.05.027>

Wheeler, J. R., McNally, M. E. (1989): Supercritical fluid extraction and chromatography of representative

agricultural products with capillary and microbore columns. *J. Chromatogr. Sci.* 27 (9), 534-539. <https://doi.org/10.1093/chromsci/27.9.534>

Supplementary data

Table S1.

UAE	TP [mg GAE/g DE*]		TF [mg CAT/g DE]		IC ₅₀ [µg/mL]	
	Residue	Raw material	Residue	Raw material	Residue	Raw material
40°C; 40 min	60.89±1.51	62.21±1.16	15.48±0.41	15.72±0.18	31.98±0.69	30.21±0.86
40°C; 20 min	70.37±1.74	64.57±1.33	17.62±0.29	16.54±0.44	24.89±0.49	25.78±1.56
50°C; 20 min	59.55±0.562	64.28±0.89	14.93±0.34	15.24±0.25	28.24±0.81	27.74±0.41

*DE – Dried extract

Table S2.

MAE	TP [mg GAE/g DE*]		TF [mg CAT/g DE]		IC ₅₀ [µg/mL]	
	Residue	Raw material	Residue	Raw material	Residue	Raw material
470 W	58.16±0.99	51.24±1.49	13.47±0.69	12.98±0.38	32.81±0.49	35.36±0.94
800 W	54.60±1.03	60.51±1.26	13.87±0.16	15.34±0.37	33.88±0.37	30.13±1.05

*DE – Dried extract