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Headspace gas chromatographic determination of β-caryophyllene in *Epilobium angustifolium* L. extracts

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Epilobium angustifolium L. is a plant widely used in folk medicine. It is rich in biologically active compounds including phenols, flavonoids, terpenes, aliphatic acids and sterols; however, until now, little attention has been paid to its volatile components. This research demonstrated that *Epilobium angustifolium* L. is rich in β-caryophyllene that is possibly responsible for its unique therapeutic properties. The leaves of *Epilobium angustifolium* L. are traditionally used as a tea. On the other hand, β-caryophyllene is soluble in oils, thus an extract of *Epilobium angustifolium* L. in edible oils should also demonstrate a healing effect. Therefore, quick and reliable methods of β-caryophyllene determination in edible oils and in water solutions are required.

In this work, headspace gas chromatography is suggested to determine β-caryophyllene. At the optimized chromatographic conditions for β-caryophyllene solutions in coconut oil the calibration curve was linear up to 10 mg g^{-1}, the detection limit was 60 μg kg^{-1}, for β-caryophyllene solutions in water the calibration curve was linear up to 40 μg l^{-1}, and the detection limit was 6 μg l^{-1}. The methods were applied for the β-caryophyllene determination in the extract of *Epilobium angustifolium* L. in sunflower oil and in *Epilobium angustifolium* L. tea.

Keywords: headspace gas chromatography, *Epilobium angustifolium* L., β-caryophyllene

INTRODUCTION

Epilobium angustifolium L. (also known as *Chamaenerion angustifolium, Chamerion angustifolium*, fireweed, rosebay willowherb and great-willowherb) is a medicinal plant that belongs to the Onagraceae family. It demonstrates anti-inflammatory, antioxidant, analgesic and anticancer properties, is widely used in folk medicine to treat gastrointestinial disorders and mucous membrane lesions, and to heal wounds, skin sores and swelling [1–7]. Leaves and grass of the plant are most commonly used as medicinal raw materials, though flowers, roots and stems are also edible. The leaves are traditionally used as a tea [8].

The relationship between the properties of *Epilobium angustifolium* L. and its composition has not been studied in detail. The healing properties of *Epilobium angustifolium* L. are thought to be due to the polyphenols, tannins and flavonoids it contains, but an insufficient attention is paid to volatile compounds, including β-caryophyllene. To our knowledge, there is only one publication mentioning the presence of β-caryophyllene in *Epilobium angustifolium* L. [9].

On the other hand, the similarity of the pharmacological properties of β-caryophyllene

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(analgesic, antioxidant, anti-inflammatory, antidepressant and cancer cell growth inhibitory effects) [10] and of *Epilobium angustifolium* L. suggests that mainly β-caryophyllene is responsible for the unique therapeutic effect of *Epilobium angustifolium* L. The correlation could be confirmed by more detailed research. This requires a rapid and reliable determination of β-caryophyllene.

β-Caryophyllene is usually determined by gas chromatography [11–13]. However, the preparation of multicomponent plant samples for gas chromatographic analysis is usually complex and time-consuming. A perfect choice for the determination of volatile compounds could be headspace sampling combined with gas chromatography. Headspace gas chromatography (HS-GC) includes the isolation of a volatile analyte in the gas phase and the subsequent automatic delivery of an aliquot of vapour to the GC system.

The aim of this work was to elaborate a fast and efficient headspace gas chromatographic method for the determination of β-caryophyllene in *Epilobium angustifolium* L. tea and in *Epilobium angustifolium* L. enriched edible oils.

EXPERIMENTAL

Reagents and samples

Glycerol (≥98%) and β-caryophyllene (≥80%) were purchased from Sigma-Aldrich (Germany). Coconut oil Naturalisimo (the Netherlands) and refined sunflower oil (Ukraine) were purchased in a local supermarket.

