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THE QUANTITATIVE DETERMINATION OF THEBAINE AND NARCOTINE BY HPLC IN SOME TURKISH *PAPAVER* SPECIES

TÜRKİYE 'DE YETIŞEN BAZI *PAPAVER* TÜRLERİNDE HPLC İLE TEBAİN VE NARKOTİN MİKTAR TAYİNİ

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The amounts of narcotine and thebaine have been determined by HPLC in some Papaver species growing wild in Turkey. P.bracteatum and P.fugax have been found to contain high yields of thebaine whereas P.cylindricum was shown to contain narcotine as the major alkaloid. The yield of thebaine in P.bracteatum of Turkish and Iranian origin has also been compared.

Bu çalışmada Türkiye'de yabani olarak yetişen bazı Papaver türlerinde, HPLC ile narkotin ve tebain miktarları tayin edilmiştir. P.bracteatum ve P.fugax türlerinin yüksek oranda tebain içerdiği buna karşılık P.cylindricum türünün ana alkaloit olarak narkotin taşıdığı saptanmıştır. Ayrıca P.bracteatum türünün Türkiye'de yetişen ve İran kökenli örnekleri tebain miktarı bakımından karşılaştırılmıştır.

Keywords : *Papaver bracteatum; Papaver fugax; Papaver cylindricum; Thebaine; Narcotine; HPLC*

Anahtar kelimeler: *Papaver bracteatum; Papaver fugax; Papaver cylindricum; Tebain; Narkotin; HPLC*

Introduction

In previous works, *P.bracteatum and P.fugax* have been found to contain high yields of thebaine whereas *P.cylindricum* was shown to contain narcotine as the major alkaloid (1-4). *P.bracteatum* growing wild in Eastern Turkey has been shown to be different in major alkaloid content, as having both thebaine and salutaridine than Iranian *P.bracteatum* containing only thebaine(2).

Narcotine, which is a powerful antitussive, is obtained from natural sources. Some Turkish *Papaver* species including *P.cylindricum* have been found to contain this alkaloid as the major one (3).

The amounts of thebaine and narcotine have been determined by HPLC to evaluate the use of *P.bracteatum* and *P.cylindricum* for the production of these alkaloids.

Materials and Methods

Plant Material: P.bracteatum, P.fugax and P.cylindricum were collected from the eastern part of Turkey in 1986. Voucher specimens are deposited in the herbarium of Faculty of Pharmacy, Istanbul (ISTE). *P.bracteatum* Arya II from Iran origin was cultivated in the gardens of the Faculty of Agriculture in Ankara. The collection data and the herbarium numbers of the specimens are given in Tables 1 and 2.

Extraction of Alkaloids

P.bracteatum: Dried capsules were powdered 0.6 g of sample was weighed accurately and moisturized with 3 ml of distilled water. 2 g of aluminium oxide (Merck, active acidic, Activity I) was added to a glass column (150X15 mm) containing 4 g of aluminium oxide held in place with a cotton plug. Thebain was eluted from the column with 5% CH_3COOH at a flow rate of 30 drops/min, until negative test with Dragendorff's reagent. The eluated was transferred into a 100 ml volumetric flask and adjusted to volume with CH_3COOH (c=6 mg/ml).

P.fugax: Dried capsules were powdered, 2.5 g of sample weighed accurately was moisturized with 5 ml of a mixture of CH_3OH-25% ammonia (3:1, v/v) and allowed to stand for 20 minutes. After the addition of 50 ml of $CHCl_3$ the mixture was shaken for 40 minutes and then filtered through a filter. The filtrat and the residue were reextracted twice with $CHCl_3$ by shaking. Combined filtrates were transferred to a glass capsule and evaporated to dryness on a water bath. The residue dissolved with the addition of 5% CH_3COOH (5 ml) was transferred into a 50 ml volumetric flask and diluted to volume with CH_3OH (c=50 mg/ml).

P.cylindricum: 1 g of dried and powdered capsules accurately weighed was extracted as in *P.fugax* (c=20 mg/ml).

