Production and Characterisation of *Taraxacum officinale* Extracts Prepared by Supercritical Fluid and Solvent Extractions

T. Sz. Kristó, É. Szőke, Á. Kéry
Department of Pharmacognosy
Semmelweis University
H-1085 Budapest, Üllői út 26.,
Hungary

P.P. Terdy, L.K. Selmeczi, B. Simándi
Budapest University of Technology and Economics
Department of Chemical Engineering
Budapest, Hungary

Keywords: dandelion, β-amyrin, β-sitosterol, quantitative data, supercritical CO_2

Abstract

There are many reports on biological activities of pentacyclic triterpenoids, which could be relevant to the pharmacological effects including anti-inflammatory properties. Dandelion (*Taraxacum officinale* Wiggers et Weber, Asteraceae) is one of the best known European medicinal plants, rich in triterpenoids, which has been used for the treatment of various inflammatory diseases such as rheumatoid arthritis and also for many infectious disorders. The aim of this work was to investigate the supercritical fluid extraction (SFE) of dandelion crude drugs (Taraxaci radix and T. folium) with carbon dioxide, to study the extraction of triterpenoids and phytosterols and to compare supercritical CO_2 extracted products and extracts made by traditional solvent extractions (*n*-hexane and ethanol 96%). Solvent extractions were carried out using a Soxhlet extractor. To define the effect of temperature and pressure on the yield of supercritical fluid extraction, a 2 factorial 3 level experiment chain was performed. The content of triterpenes and phytosterols was determined, after saponification, by thin layer chromatography-densitometry. The products gained by SFE were different from the traditional ones concerning their apparence and composition; triterpenes and their esters could be extracted quantitatively by supercritical fluid extraction using CO_2 as solvent; the extraction dynamic for β-amyrin and β-sitosterol was different; triterpenes have a higher concentration in the SFE product then in traditional ones. By means of supercritical fluid extraction of *Taraxacum* crude drugs, in function of the selectivity of the solvent, temperature, pressure and accompanying constituents, qualitatively new products can be gained. These may serve as prospective raw materials for phytopharmaceuticals.

INTRODUCTION

There is an increasing demand for natural products as curative agents and foods. Regulations in the food, pharmaceutical- and cosmetic industries are getting more rigorous so the traditional solvent extraction could cause problems. In this study we examined the extraction possibilities of the active materials of *Taraxacum officinale*. The root (Taraxaci radix) and herb (Taraxaci folium and herba) are not only traditional medicines but may serve as prospective raw materials for modern pharmaceuticals.

The anti-inflammatory activity of dandelion extracts has been recently confirmed in animal studies (Mascolo, 1987) and aqueous extracts seem to have anti-tumour activity (Nevall et al., 1996). Based on pharmacological studies, dandelion is one of the components of phytomedicines used in therapy for hepatitis and the drug also has diuretic and choleretic actions (Bisset, 1994; Bradley, 1992).

There are numerous studies about chemical composition of *Taraxacum officinale* (Czygan 1990; Hegnauer 1964; Hegnauer 1989; Komissarenco and Derkach 1981).

Roots contain several triterpenes, including taraxol, taraxerol, taraxasterol, ψ-taraxasterol, and β-amyrin; sterols (stigmasterols, β-sitosterol); inulin (ca. 25%) (Rutherford 1972); sugars (fructose, glucose, sucrose); pectin; glucosides; choline; phenolic acids e.g., caffeic and p-hydroxyphenylacetic acids; gum; vitamins; and others. The drug Taraxaci radix cum herba contains: sesquiterpenlactones, triterpenes,

phytosterols, carbohydrates, phenolic acids, flavonoids, etc (Bissett 1994; Bradley 1992; Newall et al, 1996; Williams et al., 1996). From their characteristic principles our attention has been directed to triterpenes and phytosterols with anti-inflammatory activity (Kashiwada et al., 1998; Safayhi and Sailer, 1997).

The yield of active substances during supercritical fluid (SFE) and Soxhlet extraction were compared how to produce active materials under soft conditions.

MATERIALS AND METHODS

All reagents were of reagent quality (Reanal RT, Hungary; Linde RT, Hungary). Silica gel TLC plates were from Merck KGaA (Darmstadt, Germany). The dandelion root and leaves were from Rózsahegyi Kft (Erdőkeresztes, Hungary). A herbarium specimen is deposited in the Department of Pharmacognosy, Semmelweis University.