β-Caryophyllene stock solutions in coconut oil and in glycerol (10 mg g^{-1} each) were prepared by weighting. Working β-caryophyllene solutions in coconut oil were prepared by dilution of β-caryophyllene stock solutions in coconut oil with coconut oil to a required concentration. Working β-caryophyllene solutions in water were prepared by dilution of β-caryophyllene stock solutions in glycerol with distilled water to a required concentration.

Instrumentation and conditions

Microwave assisted extraction was performed in a microwave reactor Monowave 450 (Anton Paar). Solid phase microextraction (SPME) was performed with a Supelco DVB/CAR/PDMS (50/30 μm) fibre housed in a manual holder (Supelco Bellofonte, PA, USA). SPME was carried out in a 20 ml vial closed with a silicone rubber septum placed in a cap. The vial was placed in a water-jacketed vessel on a magnetic stirrer and kept at 80°C temperature for 20 min. Desorption was carried out at 250°C for 30 s.

Headspace gas chromatographic analysis was performed on a PerkinElmer Clarus 580 series gas chromatograph (PerkinElmer, USA) equipped with a flame ionisation detector (temperature 250°C, hydrogen flow 40 ml min^{-1}, air flow 400 ml min^{-1} and auxiliary gas (helium) flow 30 ml min^{-1}). The GC system was equipped with the Rxi®-5Sil MS capillary column (30 m × 0.25 mm id, 0.25 μm film thickness).

Headspace extraction and sample introduction was performed on a PerkinElmer Headspace Sampler Turbomatrix 16 (PerkinElmer, USA) equipped with a balanced pressure system. Twenty millilitre headspace vials were used in all experiments. A headspace vial was positioned in the HS autosampler and equilibrated at selected temperature. The needle temperature and the transition line temperature was 10°C higher than the headspace vial equilibration temperature. The settings of the headspace sampler were 1 min for pressurization and 0.09 min for injection. Helium was employed as carrier gas with 16.7 psi column head pressure. The injector temperature was held at 110°C. The oven temperature was programmed as follows: 60°C for 1 min, from 60 to 250°C at 10°C min^{-1} and held for 2 min.

Gas chromatographic-mass spectrometric (GC-MS) analysis was performed on a PerkinElmer Clarus 580 series gas chromatograph equipped with a programmable temperature vaporizer injector and coupled to a PerkinElmer Clarus 560 S mass spectrometer (PerkinElmer, Shelton, USA). The system was equipped with the Elite-5MS capillary column (30 m × 0.25 mm id, 0.25 μm film thickness). Helium was employed as carrier gas with a constant flow of 1 ml min^{-1}. Injection was performed in the split mode (20:1). The oven temperature was programmed as follows: from 45 to 100°C at 2°C min^{-1}, from 100 to 250°C at 5°C min^{-1} and held for 5 min.

The transfer line temperature was 280°C. The electron ionization ion source conditions were as follows: electron energy 70 eV and temperature 180°C. GC-MS in the full scan mode was

used. Data acquisition was performed in a range of m/z 45–500. The qualitative identification of different compounds was performed by comparing their mass spectra with those stored in the NIST (National Institute of Standards and Technology) library.

Sample preparation

For *Epilobium angustifolium* L. infusion in edible oil, 10 g of sunflower oil was added to 1 g of dried and grinded leaves, and the mixture was stored for 14 days.

For *Epilobium angustifolium* L. tea, 100 ml of boiling water was added to 3 g of dried and grinded leaves, and the mixture was stored for 20 min.

RESULTS AND DISCUSSION

Preliminary analysis of *Epilobium angustifolium* L.

Epilobium angustifolium L. was first checked for β-caryophyllene using solid-phase microextraction (SPME) and gas chromatographic-mass spectrometric analysis. For this 0.2 g of dry grinded leaves was placed in a 20 ml vial and subjected to SPME for 20 min at 80°C temperature. Desorption of the SPME fibre was carried out in the GC-MS injection port at 250°C for 30 s. The results of the analysis showed (Fig. 1) that the main volatile components of *Epilobium angustifolium* L. are 2-hexenal (retention time 4.41 min), α-bourbonene (31.62 min), β-caryophyllene (32.94 min), α-caryophyllene (34.12 min), germacrene D (35.04 min), caryophyllene oxide (37.96 min) and 3-phenethylbenzonitrile (54.83 min). Thus an extract of *Epilobium angustifolium* L. is a promising source of β-caryophyllene.

