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HPLC-PDA DETERMINATION OF PSEUDO TARAXASTEROL (Ψ-TARAXASTEROL) IN DIFFERENT *COUSINIA* SPECIES

FARKLI *COUSINIA* TÜRLERİNDE PSÖDO TARAKSASTEROL (Ψ-TARAKSASTEROL) BİLEŞİĞİNİN HPLC-PDA İLE MİKTAR TAYİNİ

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ABSTRACT

Cousinia Cass. is one of the widespread genera of Asteraceae family. There is a large number ofpharmacological activity studies on this genus and it has not yet been examined phytochemically in detail. Pseudo taraxasterol (*ψ*-taraxasterol) is a pentacyclic triterpene with different pharmacological activity such as antinociceptive, anti-inflammatory. In this report, we aimed to evaluate the *ψ*-taraxasterol content, which isolated from *C. stenocephala* in seven *Cousinia* species by high-performance liquid chromatography-photodiode array detector (HPLC-PDA) method. According to quantitative analyses the highest content of *ψ*-taraxasterol was detected in *C. davisiana*, *C. stenocephala,* and *C. ramosissima* extracts (0.423±0.004, 0.395±0.004 and 0.374±0.000 g/100g$_{extract,}$ respectively). This is the first report on the quantification of pseudo taraxasterol by HPLC-PDA method in this genus, and this result will shed new lights on the advanced biological activity studies on species of this genus.

Keywords: Asteraceae, *Cousinia*, HPLC-PDA, pentacyclic triterpene.

ÖZ

Cousinia Cass. cinsi Asteraceae familyasına ait geniş yayılış alanına sahip cinslerden biridir. Bu cins üzerinde yok denecek kadar az farmakolojik etki ve fitokimyasal çalışmalar bulunmaktadır. Psödotaraksasterol (*ψ*-taraksasterol) antinosiseptif, anti-enflamatuvar aktiviteye sahip pentasikliktriterpen yapısında bileşiktir. Bu çalışmada *C. stenocephala* bitkisinden izole edilen *ψ*-taraksasterol bileşiğinin yedi farklı *Cousinia* türünde HPLC-PDA metodu ile miktar tayini yapılmıştır. Elde edilen sonuçlara gore enyüksek *ψ*-taraksasterol miktarı *C. davisiana*, *C. stenocephala* ve *C. ramosissima* ekstrelerinde bulunmuştur (sırasıyla, 0.423±0.004, 0.395±0.004 ve 0.374±0.000 g/100g$_{ekstre}$). *ψ*-taraksasterol bileşiği bu çalışmada HPLC-PDA metodu ile bu cinste ilk defa tayin edilmiş ve bu sonuçların cinsin farklı türlerinin çeşitli biyolojik aktivite araştırmalarında faydalı olacağı düşünülmektedir.

Anahtar kelimeler: Asteraceae, *Cousinia*, HPLC-PDA, pentasiklik triterpene.

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INTRODUCTION

Cousinia Cass. is one of the most diverse genera of Asteraceae family. This genus consists of 600-700 species distributed in Central and South-West Asia (1) and represented withsix sections and 38 speciesin Turkey,which is 26 of them are endemic (2,3). Some species of this genera were traditionally used for the treatment of various disorders as respiratory problems, ulcers, rheumatism and inflammation (4,5). Based on previous studies cytotoxic, antioxidant, antibacterial, and hypnotic activity of different *Cousinia*species were reported (4,6-8). To date, sesquiterpene lactones (*C. Picheriana* Bornm. ex Rech.f.*, C. Piptocephala* Bunge.*, C. canescens*DC.), triterpenes (*C. Adenostica* Bornm.), steroids (*C. canescen* DC.), and flavonoids (*C. verbascifolia* Bunge.) have been isolated from *Cousinia* species.

The species belonging to the Asteraceae family contains various types of pentacyclic triterpenoids (e.g. oleanane, ursane, and taraxastane types). Nevertheless, due to their various pharmacological activities including antiangiogenic, anti-inflammatory as well as antioxidant effects and the ability to enhance cell differentiation, they are widely used in medicine (9).There are some derivatives of pentacyclic triterpenes as ursane, gammacerane, lupane, and hopane(10).Among them, our main focus is on the pseudo taraxasterol. This compound is aursane type triterpene (urs-20-en-3-ol) also known as gamma-taraxasterol, heterolupeol, or calendol. The molecule formula of this compound is $C_{30}H_{50}O$ and containing six isoprene units (Figure I).

