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# SEED ESSENTIAL OIL ANALYSIS OF *Bunium persicum* BY HYDRODISTILLATION-HEADSPACE SOLVENT MICROEXTRACTION

**P. Salehi*, F. Mohammadi, and B. Asghari**

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*Bunium persicum* (Boiss.) B. Fedtsch. from the Umbelliferae family with the common name of wild caraway is a perennial herb with white or pink flowers and small brownish seeds which grows in warm climate zones of Iran [1]. It is also distributed in central Asia, Pakistan, Afghanistan, Keshmir, and Pamir [2].

The plant shows several therapeutic effects on digestive and urinary tract disorders and is well known as an anti-convulsion, anthelmintic, anti-asthma and anti-dyspnea in Iranian folk medicine. *B. persicu*m oil is capable of suppressing the initial stage of an inflammatory process [3].

Kalazira (*B. persicum*) is an expensive Indian spice, abaundantly used for culinary purposes and flavoring food and beverages [4]. The essential oil of *B. persicum* is used in the perfume industry and in confectionery [5]. The composition of the essential oil of *B. persicum* has been extensively investigated [3, 6].

Wide varieties of analytical methods are applicable for the extraction of the volatile compounds from plant material. The common techniques are hydrodistillation, supercritical fluid extraction (SFE), and solid phase microextraction (SPME) [6–8].

Microextraction is a technique, in which the extraction phase volume is much smaller than the sample volume; therefore, only a small fraction of sample can be extracted [9–11]. Headspace sampling for gas chromatographic analysis eliminates much of the interferences, which stem from the sample matrix [12].

Hydrodistillation-headspace solvent microextraction (HD-HSME) has already been introduced as a rapid and eco-friendly method for extraction and preconcentration of the essential oil from aromatic plants [12]. Here its application for preconcentration and analysis of the seed essential oil of *B. persicum* is reported.

Ordinary hydrodistillation for extraction of essential oils needs a large amount of plant and 2–4 h of time. If the objective of an extraction is only the analysis of the oil sample, HD-HSME could be a rapid and green variation. The present study was commenced by optimization of different factors, such as extracting solvent, sample amount, microdrop volume, extraction time, and cooling time for the HD-HSME method.

The success of HD-HSME is largely dependent on the selection of an appropriate solvent with high extraction efficiency. Moreover, the solvent should not evaporate under the extraction conditions. Among the tested solvents *n*-heptadecane showed low extraction efficiency. However, *n*-pentadecane gave the best results and its peak appeared at the end of the chromatogram with no interference with analyte peaks. *n*-Hexadecane was chosen as internal standard to correct the variation of injection volumes.

The influence of sample mass on the composition of the extracted compounds was investigated. The extracted amounts of the main constituents increased continuously with increase in sample weight up to 0.4 g and then decreased, which could be attributed to the saturation of the microdrop with volatile compounds. The optimum weight was then chosen at 0.4 g.

The study of the influence of microdrop volume on the analytical signal showed that extraction efficiency was raised up to a volume equal to 2 μL and decreased after that to reach a constant value. Therefore, the optimum microdrop volume was adjusted at 2 μL.

Study of extraction time versus relative peak area showed that the optimum time for extraction was 5 min for most of the main constituents.

Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, P. O. Box 19835-389, Evin, Tehran, Iran, e-mail: p-salehi@sbu.ac.ir. Published in Khimiya Prirodnykh Soedinenii, No. 1, pp. 87-88, January-February, 2008. Original article submitted October 18, 2006.

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TABLE 1. Essential Oil Constituents of the Seeds of *Bunium persicum* by Standard Hydrodistillation and HD-HSME Methods

| Compound | RI[a] | Percentage | | RSD, %[b] |
|---|---|---|---|---|
| | | HD | HD-HSME | |
| $\alpha$-Thujene | 927 | 0.5 | 0.2 | 10.1 |
| $\alpha$-Pinene | 936 | 1.3 | 0.5 | 19.4 |
| Sabinene | 970 | 1.2 | 0.6 | 13.3 |
| $\beta$-Pinene | 977 | 2.1 | 1.4 | 20.7 |
| Myrcene | 982 | 1.1 | 0.7 | 12.2 |
| *p*-Cymene | 1016 | 5.7 | 5.5 | 12.5 |
| Limonene | 1026 | 11.0 | 15.7 | 11.8 |
| $\gamma$-Terpinene | 1054 | 20.1 | 29.3 | 11.6 |
| *cis*-Sabinene hydrate | 1057 | 0.2 | - | - |
| $\alpha$-Terpinolene | 1082 | 2.7 | 2.3 | 18.1 |
| 4-Terpineol | 1164 | 1.0 | 0.4 | 4.7 |
| Anthemol | 1173 | 1.7 | 1.7 | 2.8 |
| Cuminic aldehyde | 1219 | 16.6 | 15.5 | 7.4 |
| *p*-Menth-2-en-7-ol | 1248 | 0.9 | 0.2 | 14.1 |
| *p*-Menth-1-en-7-al | 1253 | 0.2 | - | - |
| *p*-Mentha-1,3-dien-7-al | 1262 | 15.1 | 11.5 | 12.6 |
| *p*-Mentha-1,4-dien-7-al | 1268 | 13.2 | 13.4 | 10.9 |
| *p*-Mentha-1,4-dien-7-ol | 1302 | 1.5 | 0.5 | 8.7 |
| *trans*-Caryophyllene | 1424 | 0.6 | - | - |
| Sabinol | 1435 | 1.6 | 0.4 | 11.3 |
| Germacrene-D | 1481 | 0.2 | - | - |
| $\beta$-Bisabolene | 1500 | 0.1 | - | - |