Further a possibility of *Epilobium angustifolium* L. analysis by HS-GC was investigated as HS-GC eliminates the need to use SPME and is therefore expected to be a cheaper and more convenient method. In addition, a simple flame ionisation detector was used instead of a mass spectrometer.

HS-GC conditions were selected to allow a rapid separation of β-caryophyllene. For this 0.2 g of dry crushed leaves was placed into the headspace vial and heated at 140°C for 20 min, and subsequently the gas phase was injected for GC analysis. Various oven temperature programs have been tested. A well-separated peak of β-caryophyllene is seen in the HS-GC chromatogram obtained under optimal conditions (Fig. 2).

Two types of solutions were further investigated by HS-GC: tea (*Epilobium angustifolium* L. extract in water) and *Epilobium angustifolium* L. infusion in sunflower oil.

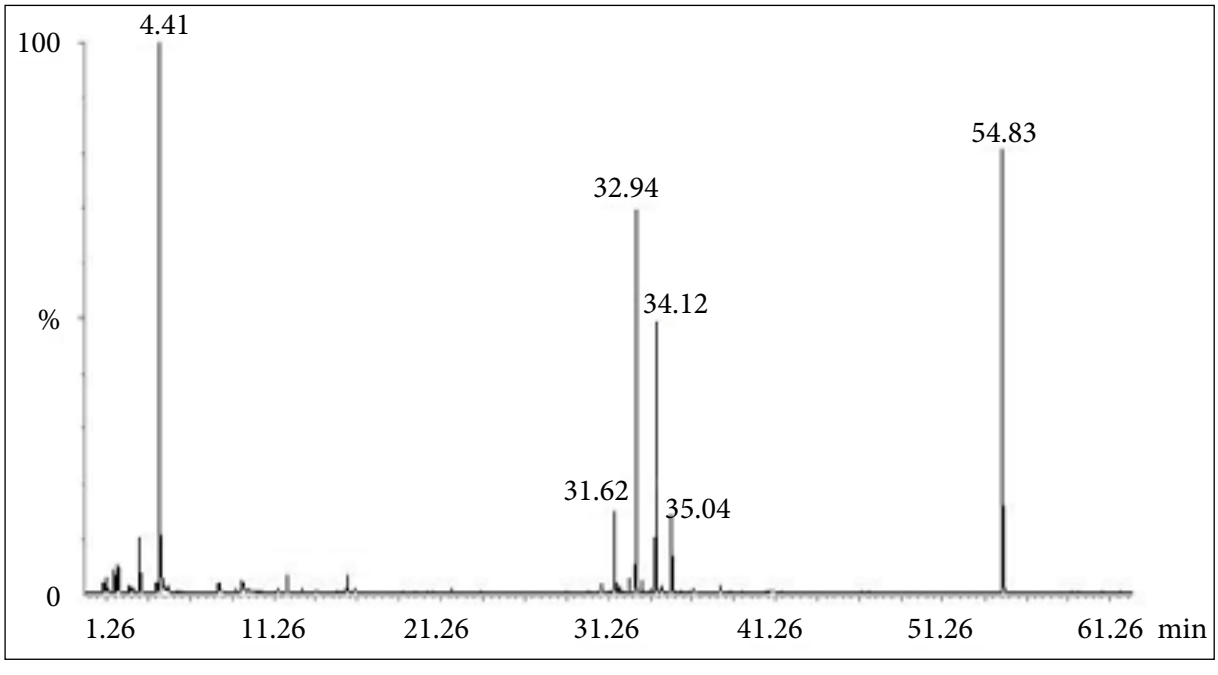

Fig. 1. Chromatogram of dry *Epilobium angustifolium* L. obtained by SPME and GC-MS analysis. For SPME and GC-MS conditions see Experimental