Soxhlet extraction. The dry drug was humected with ethanol (or *n*-hexane) at room temperature for 30 min before extraction. The extraction went until exhaution. The drug weight was 1200 g and solvent volume 2650 ml. The solvent flow rate (7 kg/h) was controlled with steam pressure adjusted by a valve and the extractor temperature (40-45^{o}C) was controlled by a thermostat. During the extraction the solvent flow rate, the vessel temperatures, and the dry content of the extracts were measured every half hour.

SFE process. One kg raw material was used for extraction. The CO_2 mass flow was kept to 7 kg/h. The extraction was considered finished when the deposit between two checks was less than 0.1 w/w % calculated on drug. Assuming that the yield is not a linear function of the extraction temperature and pressure, we set the parameters to three different levels to be able to fit a non-linear function of the yield. In the middle of the design (300 bar, 50 °C) we completed 4 experiments to determine the standard deviation.
Phytoanalytical methods. Measurements of the triterpene and phytosterol content of the extracts were carried out. Saponification was made according to our earlier reports (Kristó et al., 2000).

Thin-layer chromatography - densitometry. Standards of β-amyrin (0.05 g/mL) and β-sitosterol (0.025 g/mL), samples of SFE products and Soxhlet extracts were separated using *n*-hexane-ethyl acetate (6:2 w/w) as mobile phase on silica gel layers (Kieselgel 60 F_{254} MERCK 20 x 10 cm). Cerium sulphate reagent in acidic medium was selected as detection reagent. After heating for 10 min at 100^{o}C, measurements were performed at 600 nm, in the zig-zag mode (y = 0.2 mm, 0.2 x 1.2 mm) using a Shimadzu CS-930 densitometer. The calculation was based on calibration graphs.

RESULTS AND DISCUSSION

To evaluate the efficacy of various extraction techniques for preparing triterpene and phytosterol rich extracts from dandelion, β-amyrin and β-sitosterol were used as key compounds. Their separation was excellent (R_f values of β-amyrin and β-sitosterol were 0.58 and 0.43 respectively) in the saponified extracts. The calibration graphs used for densitometric analysis were in the range of 2.5 - 5.5 ng/mL, where r^2 was always greater than 0.9777 (Fig. 1). When the quantities of triterpenes and phytosterols were measured three times in 10 different root and leaf samples we could conclude that Taraxaci folium is a better source for triterpenes while Taraxaci radix is superior in phytosterol content (Table 1).

From Taraxaci radix the most β-amyrin was extracted by the Soxhlet method, using ethanol as solvent (889 mg/ 100 g), followed by supercritical CO_2: 450 bar, 35^{o}C (424 mg/ 100 g) and the Soxhlet method using *n*-hexane (215 mg/ 100 g). The same data for Taraxaci folium were: 677 mg/ 100 g (Soxhlet method, ethanol), 446 mg/ 100 g (supercritical CO_2: 450 bar, 65^{o}C), 496 mg/ 100 g (Soxhlet method, *n*-hexane) (Fig. 2). A possible answer for the greater quantity extracted by ethanol could be that triterpenes may be present in the roots in glycosidic form and therefore ethanol is a better solvent to extract them than the apolar supercritical CO_2. *N*-hexane extracted almost the same amount as the supercritical CO_2, because they have almost the same polarity. However, the SFE is superior to *n*-hexane extraction due to its better selectivity.

Supercritical extraction of β-sitosterol from Taraxaci radix, SFE (450 bar, 65°C) resulted in 25.3 mg/ 100 g, the Soxhlet method using ethanol and *n*-hexane in 18.1 mg/ 100 g and 19.3 mg/ 100 g respectively. The same data for Taraxaci folium were: 146 mg/ 100 g (Soxhlet method using ethanol), 134.7 mg/ 100 g Soxhlet method using *n*-hexane and 123.4 mg/ 100 g (supercritical CO_2: 450 bar 65°C). Based on these results, it seems, that phytosterols are mainly present in the free form in dandelion drugs (Fig. 3).

The supercritical fluid extracts of dandelion using different extraction parameters contained 8.938 - 24.540g/ 100 g β-amyrin; 1.498 - 2.109 g/ 100 g β-sitosterol and 7.920 - 13.750 g/100 g β-amyrin; 1.910 - 3.810 g/ 100g β-sitosterol in Taraxaci radix and T. folium respectively.

The products gained by SFE were different from the traditional extracts concerning their appearance and composition; triterpenes and their esters could be extracted quantitatively by supercritical fluid extraction using CO_2 as solvent; the extraction dynamic for β-amyrin and β-sitosterol was different; triterpenes had a higher concentration in the SFE products than in the traditional ones. By means of supercritical fluid extraction of *Taraxacum* crude drugs in function of the selectivity of the solvent, temperature, pressure and accompanying constituents qualitatively new products could be gained. Therefore they may be prospective raw materials for phytopharmaceuticals.