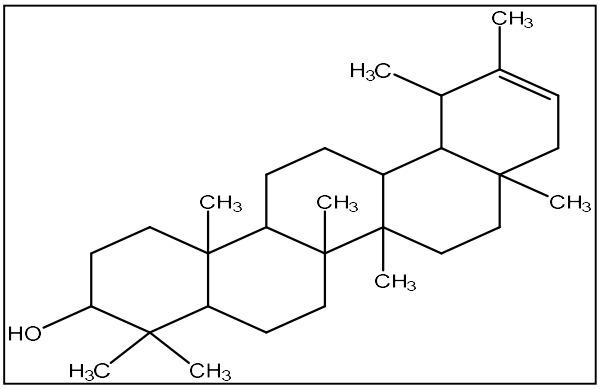

Figure I. Chemical structure of *ψ*-taraxasterol.

Pseudo taraxasterol mostly found in alcoholic beverages, dandelion root (*Taraxacum officinale*L.) and germinating seeds of *Calendula officinalis* L. (11). Although this compound was isolated from different plants the biological activity research on this compound is a few in literature. In a study, the anti-inflammatory and antinociceptive activity of thetriterpenes *β*-amyrin, taraxasterol, and pseudo-taraxasterol were investigated. As a result, it was shown that the oral administration of triterpenes to mice inhibited peritoneal leukocyte infiltration. Also, the results demonstrate the anti-inflammatory and antinociceptive activity of the triterpenes were decreased when these were acetylated, while the acetylated triterpenes in mixture with myristyloxy triterpenes improved this activity (12).

In this study, we aimed to investigate qualitative and quantitative analyses of pseudo taraxasterol in seven *Cousinia* species by HPLC-PDA method.

MATERIAL AND METHOD

Chemicals

Chromatographic grade distilled water, HPLC grade methanol analytical grade formic acid was used for HPLC analyses. Pseudo taraxasterol was isolated from *C. Stenocephala* Boiss (13).

Plant Material and Preparation of Extracts

The flowering aerial parts of *C. Aintabensis* Boiss. & Hausskn.*, C. davisiana* Hub.-Mor.*, C. ermenekensis* Hub.-Mor.*, C. foliosa* Boiss. & Bal, *C. iconica* Hub.-Mor.*, C. ramosissima* DC.*, C. stenocephala* Boiss. were harvested and identified by Prof. Dr. Osman Tugay. The specimens were stored at the Herbarium of Selçuk University. The details of the location and voucher number of plants were given in Table I.

Air-dried aerial parts of species (100 g) were powdered and extracted three times with methanol by maceration (during 24h), at room temperature. Combined macerates filtered and evaporated to dryness under reduced pressure at 37°C using a rotary evaporator. The crude extracts were stored in dark at -20°C. The yields of extracts were shown in Table I.

HPLC-PDA Assay

The high-performance liquid chromatographic apparatus (Shimadzu Prominence / LC-20A) with a degasser, pump (LC-20AT) and a controller coupled to a SPD-

Table I. Localities, voucher no and extract yields (%) of species.

Plant material	Localities of plants	Herbarium Voucher No	Yields (%)/Extract code
C. aintabensis	Mardin, Turkey; July, 2013	KNYA 11.040	10 (CA)
C. davisiana	Ermenek/Karaman, Konya, Turkey; July, 2013	KNYA 26.976	10 (CD)
C. ermenekensis	Ermenek/Karaman, Konya, Turkey; July 2013	KNYA 26.976	10 (CE)
C. foliosa	Ahir Dagı, Kahramanmaraş, Turkey; June, 2013	KNYA 26.977	8,7 (CF)
C. iconica	Ermenek/Karaman, Konya, Turkey; July 2013	KNYA 77.81	15 (CI)
C. ramosissima	Birecik/Şanlıurfa, Turkey; May, 2013	KNYA 26.978	10 (CR)
C. stenocephala	Ceylanpınar/Şanlıurfa, Turkey; July, 2013	KNYA 26.979	10 (CS)

M10Avp photodiode array detector equipped with an automatic injector interfaced to Class VP chromatography manager software. The instrument settings were performed according to the method by Sharma and Zafer (14) with slight modifications: column GL Inertsil ODS-3 (4.6mm x 250mm x 5µm), column temperature was 40°C, a flow rate of 0.8 mL/min. Detection was carried out with a sensitivity of 0.1 aufs (absorbance units full scale) between 190 and 550 nm. A mixture of methanol (A) and water: formic acid (99:1, v/v) (B) was selected as the mobile phase. The mobile phase consisted of 80% solvent A and 20% solvent B at a flow rate of 0.8 mL/min, and the injection volume was 20 µL. The retention time of the compound was 2.5 min. A 10 min equilibrium time was allowed between injections
ψ-taraxasterol and sample solutions were injected three times. Compound was prepared in methanol and seven different concentration levels (0.025-1 mg/mL) were injected for the establishment of calibration curves (y = 4642.6x – 15507; r^2 =0.9984).