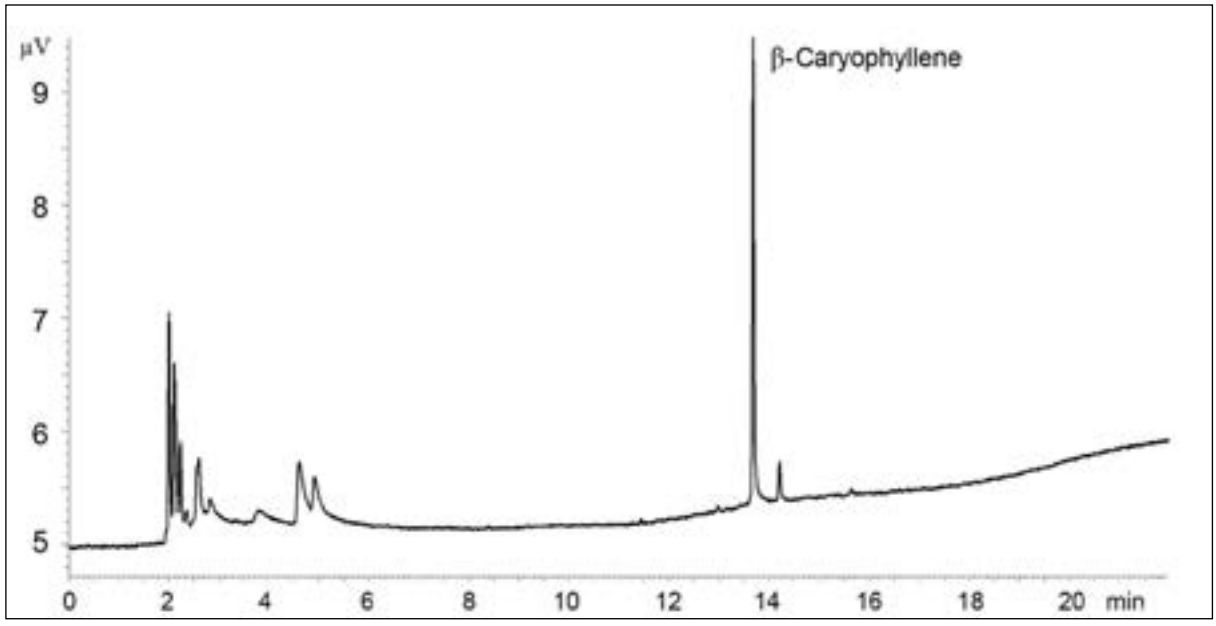

Fig. 2. HS-GC chromatogram of dry *Epilobium angustifolium* L. For HS-GC conditions see Experimental

Determination of β-caryophyllene in edible oil solution

It is known that coconut oil is stable at elevated temperatures applied for HS-GC analysis [14] thus it was used for the preparation of standard β-caryophyllene solutions. For the experiments 1 g of 1 mg g^{-1} standard β-caryophyllene solutions in coconut oil was used.

The dependence of the β-caryophyllene peak area on the heating temperature of the headspace vial was examined and it was determined that the peak area permanently increased with the temperature and reached the maximum value at 200°C (Fig. 3). Unfortunately, the investigation of the heating time influence on the peak area demonstrated that when the sample was heated at 200°C for more than 10 min, the area of the β-caryophyllene peak began to decrease (Fig. 4). This is most likely because of the decomposition of β-caryophyllene at high temperatures. When the heating temperature was reduced to 180°C, the area of the β-caryophyllene peak initially increased, then from 15 to 20 min remained stable, and slightly decreased by heating for more than 20 min. Thus, for a better repeatability of the peak area, further the sample was heated at 180°C for 15 min.

