FABAD J. Pharm. Sci., 25, 153-156, 2000

RESEARCH ARTICLES / BİLİMSEL ARAŞTIRMALAR

High Performance Liquid Chromatographic Analysis of Cucurbitacins in Some *Bryonia* Species

Gülnur TOKER*°, Nurgün ERDEMOĞLU*, M. Cihat TOKER**

High Performance Liquid Chromatographic (HPLC) Analysis of Cucurbitacins in Some Bryonia Species

Summary : *In this study, the roots of Bryonia multiflora Boiss.& Heldr. (Cucurbitaceae) growing in Central, South and East Anatolia was analysed from the aspect of cucurbitacin B and cucurbitacin I in HPLC. The results were compared with those parts of known official B. alba species. HPLC analysis were achieved on Lichrospher 100 RP C_{18} e column by using an isocratic mixture of acetonitrile: water (2:3) at a flow rate of 1ml/min as a mobile phase. The UV detection was performed at 230 nm. It was found that cucurbitacin B had a high ratio in chloroform and low in methanol extracts. On the contrary, cucurbitacin I had a high ratio in methanol and low in chloroform extracts. Cucurbitacin B was found to be 0.022% and trace and cucurbitacin I 0.021% and 0.125 % in the B. multiflora and B. alba roots respectively.*

Keywords : *Bryonia multiflora, B . alba, cucurbitacin, HPLC*

Bazı Bryonia Türlerinde Bulunan Kukurbitasinlerin Yüksek Basınçlı Sıvı Kromatografisi (YBSK) ile Analizi

Özet : *Iç, Güney ve Doğu Anadolu bölgelerinde doğal olarak yetişen ve üzerinde çalışma yapılmamış olan Bryonia multiflora Boiss& Heldr.(Cucurbitaceae) bitkisinin kökleri, kukurbitasin B ve kukurbitasin I yönünden YBSK da incelenmiş ve sonuçlar ofisinal tür olan B. alba L. ile mukayese edilmiştir. YBSK analizinde Lichrospher 100 RP C_{18} e kolon, hareketli faz olarak, akış hızı 1ml/ dak. olan asetonitril:su (2:3) karışımının izokratik olarak kullanıldığı sistemde, 230 nm dalga boyunda UV dedektör kullanılmıştır. Köklerin kloroformlu ekstrelerinde kukurbitasin B, metanollü ekstrelerinde ise kukurbitasin I miktarı fazla bulunmuştur. B. multiflora köklerinin % 0.022 kukurbitasin B, % 0.021 kukurbitasin I ; B.alba'nın ise % 0.125 kukurbitasin I ve eser miktarda kukurbitasin B ihtiva ettiği tespit edilmiştir.*

Anahtar kelimeler : *Bryonia multiflora, B. alba, kukurbitasin , YBSK*

Received : 4.5.2000
Revised : 27.7.2000
Accepted : 19.12.2000

INTRODUCTION

The roots of *Bryonia alba* and *B. dioica* (Cucurbitaceae) are well-known hydragogue cathartics in folk medicine and are also used for their antirheumatic, antiinflammatory and expectorant activities[1-8]. *B. alba* L., *B. cretica* L., *B. aspera* Stev. ex. Ledeb. and *B. multiflora* Boiss. and Heldr. grow wild in Turkey[9]. Roots have been used as a diuretic and purgative internally and for the treatment of rheumatism and hemorrhage externally[10-12].

Bryonia species have been known for a long time as a rich source of cucurbitacins which possess a wide range of biological activities. Cucurbitacin B, D, E, I, J, K, L and tetrahydro I isolated from *B. alba* roots revealed cytotoxic activity in Hela and KB human cell cultures and potent antitumour activity against 180 sarcoma and Ehrlich ascites carcinoma in mice[13-17]. Cucurbitacins also have purgative, antiinflammatory, hepatoprotective, hepatocurative, anti microbial, cardiovascular, gastric, ovulatory and antihelminthic activity[18].

* Department of Pharmacognosy, Faculty of Pharmacy, Gazi University 06330 Etiler/Ankara, TURKEY
** Department of Biology, Faculty of Science, Ankara University, 06100 Tandoğan/Ankara, TURKEY
° Correspondence

There are studies on the qualitative and quantitative analysis of cucurbitacins in some plants by HPLC and TLC[19]. But there is no reported study on *B. multiflora* and the quantitative HPLC analysis of cucurbitacins in *Bryonia* species .