RESULTS

Quantitative HPLC-PDA Analyses of Pseudo Taraxasterol

The quantitative results of the compounds are given in Table II.

Both the retention times and UV spectra were used to identify the *ψ*-taraxasterol. The quantities of the substance detected in the extracts were determined using a calibration curve. The chromatograms of *ψ*-taraxasterol and extracts of *Cousinia*species were shown in Figure II.

Table II. Content of *ψ*-taraxasterol in *Cousinia* extracts.

Cousinia species	Content*
CA	0.245±0.002
CD	0.423±0.004
CE	0.148±0.008
CF	0.214±0.005
CI	0.224±0.001
CR	0.374±0.000
CS	0.395±0.004

*Mean (g/100$g_{extract}$) ± SD (n=3); **CA:***C. aintabensis* extract, **CD:***C. davisiana* extract, **CE:***C. ermenekensis* extract, **CF:***C. foliosa* extract, **CI:***C. iconica* extract, **CR:***C. ramosissima* methanol extract, **CS:***C. stenocephala* extract.

As shown in the table II, the highest content of *ψ*-taraxasterol was found in *C. Davisiana* Hub.-Mor, *C. stenocephala* Boissand *C. ramosissima* DC extracts (0.423±0.004, 0.395±0.004 and 0.374±0.000 g/100$g_{extract}$ respectively). This is the first report of quantitative analyses of pseudo taraxasterol in *Cousinia* species by HPLC-PDA method.

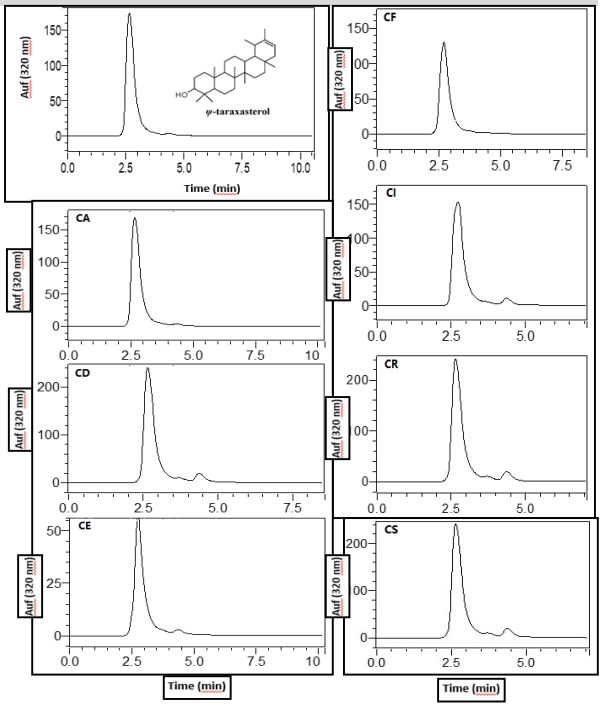

Figure II. HPLC-PDA analyses of *ψ*-taraxasterol and *Cousinia* extracts with responses at 320 nm;CA: *C. aintabensis* extract, CD: *C. davisiana* extract, CE: *C. ermenekensis* extract, CF: *C. foliosa extract*, CI: *C. iconica extract*, CR: *C. ramosissima* extract, CS: *C. stenocephala* extract

DISCUSSION

Based on chemical reports on a *Cousinia* species various chemical compounds including steroids, triterpenes, sesquiterpene lactones, and flavonoids were declared (13,15-22). *ψ*-taraxasterol is a pentacyclic triterpene that was isolated from *Pluchea quitoc* DC., *Jatropha curcas* L., *Euphorbia nematocypha* Hand.-Mazz., *Tanacetum vulgare* L., *Achillea millefolium* L., *Kalanchoe pinnata* (Lam.) Pers.,*liatris ohlingerae* (S.F.Blake) B.L.Robe, *Calotropis procera* (Aiton) Dryand., *Bryophyllum pinnatum* (Lam.) Kurz and *Sonchus arvensis* L. (23-30). Pentacyclic triterpenes have a wide spectrum of biological activities as cytotoxic, antifungal, antitumor, antiviral (31-33). To the best of our knowledge antinociceptive and anti-inflammatory effect of *ψ*-taraxasterol have been reported in the literature (34).

In this study, *ψ*-taraxasterol isolated previously from *C. stenocephala* (13) were selected and standardization of the methanol extracts of seven *Cousinia* species were achieved for the first time using a simple and efficient HPLC-PDA method. So identification and quantification of *ψ*-taraxasterol in *Cousinia* species will promote advanced studies that may help to protect against free radical damage and oxidative stress-related diseases. Moreover, because of the limited studies on *Cousinia* species and identified compounds, this qualitative and quantitative study combined with activity evaluation will shed new light on the advanced studies.

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