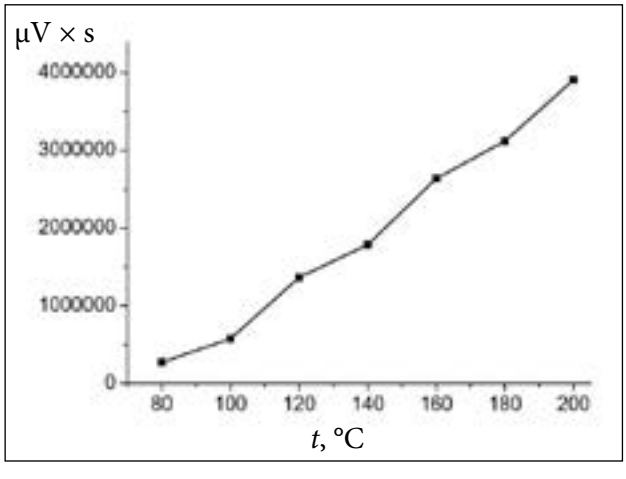

Fig. 3. Dependence of the β-caryophyllene peak area on the heating temperature of the headspace vial

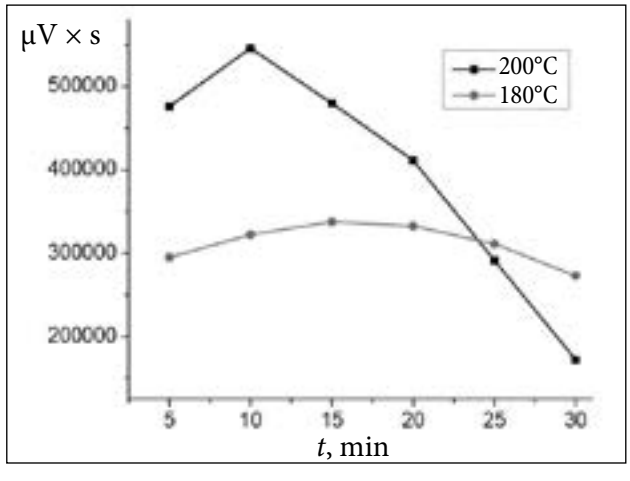

Fig. 4. Dependence of the β-caryophyllene peak area on the heating time of the headspace vial

The effect of the sample size on the β-caryophyllene peak area was investigated using 1–5 g of β-caryophyllene solution. The results demonstrated that the area of the β-caryophyllene peaks changed insignificantly with the amount of the sample (Table). The Q-test performed confirmed that neither the biggest nor the smallest peak area should be rejected as a gross error.

Table. **β-Caryophyllene peak areas obtained using different sample amount**

Sample amount, g	Peak area, mV × s
1	326367
2	304298
3	334852
4	315533
5	297426

An insignificant effect of the sample quantity on the analyte concentration in the headspace can be explained considering the formula [15]

$$c_G = c_S/(K + \beta),$$

where c_G is the concentration of the analyte in the gas (headspace) phase, c_S is the concentration of the analyte in the sample (liquid) phase, K is the partition coefficient of the analyte between the sample and gas phases, and β is the ratio of the volumes of the gas and liquid phases.

The equation shows that if K is low (the compound prefers the headspace phase), then the value of β (hence sample quantity) significantly affects the concentration in the headspace phase. Conversely, if K is high (the compound favours the sample phase), then adjusting β will have a minor effect on the concentration in the headspace phase.

β-Caryophyllene is not very volatile (its boiling point is 262°C) and is highly soluble in oils [16], therefore its partition coefficient between the oil and gas phases is large, so the phase ratio has a little effect on the concentration of β-caryophyllene in the headspace. That was confirmed by the results obtained. Based on this, 1 g of sample was used for further analysis.