In this study, the cucurbitacin contents of *B. multiflora* roots growing in Central, South and East Anatolia were investigated. Cucurbitacins in the chloroform and methanol extracts of roots were analysed by HPLC. Results were compared with those parts of *B. alba* which is an official species.

MATERIALS and METHODS

Plant Materials

Collection sites of the *Bryonia* samples used in this study are given below:

B.multiflora Boiss and Heldr. Botanical Garden of the Faculty of Science, Ankara University, September, 1998 (ANK 6209).

B.alba L. National Park of Karagöl, Ankara, September, 1998 (ANK 46915).

Voucher specimens have been deposited in the Herbarium of Faculty of Science, Ankara University (ANK).

Preparations of Samples

Chloroform extracts: 3g powdered roots were successfully extracted with petroleum ether and then with chloroform three times at room temperature.

Methanol extracts: 3g powdered roots were defatted with petroleum ether and then extracted with methanol three times at room temperature.

Combined chloroform and methanol extracts were evaporated to dryness in vacuo separately. Crude extracts were dissolved and diluted with HPLC grade methanol, passed through membrane filters (0.45 µm, Alltech) and suitable dilutions were prepared with each sample for HPLC analysis.

Chemicals

Cucurbitacin B and cucurbitacin I as authentic samples were kindly provided by Prof. Erdem YEŞİLADA (Gazi University, Faculty of Pharmacy, Ankara). The solvents used were HPLC grade and water was bidistilled. The mobile phase was degassed by immersion in an ultrasonic bath.

TLC Analysis

Chloroform and methanol extracts of plant material and cucurbitacin B and cucurbitacin I as standard were performed on TLC. Kieselgel 60 F_{254} precoated 0.2 mm thickness plastic plates (Merck) and chloroform: ethanol (95:5)(S1)[20], chloroform: ethanol (92:8) (S2) were used as solvent systems in thin layer chromatography. Spots were visualized with vanillin-phosphoric acid reagent.

HPLC Analysis

A Hewlett-Packard high performance liquid chromatograph was used which consisted of a model 1050 pump. The model 1050 UV detector was set at 230 nm. A Rheodyne 7125 injection valve was fitted with a 20 µl loop. The integrator was a Hewlett-Packard 3396A. The analytical column was a Lichrospher 100 RP C_{18} e, (5 µm) 250x4.0 mm i.d., stainless steel. The mobile phase consisted of acetonitrile : water (2:3) delivered isocratically at a flow rate of 1 ml/min resulting in a column head pressure of about 1800psi. The chromatographic analysis was performed at room temperature.

Standard Solution and Calibration Curve

1.6 mg cucurbitacin B and 1.2 mg cucurbitacin I were weighed accurately into a 25 ml volumetric flask separately and dissolved with HPLC grade methanol. Serial dilutions were prepared from these solutions using methanol. Triplicate injection of standard solutions were applied. The peak areas and the corresponding concentrations were used to construct the standard curves. Six points were used for each graph and standard linear regression was used to determine the slope and intercept.

RESULTS and DISCUSSION

In this study, cucurbitacins in the chloroform and methanol extracts of *B. multiflora* and *B. alba* roots were analyzed by HPLC. Because of the different solubility of cucurbitacins, calculations were made in two different extracts. The solubility of cucurbitacin B in chloroform is high, but that of cucurbitacin I is low. Cucurbitacin I is more soluble in water and methanol. HPLC analysis was performed on a Lichrospher 100 RP C18 e (5 µm) column using an isocratic mixture of acetonitrile: water (2:3) at a flow rate of 1ml/min as a mobile phase[22]. Chromatograms of the plant extracts showed many peaks. The peaks were identified by comparison of retention time of standard cucurbitacins I and cucurbitacin B, which were found to be 12.60 and 23.84 min. respectively. HPLC chromatograms of methanol extracts of *Bryonia* species are shown in Figure 1.

