Iranian Journal of Pharmaceutical Research (2009), 8 (1): 65-70
Received: February 2008
Accepted: June 2008

Original Article

Determination of Hyoscyamine and Scopolamine in Four *Hyoscyamus* Species from Iran

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Abstract

The purpose of the present study was to determine the tropane alkaloid content of four *Hyoscyamus* species from Iran, *i.e*. *H. pusillus* L., *H. niger* L., *H. reticulatus* L. and *H. kurdicus* Bornm. Determination of alkaloids was performed by the HPLC method. Samples were extracted with chloroform-methanol-25% ammonium hydroxide 15:5:1 (V/V/V). HPLC separation was performed on Eurospher C_{18} reversed phase column. An isocratic mixture of triethylammonium phosphate buffer (30 Mm, pH 6.2) and acetonitrile (75:25), was used as the eluent. Hyoscyamine and scopolamine were determined by the external standard method at 210 nm. All the four mentioned *Hyoscyamus* species contained hyoscyamine and scopolamine, but in different amounts. Scopolamine was the predominant tropane alkaloid in *H. pusillus, H. niger* and *H. kurdicus*, while, *H. reticulatus* contained a higher amount of hyoscyamine.

Keywords: *Hyoscyamus*; Tropane alkaloid; Hyoscyamine; Scopolamine; HPLC.

Introduction

Hyoscyamine and scopolamine are medicinally important tropane alkaloids. Because of possessing anticholinergic and central nervous system activities, they have well-established therapeutic uses (in ophthalmology, cardiology, gastroenterology, etc.). They are synthesized in several species from Solanaceous family like the genus *Atropa*, *Datura*, *Duboisia*, *Hyoscyamus* and *Scopolia*. Industrially, these natural substances are exclusively produced by plants and the demand for them is continuous (1). *Hyoscyamus* species are rich sources of tropane alkaloids, mainly hyoscyamine and scopolamine, which are widely used for their mydriatic, antispasmodic, anticholinergic, analgesic and sedative properties (2).

Due to the pharmacological and toxicological importance of tropane alkaloids, determination of these compounds, in *Hyoscyamus* species were studied. There are several articles published in the past few decades,concerning the identification and determination of these alkaloids in medicinal plants by different analytical techniques (3-6). The total alkaloid content of the leaves of *H. reticulatus* from Anatolia was determined by a colorimetric method,and found to be in the range of 0.011-0.027% (7). Parallel with the improvement of biotechnological methods (8), HPLC techniques are also widely applied for the determination of tropane alkaloids content in different plant tissues (9-13). In vivo, hyoscyamine 6β-hydroxylase (H6H) catalyzes the epoxidation of hyoscyamine to scopolamine (14). The pharmacological uses of tropane alkaloids, especially scopolamine,

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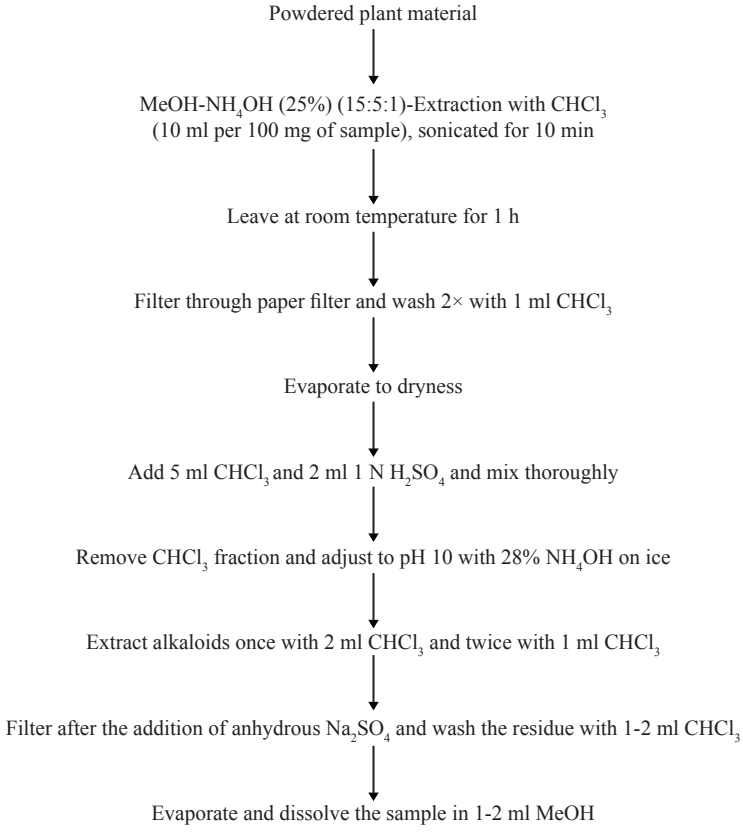

Figure 1. Scheme of the tropane extraction method used in this study, which has been described by Kamada et al. (21)

are very extensive. For example scopolamine, an antimuscarinic agent, acts as a competitive inhibitor, mimicking acetylcholine at the neural synapses and depresses the central nervous system. Its most potent activity is manifested on the iris, ciliary body, and secretory (salivary, bronchial and sweat) glands (15).