ACKNOWLEDGEMENTS

The work was supported by the Hungarian National Scientific Research Foundation: OTKA T030034; Scientific Research Support of University: ETK 84260-64 Eü. Min. 158/99 and Ministry of Welfare Hungary: ETT 02-517, ETT 03-390/1993, ETT 158/1999, ETT 250/2000. The authors wish to thank Ms Lenke Tóth for her excellent technical assistance.

Literature Cited

Bisset, N.G. (ed. and transl.) 1994. Herbal Drugs and Phytopharmaceuticals CRC Press**.** Boca Raton, Ann Arbor, London, Tokyo

Bradley, P.R. (ed) 1992. British Herbal Medicine Association, Bournemouth, British Herbal Compendium 1

Czygan, F.C. 1990. *Taraxacum officinale* Wiggers-Der Löwenzahn. *Z.* Phytother. 11:99-102.

Hegnauer, R. 1989. Chemotaxonomie der Pflanzen 8:265. Birkhäuser, Basel

Hegnauer, R. 1964. Chemotaxonomie der Pflanzen 3:265. Birkhäuser, Basel

Kashidawa, Y., Wang, H.K., Nagao, T., Kitanaka, S., Yasuda, I., Fuiioka, T. and Yamagashi, T. 1998. Anti-HIV activity of oleanolic acid, pomolic acid and structurally related triterpenoids. J. Nat. Prod. 61:1090-1095.

Komissarenco, N.F. and Derkach, A. I. 1982. *Taraxacum officinale* coumarins. Khim. Prir. Soedin. 4:519, 1982. Chem. Abstr. 96:3647.

Kristó, Sz.,T., Terdz, P., Simándi, B. and Kéry, Á. 2000. Preparation of bioactive constituents from *Taraxacum officinale* L. by means of various extraction methods J. of Oil, Soap, Cosmetics 49:93-97.

Mascolo, N., Autore, G., Capasso, F., Menhini, A. and Fasulo, M.P. 1987. Biological screening of Italian medicinal plants for anti-inflammatory activity. Phytother. Res. 1:28-31.

Newall, C.A., Anderson, S.A. and Phillipson, J.D. 1996. Herbal Medicines. The Pharmaceutical Press, London

Rácz-Kotilla, E., Rácz, G. and Solomon, A. 1974. The action of *Taraxacum officinale* extracts on the body weight and diuresis of laboratory animals. Planta Med. 26:212-217.

Rutherford, P.P. and Deacon, A.C. 1972. ß-Fructofurasonidase from roots of dandelion (*Taraxacum officinale* Weber). Biochem. J. 126:569-573

Safayhi, H. and Sailer, E.R. 1997. Anti-inflammatory actions of pentacyclic triterpenes. Planta Medica 63:487-493.

Williams, Ch.A., Goldstone, F. and Greenham, J. 1996. Flavonoids and cinnamic acids and coumarins from different tissues and medicinal preparations of *Taraxacum officinale*. Phytochemistry 42:121-127

Tables

Table 1. Triterpene and phytosterol content of the dandelion drugs

	***Taraxaci radix*[1]**	***Taraxaci folium*[2]**
β-amyrin mg/100g	340.9	551.1
	321.2	528.0
	343.0	557.6
β-sitosterol mg/100g	41.2	38.2
	46.7	34.3
	43.4	38.5

[1,2] Data represent the average content of active substance in three times 10 different root and leaf samples