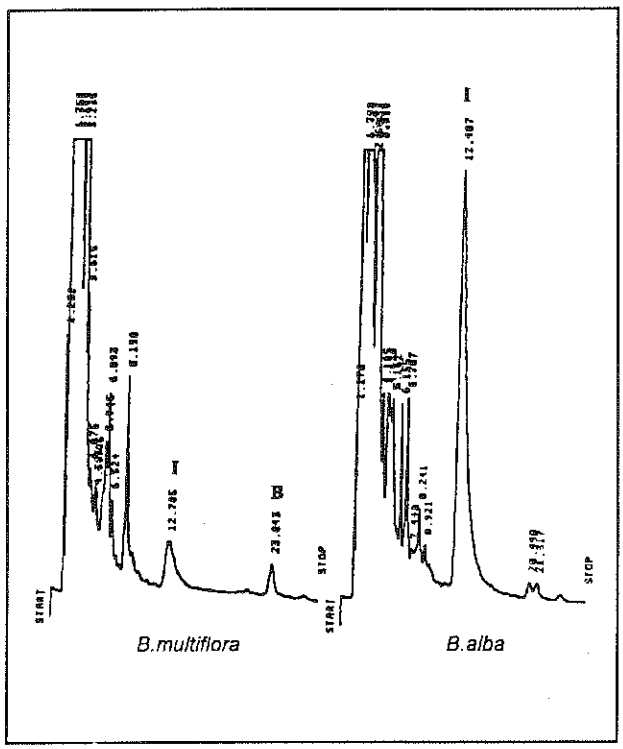

Figure 1. HPLC chromatograms of methanol extracts of *Bryonia* species I- cucurbitacin I, B-cucurbitacin B.

The detector response correlated linearly with concentrations in the ranges 1.92-38.4 µg/ml for the cucurbitacin B and 3-48 µg/ml for cucurbitacin I. The regression equations and correlation coefficients were determined Y= 1618.4X-2261.5 (r=1.000) for cucurbitacin B and Y=1250.1X-3911.5 (r=0.997) for cucurbitacin I. Y is peak height and X is concentration of standards in µg/ml. The precision of the system was tested with three successive injections of standard solutions and plant samples. Table 1 shows the cucurbitacin B and I contents of *Bryonia* species. Statistical evaluation revealed relative standard deviations at different values for three injections which are shown in Table 2.

Table1: Cucurbitacin B and cucurbitacin I amounts in *Bryonia* roots (% g dry weight)

	B.multiflora		*B.alba*	
	Methanol ext.	Chloroform ext.	Methanol ext.	Chloroform ext.
Cucurbitacin B	0.010	0.022	Trace	Trace
Cucurbitacin I	0.021	0.0098	0.125	0.0348

Table 2: Cucurbitacin B and cucurbitacin I contents (µg/ml) in *Bryonia* species with relative standard deviation (RSD)

	Cucurbitacin B			Cucurbitacin I		
Samples	Mean±SEM*	SD**	RSD(%)	Mean±SEM*	SD**	RSD(%)
B.multiflora						
Methanol ext.	4.24±0.06	0.10	2.36	8.50±0.08	0.15	1.76
B.multiflora						
Chloroform ext.	22.30±0.34	0.59	2.65	9.80±0.08	0.11	1.12
B.alba						
Methanol ext.	--	--	--	50.20±0.24	0.34	0.68
B.alba						
Chloroform ext.	--	--	--	37.57±0.01	0.01	0.026

*SEM: Standard error of mean

**SD: Standard deviation , ext: extract.

TLC analysis of samples were performed using S_1 and S_2 solvent systems. Rf values of cucurbitacin B and cucurbitacin I were 0.35 and 0.25 in system S_1 and 0.66 and 0.60 in system S_2 respectively.

HPLC analysis of the roots of *B. alba* revealed that cucurbitacin I was the main component and determined in high concentration (0.125%) in methanol extracts and low concentration (0.034%) in chloroform extracts. Cucurbitacin B was found almost in trace amounts in both extracts.

The main cucurbitacin of *B. multiflora* roots is cucurbitacin B in chloroform extracts (0.022%) which is in low concentration in methanol extracts (0.010%).

Cucurbitacin I was also found as the main cucurbitacin in methanol extracts (0.021%) and in low concentration in chloroform extracts(0.010%). The results obtained from HPLC analysis were compared with those of TLC.

It is generally accepted that cucurbitacins are highly cytotoxic, particularly cucurbitacin I. Cucurbitacin B significantly inhibited both serotonin and bradykinin induced-edemas in mice and showed a potent antiinflammatory activity[21-22] and liver protective effects have been shown on rats with experimentally induced liver damage[23].

This is the first study about the chemical composition of *B. multiflora* and also *B. alba* growing in Turkey. It was proved that cucurbitacin B is present in addition to cucurbitacin I in B. multiflora roots. The great spectrum of biological activities of cucurbitacin B is of special interest considering the medicinal use of *B. multiflora* for the treatment of liver diseases, rheumatism and arthritis. However cucurbitacin B was found in low concentration.

Acknowledgement: This work was supported by Research Foundation of Gazi University (Project code No: SBE11/ 96-20)

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