In this study the extraction and determination of hyoscyamine and scopolamine from *H. pusillus* L., *H. niger* L., *H. reticulatus* L. and *H. kurdicus* Bornm. as four medicinal plants of Iran were investigated.

Experimenal

Plant material

Different parts of four *Hyoscyamus* species, i.e. *H. pusillus* L., *H. niger* L., *H. reticulatus* L. and *H. kurdicus* Bornm. were collected from their natural habitats in Iran. The collecting date, locality information and voucher numbers of the studied species have been listed in Table 1.

Voucher specimens have been deposited at the herbarium of Medicinal Plants and Drugs Research Institute of Shahid Beheshti University (Tehran, Iran).

Extraction of total alkaloids

In order to ensure that the collected plants have tropane alkaloids, the Vitali-Morin test was used (16). Four different methods of alkaloid extraction were tested on each plant material and then the extracts analyzed using with HPLC (17-21). The best results are based on the reported procedure by Kamada at al (21), In this method higher amounts of tropane alkaloids were obtained compared to the other methods reported in the literature. However, this method detected a limited amount of additional and interference HPLC. The test procedure has been summarized in Figure 1. The resulting extract was directly subjected to HPLC analysis.

Table 1. The collecting date, locality information and voucher numbers of studied *Hyoscyamus* species.

Species	Collecting date	Locality	Voucher number
H. pusillus L.	Full flowering, May 2006	Tehran: Tehran-Qom highway, 50 Km to Qom, 1300 m	MP-1007
H. niger L.	End of flowering, beginning of fruit formation, with unripe fruits, January 2006	Tehran: Tehran-Dizin road, 2700 m	MP-1006
H. reticulatus L.	End of flowering, beginning of fruit formation, with unripe fruits, May 2006	Tehran: Tehran-Ghazvin, Barajin road, the mountain around Barajin, 1480 m	MP-1015
H. reticulatus L.	End of flowering, beginning of fruit formation, with unripe fruits, May 2006	Tehran: Tehran, after the Latyan Dam, befor Lavasan, near to Lim mineral, 1750 m	MP-1005
H. kurdicus Bornm.	Full flowering, May 2006	Tehran: Tehran-Ghazvin, Alamut road, Razjerd to Ghostinlar, 2300 m	MP-1027

Recovery studies

The standard methanolic solution of hyoscyamine and scopolamine (50 ppm) was prepared. Tropane alkaloids were recovered with 5ml aliquots of chloroform-25% and ammonium hydroxide (15:1 V/V), and then entered in to the previously described procedure shown in Figure 1.

HPLC analysis

HPLC analysis was carried out on a Knauer HPLC system (Berlin, Germany) equipped with a Eurospher C_{18} column (25 cm × 4 mm i.d., RP) and a UV detector. Elution was monitored at 210 nm.

Isocratic elution with a mixture of triethylammonium phosphate buffer (30 Mm, pH 6.2) and acetonitrile (75:25) at a flow rate of 1.0 ml/min was selected to achieve maximum separation and sensitivity. Hyoscyamine and scopolamine hydrobromide were obtained from Sigma-Aldrich. The calibration graphs for standard samples were constructed by plotting the peak area of the alkaloids against their concentrations. Linear calibration graphs were obtained with good correlation for standard solutions (Table 2).

Results and Discussion

Quantitative analysis of hyoscyamine and scopolamine were determined on various parts of *Hyoscyamus pusillus*, *H. niger*, *H. reticulatus* (in two regions) and *H. kurdicus*. The results have been summarized in Table 3, where the amount of tropane alkaloids is reported in ppm (g/g) based on the weight of the ground dry plants. As shown, all four *hyoscyamus* species contained both the above mentioned tropane alkaloids. Our findings indicated that the distribution of tropane alkaloids was different in various parts of the *hyoscyamus* species investingated.