Figures

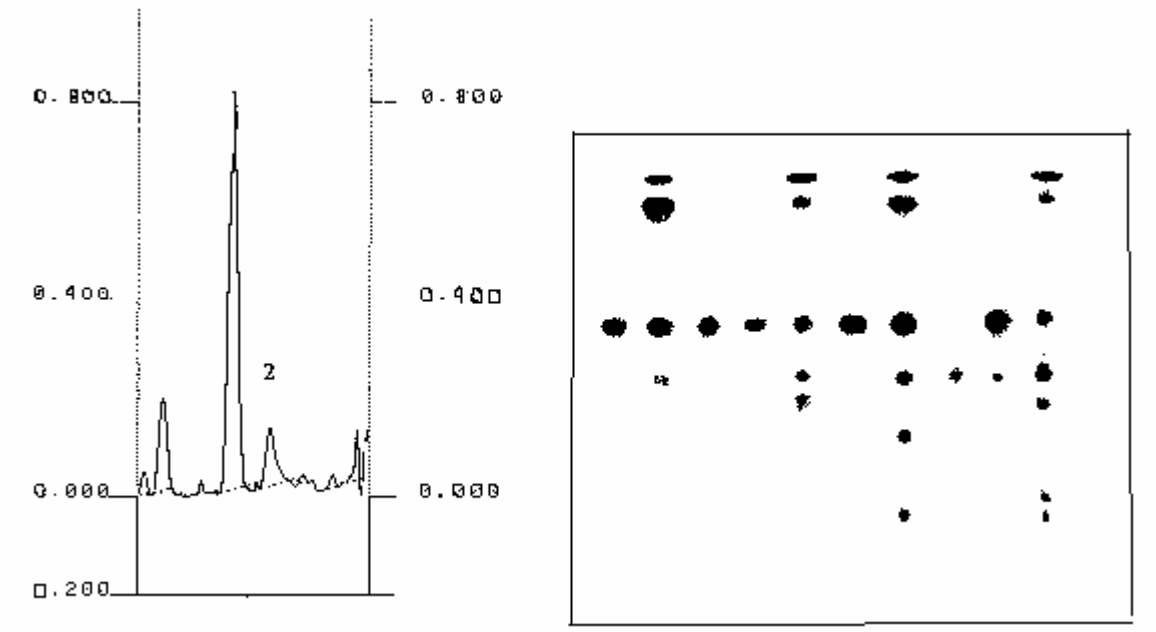

Fig.1. The TLC-densitometric measurment of β-amyrin and β-sitosterol content of different dandelion root and leaf extracts (1=β-amyrin; 2=β-sitosterol)

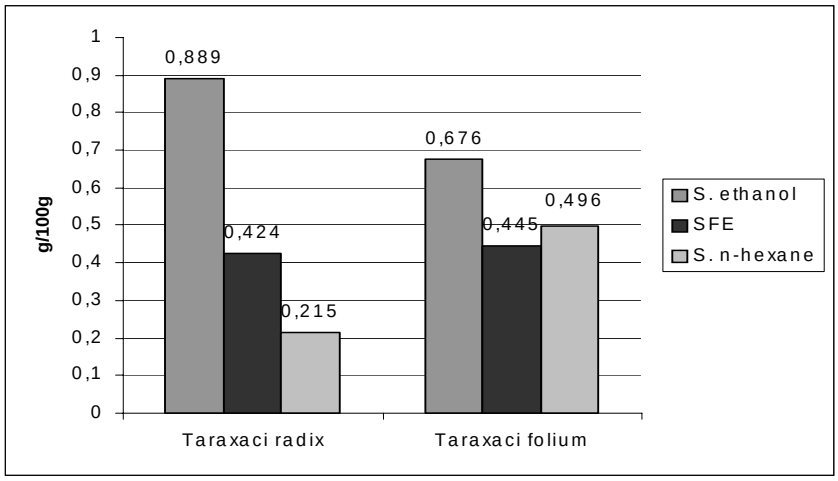

Fig. 2. β-amyrin yield with SFE and solvent extraction from *Taraxaci radix* and *T. folium*

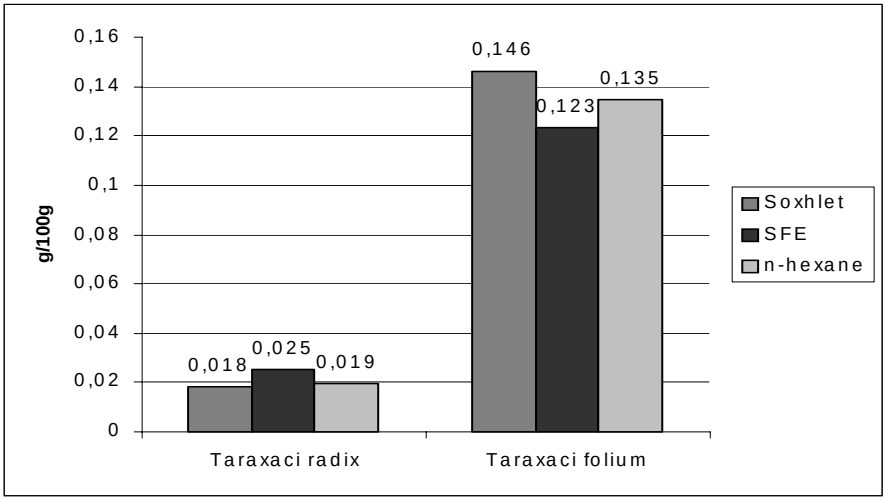

Fig. 3. β-sitosterol yield with SFE and solvent extraction from *Taraxaci radix* and *T. folium*