STUDIA UNIVERSITATIS BABEŞ-BOLYAI, CHEMIA, LIV, Sp.Iss. 2, 2009

OBTAINING AND IDENTIFICATION OF BIOACTIVE COMPOUNDS FROM *Ligularia sibirica* (L.) CASS

ÁRPÁD KAPÁS[a,b], BEÁTA ÁBRAHÁM[b], CSABA D. ANDRÁS[b], SZABOLCS LÁNYI[b], TĂNASE GH. DOBRE[a]

ABSTRACT. Secondary metabolites were obtained from Chinese medicinal plant, *Ligularia sibirica* by using coupled hydrodistillation-extraction method. The essential oil was extracted by microwave assisted hydrodistillation, analysis of these volatile compounds was made with GC-MS. The ethanolic extract was re-extracted with chloroform; the identification of the pyrrolizidine alkaloids were made with thin layer chromatography. The main identified volatile compounds were sabinene, limonene, and terpinolene, and the alkaloids in the extract were most probably, tussilagine and iso-tussilagine.

Keywords: Ligularia sibirica, microwave assisted hydrodistillation, essential oils, pyrrolizidine alkaloids

INTRODUCTION

Some of the species from vegetal kingdom can be important sources of bioactive secondary metabolites for pharmaceutical industry, however studying chemical composition of different, practically unknown plant species, can result solutions for developing new medicines and new therapies.

Twenty to forty *Ligularia* species (*Compositae*) have been used in traditional Chinese medicine due to their positive impact on health [1]. The most frequently identified bioactive compounds of these plants are eremophilane-type sesquiterpenes (ETS) and pyrrolizidine alkaloids (PA). Many isolated ETS have cytotoxic [2-4], antibacterial, antitumor [5] and anti-HIV [6] effect.

Four ETS and two PAs have been isolated from *L. sibirica*. Ligularol (1) and ligularine A (2) were extracted with supercritical carbon dioxide [7]. Ligularenolide and ligularone (3 and 4) were not tested from bioactivity viewpoint until now. In addition tussilagine and iso-tussilagine (5 and 6) PAs are possessing antimicrobial and immune system stimulation effect and are used for anti HIV-1 [8], HSV-1 and HSV-2 treatments [5, 9]. Tussilagine and iso-tussilagine were isolated from *L. sibirica* by Wiedenfeld et al. [10].

[a] *Universitatea Politehnica din Bucureşti, Facultatea Ştiinţa şi Ingineria Materialelor, Str. Polizu, Nr. 1-7, RO-010737, Bucureşti, Romania, tghdobre@gmail.com*

[b] *Universitatea Sapientia, Cluj-Napoca, Facultatea Miercurea Ciuc, Departamentul Ştiinţe Tehnice, Pţa. Libertăţii, Nr. 1, RO-530104, Miercurea Ciuc, Romania, kapasarpad@sapientia.siculorum.ro*

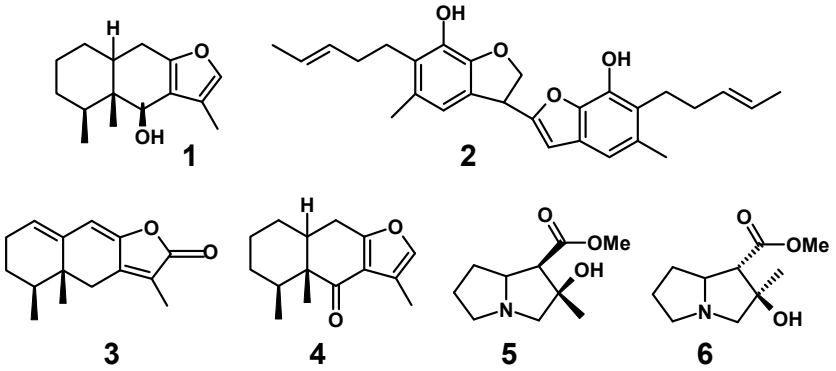

Figure 1. Identified compounds from *L. sibirica*

Chemical compositions of volatile oils obtained from this plant are unknown.

Commonly used extraction methods to obtaining volatile oils are hydrodistillation, supercritical carbon dioxide extraction, solvent extraction and simultaneous distillation-extraction method. All the methods have advantages and also disadvantages from energetical, analytical and ecological viewpoint.