Scopolamine was the predominant tropane alkaloid in *H. pusillus*, *H. niger* and *H. kurdicus*. This findig is in agreement with data reported studies by Supria on *H. niger* and *H. muticus* (22). These data also indicated the presence of a higher hyoscyamine content in *H. reticulates* in both regions studied. That finding is in accordance with the results reported by Jonkova (23). Higher amounts of scopolamine have been observed in the leaves of *H. pusillus* and *H. kurdicus*, as well as the seeds of *H. niger* (Figures 2-4). The seeds of *H. reticulates*, in

Table 2. Linear regression equation and correlation coefficient for hyoscyamine and scopolamine (n=3).

Compounds	Linear regression equation[a]	Correlation coefficient	LOD (mg/L)	LOQ (mg/L)
Hyoscyamine	Y= 0.3307X+24.463	0.9972	0.5	1.0
Scopolamine hydrobromide	Y= 0.3036X+8.461	0.9960	0.5	1.0

[a] Y: Area; X: Concentration

Table 3. Hyoscyamine and scopolamine contents of the four investigated native *Hyoscyamus* species from Iran.

Collection place	Plant material	Plant organ	Hyoscyamine			Scopolamine			Hyoscyamine to Scopolamine ratio
			Amount[a]	Recovery %	RSD%[b] (n=3)	Amount[a]	Recovery%	RSD%[b] (n=3)	
Tehran-Qom	*H. pusillus*	Root	0.0205	92	4.607	0.0876	96	2.768	0.2346
		Stem	0.0182	85	8.947	0.0573	84	7.053	0.3176
		Leaf	0.0385	92	1.145	0.0903	86	2.764	0.4263
Tehran-Dizin	*H. niger*	Stem	0.0173	91	8.1215	0.0529	96	3.6756	0.3271
		Leaf	0.0440	94	1.6670	0.0605	92	2.5140	0.7266
		Flower	0.0945	94	5.6043	0.1294	85	7.8112	0.7304
		Seed	0.1097	86	3.5786	0.1887	92	1.2590	0.5813
Ghazvin-Barajin	*H. reticulatus*	Root	0.0189	85	5.1570	0.0097	93	8.7840	1.9484
		Stem	0.0518	84	5.785	0.0386	86	7.8090	1.3419
		Leaf	0.0965	92	8.2040	0.0585	83	6.3150	1.6495
		Flower	0.0873	87	3.2170	0.0434	94	8.3560	2.0115
		Seed	0.1168	92	1.6070	0.0755	85	2.5680	1.5470
Tehran-Latyan Dam	*H. reticulatus*	Stem	0.1081	84	1.9580	0.0691	92	2.3940	1.5643
		Leaf	0.1214	86	7.5680	0.0813	91	8.7140	1.4932
		Seed	0.1914	92	6.4320	0.1647	94	4.0640	1.1621
Ghazvin-Razjerd	*H. kurdicus*	Root	0.0455	86	1.2145	0.0787	84	3.7880	0.5781
		Stem	0.0093	92	1.6453	0.0354	83	6.5642	0.2627
		Leaf	0.0894	93	2.8132	0.1285	94	1.6450	0.6957
		Flower	0.1031	91	3.4175	0.1277	94	8.5160	0.8073

[a] the calculated mean amount of alkaloid (g/g) , based on the weight of the ground dry plants in three replicates
[b] Relative standard deviation

both the studied regions, contained hyoscyamine in higher amounts that the other plant parts (Figures 5 and 6).

It can be concluded that, among the four studied *hyoscyamus* species, *H. pusillus*, *H. niger* and *H. kurdicus* due to the higher amount of scopolamine and *H. reticulatus* with a higher content of hyoscyamine, could be used as a rich source of these tropane alkaloids for medicinal purposes.

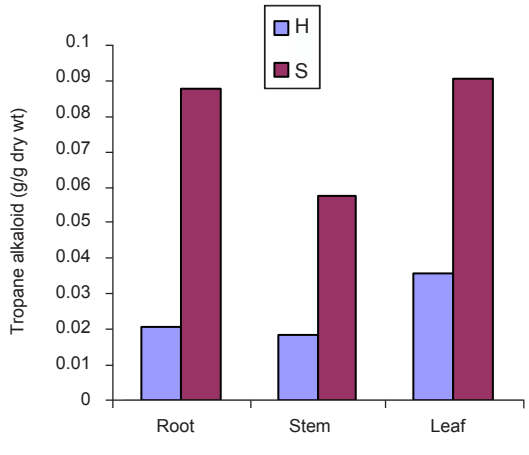

Figure 2. Amount of tropane alkaloids (**H**: Hyoscyamine and **S**: Scopolamine) determined in different tissues of *H. pusillus*.