Quality parameters were determined under the optimized conditions. The calibration curve was drawn with 6 calibration points with three replicate injections and was linear up to 10 mg g^{-1} with the correlation coefficient 0.999. The limit of detection was calculated as three times the baseline noise and was 60 μg kg^{-1}. The relative standard deviation was determined by five replication analysis of the sample with the β-caryophyllene concentration 0.1 mg g^{-1} and was 3.8%.

The method was applied for the determination of β-caryophyllene in *Epilobium angustifolium* L. infusion in sunflower oil. A headspace chromatogram of the infusion is shown in Fig. 5. It was found that the infusion contained 1.3 mg kg^{-1} of β-caryophyllene.

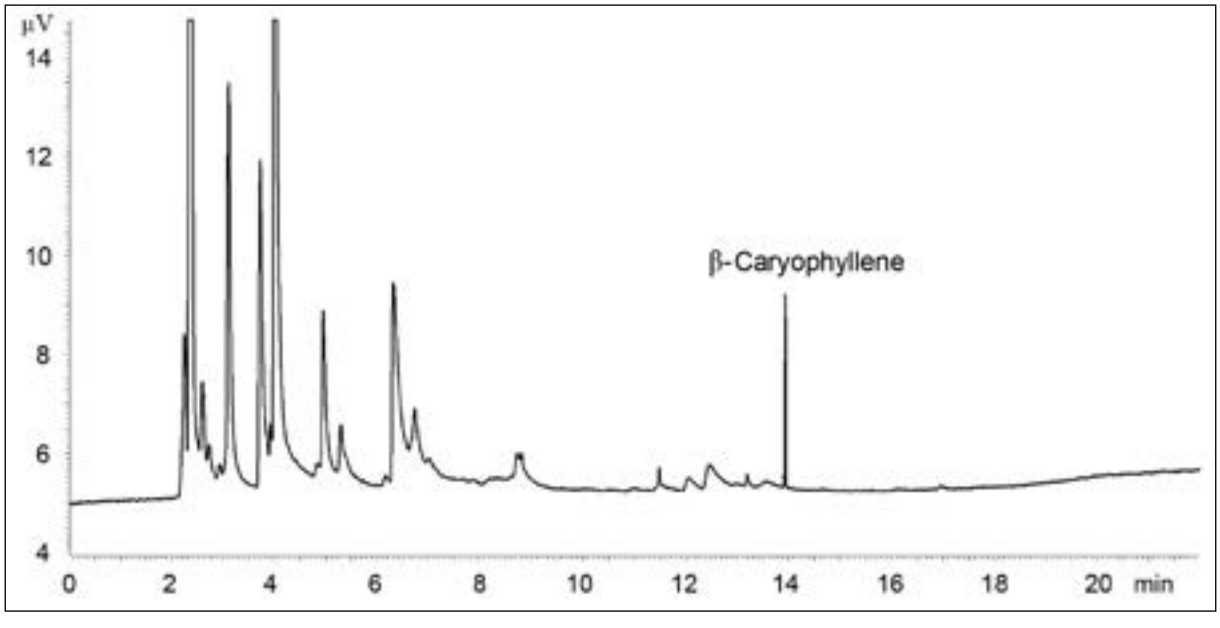

Fig. 5. HS-GC chromatogram of *Epilobium angustifolium* L. infusion in sunflower oil. For HS-GC conditions see Experimental

Determination of β-caryophyllene in aqueous solution

The solubility of β-caryophyllene in water is rather low, at 25°C it is 50.11 μg l^{-1} [17]. Therefore, for the preliminary study an aqueous solution containing 40 μg l^{-1} of β-caryophyllene was used.

For HS-GC, the heating temperature of the sample must be lower than the boiling point of the main matrix component. Since in the case of aqueous solutions the main component of the matrix is water, the headspace vial was heated to 95°C temperature. Unfortunately, the headspace chromatogram did not show a peak of β-caryophyllene even when 10 ml of the solution was analysed.