[a]Retention indices relative to $C_6$-$C_{24}$ on the DB-1 column.
[b]Relative standard deriation.

In the first report on HD-HSME, the extraction was made during the hydrodistillation process (hot method). However, we found that in the case of oils containing heavier terpenoids, it was better to start the extraction a while after turning off the heater (cold method). Therefore, the effect of cooling time on the extraction efficiency was studied. By paying careful attention we found that the optimum cooling time was 3 minutes.

The repeatability of the method performed under optimized conditions (i.e., sample weight 0.4 g; extraction time 5 min; drop volume 2 μL) was determined by analyzing the samples in triplicate. The precision is defined as the relative standard deviation (RSD) (Table 1).

The same procedure that has been reported previously was used [13]. Briefly, 0.4 g of the dried plant in 80 mL of water was heated by a mantle in a 100 mL round bottom flask. The mixture was refluxed for 15 min. After uptake of 2 μL of *n*-pentadecane containing *n*-hexadecane (as internal standard, 200 ppm), the needle of the syringe was inserted into the headspace of the plant sample. For initiating the extraction, the syringe plunger was depressed and a microdrop of extracting solvent was suspended from the needle tip. After extraction for an optimized period, the plunger was withdrawn and the needle was removed from the headspace and injected into the GC or GC-MS injection port. The response was calculated as the relative peak area of the analytes to the internal standard in the GC chromatogram [14 – 16].

HD-HSME is a simple, rapid, and rather cheap method for preconcentration and analysis of the essential oil from aromatic plants and their seeds. It is also environmentally benign because only a few microliter of the solvent is used. The application of HD-HSME in the rapid and quantitative analysis of volatile compounds in foods, cosmetics, medicines, and perfumes is under investigation.

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**REFERENCES**

1. A. Ghahraman, *Plant Systematics. Cormophytes of Iran*, Vol. **2**, Nashre Daneshgahi Publisher, Tehran, 1993, p. 671.
2. A. Zargari, *Medicinal Plants*, Vol. **2**, Tehran University Press., Tehran, 1996, pp. 509–515.
3. M. H. Booskabaky and A. Moghaddas, *Iran Biomed. J.*, **8**, 149 (2004).
4. R. K. Thappa, S. Ghosh, S. G. Agarwal, A. K. Raina, and P. S. Jamwal, *Food Chem.*, **41**, 129 (1991).
5. M. H. Salehi Sormaghi, G. Amin, and S. Kaveh, *Bunium persicum*, in: *Iranian Herbal Pharmacopoeia*, N. Ghasemi Dehkordi (ed.), No. 2, Ministry of Health and Medical Education, Tehran, 2002, pp. 426–432.
6. A. Foroumadi, A. Asadipour, F. Arabpour, and Y. Amanzadeh, *J. Essent. Oil Res.*, **14**, 161 (2002).
7. K. K. Khaidarov, Y. D. Sadykov, L. D. Lebedeva, and M. B. Ismailova, *Khim.-Farm. Zh.*, **25**, 73 (1991).
8. S. M. Pourmortazavi, M. Ghadiri, and S. S. Hajimirsadeghi, *J. Food Compos. Anal.*, **18**, 439 (2005).
9. H. Lord and J. Pawliszyn, *J. Chromatogr. A.*, **885**, 153 (2000).
10. R. G. Belardi and J. Pawliszyn, *Water Pollut. Res. J. Can.*, **24**, 179 (1989).
11. C. L. Arthur and J. Pawliszyn, *Anal. Chem.*, **62,** 2145 (1990).
12. A. R. Fakhari, P. Salehi, R. Heydari, S. N. Ebrahimi, and P. R. Haddad, *J. Chromatogr. A*, **1098**, 14 (2005).
13. P. Salehi, B. Asghari, and F. Mohammadi, *Chromatographia*, **65**, 119 (2007).
14. T. Shibamoto, *Retention Indices in Essential Oil Analysis*, in: *Capillary Gas Chromatography in Essential Oil Analysis*, P. Sandra, C. Bicchi (eds.), Huethig Verlag, New York, 1987.
15. N. N. Davis, *J. Chromatogr.*, **503**, 1 (1990).
16. R. Adams, *Identification of Essential Oil Components by Gas Chromatography/Quadropole Mass Spectroscopy*, Allured, Carol Stream, U.S.A., 2001.