Microwave assisted hydrodistillation (MWHD) became a widely used method for essential oils extraction from plants, due to its advantage of homogenous heating which results a more effective obtaining of heat sensible compounds [11,12]. During the MWHD process a pressure difference occurs between the inner and outer side of the plant cells, therefore the compounds are more easily released to the surrounding solution by breaking the external cell wall and resulting a higher mass transport of compounds [13].

Volatile oils have been reported as having good influence on human health due to their bioactive proprieties (for example: antitumor, cytotoxic, anti proliferative, anti-inflammatorily effect). The influence of oils on health depends on the bioactivity of different components and on the type of interactions between the compounds [17-31].

PAs obtaining method described in literature is based on extraction with ethanol or methanol followed by acid-soluble and acid-insoluble partition, which is based on PAs-salts high solubility in acid solutions. The final extraction with chloroform is based on neutralization at pH values from 9 to 10 [14,15]. Separation and purification of PAs can be proceeded by using silica gel column chromatography and/or thin layer chromatography (TLC) with eluent mixture of chloroform (in some case dichlormethane), methanol and ammonium hydroxide [14-16].

The aims of this paper are: I) extraction of essential oils from *L. sibirica* with microwave assisted hydrodistillation, II) use of chromatographic analysis to choose an adequate oil separation method and III) extraction of PAs from leaves with coupled extraction method to reduce compounds diversity in extracts.

RESULTS AND DISCUSSION

Gas chromatography analysis

Gas chromatography analysis resulted identification of forty two compounds, most of them being identified previously from another plant species and several being rare substances. The main compounds with good identification quality were sabinene (15.05%), limonene (10.95%), terpinolene (13.68%), γ-terpinene (5.56%), β-cariophylene (5.30%), α-pinene (2.85%), *d*-germacrene (1.44%), α-humulene (0.94%), α-phellandrene (0.84%), β-elemene (0.49%), β-cubanene (0.42%), δ-cadinene (0.39%) and α-copaene (0.18%). Notable quantity of indefinite identified compounds were *o*-toluamide (3.93%), 3-caren (0.55%), z,e-α-fernasene (0.58%), 1,z-5,e-7-dodecatriene (0.52%) theaspirane A (0.47). Other components were present in trace quantities and low identification quality.

TLC analysis results

TLC separation method resulted eight uncolored spots at visible light (fig. 2). The spots could be distinguished better at 254 nm than at 315 nm wavelength UV light. Spots 5 and 8 showed better spectrophotometric absorption at 220 nm wavelength, this value being known as maximal absorption value of pyrrolizidine alkaloids N-oxide form.

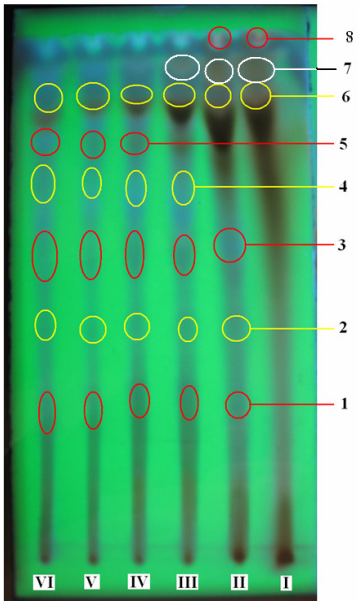

Figure 2. TLC plate photograph

CONCLUSIONS

Volatile oils from leaves of *Ligularia sibirica* were successful extracted with microwave assisted hydrodistillation method. The extract analysis with gas chromatography and mass spectrometry resulted identification of 42 components. Coupled extraction and TLC showed adequacy for obtaining PAs components.

EXPERIMENTAL SECTION

Materials and apparatus

The plant (*L. sibirica*) was collected in September of 2009 and stored in a cool and dry location. TLC G_{254} plates, dichloromethane, absolute methanol, 25% ammonium hydroxide solution, sulfuric acid were produced by Merck (Darmstadt, Germany).

The used microwave assisted hydrodistillation apparatus is a combination of a modified microwave oven with a Clevenger extension (fig. 3) and with a coupled rotation head.