The sensitivity of HS-GC can be increased by raising the heating temperature of the sample. In the case of aqueous solutions the heating temperature cannot exceed 100°C. To overcome the limitation, a possibility to transfer β-caryophyllene to a matrix with a higher boiling point has been investigated. Coconut oil was chosen as the extraction agent. As β-caryophyllene is highly soluble in oils, coconut oil should quantitatively extract it from the aqueous solution. After, as shown above, a solution of β-caryophyllene in coconut oil can be heated to 180°C resulting in easier transfer of β-caryophyllene to the headspace.

Extraction of β-caryophyllene was performed in a microwave extractor at 80°C for 5 min. Microwave assisted extraction is very fast. Additionally, at elevated temperatures the coconut oil is liquid and can be easily taken for further analysis. For the extraction 20 ml of aqueous β-caryophyllene solution and 2 g of coconut oil were taken. After extraction, 1 g of the extract was transferred to a headspace vial and subjected to HS-GC analysis under the conditions described above.

The calibration curve was drawn with 6 calibration points with three replicate injections and was linear up to 40 μg l^{-1} with the correlation coefficient 0.987. The limit of detection was calculated as three times the baseline noise and was 6 μg l^{-1}. The relative standard deviation was determined by five replication analysis of the sample with the β-caryophyllene concentration 20 μg l^{-1} and was 11.7%.

The method was applied for the determination of β-caryophyllene in *Epilobium angustifolium* L. tea. It was found that the tea contained 31 μg/l^{-1} of β-caryophyllene.

CONCLUSIONS

Edible oils with a high content of unsaturated fatty acids are susceptible to oxidation. In the literature there are a lot of data concerning the improvement of the oxidation stability of vegetable oils by addition of essential oils from plants used as condiments such as mint, laurel, thyme, rosemary, basil, clove, cinnamon and others [18–21]. However, to the best of our knowledge, there is no information on the use of *Epilobium angustifolium* L. for the quality improvement of edible oils. Since *Epilobium angustifolium* L. and one of its main volatile components β-caryophyllene are known to have good antioxidant properties, we propose to use *Epilobium angustifolium* L. as an additive for edible oils. β-caryophyllene enriched edible oils are also of a good taste and should demonstrate favourable pharmacological properties.

The results presented in this article demonstrate that HS-GC analysis can be successfully applied for the determination of β-caryophyllene in *Epilobium angustifolium* L. infusions in edible oils. The influence of *Epilobium angustifolium* L. on the oxidation stability of vegetable oils is in progress.

The leaves of *Epilobium angustifolium* L. are traditionally used as a tea. The tea not only has a pleasant specific aroma, but also has healing properties. However, the solubility of β-caryophyllene in water is relatively low, so its concentration in tea is close to the detection limit of the proposed method. To determine lower concentrations of β-caryophyllene, the method of choice should be SPME followed by GC.

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References

1. S. Granica, J. P. Piwowarski, M. E. Czerwinska, A. K. Kiss, *J. Ethnopharmacol.*, **156**, 316 (2014).
2. H. Ostrovska, O. Oleshchuk, S. Vannini, et al., *EuroBiotech J.*, **1**, 126 (2017).
3. B. Tita, H. Abdel-Haq, A. Vitalone, G. Mazzanti, L. Saso, *Il Farmaco*, **56**, 341 (2001).
4. L. Battinelli, B. Tita, M. G. Evandri, G. Mazzanti, *Il Farmaco*, **56**, 345 (2001).
5. A. Vitalone, F. Bordi, C. Baldazzi, G. Mazzanti, L. Saso, B. Tita, *Il Farmaco*, **56**, 483 (2001).

