ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXII, N 4, 3 SECTIO DDD 2009

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Herbal volatiles from Cretan barberry (Berberis cretica) obtained by supercritical fluid extraction

Analiza składu związków lotnych w zielu berberysu kreteńskiego (*Berberis cretica*) metodą ekstrakcji z płynem w stanie nadkrytycznym

Cretan barberry (*Berberis cretica*) is one of the representatives of *Berberidaceae* family. These are mainly shrubs spread worldwide – in Europe, Asia, Africa and America. The only species naturally occurring in Poland is the common barberry (*Berberis vulgaris*), although many other shrubs from its family are used for decorative purposes and are planted in gardens.

Cretan barberry, whose constituents are investigated in the course of the current study, is derived from the island of Crete in Greece. It naturally occurs in rocky places at the altitude of more than 900 m above sea level. *Berberis cretica* is a thorny shrub growing in the mountainous regions of Greece at the heights over 900 m. above sea level. It occurs naturally in the sunny, rocky locations and in flat valley bottoms, although it can also be planted in gardens [11].

The leaves of Cretan barberry are small, oval, sharpened and mallow. The multitudes of tiny yellow flowers are characteristic of *Berberis cretica* as well as dark blue berries which appear at the end of the summer and are valuable sources of vitamine C and phenolic compounds [4].

The shrub was commonly used in the medicine of the Far East to help people suffering from indigestion and diarrhea [3]. Extracts of berberis are being examined thoroughly these days, revealing its different medicinal properties. These plants are rich sources of tannins, waxes, mucus, mineral salts and pectines as well.

A high concentration of isoquinoline alkaloids (major active constituents) with berberine – their main representative – affects barberry's activity. Currently, protoberberine alkaloids are known for their ability to block the synthesis of DNA and to inhibit cellular permeability. The chief activity of berberine is the ability to block the development of malaria. Moreover, different species of barberry are potent acetylcholinesterase and monoaminoxidase inhibitors. Extracts from berberis species are characterized by analgesic, antibacterial, antiviral, antiarrhythmic, antiinflammatory, cholagogic and ossific properties [4, 10]. A broad spectrum of its properties encourages to do further research on it.

The current study focuses on barberry's essential oils. They are natural mixtures of terpenes. Every species is characterized by a different qualitative composition of volatiles. The identification of individual essential oils can be performed using different methods, out of which GC-MS is the most common one. It enables fast and accurate analysis of different compounds, comparing their relative retention times and mechanisms of fragmentation [9].

In the course of our study a novel extraction method was used to obtain the constituents of essential oil coming from herb of *Berberis cretica* – for the first time in this gender.

SFE (*Supercritical Fluid Extraction*) method uses the ability of gases to be transformed into supercritical liquid. Gases are liquefied under certain pressure and compression values. On the other hand, if gas is being heated up to a certain temperature, no pressure is able to transform it into liquid. The value of such temperature is called critical temperature, whereas the corresponding vapour pressure is defined as critical pressure. Both of the values above – critical pressure and critical temperature – determine the critical point, which is characteristic of every substance. Crossing these values turns the substance into the critical state – into a supercritical fluid (SCF).

The properties of both gas and liquid characterize this so called "fluid". It is described by high penetrability, small viscosity and low surace tension, which results in faster cell permeability and more efficient extraction of active constituents. From another point of view, according to the properties of a liquid, the fluid in the supercritical state washes out active compounds more thoroughly. Furthermore, it is possible to wash out even a chosen substance or to get rid of a certain constituent. Supercritical Fluid Extraction uses the following solvents: carbon dioxide, ethane, benzene, toluene, ammonia, trichlorofluoromethane or water [5].

Carbon dioxide is the most common solvent used in SFE according to its high availability, low price, odourlessness and flavourlessness. Additionally, it is environmentally friendly and non-toxic, which enables its usage in food industry. As a result, supercritical fluid extraction can be used in decaffeination of coffee, production of dietary (low fat) food or extraction of naturally occurring pesticides [6].

