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Investigation of Podophyllotoxin in some Plants in Lamiaceae Using HPLC

Türkiye'de Yetişen Lamiaceae Familyasındaki Bazı Türlerde Podofilotoksin'in Yüksek Basınçlı Sıvı Kromatografisi ile Tespiti

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SUMMARY

High pressure liquid chromatography has been used for the separation and the quantitation of podophyllotoxin from crude extracts of *Teucrium polium, Teucrium chamaedrys, Nepeta nuda* ssp. *gladulifera. Thymus capitatus, Phlomis nissolii* and *Salvia cilicica.* The percentage of podophyllotoxin is highest in *Teucrium chamaedrys* (0.14%) and *Nepeta nuda* ssp. *gladulifera* (0.11%) respectively.

ÖZET

Teucrium polium, Teucrium chamaedrys, Nepeta nuda ssp. *glandulifera, Thymus capitatus, Phylomis nissolii* and *Salvia cilicica'dan* elde edilen ekstreler yüksek basınçlı sıvı kromatografisi ile podofilotoksin miktarı açısından incelendi. *Teucrium chamaedrys* (0.14%) ve *Nepeta nuda* ssp. *glandulifera* (0.11%) incelenen türler arasında yüksek oranda bu etken maddeyi taşıdığı saptandı.

Key **Words:** Aryltetralin lignans, HPLC, Lamiaceae, Podophyllum lignans, podophyllotoxin.

INTRODUCTION

Lignan is the name *given* to a large family of natural products that contain the 2,3 dibenzylbutane skeleton. Lignans especially podophyllotoxin, is of interest to scientists because of their antimitotic and antitumour activity (1). The podophyllotoxin derivatives teniposide and etipo-

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side have been used in the chemotherapy of cancers (2). Podophyllotoxin is stili extracted from the roots of *Podophyllun peltatum* and *P. hexandrum* plants collected in the wild. Chemical synthesis is possible, but complex and uneconomic (2). Therefore there is an increasing interest in additional sources for supply of podophyllotoxin.

The interest of lignans has been greatly increased because of isolation and characterization of lignans from animal sources including human urine (3-6). This research is concerned with the detection of podophyllotoxin from some plant species of Family Lamiaceae (genus *Nepeta, Salvia, Teucrium, Thymus, Phlomis)*. Lamiaceae is a family of herbs or shrubs usually glandular and aromatic, comprising about 400 species divided into 45 genera in Turkey. Only three phytochemical studies dealing with lignans content of *Hyptis tomentosa* and *H. verticillata* have been reported to date (7-9).

The reason of choosing the phytochemical analysis was due to the fact that there had not been any studies on aryltetralin lignans in these Turkish species.

TLC have been useful but not entirely definitive for the analysis of the aryltetralin group lignans, therefore HPLC method was selected as the metod of choice for this determination.

The analytical separation of some aryltetralin lignans by high-pressure liquid chromatography (HPLC) has been described by using reserve- phase column in the literature (10-15). In previous study was found that acetonitrile -water (35:65) system gave the best separation on C18 column (16).

EXPERIMENTAL

Plant material: Plants were collected from different regions in Turkey. The voucher specimen of samples have been deposited in the private Herbarium of Prof. E. Şarer and Herbarium of Faculty of Pharmacy, Ankara University (AEF 18709, 18711, 19565).

Chromatographic Conditions:

High-pressure liquid chromatography-Waters Assoc. equipped with U6K Universal injector, Waters 6000 A pumps,

Column: Spherisorb- ODS (25 mm x 4.6 mm i.d).

Mobil phase: Acetonitrile: water (35:65) isocratic system

Flow rate: 1 ml/min

Detection: Hewlett-Packard photodiod-array detector coupled with computer wavelength: 238-242 nm

HPLC separation:

Standard preparation: 0.7 mg podophyllotoxin was weighed into a 25 ml of volumetric flask and dissolved in HPLC methanol. The sample was injected under the described chromatographic conditions and the retention time was recorded.

Series dilutions were prepared from these solutions into 10 ml of volumetric flask and they were used for the calibration curve. Least-squares linear regression analysis was used for the quantitative determination. The equation of linear regression of the calibration line and correlation coefficient were determined for podophyllotoxin where Y present the peak area and X the amount (u.g) of podophyllotoxin (Y=33.826+33.974).

Sample preparation: Plant samples (aerial) were analyzed after enzymatic hydrolyses. Dried and powdered plant samples (200 mg) were hydrolyses by using 6-glucosides in 9 ml water was added and the mixture was stirred at 37°C. After 4 hours incubation the solution was extracted with dichlorometan (10x2). The dicholorometan extract was trasferred to a flask and evaporated to dryness. 1.5 ml HPLC grade methanol was added and the sample was prepared for analysis.

RESULTS AND DISCUSSION

Lim and Ayres stated that "the purification of lignans by recrystalization is often difficult because of the tendency of lignans to aggregate or complex with each other; HPLC provides on effective and powerful technique for their separation" (12). The usefulness of the method, is detecting the presence of aryltetralin lignans in new plant samples. Since podophyllotoxin is one of the more important lignan, it was selected for the initial study. This technique represents a rapid and effective method for detecting the presence of podophyllotoxin and other lignans in a plant extract. Direct proof of the presence of a particular lignan will, depend on its isolation and characterization, but this rapid test will serve to detect these compounds in crude extract.

in this study, podophyllotoxin content in six species from five genus has been investigated using HPLC. Quantitative analysis was performed on C-18 bonded silica gel phase using acetonitrile-water (35:65) as mobile phase and isocratic elution. The percentage of podophylotoxin is given in Table 1. According to the quantitative results, the content of podo-

phyllotoxin is highest in *Teucrium chamaedrys* (0.14%) and *Nepeta nuda* ssp. *glandulifera*

(0.11%) respectively.

This is the first report of these five genus for podophyllotoxin content. The aim of this study was to state whether podophyllotoxin exists in the other species in Lamiaceae where there are only three studies (6-8) from the point of view of lignan. According to these results, further studyies on these species which are commonly found in Turkey;

1- To make use of the species high in podophyllotoxin for economic purposes,

2- To increase the rate of podophyllotoxin by the was of cell culture of the species high in podophyllotoxin.

Table 1: Podophyllotoxin percentages in six from Fam. Lamiaceae growing in Turkey.

Genus name	Species name	*(%)*
Nepeta	**N.nuda* ssp. *glandulifera*	0.11
Phlomis	**P. nissoli*	0.05
Salvia	**S. cilicica*	0.08
Teucrium	*T.polium*	0.09
	T. chamaedrys	0.14
Thymus	*T. capitatus*	0.05

* endemic species

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