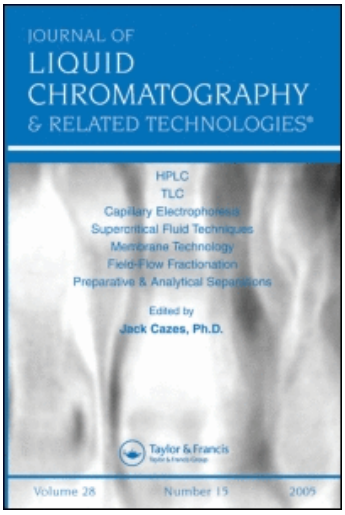

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High-Performance Liquid Chromatographic Determination of Iridoids in Cruciata Taurica

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF IRIDOIDS IN CRUCIATA TAURICA

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ABSTRACT

The aerial and underground parts of Cruciata taurica (Pallas ex Willd.) Ehrend. s.l.(Rubiaceae) yielded three iridoids. Of these, Monotropein, Asperuloside and Aucubin. These compounds were separated and quantitated by reversed-phase HPLC.

INTRODUCTION

In the previous papers(1,2), the plant Cruciata taurica was investigated in regard to botanical properties and chemical constituents. Continuing our work on iridoids from C.taurica, we have now detected three iridoid glycosides. Of the possible methods for the analysis of iridoids(3), high-performance liquid chromatography is becoming the most widely used because it is fast, accurate, reproducible and can be used to determine both individual and total iridoids. Recently, there have been several HPLC methods developed for iridoid analysis(4-9).

In this paper, a separation and determination of iridoids of C.taurica using HPLC is reported.

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EXPERIMENTAL

The plant material of Cruciata taurica (Pallas ex Willd.) Ehrend.s.l.(Rubiaceae) used in this research were collected in May from Ahlatlıbel,Ankara,Turkey. Herbarium specimens are stored in the herbarium,Faculty of Pharmacy, University of Ankara.

The powdered aerial and underground parts of C.taurica (1 g) were extracted with methanol under reflux for 3 hr. The methanolic extract was concentrated to dryness and the residue was dissolved in bidistilled water and diluted with bidistilled water to 20 ml*. The aqueous layer was filtered through "Sep-Pak" silica cartridge. 5 Ml of filtrat were injected to HPLC column.

High-performance liquid chromatography was carried out in an M 730 Waters Liquid Chromatograph Data Module equipped with model 6000 A pump dual reciprocating piston heads model U6 K septumless injector, a 660 Solvent programmer, M 450 variable-wavelength detector and M Bondapak C_{18} column(300 x 3.9 mm). The HPLC mobile phase was methanol-water(20-80 % by volume). The mobile phase components were degassed by immersion in an ultrasonic bath and filtered through a Millipore HA(0.45 Mm) membrane filter. Flow rate was 2 ml/min. The effluent was monitored at 230 nm and detector sensitivity 0.04 aufs. Chart speed was 0.5 cm/min.

*Full details of the isolation of the iridoids are available on request to the authors.

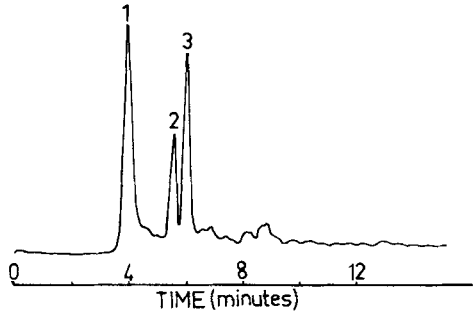

Figure 1. Isocratic analysis of iridoids derived from aerial parts of Cruciata taurica (Peak 1:Monotropein, Peak 2:Aucubin, Peak 3:Asperuloside)

RESULTS AND DISCUSSION

The following separation was achieved by using the mixed iridoids of C.taurica. An isocratic HPLC chromatograms are illustrated in figure 1 and 2.

Retention times obtained for monotropein, aucubin and asperuloside using this method were 3.95, 5.55 and 6.00 minutes, respectively. The values were also obtained by iridoids isolated from this plant. All isolated iridoids were identified by standart spectral data(6,10,11), as well as by authentic sample comparison.

The HPLC chromatogram of the aerial parts of C.taurica showed three distinct peaks: These are monotropein(38.1 %), asperuloside(26.3 %) and aucubin(18.6 %). The underground parts of C.taurica contained two iridoid compounds, monotropein(51.7 %) and asperuloside(4.4 %) but not aucubin.

The HPLC analysis of iridoid constituents of plants has been used extensively.

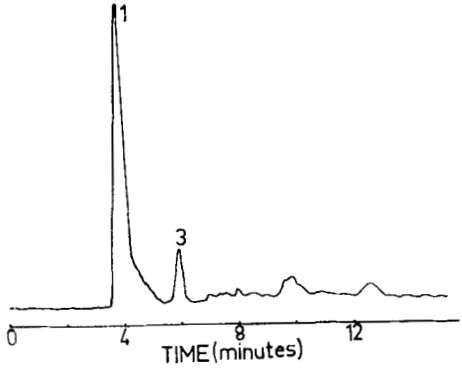

Figure 2. Isocratic analysis of iridoids derived from underground parts of Cruciata taurica.

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