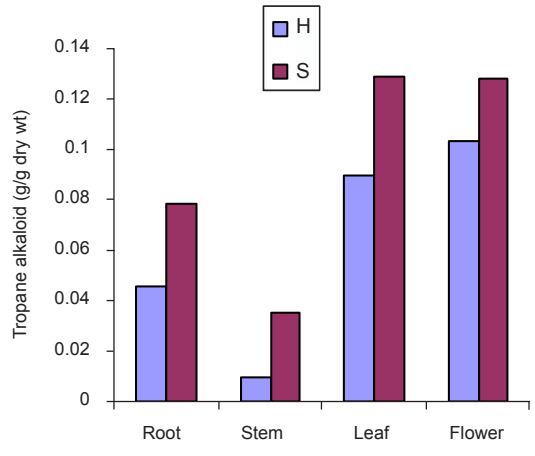

Figure 3. Amount of tropane alkaloids (**H**: Hyoscyamine and **S**: Scopolamine) determined in different tissues of *H. kurdicus*.

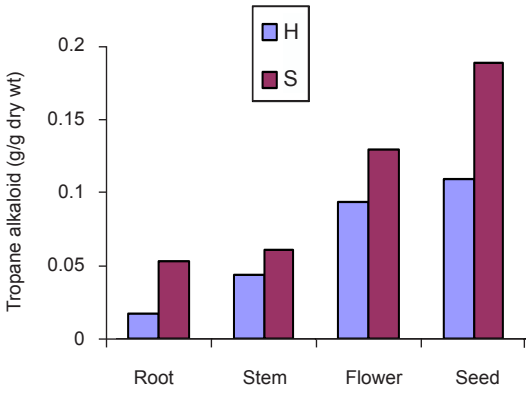

Figure 4. Amount of tropane alkaloids (**H**: Hyoscyamine and **S**: Scopolamine) determined in different tissues of *H. niger*.

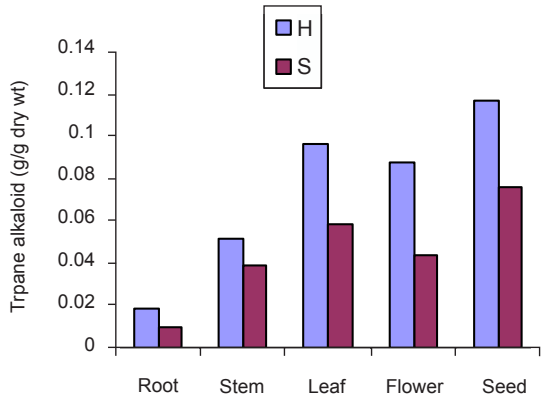

Figure 5. Amount of tropane alkaloids (**H**: Hyoscyamine and **S**: Scopolamine) determined in different tissues of *H. reticulatus* in Ghazvin-Barajin region.

In terms of the mean amount of hyoscyamine to scopolamine ratio, *H. pusillus* was the predominant species. This the due to high amount of conversion hyoscyamine to scopolamine, making it a suitable species for transportation of the H6H enzyme.

Acknowledgments

The authors would like to thank Dr. Jaimand from the Research Institute of Forests and Rangelands and also the Department of Phytochemistry, Shahid Beheshti University for their technical and financial assistances.

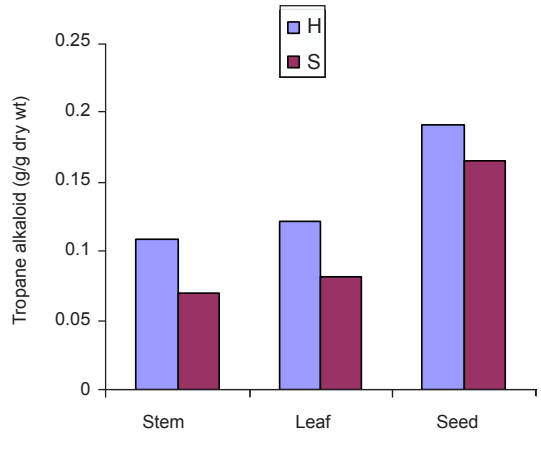

Figure 6. Amount of tropane alkaloids (**H**: Hyoscyamine and **S**: Scopolamine) determined in different tissues of *H. reticulatus* in Tehran-Latyan dam region.

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