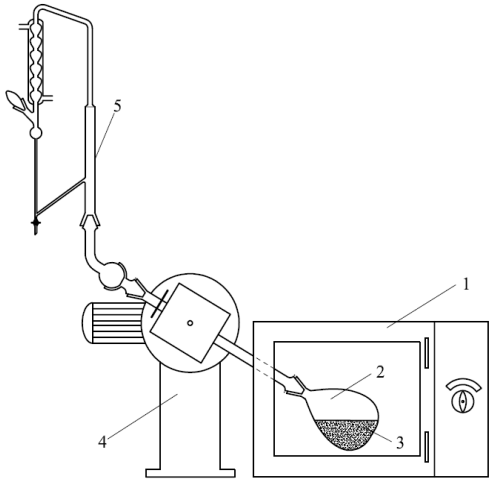

Figure 3. Microwave assisted hydrodistillation apparatus: 1-microwave oven, 2-balloon, 3-plant material, 4-rotation head and 5-Clevenger extension.

Microwave assisted hydrodistillation method

350 g of fresh and cracked leaves of *L. sibirica* and 500 ml distillated water were placed in the apparatus balloon. The electric power was set to 800 W. After 12 minutes of heating time the first drop appeared in the collector tube of Clevenger extension, following by 30 minutes of hydrodistillation procedure, which resulted 0.855 ml of essential oil.

Extraction procedure

After MWHD procedure the residue of plant material was dried in microwave oven under reduced pressure and extracted three times with 1.5 l of 80 %v/v ethanol at temperatures of 25, 40 and 70 °C. The resulted extracts were evaporated in vacuum at 40 °C by giving approximate 55 g of material which was dissolved in 300 ml 1 N sulfuric acid solution. The alkaloids-containing fraction was extracted five times with 50 of dichloromethane in all cases (extracts I to VI in fig. 2).

Gas chromatography with mass spectrometry detection

GC analyses were performed by HP 5890 II apparatus with HP 5971 A EI-MS detector, PONA 30 m × 0.25 mm × 0.25 μm column. The oil was diluted in hexane (1:100) and 1 μl from this was injected for separation. The carrier gas was hydrogen at 5 psi inlet pressure. Injector and detector temperatures were set to 250 °C and 280 °C respectively. The column temperature was programmed from 40 to 260 °C at 4 °C/min temperature gradient.

Thin layer chromatography (TLC)

TLC G_{254} 10 cm × 20 cm plates and dichloromethane/methanol/25% ammonium hydroxide solution (85:28:3 v/v) as eluent were used for the separation of alkaloid fraction. One ml from each dichloromethane extract was evaporated in vacuum, dissolved in 5 μl dichlormethane and introduced in the plates. The chromatographic time was 60 minutes.

Visualization and spectrophotometric detection (SD)

After separation was finished, the plate was dried at room temperature and the spots were visualized at 254 and 315 nm wavelength. The developed spots were dissolved in methanol and subjected of spectrophotometric detection in scan mode at 200 to 350 nm wavelength.

Varian Cary 50 UV-Vis spectrophotometer was used for detection.

REFERENCES

1. P. L. Li, C. M. Wang, Z. X. Zhang, Z. J. Jia, *Tetrahedron* **2007**, *63*, 12665.
2. H. Baba, Y. Yaoita, M. Kikuchi, *Journal of Natural Medicines,* **2007**, *61*, 472.
3. H. Baba, Y. Yaoita, M. Kikuchi, *Helvetica Chimica Acta*, **2007**, *90*, 1028.
4. K. Gao, W. Shu, W. Jian Jia, Z. Jian Jia, *Phytochemistry*, **2007**, *47*, 269.
5. J. Q. Liu, M. Zhang, C. F. Zhang, H. Y. Qi, A. Bashall, S. W. A. Bligh, Z. T. Wang, *Phytochemistry*, **2008**, *69*, 2231.
6. S. B. Singh, D. Zink, J. Polishook, D. Valentino, A. Shafiee, K. Silverman, P. Felock, A. Teran, D. Vilella, D. J. Hazuda, R. B. Lingham, *Tetrahedron Letters*, **1999**, *40*, 8775.