According to the unpolar character of carbon dioxide, extraction of polar compounds remains insufficient. Therefore, gradient of a polar solvent (methanol, ethanol or water) is introduced in the course of the analysis.

Fractions obtained from SFE (collected every hour) are dry, as the CO_2 evapourates in the moment of fractions' collection. It shortens the process of extraction considerably. Thanks to SFE more reach extracts originated without toxic solvents are obtained [1, 5, 6].

MATERIAL AND METHODS

Plant material – herb of Cretan barberry (*Berberis cretica)* – was collected before flowering on the rocky slope of Rouvas forest, Crete, Greece. 220g of air-dried and powdered herb was extracted using SFE method (Supercritical Fluid Extraction) in the conditions of elevated pressure (pressure of extractor: 100 ba, pressure of collectors: 50 ba) and temperature (temperature of both extractor and collectors: 40°C), using carbon dioxide as the main solvent (CO_2 flow: 5kg/l). Fractions were collected every hour. In the above-mentioned conditions, carbon dioxide exists as the supercritical liquid, preserving the properties of both gas and liquid.

The obtained fractions were analyzed using HPLC (High Performance Liquid Chromatography) and GC-MS (Gas Chromatography-Mass Spectrometry) in order to elucidate the qualitative composition of essential oils present in the Cretan barberry. The oil was taken by dissolving in gradient grade dichloromethane (0.5 ml) and kept at 4°C in a sealed brown vial. Then 10 µl of the extract was directly injected into the GC-MS instrument.

GC-MS analysis was performed using Hewlett-Packard 5973-6890 with a capillary column (30m x 0.25mm x 0.25 µm). The column's temperature was increased from 60°C to 280°C with a 3°C/min heating ramp. As the mobile phase, helium with flow 1ml/min was used. The relative percentage amounts of the separated compounds were calculated from total ion chromatograms by a computerized integrator.

Identification of essential oils was conducted on the basis of the comparison of their MS spectra with those present in Wiley and NBS libraries [7, 9]. Furthermore, conformity of the obtained results was checked with numerous references.

RESULTS

In the course of the current study, the composition of volatiles present in two first fractions (fraction A and fraction B) obtained from supercritical fluid extraction was determined. Two injections to GC-MS were made and subsequently their chromatograms were compared using Wiley and NBS libraries. The obtained results are shown in Table 1.

Table 1. Constituents of essential oils from fraction A and B obtained with SFE

No	Retention time [min]	Percentage amount [%]	Constituent
Qualitative composition of FRACTION A			
1	35.44	1.95	5,6,7,7a-tetrahydro-2(4h)-benzofuranon,
2	46.85	2.26	isopropyl myristate
3	47.26	2.63	2,6,10-trimethylneophytadiene
4	47.44	5.85	6,10,14-trimethyl-2-pentadecanone,
5	49.50	1.20	n-eicosane
6	50.32	3.83	hexadecanoic acid, ethyl ester
7	51.20	6.40	1,2-benzenedicarboxylic acid, dibuthyl ester
8	52.59	1.25	hexadecanoic acid, ethyl ester
9	55.70	1.13	9,12-octadecadienoic acid, metyl ester
10	55.87	0.90	9,12,15-octadecatrienoic acid
11	56.00	1.30	n-heneicosane
12	57.09	7.26	linoleic acid
13	61.97	1.64	n-nonadecane
14	64.77	0.81	n-tetracosane
15	67.46	3.70	n-pentacosane
16	70.05	0.94	n-hexacosane
17	72.56	5.79	n-heptacosane
18	77.30	11.91	n-nonacosane
19	78.00	0.86	hexacosanoic acid, metyl ester
20	82.25	3.19	n-heneicosane
Qualitative composition of FRACTION B			
1	29,79	4,23	4-hydroxy-3-methoxybenzaldehyde,
2	51,20	6,40	1,2-benzeneidcarboxylic acid, dibu
3	72,56	5,79	n-heptacosane
4	74,95	2,50	n-octacosane
5	77,30	11,91	n-nonacosane
6	82,25	3,19	n-heneicosane