6. A. Vitalone, M. Guizzetti, L. G. Costa, B. Tita, *J. Pharm. Pharmacol*., **55**, 683 (2003).
7. A. Kavaz Yuksel, E. Dikici, M. Yuksel, M. Isık, F. Tozoglu, E. Koksal, *J. Food Meas. Charact.* (2021).
8. *Aegean Edibles* [https//aegeanedibles.weebly.com/epilobium-angustifolium.html].
9. V. Kaskoniene, A. Maruska, I. Akuneca, et al., *Nat. Prod. Res*., **30** 1373 (2016).
10. F. Francomano, A. Caruso, A. Barbarossa, et al., *Appl. Sci.*, **9**, 5420 (2019).
11. M. H. Park, C. J. Kim, J. Y. Lee, I. S. Kim, S. K. Kim, *Sci. Rep.*, **11**, 13853 (2021).
12. M. N. L. Ngo-Mback, E. B. Famewo, D. MubarakAli, et al., *Biocatal. Agric. Biotechnol*., **22**, 101412 (2019).
13. S. Mokhtarzadeh, B. Demirci, K. M. Khawar, N. Kirimer, *J. Essent. Oil-Bear. Plants*, **21**, 277 (2018).
14. V. Vičkačkaitė, G. Pipiraitė, V. Poškus, I. Jurkutė, B. Bugelytė, *Chemija*, **31**, 95 (2020).
15. A. Tipler, *An Introduction to Headspace Sampling in Gas Chromatography*, PerkinElmer, Inc. (2014).
16. *Beta-Caryophyllene | $C_{15}H_{24}$ – PubChem* [https://pubchem.ncbi.nlm.nih.gov/compound/beta-caryophyllene].
17. *Beta-Caryophyllene, 87-44-5 – The Good Scents Company* [https://thegoodscentscompany.com/data/rw1060851.html].
18. G. Beretta, R. Artali, R. M. Facino, F. Gelmini, *J. Pharm. Biomed. Anal*., **55**, 1255 (2011).
19. M. M. Ozcan, D. Arslan, *Food Chem*., **129**, 171 (2011).
20. G. Ozkan, O. Sagdic, R. S. Gokturk, O. Unal, S. Albayrak, *Food Sci. Technol*., **43**, 186 (2010).
21. B. Tepe, D. Daferera, A. S. Tepe, M. Polissiou, A. Sokmen, *Food Chem*., **103**, 1358 (2007).

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β-KARIOFILENO NUSTATYMAS *EPILOBIUM ANGUSTIFOLIUM* L. EKSTRAKTUOSE VIRŠERDVĖS DUJŲ CHROMATOGRAFIJOS METODU

S a n t r a u k a

Epilobium angustifolium L. augalas yra plačiai naudojamas liaudies medicinoje. Jame gausu biologiškai aktyvių junginių, tarp jų fenolių, flavonoidų, terpenų, alifatinių rūgščių, sterolių, tačiau iki šiol mažai dėmesio buvo skiriama augalo lakiesiems komponentams. Šis tyrimas atskleidė, kad *Epilobium angustifolium* L. turi daug β-kariofileno, kuris galbūt yra atsakingas už unikalias *Epilobium angustifolium* L. terapines savybes. Tradiciškai *Epilobium angustifolium* L. lapai naudojami arbatai. Kita vertus, β-kariofilenas tirpsta aliejuje, todėl tikėtina, kad *Epilobium angustifolium* L. ekstraktas valgomajame aliejuje taip pat pasižymi gydomuoju poveikiu. Reikalingi greiti ir patikimi β-kariofileno nustatymo valgomajame aliejuje ir arbatoje metodai.

Šiame darbe β-kariofileną siūloma nustatyti viršerdvės dujų chromatografijos metodu. Optimaliomis chromatografinėmis sąlygomis gauta β-kariofileno tirpalų kokosų aliejuje kalibravimo kreivė tiesinė iki 10 mg g^{-1}, aptikimo riba 60 μg kg^{-1}, β-kariofileno vandeninių tirpalų kalibravimo kreivė tiesinė iki 40 μg l^{-1}, aptikimo riba 6 μg l^{-1}. Parengtos metodikos leidžia nustatyti β-kariofileną *Epilobium angustifolium* L. ekstrakte saulėgrąžų aliejuje ir arbatoje.