Chemicals: All solvents (Merck) were HPLC grade. n-Heptanesulphonic acid was purchased from Fluka

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Table 1. Amount of thebaine and salutaridine in *P.bracteatum* and *P.fugax*

Sample (ISTE No.)	Collection Data	Thebaine % SD (n=6)	Salutaridine % SD (n=6)
P.bracteatum (ISTE 57106)	Munzur/Tunceli, 2150 m (28-31.7.1986)	1.42 0.10	0.52 0.04
P.bracteatum Arya II	Cultivated, Fac. of Agriculture, Ankara (25.8.1987)	4.15 0.19	---
P.fugax (ISTE 57051)	Toklular/Karlıova, 1400-1700 m (21-23.7.1986)	0.77 0.18	---

Table 2. Amount of narcotine in *P.cylindricum*

Sample (ISTE No.)	Collection Data	Narcotine % SD (n=6)
P.cylindricum (ISTE 57054)	Aktuzla köprüsü /Malazgirt 1730 m (23-24.7.1986)	0.43 0.03
P.cylindricum (ISTE 57055)	Bozdağ/Tutak, 1900-2200 m (24-26.7.1986)	0.80 0.10

and narcotine standard was purchased from Mallinckrodt, St-Louis, MD, USA. Thebaine and salutaridine isolated and purified during previous investigations were used as standards.

Apparatus and Chromatographic Conditions of HPLC: A Waters Liquid Chromatograph was used, equipped with a Model 510 pump, a Model U6K Universal Injector and 7127 Rheodyne Injector, a Model 481 UV Detector and Data Module Model 740 (Waters Assoc., Milford, Mass., USA). The separations were carried out using a µBondapak C 18 column (300x3.9 mm, 10 µm ID, Waters). The operating conditions of HPLC were: ambient temperature; the flow rate of eluting solvent 1.5 ml/min.; wavelength of UV detector 254 nm; sensibility of UV detector (AUFS) 0.2; pressure 2000 Psi; chart speed 2 cm/min. Mobile phase was a mixture of ammonium acetate (1%, pH=5-6)-acetonitril-tetrahydrofuran (74:25:1, v/v) containing 0.5 g n-heptanesulphonic acid (5). It was used after filtration through a HA-0.45 µm Millipore filter and degassing in an ultrasonic bath. The sample were filtered through a Millex HA-0.10 µm filter before injecting. The injection volume was 10 µl.

Preparation of the standard solutions
Thebaine: 100 mg of thebain was dissolved with the addition of CH_3OH in a 100 ml volumetric flask and adjusted to volume with CH_3OH (stock solution, c=1 mg/ml). 6 ml of the stock solution was transferred to a 10 ml volumetric flask and diluted to volume with CH_3OH (c=0.6 mg/ml).
Narcotine : 100 mg of narcotin was dissolved with the addition of 3 ml of CH_3COOH in a 100 ml volumetric flask and diluted to volume with CH_3OH (c=1 mg/ml).
Salutaridine: 100 mg of salutaridin was dissolved with the addition of CH_3OH in 100 ml volumetric flask and adjusted to volume with CH_3OH (stock solution, c=1 mg/ml). 1 ml of the stock solution was transferred to a 10 ml volumetric flask and diluted to volume with CH_3OH (c=0.1 mg/ml).

Results and Discussion

1.42% thebaine and 0.52% salutaridine have been found by HPLC in the capsules of the Turkish sample whereas 4.15% of thebaine has been determined in the capsules of the Iranian origin (population Arya II) cultivated in the Faculty of Agriculture in Ankara (Table 1). Although the amount of thebaine is lower in the Turkish sample, this can be increased by cultivation under controlled

conditions as in the case of the Iranian sample. However it can still be used for the production of thebaine since the yield of morphine is only 0.3-0.5% in the capsules of *P.somniferum* which is used for manufacturing morphine in Turkey.

HPLC analysis of the aerial parts of *P.cylindricum* collected from two different regions have revealed 0.47% and 0.80% narcotine (Table 2). This results shows that Malazgirt sample can be used for the production of narcotine since it is known that the capsules of *P.somniferum*, the main source for this alkaloid, contains narcotine up to 0.8%.

Previously we have reported the existence of thebaine as the major alkaloid in *P.fugax* of Turkish origin (1). In this work the amount of thebaine has been found as 0.76% by HPLC. These results show that *P.bracteatum* and *P.cylindricum* can be used for the production of thebaine and narcotine.

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