The current research done on the constituents of Cretan barberry has not yet revealed its essential oil composition. The present study led to the identification of 18 volatile substances present in the

first fraction obtained from SFE and 6 compounds which belong to the second fraction. The chief constituents of fraction A are n-nonacosane (11.91%), linoleic acid (7.26%) and 6,10,14-trimethyl-2-pentadecanone (5.85%). Fraction B contains primarily: n-nonacosane (11.91%), n-heptacosane (5.79%) and 4-hydroxy-3-methoxy-benzaldehyde (4.23%). Volatiles listed in Table 1 make 64.8% and 34.02% of the total content of fraction A and fraction B, respectively.

Composition of both fractions differs considerably. Fraction A is richer in volatiles. Among all compounds present in fraction B, only 4-hydroxy-3-methoxy-benzaldehyde does not occur in fraction A. The latter contains as much as 64.8% essential oils.

DISCUSSION

Current reports on essential oil composition of *Mahonia japonica* [8] – the plant which belongs to the *Berberidaceae* family, the same with Cretan barberry – do not coincide with results of the present survey. It confirms the variety of volatile composition among different species – even those belonging to the same family.

Results obtained in our investigation confirm the efficacy of the applied method. Supercritical Solvent Extraction is fast, simple and non-toxic. It enabled to obtain a rich extract, also in volatile compounds. Furthermore, SFE is characterized by high efficiency and repeatability of research results, which in case of secondary metabolites' analysis is highly significant.

It is worth noticing that both analyzed fractions are rich in ftalates. It is probable that those are rare naturally occurring substances. Further investigation to confirm their character is needed.

CONCLUSIONS

1. Use of Supercritical Fluid Extraction in the course of the current inquiry led to elucidation of qualitative composition of volatiles present in the herb of Cretan barberry (*Berberis cretica*). Due to its low toxicity, low costs of analysis and renewal of gaseous phase, SFE is worth recommendation in the course of plant material extraction.

2. The analyzed fractions coming from SFE are rich in essential oils and contain: Fraction A – 18 volatile compounds, making together 64.8% of the analyzed sample; Fraction B – 6 substances, making 34.02% of the sample.

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SUMMARY

Cretan barberry (*Berberis cretica*) is one of the representatives of *Berberidaceae* family, which is composed of over 450 species. A broad spectrum of barberry's activity stimulates further research on this plant. Modern scientific journals give no information on the constituents and properties of essential oils produced by any species of barberry, which is why it became the topic of the current study. The essential oils fraction was obtained in the course of SFE (*Supercritical Fluid Extraction*) – for the first time in the family of *Berberidaceae*. Analysis of the results was conducted with the help of GC-MS method (*Gas Chromatography-Mass Spectrometry*), revealing the presence of 18 different terpenes.

STRESZCZENIE

Berberys kreteński (*Berberis cretica*) jest jednym z przedstawicieli rodziny *Berberidaceae*, do którego zaliczamy około 450 gatunków roślin. Szereg zastosowań leczniczych ciał czynnych występujących w berberysie czyni tę roślinę uniwersalną w etnomedycynie Grecji oraz zachęca do dalszego jej badania. Brak doniesień naukowych dotyczących olejku eterycznego zawartego w tej roślinie skłonił do przeprowadzenia szczegółowych badań jakościowych związków lotnych berberysu kreteńskiego. Frakcję substancji lotnych otrzymano dzięki metodzie SFE (*Supercritical Fluid Extraction*) – ekstrakcji w stanie nadkrytycznym, zastosowanej po raz pierwszy w rodzinie *Berberidaceae*. Analizę wyników przeprowadzono stosując spektrometrię mas sprzężon z chromatografią gazową GC-MS (*Gas Chromatography – Mass Spectrometry*), co potwierdziło obecność 18 różnych związków należących do grupy terpenów.