7. C. Bicchi, F. R. A. Rubioloc, *Journal of Natural Products*, **1991**, *54*, 941.
8. O. Rangel, J. Angel, US Patent, 7604823, **2006**.
9. S. Meryl, US Patent, 6355684, **2002**.
10. H. Wiedenfeld, S. Narantuya, M. Duma, A. Monhbaatar, *Scientia Pharmaceutica*, **2003**, *71*, 129.
11. M. Kosar, T. Özek, M. Kürkçüoglu, K. H. C. Baser, *Journal of Essential Oil Research*, **2007**, *19*, 426.
12. M. Lucchesi, F. Chemat, J. Smajda, *Journal of Chromatography A*, **2004**, *1043,* 323.
13. S. Ž. Milojević, T. D. Stojanović, R. Palić, M. L. Lazić, V. B. Veljković, *Biochemical Engineering Journal* vol. 39, no.3, May **2008**, *39,* 547.
14. J. Q. Xu, Y. S. Li, Y. M. Li, S. H. Jiang, C. H. Tan, D.Y. Zhu, *Planta Medica*, **2006**, *72*, 563.
15. J. Q. Liu, M. Zhang, C. F. Zhang, H. Y Qi, A. Bashall, S.W. A. Bligh, Z. T. Wang, *Phytochemistry*, **2008**, *69*, 2231.
16. C. M. Passreiter, *Biochemical Systematics and Ecology*, **1998**, *26*, 839.
17. A. L. Medina-Holguín, F. O. Holguín, S. Micheletto, S. Goehle, J. A. Simon, M. A. O'Connell, *Phytochemistry* **2008**, *69,* 919.
18. M. A. Apel, V. L. Sardá Ribeiro, S. A. L. Bordignon, A. T. Henriques, G. Poser, *Parasitology Research*, **2009**, *105*, 863.
19. S. R. Fuselli, S. B. G. Rosa, M. J. Eguaras, R. Fritz, *World Journal of Microbiology and Biotechnology*, **2008**, *24*, 2067.
20. L. Alejandro, L. Susana, Z. Eduardo, M. Héctor, *Entomologia Experimentalis et Applicata*, **2008**, *129*, 107.
21. F. Menichini, R. Tundis, M. R. Loizzo, M. Bonesi, M. Marrelli, G. A. Statti, F. Menichini, F. Conforti, *Fitoterapia*, **2009**, *80*, 297.
22. S. Pepeljnjak, I. Kosalec, Z. Kalođera, N. Blažević, *Acta Pharmaceutica*, **2005**, *55*, 417.
23. N. Lukwa, P. Mølgaard, P. Furu, C. Bøgh, *Tropical Biomedicine*, **2009**, *26*, 85.
24. K. Boehme, J. A. Noletto, W. A. Haber, W. N. Setzer, *Natural Product Research,* **2008**, *10*, 31.
25. W. A. Haber, B. R. Agius, S. L. Stokes, W. N. Setzer, *Records of Natural Products*, **2008**, *2*, 1.
26. P. Angelini, R. Pagiotti, B. Granetti, *World Journal of Microbiology* and *Biotechnology,* **2008**, *24*, 197.
27. A. Garozzo, R. Timpanaro, B. Bisignano, P. M. Furneri, G. Bisignano, A. Castro, *Letters in Applied Microbiology*, **2009**, *49*, 806.
28. J. L. Wang, Y. Li, C. L. Lei, *Natural Product Research,* **2009**, *23*, 1080.
29. A. L. Medina-Holguín, F. O. Holguín, S. Micheletto, S. Goehle, J. A. Simon, M. A. O'Connell, *Phytochemistry*, **2008**, *69*, 919.
30. S. Sibanda, G. Chigwada, M. Poole, E. T. Gwebu, J. A. Noletto, J. M. Schmidt, A. I. Reac, W. N. Setzer, *Journal of Ethnopharmacology*, **2004**, *292*, 107.
31. I. Orhan, E. Küpeli, M. Aslan, M. Kartal, E. Yesilada, *Journal of Ethnopharmacology*, **2006**, *105*, 235.
32. J. Gonçalves, S.C. Rosa, A. Neves, F. Judas, L. Salgueiro, C. Cavaleiro, M.C. Lopes, A.F. Mendes, *Osteoarthritis and Cartilage*, **2008**, *16*, S232.